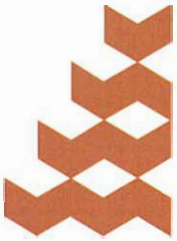
DOCTOR OF PHILOSOPHY

Mechanisms of speciation and coexistence in corydoradinae catfishes

Alexandrou, Markos

Award date:
2011

Awarding institution:
Bangor University



NATURAL ENVIRONMENT RESEARCH COUNCIL

PRIFYSGOL BANGOR UNIVERSITY

Mechanisms of Speciation and Coexistence in Corydoradinae Catfishes

A thesis submitted to Bangor University for the degree of Doctor of Philosophy

By Markos A. Alexandrou, B.Sc., MRes

March 2011

Molecular Ecology and Fisheries Genetics Laboratory

School of Biological Sciences

Environment Centre Wales

Bangor University

BANGOR

Gwynedd, LL57 2UW

Dedication

To my parents, who both inspired me to be where I am today.

Abstract

Nothing in evolutionary ecology makes sense except in the light of comparative multidisciplinary evidence. In light of this view, I employed a variety of techniques to study the mechanisms of speciation and coexistence in Corydoradinae catfishes. Specifically I used a combination of molecular phylogenetics (using mitochondrial and nuclear markers), stable isotope analyses (of carbon and nitrogen), geometric morphometrics, colour pattern analyses, and quantification of total nuclear DNA content. These data have been combined and analyzed using comparative frameworks in order to address the following hypotheses: (a) that positive interactions among mimetic species outweigh the negative effects of competition for resources; (b) that genome duplications drive diversification; (c) a number of available hypothesis that account for Corydoradinae patterns of historical biogeography. I establish the first comprehensive molecular phylogenetic framework for the Corydoradinae supporting the existence of nine well supported lineages. I show that in at least 24 sympatric assemblages, Corydoradinae catfishes form Müllerian mimicry rings composed of ecologically, morphologically and phylogenetically differentiated species. Genome size estimation and phylogenetic analysis revealed that polyploid lineages are derived in comparison to diploid lineages, and that polyploids are significantly more species rich than diploids. Furthermore, biogeographic analyses show that the majority of cladogenesis occurred within basins and not as a result of paleobasin vicariance. These results support the following conclusions: (a) that negative interactions, such as competition for resources, and phylogeny determine community structure among mimetic species thereby outweighing the effects of positive interactions such as mimicry; (b) that ancestral genome duplication within the Corydoradinae accelerates diversification rates; (c) Taxon Pulses, Paleogeography and Hydrology, and Phylogenetic Niche Conservatism account for spatiotemporal patterns of distribution. Moreover, I discuss a number of different mechanisms of speciation (colour pattern convergence, morphological divergence, genome duplication and allopatric speciation) and coexistence (resource partitioning, mutual defence through shoaling and colour pattern convergence in sympatry) relating to the Corydoradinae. All results are discussed in the context of evolutionary ecology at different temporal and spatial scales, in order to shed more light on the complex history of these fascinating catfishes.

List of Publications

Alexandrou MA, Oliveira C, Maillard M, McGill RA, Newton J, Creer S, Taylor MI. 2011. Competition and phylogeny determine community structure in Müllerian co-mimics. *Nature* 469: 84-88.

Mable B.K., Alexandrou M.A. and Taylor M.I. 2011. Genome duplication in amphibians and fish: An extended synthesis. *Journal of Zoology* (Invited Review).

Alexandrou MA and Taylor MI. 2011. Evolution, ecology and taxonomy of the Corydoradinae revisited. In Identifying Corydoradinae Catfish: *Aspidoras-Brochis-Corydoras-Scleromystax*-C-numbers & CW-numbers. Supplement No1 (Eds Ian A. M. Fuller and Hans-Georg Evers). Ian Fuller Enterprises.

Acknowledgements

Firstly and foremost, I thank Martin Taylor for being an amazing supervisor and putting up with my incessant questioning. I thank Claudio Oliveira, without whom this project would have been impossible. I thank Juan Montoya-Burgos, Mark Sabaj Perez, Ian Fuller, Hans-Georg Evers, Mark Walters and Kim Mathiesen for providing tissue samples and photographs, and Amanda Orchard for photographing preserved specimens. I would also like to thank Brent Emerson, Graeme D. Ruxton, for their suggestions and critical evaluation of some of the work. I thank Gary R. Carvalho, Simon Creer, Wolgang Wüster, Roger Thorpe, Jenny Rock, Marjorie Maillard, Phil Donoghue, John Lundberg, Luke Harmon, Michael Alfaro, Petr Rab, Michael Hardman, Lukas Ruber, Yann Surget-Groba, Dunja K. Lamatsch, Ralf Britz, and Giacomo Bernardi, for their advice and help throughout the project. I would like to thank the staff and students of UNESP, Botucatu, Brazil for facilities and research support during fieldwork including Paolo Venere and Guilherme Lopes for helping to collect samples in the Rio Araguaia, and Marcello Britto for identifying samples.

I thank Barbara K. Mable for giving me permission to include the polyploidy review in my appendices, as she is lead author on the publication and contributed the majority of the writing (with the exception of the sections on fish which I co-authored with Martin Taylor). I thank Marjorie Maillard for her help on PCRs and the amplification and sequencing of a large number of samples. I thank Rona McGill and Jason Newton for their help with the stable isotope analyses. I thank Richard FitzJohn for his invaluable help with the QuaSSE analyses, and advice on the diversification rates analyses. I thank all staff and members of MEFGL for their help and support, as well as all the enthusiastic members of the Catfish Study Group. I thank Danni Shore for putting up with me and being a most wonderful girlfriend. Furthermore, I thank my family for their care and support, as well all the former residents of 1 Nant Cottages and the current residents of 3 Garth Terrace for being fantastic housemates.

Finally, this research was funded by NERC small grant (NE/C001168/1), NERC Mass Spectrometry access grant (NE/F007205/1) awarded to Martin I. Taylor., and a NERC PhD studentship (NE/F007205/1) awarded to myself. Research was also supported by UNESP, Brazil and a Santander Mobility Scholarship.

Table of Contents

List of Tables

List of Figures

Preface

"Nothing in biology makes sense except in the light of evolution"

(Dobzhansky, 1964)

"Nothing in evolutionary biology makes sense except in the light of ecology"

(Grant and Grant, 2008)

Nothing in evolutionary ecology makes sense except in the light of comparative multidisciplinary evidence

One of the main goals of evolutionary biologists is to study the mechanisms driving speciation (cladogenesis) and extinction, and the cumulative effect this has on the Tree of Life over extended temporal scales. Ecologists have traditionally focused on the interactions of organisms with each other and their environment. More recently, the field of evolutionary ecology has sought to unite ecology and evolutionary biology in order to investigate the evolutionary history of species and the consequences of their interactions (Mayhew, 2006). Some of the main themes of evolutionary ecology include the evolution of behaviour, life history, interspecific interactions, biodiversity, and community structure (Fox, 2001; Mayhew, 2006; Pianka, 2000). One of the central questions for evolutionary ecology since Darwin has been: Why are some clades more species rich than others? Natural selection accounts for adaptation, but how do we explain diversity and complexity, and what are the mechanisms that generate and maintain them? Mutation and genetic drift have been proposed as background forces acting on diversity and complexity. This has recently been proposed as Biology's First Law:

"Zero-Force Evolutionary Law: In any evolutionary system in which there is variation and heredity, there is a tendency for diversity and complexity to increase, one that is always present but may be opposed or augmented by natural selection, other forces, or constraints acting on diversity and complexity."

(McShea and Brandon, 2010)

Despite the fact that neutral processes can give rise to complexity and diversity when there is heredity and variation, diversity can also be explained by natural selection

driving speciation of closely related species (Darwin, 1859), and/or selection favouring certain species and lineages with more potential for speciation (Stanley, 1979). Complexity can arise when there are selective advantages associated with symbioses, while selection may favour complexity when it is positively correlated with ecological specialization (McShea and Brandon, 2010). Furthermore, with the accumulation of new species, niche complexity can increase (Waddington, 1969). Thus complexity and diversity are mutually reinforcing, and this principal reveals the tight links between ecology and evolution.

We already know that evolution affects ecology, and that ecology affects evolution. So is there a feedback loop? According to Kokko and Lopez-Sepulcre (Kokko and Lopez-Sepulcre, 2007), ecogenetic feedback is described as follows: If fluctuations in population density have an effect on individual fitness, and this effect varies depending on phenotype, then behaviour and/or life history characteristics (as determined by the genotype) directly influence and potentially change population dynamics. The change in the population may subsequently favour different genotypes under different conditions. This hypothesis, now widely referred to as eco-evolutionary dynamics, has been used to experimentally examine ecosystem effects of the evolution (and co-evolution) of populations in the wild (Hendry et al., 2011; Palkovacs and Post, 2009; Schoener, 2011). Although much progress has been made in understanding these dynamics, many questions remain, including whether the maintenance of interactions and stability of communities rely on accelerated diversification, or whether such diversification is ecologically insignificant (Schoener, 2011; Thompson et al., 1998).

To answer this question, one must inevitably employ a multidisciplinary approach, compiling data from a variety of different fields in order to shed light on the mechanisms responsible for the origins and maintenance of biodiversity.

Glossary

Adaptive radiation- the diversification of a group of organisms into forms filling different ecological niches

Advergence- if unequally protected, the less-protected species will be more strongly selected to change its appearance, which might speed up its rate of evolution, while the better-protected species might evolve more slowly or perhaps not at all

Aggressive mimicry- a form of mimicry where predators, parasites or parasitoids share similar signals with a harmless model, allowing them to avoid being correctly identified by their prey or host

Allopatric speciation- speciation that occurs when biological populations of the same species become isolated due to geographical or social changes

Allopolyploid- polyploid in which the chromosome sets are derived from more than one species through hybridization

Aposematism- warning coloration that advertises unpalatibility of prey to predators

Apostatic selection- frequency-dependent selection by predators, particularly in regard to prey that are different morphs of a polymorphic species

Assortative mating- a form of non-random mating in which individuals select mates with a similar phenotype to themselves

Automimicry- the possibility that less well protected individuals may gain protection from predators by resembling better defended conspecifics; assuming almost all defended species will show some form of intraspecific variation in defence, and that this variation may sometimes be sufficient to confer protection to more weakly defended individuals that would not otherwise arise

Autopolyploid- non-hybrid, increase in ploidy within species. Multiplication of the same chromosome set

Batesian mimicry- when members of a palatable species gain a degree of protection from predators resembling an unpalatable or otherwise defended species

Biodiversity hotspot- a biogeographic region with a significant reservoir of biodiversity

C-Value- the amount of DNA contained within a haploid nucleus

Camouflage- an animal's natural colouring or form that enables it to blend in with its surroundings

Character displacement- the phenomenon where differences among similar species whose distributions overlap geographically are accentuated in regions where the species co-occur but are minimized or lost where the species' distributions do not overlap

Cladogenesis- the formation of a new group of organisms or higher taxon by evolutionary divergence from an ancestral form

Confusion effect- one of several mechanisms that reduce predation risk in group-living prey, others include group defence, increased vigilance, and attack abatement

Convergence- the acquisition of the same biological trait in unrelated lineages

Countershading- protective coloration of some animals in which parts normally in shadow are light and those exposed to the sky are dark

Crypsis - organisms that come close to perfectly matching the environment so that detection of them is challenging

DEC- a model describing the processes of dispersal, extinction and cladogenesis with a phylogeographic context

Diploidization- gradual conversion from polyploidy to diploidy through genetic changes that differentiate duplicated loci

DIVA- a model describing the processes of dispersal and vicariance within a phylogeographic context

Divergent resolution- reciprocal silencing of duplicated genes, leading to reproductive isolation upon secondary contact

Ecoregion- an ecologically and geographically defined area that is smaller than an ecozone and larger than an ecosystem

Ecosystem services- the benefits for humankind based on the multitude of resources and process supplied by natural ecosystems

MicroRNA- a cellular RNA fragment that prevents the production of a particular protein by binding to and destroying the messenger RNA that would have produced the protein

Mimicry ring- a group of species all mimicking the same colour pattern

Müllerian mimicry- two or more unpalatable species that converge (or adverge) on the same warning colour pattern

Multivalent- meiotic association of more than two chromosomes, resulting in synapsis and recombination between partners

Negative interactions- mutually detrimental interaction between individuals, populations or species, or interactions where one species benefits at the expense of another

Neofunctionalization- acquisition of novel function by a duplicated gene

Neopolyploid- newly generated polyploid individuals, often induced through artificial means

Neotropics- denoting a zoogeographical region comprising Central and South America, including the tropical southern part of Mexico and the Caribbean.

Net diversification- the net effect of speciation minus extinction

Niche differentiation- the process by which natural selection drives competing species into different patterns of resource use or different niches

Niche- the relational position of a species or population in its ecosystem to each other

Oddity effect- when ''odd'' individuals suffer disproportionately high rates of predation, and solitary individuals join groups whose members are most similar to themselves in appearance

Parallel evolution- the development of a similar trait in related, but distinct, species descending from the same ancestor, but from different clades

Parapatric speciation- speciation that occurs due to variation in the mating habits of a population within a continuous geographical area

Phylogenetic niche conservatism- a pattern that of species coexistence that results when closely related species are more ecologically similar than would be expected based on their phylogenetic relationships

Phylogenetic overdispersion- a pattern of species coexistence based on a long history of competitive interactions which causes evolutionary divergence in species niches

Polyploid- an organism or cell containing more than two homologous sets of chromosomes

Positive interactions- species interactions that benefit at least one of the participants and cause no harm to either

Primary defence- operates before a predator initiates any prey-catching behaviour

Search image- a transitory enhancement of detection ability for particular cryptic prey types or characteristics

Secondary defence- operates once a predator initiates an attack and functions by increasing the chances of an individual surviving the prey capture process

Social mimicry- convergence of bright or otherwise conspicuous colours (including black and white) among not particularly closely related species that often associate with one another in mixed flocks

Stable isotopes- naturally occurring stable forms of elements with differing nuclear masses, which confer disparate physical properties that cause such isotopes to behave differentially in biogeochemical processes

Subfunctionalization- separation of function of duplicated genes into different tissues or developmental stages

Sympatric speciation- is the process through which new species evolve from a single ancestral species while inhabiting the same geographic region

Weberian apparatus- an anatomical structure that connects the swim bladder to the auditory system in fishes belonging to the Superorder Ostariophysi

Chapter 1: Introduction

1.1 The Corydoradinae

1.1.1 Taxonomic Background

Fish represent the greatest diversity of currently recognized extant vertebrate taxa, with a total of 27,977 described species (Nelson, 2006). Within the ray-finned fishes (Actinopterygii), the majority of species belong to three main orders: Perciformes (10,033 species; 36%), Cypriniformes (3,268 species; 12%), and Siluriformes (2,867 species; 10%) (Nelson, 2006). The family Callichthyidae (Bonaparte, 1838), commonly known as the armoured catfishes, is characterized by two rows of bony plates covering the full length of the body (subdivided at the lateral line). The name callichthyis is Greek in origin, derived from the words kallis (beauty) and ichthys (fish). The family contains two subfamilies, the Callichthyinae (genera: *Callichthys*, *Dianema*, *Hoplosternum*, *Lepthoplosternum* and *Megalechis*) and the Corydoradinae (*Aspidoras*, *Scleromystax*, *Corydoras*, and *Brochis*). The Corydoradinae includes 90% of the diversity within the Callichthyidae with more than 170 valid species, and more being described annually. *Corydoras* are bottom-dwelling insectivores/detritivores, currently distributed throughout the continent of South America (with the exception of Chile). Their northern most limit is on the island of Trinidad (*Corydoras aeneus*, 11°N), while in the south *Corydoras* have been reported as far as the Chubut basin of Argentina (*Corydoras paleatus*; 42°S). A remarkable fossil discovered in the Juyuy Province of Argentina (*Corydoras revelatus*) suggests that the origins of these catfish date back to the Paleocene, by which time they already resembled extant species (Cockerell, 1925).

Between the description of the first callichthyid, *Silurus callichthys* (*Callichthys callichthys)*, by Linnaeus (Linnaeus, 1758), up until the time of Hoedeman's (Hoedeman, 1952) definition of the subfamilies Callichthyinae and Corydoradinae, there has been almost continuous systematic revision of the group. The genus *Corydoras* was first established by Lacépède in his Histoire Naturelle des Poissons (Lacépède, 1803), designating *Corydoras geoffroyi* as the type species. In comparison with *Brochis* (Cope, 1872) and *Aspidoras* (Ihering, 1907), *Corydoras* differs by its laterally compressed head, short rictal barbels, and a single pair of short mental barbels at the lower lip. The genus *Brochis* can be distinguished by virtue of it s

multiple soft dorsal rays (up to 12; 6-8 in *Corydoras* and *Aspidoras*). *Aspidoras* differs from *Corydoras* by having a short supra-occipital crest, which does not separate the nuchal plates (Nijssen, 1970). Over 40% of the species currently recognized under the genus *Corydoras* have been taxonomically characterized by Nijssen (Nijssen, 1970) and Nijssen & Isbrucker (Nijssen and Isbrucker, 1980b).

With the exception of a recent molecular phylogeny, the majority of the taxonomic and systematic investigations of Callichthyidae inter-relationships have been based on morphological characters and cladistic morphometric analyses. The morphological characteristics that define the family are as follows:

1. Two longitudinal rows of lateral body plates that completely cover the sides.
2. Two or three (basal) barbels at the junction of the lips on either side of the mouth (Gosline, 1940)

1.1.2 Systematics

From the early 19th century to this very day, taxonomic descriptions of *Corydoras* have been primarily based on colour patterns. These tend to be variable both intra- and inter-specifically, thus making them difficult to quantify objectively (Nijssen, 1970). There is little or no information on the genetic structure of populations, and there exist very few comprehensive collections of *Corydoras*. Most morphological descriptions rely heavily on proportional differences, where specific measurements are expressed as ratios of each other. These ratios are of questionable phylogenetic utility due to variation across geographically disparate populations and within populations due to growth allometry (Nijssen, 1970). There is considerable overlap in most species groups originally proposed by Nijssen and Isbrucker (Nijssen and Isbrucker, 1980b), especially in overall body form and meristics. Thus, elucidating the systematic relationships of *Corydoras* based on meristics and colour has proven a very challenging task, offering limited insight into the systematic relationships of this species rich genus.

One of the routes taken prior to the advent of molecular phylogenetic techniques was the examination of allometric variation in *Corydoras.* Using the tables of counts and measurements from Nijssen and Isbrucker's taxonomic review of *Corydoras,* Strauss

(Strauss, 1985) performed a multivariate statistical analysis using slopes, intercepts, principal components and corresponding confidence intervals. Results from this analysis indicate that changes in morphology are the result of subtle, perhaps heterochronic changes in relative growth rates among body structures (Strauss, 1985). The author found that four out of the five groups proposed by Nijssen couldn't be discriminated properly due to overlap in body form and meristic measurements. These data suggest that regression coefficients of logarithmically transformed body measurements would be much more informative than ratios for taxonomic descriptions (Strauss, 1985). The results are in direct conflict with Nijssen's original proposed species groups that rely heavily upon ratios and colour patterns.

The subject of allometry in body shape was advanced further in two studies focusing on ontogeny in the most basal callichthyid genera (*Hoplosternum, Megalechis, Lepthoplosternum*, and *Callichthys*) (Reis, 1998a; Reis et al., 1998). Using a landmark morphometric approach, the authors examined 25 specimens ranging from small post-transformational juveniles to fully grown, large adults (Reis et al., 1998). Their statistical findings support the conclusion that there is allometric shape change during ontogeny in *C. callichthys*, which is dominated by an elongation of the postcranial region relative to the head. The deepening of the mid body and elongation of the postcranial in *C. callichthys* (Reis et al., 1998) are consistent with findings for *Hoplosternum, Megalechis*, and *Lepthoplosternum* (Reis, 1998a). Once again, this study raises concerns about the validity of species descriptions and groupings based on limited meristic measurements.

The otophysan Weberian apparatus, consisting of modifications to the anterior vertebrae, their appendages, and associated connective tissues, is shared by an amazing array of more than 6,500 species of Cypriniformes, Characiformes, Gymnotiformes, and Siluriformes (Nelson, 2006). An investigation of the ontogenetic development of the Weberian apparatus in *Corydoras paleatus,* revealed a significantly reduced state of the character (Coburn and Grubach, 1998). Their most important finding is that myoseptae can be used to discriminate between loss and fusion of vertebral segments, and that the Weberian apparatus of *C. paleatus* has three rather than the typical five fused vertebrae. The authors conclude that *Corydoras* lack nearly all the elements forming the typical siluriform Weberian apparatus (Coburn

and Grubach, 1998).

A morphologically based approach constituted the first modern phylogenetic analysis covering all callichthyid genera within a siluriform framework (Reis, 1998a). The author set out to describe the skeletal anatomy of the Callichthyidae, to study the phylogenetic interrelationships among species and to test the monophyly of the family and its genera. Some of the characters considered for this analysis include the: neurocranium, latero-sensory canals, suspensorium and mandibular arch, infraorbital series, opercular series, hyoid arch, branchial arches, Weberian apparatus and axial skeleton, unpaired fins, pectoral fin and girdle, and the pelvic fin and girdle. The resulting cladogram supports the monophyly of the family Callichthyidae, and the division of the subfamilies Callichthyinae and Corydoradinae (Reis, 1998a). Within the latter subfamily, the genus *Aspidoras* is sister-group of a clade formed by *Corydoras* and *Brochis*. There were no characters that supported the monophyly of *Corydoras,* whereas the monophyly of *Brochis* was supported by four derived features. The author provides a key to all callichthyid genera based on the morphological features described, and discusses previously proposed species groupings (Nijssen, 1970). Although the analysis was robust, it lacked taxonomic sampling within the Corydoradinae; most notably within the genus *Corydoras* and the later described *Scleromystax*.

The morphological phylogeny of the subfamily Corydoradinae was subsequently re-analyzed and re-defined on the basis of 83 morphological characters (Britto, 2003). This study expanded the list of morphological characters and total taxonomic coverage within the genera: *Corydoras*, *Scleromystax,* and *Aspidoras*. One of the main results was the non-monophyletic nature of the genus *Corydoras* as currently defined (Britto, 2003). Instead of *Brochis* and *Corydoras* forming a group with *Aspidoras* as its sister assemblage, Britto proposed a clade consisting of *Aspidoras* and *Scleromystax* (Britto, 2003). The author also proposed a new classification scheme encompassing the monophyletic assemblages as defined by the resulting cladograms.

Finally, by the 21st century, the first molecular phylogeny of the family Callichthyidae was published (Shimabukuro-Dias et al., 2004b). The authors sequenced mitochondrial rRNA, ND4, and tRNA markers for 28 representative

callichthyid species (Genera: *Corydoras* (12), *Aspidoras* (3), *Brochis* (2), *Dianema* (2), *Lepthoplosternum* (2), *Megalechis* (2), *Callichthys* (2), and *Hoplosternum* (2). Outgroups were included from the Nematogenyidae, Trichomycteridae, Astroblepidae, and Loricariidae. Phylogenetic analysis was performed using maximum parsimony and maximum likelihood, and the authors compared and combined their results with available morphological data. Cytogenetic data on chromosome counts were mapped onto the phylogeny, showing some very interesting cases of genome duplication amongst *Corydoras* and *Brochis* lineages (Shimabukuro-Dias et al., 2004b). The authors conclude that the Callichthyidae form a monophyletic assemblage that comprises two natural groups: subfamily Corydoradinae (*Aspidoras, Brochis, and Corydoras*) and subfamily Callichthyinae (*Callichthys, Dianema, Hoplosternum, Lepthoplosternum,* and *Megalechis*) (Shimabukuro-Dias et al., 2004b). This molecular phylogeny conflicts with proposed morphological relationships as far as sister-group relationships are concerned. The addition of karyotypic data added a unique phylogenetic perspective, supporting basal relationships of diploid progenitors and the monophyly of polyploid species groups within *Corydoras* (*C. metae* & *C. araguaiensis).*

1.1.3 Cytogenetics

The first paper reporting karyotypic and cytogenetic diversity within *Corydoras* species uncovered some very exciting results (Scheel et al., 1972). The authors present chromosome counts of 14 species of *Corydoras* showing major karyotypic variation resulting from genome duplications, ranging from 2n=44-132 (Scheel et al., 1972). The authors also examined electrophoretic patterns and demonstrated extreme inter-specific variation (Scheel et al., 1972). Results were compared to the groups proposed by Nijssen, yet there was no discernable relationship between the karyotypic/electrophoretic data and previously proposed groupings. This is not surprising considering that Nijssen's groupings are based on a combination of colour patterns and morphology, loosely put together with no phylogenetic framework.

Additional research on the cytogenetics of *Corydoras* focused on *C. aeneus* from four rivers in southern Brazil (Oliveira et al., 1988). Chromosome number variation within the *C. aeneus* populations ranged from 58-64, with multiple cases of robertsonian rearrangement and B chromosomes (Oliveira et al., 1988). Robertsonian

polymorphism, which may result from centric fusions and dissociations, is a relatively rare phenomenon requiring special preconditions for persistence in natural populations (White, 1973). B chromosomes on the other hand are widely distributed in plants and animals (Jones and Rees, 1982), yet within fish, supernumerary chromosomes are restricted to a few species (Foresti et al., 1989). When compared to previous results, differences in *C. aeneus* karyotypes indicate the existence of a diploid-tetraploid system within the species group (Oliveira et al., 1988). Further investigations also examined three allopatric populations of *C. nattereri* from the southeast coast of Brazil, in the state of Sao Paulo (Oliveira et al., 1990). These three populations of the 'same species' exhibit three different chromosome counts (40, 42, 44), thus suggesting reproductive isolation partly due to karyotypic differentiation (Oliveira et al., 1990). The authors explain the cytogenetic difference by suggesting that the occurrence of tandem translocation events was responsible for the observed variability (Oliveira et al., 1990). Their analysis shows that chromosomal rearrangements were apparently more frequent than morphological modifications in *C. nattereri* since Nijssen and Isbrucker were unable to distinguish different morphological groups in this species (Nijssen and Isbrucker, 1980a; Nijssen and Isbrucker, 1980b). Oliveira et al. continued their investigation of cytogenetic diversity within *Corydoras* (Oliveira et al., 1992). Chromosome counts, genome sizes, nuclear organizing regions, and diploid chromosome arm numbers were examined in 11 species from a variety of localities across the South American continent, increasing the total number of *Corydoras* species with known karyotypic constitution to 30. The research demonstrated that there are at least five groups of species sharing similar chromosome morphology, diploid numbers, and DNA content (Oliveira et al., 1992). A diagram suggesting hypothetical cytogenetic interrelationships in the genus *Corydoras* was presented which also conflicted with previously proposed groupings (Oliveira et al., 1992). More cytogenetic and genome size data was presented in a couple of studies reporting data for six genera of the family Callichthyidae (Oliveira et al., 1993a; Oliveira et al., 1993b). Diploid numbers ranged from 44-100, and C-values from 1.18-2.77 pg/nucleus. A new, updated hypothesis was presented concerning cytogenetic interrelationships in the family. Following the initial cytogenetic investigations of Oliveira et al., two subsequent studies were published on karyotype variability (44-102) in *Corydoras, Aspidoras,* and *Brochis* (Fenerich et al., 2004; Shimabukuro-Dias et al., 2004a).

1.1.4 Behaviour, Ecology and Toxicity

Behavioral and ecological research on *Corydoras* has not been widely conducted. Some work has been published, but mostly in aquarist magazines for the interest of keen hobbyists. Yet a few of these studies still carry scientific value and test interesting hypotheses. One such example is the doctoral work of David Sands on crypsis and camouflage in *Corydoras* (Sands, 1994). This work was focused on *C. adolfoi* and *C. imitator,* sympatric in tributaries of the upper Rio Negro. The two species share a remarkably similar colour pattern, prompting an investigation of the evolution of colour patterns alongside cryptic behaviour. In order to observe behavioral reactions, a setup was constructed with a series of model and live predator experiments, recording and filming responses to predation (primarily freeze behaviour) (Sands, 1994). The predator of choice was *Hoplias malabaricus* (a common predator throughout the Neotropics belonging to the order Characiformes), and a 'tank within a tank' system was used to restrict the movements of the predator while retaining visual contact with the *Corydoras* (Sands, 1994). In both experiments (live and model predators), *C. imitator* was found to freeze significantly longer than *C. adolfoi*, while groups of different species would compact quickly and form bi-specific shoals (Sands, 1994). Based on the laboratory experiments, and observations in the field, the author concludes that it is likely *Corydoras* patterns have developed alongside cryptic behaviour to reduce conspicuousness within their habitats, and that the genus may have adapted towards close interspecific and bi-specific shoaling (Sands, 1994). Shoaling behaviour in *Corydoras* was further investigated by Paxton, in a series of experiments measuring activity, depth preference and intra-specific assembly of *C. pygmaeus* and *C. ambiacus* (Paxton, 1997). Results from these experiments revealed that *C. pygmaeus* does not show a clear open-water preference, and that both species were most active and highest in the water column at twilight, while the larger of the two, *C. ambiacus*, formed more cohesive shoals when compared to the dwarf *C. pygmaeus* (Paxton, 1997).

In relation to reproductive behaviour, the production of sound is well documented among a variety of actinopterygiian families (Ladich, 2000). Sound production of *C. paleatus* was investigated in relation to reproductive behaviour, offering the first reliable data for the Corydoradinae (Pruzsinszky and Ladich, 1998). The sounds

recorded were broad-band, pulsed, acoustic signals produced during abduction of the pectoral spines (Pruzsinszky and Ladich, 1998). Sound production was primarily observed in the males during courting or dyadic encounters, while all individuals were found to produce sound when taken out of the water (under stressful conditions) (Pruzsinszky and Ladich, 1998). Sound production was further investigated using a comparative approach to examine acoustic behaviour in relation to reproduction for five species of *Corydoras* (Kaatz and Lobel, 1999; Kaatz and Lobel, 2001). The authors grouped sounds under four predetermined categories (courtship, agonistic chase, agonistic pre-chase, and startle). Results show that courtship-associated sounds were produced by all species (*C. aeneus, C. arcuatus, C. leopardus, C. paleatus, C. reticulatus*) prior to spawning, when males hovered near females beating pectoral and dorsal fins (Kaatz and Lobel, 1999; Kaatz and Lobel, 2001). The authors suggest that *Corydoras* produce sounds in at least four behavioral contexts, and that acoustic activity is low for non-reproductive fishes but is higher when reproducing (Kaatz and Lobel, 1999; Kaatz and Lobel, 2001).

A closer look at the reproductive behaviour of Corydoradinae revealed some very surprising reproductive adaptations, notably for *C. aeneus* females that appear to be sperm drinkers (Kohda et al., 1995). The authors report a unique reproductive behaviour and a new mode of egg insemination where during courtship, the female attaches her mouth to the male's genital opening and directly drinks his sperm (Kohda et al., 1995). The sperm then pass though her intestine and are discharged together with eggs into the pouch formed by her pelvic fins (Kohda et al., 1995). Although the methods and controls are lacking in the experiments performed, results seem to suggest that this may be a valid mode of insemination, unique within all the animal kingdom (although many researchers remain skeptical). Investigations into *C. aeneus* reproductive biology continued with the examination male reproductive success (Kohda et al., 2002). Results support non-territoriality in males and lack of aggression towards conspecifics, while there was no evidence to suggest female preference for particular morphological traits, yet the females tended to prefer males with a high relative courtship frequency indicating a random mating strategy (Kohda et al., 2002). Another interesting aspect of Corydoradinae reproductive biology was revealed through thorough examination of the ultrastructure of *C. aeneus* eggs (Huysentruyt and Adriaens, 2005). Their surface structure was shown to be unique among teleosts,

with small villi-like protuberances that resemble attaching-filaments, while they were also found to be very adhesive, leading the authors to interpret this structure as an adaptation to fast flowing turbid waters (Huysentruyt and Adriaens, 2005).

The Corydoradinae, among many other actinopterygiian fishes have evolved (or retained) the ability to breath air. Using *C. aeneus* as a model, researchers tested the transit cost of aerial respiration by calculating time budgets and estimating energy budgets (Kramer and McClure, 1981). Key findings include that air breathing is substantially more costly than has previously been assumed on the basis of comparisons between air and water as respiratory media (Kramer and McClure, 1981). They go on to suggest that these costs greatly reduce the supposed advantages of using air as a respiratory medium and favor the continued use of dissolved oxygen, even by organisms with the capacity for aerial respiration (Kramer and McClure, 1981). Although, the authors do not consider this, there is a cost for continuous air breathing that is not related to physiology. When small fish surface to breath air, they are exposed to aerial predators much more than if they were on the benthos (exposing their position), which would inevitably introduce a mortality cost likely to outweigh physiology.

It has been well known by fish keepers that Corydoradinae (among many other catfishes) are capable of producing toxins. However, this observation was based on anecdotal evidence, scientifically unconfirmed until some relatively recent investigations (Greven et al., 2006). Two papers, one on *C. sterbai* (Kiehl et al., 2006) and one on *C. aeneus* (Greven et al., 2006), demonstrated the production of toxins via the axillary glands at the base of the pectoral spine in both species. The ultrastructure and general histology of *C. aeneus* axillary glands were examined in detail (Greven et al., 2006), while subsequent work revealed that stress induced secretions from the axillary gland are definitely toxic, containing a variety of bactericidal substances (Greven et al., 2006). More recently, Wright has shown that many more Corydoradinae species are toxic than previously hypothesized (Wright, 2009). These results are of particular interest due to the observation of sympatric mimetic species in the wild. Although all *Corydoras* may possess the capability to produce toxins, some species are likely to be more toxic than others, and a comparative genera-wide approach may reveal interesting evolutionary relationships based on levels of toxicity.

1.1.5 Paleontology

One final note on the biology of this remarkable group of fish relates to their ancient evolutionary origins. Fossil remains of many early actinopterygiian fishes are notoriously difficult to classify due to their fragmentation and poor state of preservation. Callichthyidae fossils are far from common within Neotropical formations, yet a remarkably preserved specimen named *Corydoras revelatus* was described in the early 20th century (Cockerell, 1925) from the Maiz Gordo Formation of Argentina, placing the origins of the subfamily within the late Paleocene. More recent remains of callichthyids have been discovered in the La Venta Formation from the Middle Miocene of Columbia (Lundberg, 1997). These fragments consist of a skull and predorsal plates attributed to *Hoplosternum sp.* Remains of clearly distinguishable callichthyid pectoral spines have been identified from the Solimoes formation (Reis, 1998b), suggesting a common origin ancient of fauna from different sites across South America (Lundberg et al., 1998).

1.2 Thesis Focus

Corydoras catfish comprise a significant proportion of the freshwater ichthyofaunal diversity of South America. They are of particular interest evolutionarily due to the genetic and ecological mechanisms that have enabled their diversification. Variation in genome sizes and chromosome numbers suggest several independent genome duplication events amongst the species rich clades of *Corydoras* (Oliveira et al., 1992). Genome duplication and polyploidy in ancient fish lineages is regarded to be one of the key genetic mechanisms responsible for the rapid evolution of vertebrates. Although polyploidy is common in plants, there are few well-documented examples of recent polyploid events within animal lineages. Thus these fishes can be used as model group for the study of vertebrate genome evolution. Furthermore, the rivers and streams of South America constitute a kaleidoscope of habitats, climates and niches. It is this environmental heterogeneity that sets the stage for evolution to be influenced by ecology. Inter-specific interactions of *Corydoras* are of particular interest because different species coexist throughout their distribution (usually belonging to different lineages). These species assemblages tend to display identical colour patterns suggesting possible mimetic relationships. Different species coexisting also tend to vary in snout morphology, some being blunt while others are elongated (Nijssen, 1970). This variability in snouts may be due to competition for food and resources, thus enabling niche differentiation between species. Therefore, studying coexistence of sympatric pairs of *Corydoras* offers a unique opportunity to test ecological theories of mimicry, competitive exclusion and dietary overlap in the wild.

A multidisciplinary approach was necessary in order to investigate the mechanisms of speciation and coexistence in Corydoradinae, considering that the latter processes are not mutually exclusive and lie at the interface of ecology and evolution. The subject therefore merited the use of molecular phylogenetics to elucidate inter-relationships, stable isotopes to investigate dietary overlap, morphometrics to identify shape change associated with diet, colour pattern analyses to quantify and compare different mimetic patterns, genome size quantification to evaluate the role of polyploidy in diversification, and a comparative biogeographic approach to assess likely patterns of geographic diversification. These data were combined and analyzed using a variety of phylogenetic comparative methods, including relaxed molecular clock analyses, ancestral reconstructions, diversification rate tests and dispersal-extinction

cladogenesis models. Furthermore, a consideration of a wide body of literature including mimicry, ecology, whole genome duplication, diversification, biogeography, conservation and comparative methodology was also necessary in order for the results presented herein to fit into the appropriate context. This introductory Chapter attempts to review the important literature in each of these broad areas, but due to the breadth of the topics covered does not (and cannot) cover all areas exhaustively (see review in appendix for detailed coverage of polyploidy in fishes).

1.3 Mimicry & Colour Pattern Convergence

Sympatric Corydoradinae taxa frequently share colour patterns, which have evolved through mimicry (Chapter 2). Although their colour patterns are aposematic, they are made up of cryptic and/or disruptive elements. Here I review the relevant literature on mimicry with particular emphasis on Müllerian, Batesian and social mechanisms as well as crypsis and disruptive coloration.

1.3.1 Müllerian, Batesian and Social Mimicry

The observation that unrelated sympatric species sometimes adopt almost identical colour patterns was first reported by Walter Henry Bates in 1862 after lengthy investigations in the Amazon (Bates, 1862). Bates suggested that palatable butterfly species may gain protection from predation by resembling unpalatable species ('Batesian mimicry'), a widely accepted explanation for the close resemblance between distantly related sympatric species when at least one is unpalatable to predators. This unpalatibility is signalled to predators using aposematic coloration (Sherratt and Beatty, 2003). Müllerian mimicry, occurs when two or more species with effective secondary defences share a similar appearance which becomes recognized by predators (Müller, 1878). Mimetic relationships involving aposematism have been reported in a wide variety of taxa including bumble bees (Williams, 2007), butterflies (Bates, 1862; Brower, 1996), moths (Jones et al., 1962), beetles (Poulton, 1890), millipedes (Marek and Bond, 2009), frogs (Darst and Cummings, 2006), snakes (Sanders et al., 2006) and fish (Caley and Schluter, 2003; Wright, 2011).

The majority of the research into colour pattern mimicry has focused on insects, yet there are a substantial number of examples from vertebrate taxa. For example, in marine fish (comprising the vast majority of vertebrates) mimics are plentiful, including examples of species with protective resemblance, Batesian mimicry, Müllerian mimicry, aggressive mimicry, and social mimicry (Randall, 2005). Most of these examples come from the teleost lineages (labrids, frogfish, blennies, cichlids, flatfish, Perciformes, scorpionfish, pipefish, carangids), with some examples from the eels and catfish (Randall, 2005). Within the coral reef communities, examples of mimicry in fishes takes many guises and are not rare phenomena (Randall, 2005). One particularly interesting example of Batesian mimicry was demonstrated in a field study where Caley & Schluter measured the protection from predators generated by a

conspicuous unpalatable pufferfish species (*Canthigaster valentini*), to colour patterns that resemble it (Caley and Schluter, 2003). Results suggest that piscivorous fishes on the reef are educated regarding the toxicity of puffers, and that avoidance of fish having the pufferfish pattern (*Paraluteres prionurus*) has generated selection favouring mimetic resemblance by palatable species (Caley and Schluter, 2003). Another case from the Siluriformes in South America includes the mimetic association between *Corydoras diphyes* and *Otocinclus mimulus*, unusual considering that the two species occupy different microhabitats, thus likely to be controlled by a predator operating between these habitats (Axenrot and Kullander, 2003).

Mimics are also common in frogs and snakes. Within the Dendrobates poison frogs, there are multiple examples of Batesian and Müllerian mimics (Darst and Cummings, 2006; Symula et al., 2001). In the case of the Peruvian *Dendrobates imitator*, evidence supports a Müllerian mimetic radiation in which a single species mimics different sympatric species in different geographic regions (Symula et al., 2001). In Ecuador, *Epipedobates bilinguis* and *E. parvulus* are Batesian models mimicked by *Allobates zaparo*. The two models (*E. bilinguis* and *E. parvulus*) are able to coexist due to differences in predator avoidance based on toxicity, which confers greater protection to mimics resembling the less toxic model (Darst and Cummings, 2006). Although less diverse than dendrobatids, there are also multiple cases of mimicry in snakes, both Batesian in the case of coral snakes (Harper and Pfennig, 2007; Kikuchi and Pfennig, 2010a; Kikuchi and Pfennig, 2010b) and Müllerian examples from Asian pitvipers (Sanders et al., 2006). Interestingly enough, in the case of some European vipers, colour patterns are seemingly cryptic yet also aposematic, suggesting that the two mechanisms are not mutually exclusive (Wuster et al., 2004).

Sympatric species may also evolve to resemble one another if they live in mixed species aggregations through 'social mimicry' (Alevizon, 1976; Barnard, 1979; Ehrlich and Ehrlich, 1973). Social mimicry differs from classical Batesian and Müllerian mimicry in that species need not be unpalatable, and unprofitability is not signalled to predators. Instead, predators may focus on the most abundant prey species causing apostatic selection, or on less abundant prey creating an anti-apostatic selection effect. Experimental evidence suggests that solitary prey will be predated upon more frequently than prey which live in groups (Lindstrom et al., 2001), resulting in a reduction in per capita mortality as aggregation size increases (Sandin

and Pacala, 2005). This is known as the 'confusion effect' (Krause and Ruxton, 2002), most powerful when prey share colour pattern (Theodorakis, 1989), making it increasingly hard for predators to single out individual prey as aggregation size increases (Godin, 1986). Predators may combat the 'confusion effect' by targeting odd-looking individuals - known as the 'oddity effect' (Mathis and Chivers, 2003). Groups containing odd coloured individuals suffer higher rates of predation than groups of similar individuals, and odd individuals suffer higher rates of predation than the majority colour (Krakauer, 1995; Landeau and Terborgh, 1986; Ohguchi, 1981; Theodorakis, 1989; Tosh et al., 2006). Oddity effects are anti-apostatic, proving most effective when the prey are at high densities and very mobile (Wilson et al., 1990).

There are both differences and similarities in some manifestations of mimicry among insects and vertebrates which appear to be both quantitative and qualitative (Pough, 1988). In some cases, mimicry systems are considered tripartite, involving a model (living or material agent with perceptible characteristics), a mimic (organism that plagiarizes the characteristics of the model), and a dupe (organism deceived by the mimic) (Pasteur, 1982). Such an example has been documented in cases of mimicry of snakes for which the selective agents appear to be birds, and probably even some species of birds that are the selective agents of mimetic insects (Pough, 1988). The examples of mimicry among vertebrates are numerically fewer than those involving insects, possibly explained by the vast diversity of insects in comparison to vertebrates. Given our limited understanding of the chemosensory, auditory, and tactile worlds of most vertebrates, more examples of mimicry in vertebrates will be revealed as we continue to investigate its many manifestations (Pough, 1988).

1.3.2 Mimicry and Speciation

Bates, Wallace, and Darwin all realised that the strong natural selection pressures among co-mimics, could also lead to speciation (Mallet and Joron, 1999). A particularly well documented example involves distantly related sympatric species of *Heliconius* butterflies which adopt identical colour patterns, while sister species differ significantly in pattern (Jiggins et al., 2004; Turner, 1976). This suggests a mechanism for reproductive isolation through colour pattern convergence and assortative mating. Mimicry also leads to strong selection against non-mimetic hybrids or intermediates and should therefore contribute strongly to speciation and

species maintenance, by acting as a form of ecologically mediated post-mating isolation (Mallet and Joron, 1999). If mimicry contributes to speciation, mimetic shifts should often be associated with speciation within phylogenies, possibly accounting for the adaptive radiation in *Heliconius* butterflies. Mimetic pattern has been switched between eight of nine pairs of terminal *Heliconius* sister taxa (Mallet et al., 1996), a phenomena which has been observed in other lepidopterans as well (Ritland, 1991). Müllerian co-mimics are usually unrelated, while closely related species almost always belong to different mimicry rings (Turner, 1984).

The weakness of purifying selection in polymorphic populations can help explain why puzzling polymorphisms persist in some Müllerian mimics, and how they enable populations to explore the selective landscape, which can increase the chances of shifting balance. This is one of the few ways to explain the empirical observation that utterly novel colour patterns evolve continually in warning-coloured and mimetic butterflies (Mallet and Joron, 1999). In a similar way, weak selection against multiple rings may be partially responsible for the diversity of mimicry in any given area (Mallet and Joron, 1999). Theory suggests that all Müllerian mimics should converge into one large ring, yet simulations have shown that complete convergence is not observed, and Batesian mimicry is shown to be an important factor in the origin of mimicry rings (Franks and Noble, 2004). Redundancy in the outcome of natural selection is most evident in the examples of butterfly mimics, whose traits can now be mapped with the advent of developmental genetics and comparative genetic linkage analyses capable of linking the genomic region for matching colour patterns (mimicry locus) with that for mate preference (Joron et al., 2006; Kronforst et al., 2006; Naisbit et al., 2003).

The evolution of warning colouration is still widely considered paradoxical in the sense that very brightly coloured individuals would be at a selective disadvantage because of their greater conspicuousness to predators that are naive to the meaning of the signal (Marples et al., 2005). These ideas rest on assumptions of how predators forage in the wild, and thus have been difficult to test. Some studies have approached the issue and come to two conclusions: 1) Many predators are so conservative in their food preferences that even very conspicuous novel prey morphs are not necessarily at a selective disadvantage; and 2) The survival and spread of novel colour morphs can be simulated in field and aviary experiments using real predators foraging on

successive generations of artificial prey populations (Marples et al., 2005). Thus the conservative foraging of certain predators may allow for conspicuousness to become fixed within a population relatively rapidly.

Using a phylogeographic approach to study the evolution of colour patterns (and whether they have evolved neutrally or under selection) is a different methodological approach than those mentioned above, yet it has proven successful for many different species. Using digital photographs, principal components analyses, partial Mantel tests, and testing variation parameters, a recent study showed that the concealment pattern in poison dart frogs had evolved under genetic drift, hence regarding these patterns as a multicomponent signal system (Wollenberg et al., 2008). The Mantel test has also been used to investigate a Müllerian mimetic radiation in Asian pitvipers, where a conspicuous red colour pattern is associated with sympatric and parapatric populations in four genera (Sanders et al., 2006). In another case using chemically defended bird species, researchers tested for convergent evolution in colouration, showing that the mimetic phenotype is ancestral to both species and that the resemblance in most races is better explained by a shared ancestry (Dumbacher and Fleischer, 2001).

Experimental research has suggested that diet specialization and resultant toxicity may play a role in facilitating the evolution and persistence of warning colouration (Darst et al., 2005; Santos et al., 2003). This hypothesis was tested in the genus *Papilio* using a phylogenetic approach, and results indicate at least four independent origins of aposematic larval colouration within these lepidopterans (Prudic et al., 2007). The authors conclude that neither diet nor chemical specialization facilitated the origin of these aposematic larvae, yet there was a significant relationship between the signal environment and aposematism (Prudic et al., 2007). Although these phylogenetic approaches have proven relatively powerful and robust, those which rely entirely one partial mantel tests (designed to test the correlation among three matrices of pairwise distances), could be inadequate because the associated p-value is not indicative of type I statistical errors (Raufaste and Rousset, 2001).

1.3.3 Aposematism, Crypsis & Disruptive Colouration

The functions of warning colourations (aposematism) are relatively well described and understood, despite the gap of knowledge concerning the origin and maintenance

of such functions (Mappes et al., 2005). Aposematism should not be seen as an anti-predator strategy that is an alternative to crypsis, considering that the two are not always mutually exclusive, but rather part of a continuum of strategies from very protected highly conspicuous to weakly protected less conspicuous forms (Mappes et al., 2005). Considering that nothing in evolution is complete, much of what we see today is in some intermediate stage which can shift depending on selection pressures (Mappes et al., 2005). Camouflage may be achieved in three ways: crypsis, disruptive colouration, and masquerade (Endler, 1981). Cryptic prey resemble random samples of the visual background (Endler, 1981), minimizing their signal/noise ratio. Disruptively coloured prey contain some highly conspicuous as well as cryptic pattern elements, while in masquerade, the prey is detected as distinct from the visual background but not recognized as edible (Endler, 1981; Endler, 2006). According to some authors, there is a contradiction in two of the ways that animals avoid detection (crypsis and disruptive colouration), because crypsis relies on minimizing the signal/noise ratio, while disruptive colouration relies on keeping it high (Merilaita and Lind, 2006). The underlying problem is that most observations do not consider the importance of the visual and cognitive abilities of both predators and prey in order to understand the evolution of prey colouration in the wild (Endler, 2006).

Research on background matching and natural selection in Trinidadian guppies produced clear results demonstrating that populations living on coarse gravel had larger spots than populations living on fine gravel, with a given predation intensity (Endler, 1980). Moreover, results also suggest that sexual selection increases conspicuousness and colour pattern diversity, and that the complexity of colour pattern polymorphism in these guppies may result from the complex backgrounds, rare sympatry with congeners and automimicry among different kinds of colour spots (Endler, 1980). Results from investigations of the convergent and divergent effects of natural selection on colour patterns in two species - *Poecilia* (South America) and *Phalloceros* (North America)- suggest that there may not be a single optimum design for colour pattern because (a) there is more than one way to be cryptic to predators and attractive to mates, and (b) predation intensity changes from place to place, so the optimum colour pattern parameters vary geographically (Endler, 1982b).

The interaction between the spectral composition of ambient light and the reflectance spectra of colour pattern elements affects the conspicuousness of colour patterns to

conspecifics, predators, and prey (Endler, 1990; Endler, 1992; Endler, 1993). Applying some of the same theory to different vertebrate models, subsequent investigations focused on the interacting effects of lek placement, display behaviour, ambient light, and colour patterns in three forest-dwelling birds from French Guiana (Endler and Thery, 1996). The colour patterns and behaviour of each species maximize its visual contrast during its display and reduce it off the lek or on the lek but not displaying (Endler and Thery, 1996). More recent work on bowerbirds has revealed that their colour pattern evolution is at least partially predictable from the function of the visual system and from knowledge of different functions of different components of the colour patterns (Endler et al., 2005). Convergent evolution of cryptic coloration, where two or more unrelated species independently evolve the most cryptic colour pattern for a particular habitat / predator combination is theoretically plausible and has been reported among North American fishes (Armbruster and Page, 1996). In South America, various species of Siluriformes and Gymnotiformes have been recorded mimicking fallen leaves in rivers, and their leaf-like shape, cryptic colour, and escape movements are regarded as a convergent defensive response to diurnal piscivorous predators (Sazima et al., 2006).

Polymorphic cryptic colouration has been recorded in a variety of prey species, and can be maintained in a population by frequency dependent selection (in which more common prey types are attacked disproportionately often) (Allen, 1988; Bond and Kamil, 2002). Since frequency-dependent selection operates against common, familiar phenotypes, it should also lead to the proliferation of new, disparate phenotypes in an initially monomorphic prey population (Allen, 1988). This hypothesis was tested in an experiment during which blue jays (*Cyanocitta cristata*) searched for digital moths on computer monitors (Bond and Kamil, 2002). Moth phenotypes evolved under simulation controlled by a simple genetic algorithm in which individuals that were selected by the jays were less likely to reproduce (Bond and Kamil, 2002). The jays had difficulty detecting atypical moth patterns, and over successive generations the moths evolved to become significantly harder to detect, exhibiting greater phenotypic variance than non-selected or frequency-independent selected controls (Bond and Kamil, 2002). These results confirmed frequency-dependent selection and suggest the use of searching images, which enhance the detection of common prey (Bond and Kamil, 2002).

Our understanding of disruptive colouration and background matching has been largely based on theory, with a complete absence of experimental fieldwork. Given this absence, researchers set out to test two key predictions using an experimental approach: that patterns on the body's outline should be particularly effective in promoting concealment and that highly contrasting colours should enhance this disruptive effect (Cuthill et al., 2005). Based on artificial moth-like targets and exposure to bird predation in the field, survival analysis supported the predictions, indicating the effectiveness of disruptive colouration as a mechanism of camouflage in itself, thus making the distinction with background matching (Cuthill et al., 2005). The principle of disruptive colouration gained further support from subsequent experimentation which presented great tits (*Parus major*) with artificial background-matching and disruptive prey (Merilaita and Lind, 2006). Their results suggest that the two different types of prey were equally cryptic, leading the authors to conclude that resemblance of the background is an important aspect of concealment, but that coloration matching a random visual sample of the background is neither sufficient nor necessary to minimize the probability of detection (Merilaita and Lind, 2006).

Using artificial moth-like prey items and avian predators has become a successful and popular way of testing theories on detection of disruptive and background-matching colouration, while even human predators have been used in controlled environments to test such ideas (Fraser et al., 2007; McGuire et al., 2006). Using this approach, researchers were able to test whether bilateral symmetry present in natural prey may compromise the efficiency of camouflage (Cuthill et al., 2006). The authors found that symmetry reduced the effectiveness of both non-disruptive and disruptive background-matching coloration to a similar degree so that the negative effects of symmetry on concealment are no greater for disruptive than non-disruptive patterns (Cuthill et al., 2006). Results from an independent investigation found further support for the latter conclusions, suggesting that two proximate mechanisms explain the diversity of visual anti-predator defences: 1) disruptive colouration on the body outline provides camouflage independent of the background, and 2) background matching and disruptive colouration on the body interior provide camouflage, but their protection is background specific (Schaefer and Stobbe, 2006). Thus disruptive colouration may allow animals to exploit backgrounds on which they are not perfectly

matched, and to possess conspicuous markings while sill retaining a degree of camouflage (Stevens et al., 2006).

Cephalopod camouflage is considered unrivalled in the animal kingdom. In comparison with most animals that have fixed or slightly changeable camouflage patterns, cephalopods can show a variety of camouflage patterns, and they can instantly change them using their neurally controlled chromatophore system in the skin (Hanlon and Messenger, 1988; Messenger, 2001). Recent results from an investigation of the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns, suggest that: 1) at high contrast levels, cuttlefish body patterning depended on check size; 2) for low contrast levels, body patterning was independent of check size; and 3) on the same check size, cuttlefish fine-tuned the contrast and fine structure of their body patterns in response to small contrast changes in the background (Barbosa et al., 2008). For instance, the mimic octopus species of Indonesia resembles the local flounder in aspects of shape, swimming actions, speed, duration, and colouration (Hanlon et al., 2008). Field observations have shown that their behaviour turns cryptic as the octopus remains motionless, assuming body patterns and postures that resembled sponges, tube-worm tubes, and colonial tunicates (Hanlon et al., 2008).

1.4 Mutualism, Competition and Phylogenetic Community Ecology

Communities composed of distantly related mimetic Corydoradinae catfishes are highly structured. This structure is determined by phylogenetic relatedness and ecological traits that enable mimics to partition resources. In the meantime they also benefit from mutualistic interactions. In this section I consider the literature on the respective roles of positive (mutualisms) and negative (antagonistic) interactions, and phylogenetic distance in determining community composition.

> "As species of the same genus have usually, though by no means invariably, some similarity in habits and constitution, and always in structure, the struggle will generally be more severe between species of the same genus, when they come into competition with each other, than between species of distinct genera"
>
> (Darwin, 1859)

From Darwin until relatively recently, competition, predation, parasitism and environmental stressors have dominated ecological investigations of community composition and maintenance (Darwin, 1859; Guase, 1934; Hutchinson, 1947). Theoretical and empirical studies have provided strong support for the ecological and evolutionary role of such negative interactions. On the other hand, positive interactions, such as mutualisms and symbiotic relationships have been historically neglected and have been considered as playing a lesser role in community ecology, restricted to a handful of specialized cases. Following the establishment of ecological theory (May, 1981), ideas concerning positive interactions and their potential to structure ecological communities have received much more attention (Bertness and Callaway, 1994; Boucher, 1985; Bronstein, 1994; Bruno et al., 2003; Callaway, 1995; Stachowicz, 2001; Valiente-Banuet et al., 2006). Recent empirical and theoretical research suggests that mutualisms can increase the phylogenetic diversity of some communities (Valiente-Banuet and Verdu, 2007), and that such positive interactions are capable of generating and maintaining diversity within communities even when co-existing species compete (Elias et al., 2008; Elias et al., 2009; Filotas et al., 2010a; Filotas et al., 2010b; Gross, 2008). However, until now, the vast majority of this

research has focused on plant communities (Brooker et al., 2008), with very few examples from the animal kingdom (Cardinale and Palmer, 2002; Colwell, 1995; Elias et al., 2008). Furthermore, there is a notable absence of literature on the interplay between positive and negative interactions from a phylogenetic perspective (Elias et al., 2009), and the impact the latter forces have on the origins and maintenance of biodiversity.

Recently, the rapidly expanding field of phylogenetic community ecology has made an important contribution towards the integration of evolutionary biology and community ecology (Losos, 1996; Schluter, 2000; Webb et al., 2002). This has boosted interest in understanding the phylogenetic structure of communities and niches, the evolution of ecologically relevant morphological traits and the biogeographic patterns of community assembly (Webb et al., 2002; Wiens and Graham, 2005). A variety of metrics have been developed in order to assess the importance of different mechanisms responsible for community assembly, such as competition (as indicated by phylogenetic over-dispersion) and habitat filtering (niche conservatism), food-web interactions, predation, pathogen-host interactions and even neutral processes (Cooper et al., 2010; Ives and Helmus, 2010; Pausas and Verdu, 2010; Vamosi et al., 2009). Many challenges remain in terms of detecting ecologically relevant signals in phylogenetic trees, notably in the development of null models capable of distinguishing clustered or overdispersed communities, and determining the extent to which ecologically relevant phenotypic traits are conserved over different timescales (Cavender-Bares et al., 2009; Emerson and Gillespie, 2008). Also, despite recent interest in phylogenetic community ecology, studies are still heavily biased towards plants and terrestrial communities (Anderson and Cairney, 2004; Cavender-Bares et al., 2004; Vamosi et al., 2009), with few examples from the animal kingdom (Elias et al., 2008; Kozak and Wiens, 2010; Losos et al., 2003; Parra et al., 2010). Nevertheless, empirical evidence suggests that micro- and macroevolutionary processes can determine the assembly and maintenance of communities, while ecological interactions amongst co-existing species are capable of influencing micro and macroevolutionary processes (Johnson and Stinchcombe, 2007).

1.5 Niche Partitioning, Ecologically Relevant Morphological Traits, & Stable Isotopes

Corydoradinae communities are typically composed of species with different snout morphology and body size. These traits are ecologically relevant because they are linked to resource acquisition and enable species to partition resources thereby avoiding competition. Here I review the relevant theoretical and empirical literature on niche partitioning and competition in vertebrates, and how stable isotopes have been applied to investigate these phenomena.

1.5.1 Resource partitioning & Competition

Defining any given niche and determining its potential size or width has proven a tricky endeavour, yet it remains key to understanding evolutionary processes. Despite the difficulty in semantics, niche is traditionally considered as the ecological space occupied by a species within an environment (Chase, 2003; Holt, 2009). Niche width can be expressed by calculating the heterogeneity within a set of ecological measurements, often borrowing indices derived as measures of evenness and richness (Shannon and Weaver, 1949; Simpson, 1949). Niche partitioning has been shown to occur in relation to the physical environment, resources, behaviour and the traits of co-existing species (Ricklefs, 2010). As such, species coexistence often relies heavily on niche differentiation via resource partitioning as a stabilizing mechanism capable of maintaining species diversity over long time periods (Chesson, 2000). Competition for resources on the other hand is not viewed as a stable mechanism of coexistence, often leading to extinction via competition exclusion, or ecological character displacement (Losos and Schluter, 2000; Schluter, 2000) and cladogenesis (Bridle and Jiggins, 2000). Niche conservatism has also been shown in a variety of vertebrate communities, and can have important consequences for patterns of speciation, biogeography, coexistence and for conservation (Wiens et al., 2010; Wiens and Graham, 2005).

In cases of resource partitioning, a separation in niche is usually tightly linked with ecologically relevant morphological traits, or differences arising in behavioural attributes of the interacting species. Many species also avoid competition by using the same resource at different times or in different areas. Changes in the beaks of Galapagos ground finches in response to climatic cycles (Grant and Grant, 2008), the

repeated evolution of benthic and limnetic stickleback forms (Schluter, 2000), highly adapted jaw morphology in rift lake cichlids (Ruber et al., 1999), and spatial partitioning of *Anolis* lizards (Losos and Schluter, 2000) are all celebrated examples from the natural world. Identifying the specific traits responsible for resource partitioning and quantifying them is of key importance to understanding the mechanisms of niche differentiation (Schluter, 2000). For organisms whose morphospace can be analysed in two dimensions, the landmark based geometric morphometric approach has shown great promise in distinguishing axes of morphological differentiation and identifying ecologically relevant characters (Clabaut et al., 2007; Zelditch et al., 2004). As closely related species are expected to be more similar in shape than distantly related ones, the combination of morphometrics with phylogenetic information can help to determine the extent to which morphological traits have been uniformly or randomly inherited within a given lineage and how this may have affected the diversification process (Alfaro et al., 2009a; Linde et al., 2004; Price et al., 2010).

1.5.2 Stable Isotopes & Applications

Stable isotopes have been traditionally used to solve large-scale biogeochemical problems by measuring and comparing isotope composition changes in global elemental cycles (Peterson and Fry, 1987). The measurement of isotopic composition in elements such as Carbon, Nitrogen, and Sulfur has proven applicable and useful for the understanding of element cycles within ecosystems (Peterson and Fry, 1987). Some ecological applications of stable isotopes include the use of isotope ratios as 'recorders' in biotic and abiotic molecules, applied to reconstruct ecological processes or trace ecological activities (West et al., 2006). Among the most numerous uses of stable isotopes in ecology have been the applications to dietary patterns, primarily aiming to unravel dietary inputs of animals and providing semi-quantitative information about dietary preferences (West et al., 2006). On the other hand, biologists have also used stable isotopes to infer geographical origins (tracking movement patterns) and to differentiate among populations of animals (Rubenstein and Hobson, 2004).

Applying stable isotope analyses to these problems has proven the most promising step forward in ecological studies attempting to examine diet, niche and food web

composition. The carbon and nitrogen isotopic composition of consumer tissues can be defined as a function of: $\delta^{15}N$ and $\delta^{13}C$ of each prey species; the relative proportions of each prey species assimilated; the isotopic fractionation associated with converting prey tissue into consumer tissue; and foraging location (Bearhop et al., 2004). The stable isotope signatures of tissues generally reflect diet over the period of tissue synthesis, so that tissues with different turnover rates will integrate dietary information over different temporal periods (Bearhop et al., 2002) (i.e. blood being short term, bone long term). These are clear advantages over standard dietary analysis techniques, with potential for much more information which was previously inaccessible. Bearhop et al. (Bearhop et al., 2002) outline the main practical problems associated with quantifying trophic niche width using conventional dietary analysis:

1. Difficulty of getting an accurate measure of abundance of different prey items. Measurements are often subject to unreliable estimation.

2. Temporal integration of dietary information is often difficult to quantify, making any standard dietary analyses limited to a very short period of time (snapshot).

3. Variation in prey assimilation rates cannot be considered.

Although stable isotopes have been applied to populations of many vertebrate and invertebrate species (both in terrestrial and aquatic systems), this review focuses primarily on research conducted on fish. Fish, unlike vertebrate endotherms, have a discontinuous pattern of growth depending on the availability of their food source. Isotope turnover is defined as the change in tissue isotope composition attributable to growth and metabolic tissue replacement (MacAvoy et al., 2001). For ectotherms, like fish, isotope turnover in the muscle depends primarily on growth rather than metabolic replacement. However, the liver being a regulatory tissue with a continuous protein turnover may retain isotopic variation in times of limited growth (Perga and Gerdeaux, 2005). This idea was investigated using populations of whitefish in Lake Geneva, comparing muscle, liver, and food isotope compositions (Perga and Gerdeaux, 2005). The seasonal amplitude of isotope variation was found to be two to three times higher in liver compared to muscle tissue; and that during autumn and winter, the liver isotope signature was the only one responding to changes in isotope composition of food sources, while the muscle tissue only reflected food consumption during the spring and summer (Perga and Gerdeaux, 2005).

Currently, three main methods are employed in order to assess temporal diet variation using naturally occurring stable isotopes: 1) Compare samples from the same type of tissue that has been sampled over time, 2) Compare tissues with different metabolic rates, 3) Compare sections from tissues with progressive growth. According to Dalerum & Angerbjorn, knowledge of tissue specific metabolic rates, which determine the molecular turnover for a specific tissue, is of central importance for all these comparisons (Dalerum and Angerbjorn, 2005). When comparing different tissues, estimates of isotopic fractionation or differences between the tissues would be required. Any study of this nature would benefit from the use of multiple tissues with different turnover rates (Dalerum and Angerbjorn, 2005). Fish scale collagen for example, has been shown to contain a carbon isotope signature that derives from later formed collagen (effectively restricted the last season of growth), rather than exhibiting a progressive growth record similar to hair, feathers, claws, or teeth (Hutchinson and Trueman, 2006).

Other problems can arise when stable isotopes are used specifically to assess diet, trophic level and niche width in wild populations. This is due to variations in isotopic signatures among individuals, which depend on inherent variability as well as differences in feeding habit (Barnes et al., 2008). Essentially, this error can be corrected by measuring the inherent variability in a wild population on a case-by-case basis, thus establishing the baseline variation necessary for absolute assessment of dietary habits (Barnes et al., 2008). Dealing with lipids in stable isotope analyses provides further challenges because they are depleted in carbon relative to proteins and carbohydrates, and variation in lipid content among organisms or among tissue types has the potential to introduce considerable bias in studies focusing on carbon (Post et al., 2007). This problem can be resolved with direct lipid extraction and mathematical normalization.

A number of studies have been successful in determining niche segregation, dietary life history variation, ontogenetic dietary changes, migration, and resource acquisition using stable isotope signatures from vertebrate tissues. A celebrated example of vertebrate niche differentiation and speciation are the cichlid fishes inhabiting Lake Malawi in Africa. Lake Malawi is inhabited by over 500 cichlid species, many of which have similar morphology and appear to utilise similar resources. An examination of stable isotope signatures from the muscle tissue of five sympatric

species revealed that while two species had segregated, the remaining three exhibited considerable overlap (Genner et al., 1999). These species were also anatomically indistinguishable, and the dietary similarity was confirmed by stomach content analysis, thus suggesting that dietary segregation was not necessarily key to coexistence in this case (Genner et al., 1999). The authors followed on to show that isotopic signatures in Malawi cichlids change throughout their life history, with an ontogenetic dietary shift from planktonic to benthic food sources (Genner et al., 2003).

Ontogenetic dietary changes have also been shown in coral reef fishes in the Caribbean. In this case juveniles seek refuge in the mangrove nursery grounds, progressively moving out towards the seagrass beds and coral reefs as the get older (Parrish, 1989). Stable isotopes in combination with gut content analysis of herbivorous and carnivorous fish revealed that juveniles and adults are separated ecologically and spatially for a significant amount of time, and that herbivorous fish do not change their trophic status with increasing size, whereas carnivorous fishes feed on increasingly larger prey at increasingly higher trophic levels prior to their migration from the nursery to the reef (de la Moriniere et al., 2003). Migration and resource use has been determined for many salmonids, famous for their shift through riverine, lacustrine and marine environments throughout their life history. Results based on stable isotope analysis suggest better growth conditions in the lacustrine system and marine environments, indicating possible adaptive advantages for salmonid movement away from natal streams and brooks (Jardine et al., 2005).

The habitat of the European eel (*Anguilla anguilla*) spans untold distances including changes from the marine to freshwater system. Using stable isotopes, Harrod et al. found significant differences in carbon, nitrogen and C:N ratios of eels when comparing between brackish, freshwater and marine habitats (Harrod et al., 2005). A discriminates analysis suggested considerable movement and connectivity between freshwater and brackish habitats, including a growth correlation (mean condition and estimated age was significantly lower in marine eels, whilst observed length at age was significantly higher in marine eels, intermediate in brackish and lowest in freshwater) (Harrod et al., 2005). Combining stable isotope analysis with gut contents analysis provides a powerful tool for describing complex and fine scale trophic relationships (Woodward and Hildrew, 2002). Using this combination to assess

trophic position and identify diets of six freshwater fish led to the conclusion that both methods were equally robust at discriminating trophic groups of fishes (Rybczynski et al., 2008). While neither method detected seasonal changes in omnivore diets, overall stable isotopes are less laborious and allow for comparisons of food web structure and element cycling (Rybczynski et al., 2008).

Research on niche differentiation in sticklebacks has considered fluctuating asymmetry of morphological traits (fluctuating asymmetry of a dimensional or meristic trait is characterized by the left minus tight difference and has a normal distribution with a mean equal to zero) in relation to trophic level using stable isotope analysis and gut contents (Eric et al., 2007). Results strongly suggest that fluctuating asymmetry of pectoral fins affects foraging behaviour, and that stable isotopes of individuals phenotypes provide a useful tool for assessing the ecological consequences of morphological variation (Eric et al., 2007). On the level of fish communities, it has been hypothesized that inter-specific competition will reduce niche utilization and drive morphological evolution in character displacement (Brown and Wilson, 1956).

In a study on populations of roach and perch in Swedish lakes, morphometrics, stomach contents, and stable isotope analyses were used to show that resource polymorphism and adaptive morphological variation arise as a function of tradeoffs between general body form and ecological performance (Svanback et al., 2008). This is driven by different optimal morphologies for searching and attacking prey in littoral and pelagic habitats of lakes (Svanback and Eklov, 2003; Svanback and Eklov, 2004; Svanback et al., 2008), and confirms the hypothesis that resource polymorphism is very common in fish populations. Stable isotope analysis have been combined with phylogenetics, geometric morphometrics and diet to determine whether resource partitioning is the critical ecological pressure that drives adaptive radiations in isolated freshwater systems. Using endemic *Telmatherina* species isolated in Lake Matano of Sulawesi, researchers demonstrated that all species in the lake can categorized intro three lineages each possessing specialized skull shapes and pharyngeal jaw bones allowing them to exploit different resources (Roy et al., 2007). The study provides genetic, morphological, diet and trophic data that are consistent with the ecological seeding of adaptive radiation among the *Telmatherina* in Lake

Matano and provides an independent data set, demonstrating the ecological basis of this process (Roy et al., 2007).

1.6 Whole Genome Duplication (See Appendix 2 for Review)

As noted in the background on Corydoradinae biology, many species of Corydoras have undergone whole genome duplications. Here I review the phenomena of genome duplication, with particular focus on fish lineages.

When an organism's cells contain more than two homologous sets of chromosomes, that organism is referred to as a polyploid. Polyploidy is a mutation at the genomic level, and can occur in plants and animals via genome doubling, gametic non-reduction (mostly in plants), and polyspermy (Otto and Whitton, 2000). Throughout evolutionary time, there have been multiple rounds of whole genome duplications (polyploidization events), more so within the plant rather than animal lineages (Otto, 2007; Otto and Whitton, 2000; Soltis et al., 2010; Soltis and Soltis, 1995; Soltis and Soltis, 1999). Genome duplication is both an ancient and ongoing process in yeast, plants, fungi, and animals (Crow and Wagner, 2006). Hypotheses concerning the time and placement of these events within the animal phylogeny suggest that two rounds of duplication within early vertebrates provided the raw genetic material needed for the rapid diversification of those lineages (Donoghue and Purnell, 2005; Ohno, 1970; Otto and Whitton, 2000; Zhang, 2003). The exact process by which genomes duplicate is still being unravelled, yet two main mechanisms have emerged: Autopolyploidy, originating endogenously with all alleles at a locus derived from the same species; Allopolyploidy, arising through hybridization (Lynch, 2007). Part of the process is often determined by the presence or absence of unreduced gametes. There are many potential problems for a newly formed polyploid individual, particularly the fact that it will find itself primarily amongst diploid relatives, with which it is most likely to produce inviable offspring of intermediate ploidy level. Since a new population of polyploids is necessarily sympatric or parapatric with its ancestors, it can persist only if it has acquired sufficient ecological difference to coexist with and reduce gene flow from its diploid ancestor (Ramsey and Schemske, 2002). For plants, autopolyploidy via self-fertilization presents an alternative pathway, yet since many animals reproduce sexually allopolyploidy may be the more common mechanism.

Some theories from the plant literature predict that: (i) polyploidy should be more common in temperate than in tropical breeders because environmental fluctuations

may promote unreduced gamete formation; (ii) polyploidy should be most common in organisms with sufficient numbers of gametes that random meiotic problems can be overcome; (iii) and that polyploidy should be more frequent when mechanisms to promote assortative mating are a direct byproduct of genome duplication (Mable, 2004). Interspecific variation in DNA amount is positively correlated with cell size and mass rate of development, total length and/or volume of chromosomes at metaphase, total volume of nucleolar material, cell area and volume, and minimum cell doubling time (Bennett, 1987). Thus the C-value has considerable adaptive significance in plants, with a wide range of important phenotypic and phenological characters at levels from the cell to the organism and many of its relationships are strikingly precise because of their biophysical nature (Bennett, 1987). Considering changes in chromosome numbers and the subsequent reproductive barriers this can cause, it is postulated that polyploidy can be considered a borderline mechanism of rapid speciation, requiring only one or two generations (Stebbins and Ayala, 1981).

Although polyploidy is less frequent in vertebrates, it does occur and is most common among the fishes. Since the advent of karyotyping techniques, and the introduction of methods to quantify total genomic DNA content within the nucleus (C-value), certain lineages of fishes have revealed extreme cytogenetic variation (Hinegardner, 1968; Hinegardner and Rosen, 1972; Venkatesh, 2003). Results from sequencing data have revealed seven HOX clusters (involved in the control of segmental development) in the zebrafish genome, compared to four in tetrapods (Amores et al., 1998). Comparative analyses of several model genomes have uncovered that polyploidization events are likely to have occurred after the divergence of amphioxus and the vertebrates (Furlong and Holland, 2004), within the cartilaginous fishes (Robinson-Rechavi et al., 2004), at some point prior to the diversification of actinopterygiian and sarcopterygian lineages (Christoffels et al., 2004; Crow et al., 2006; Taylor et al., 2003), and within the Eutelost (Robinson-Rechavi et al., 2001) and Teleost lineages (Hoegg et al., 2004). Some of these events have been recorded within certain actinopterygiian families, such as the Salmonidae (Johnson et al., 1987), Catostomidae (Ferris and Whitt, 1979), Acipenseridae (Ludwig et al., 2001), Cyprinidae (David et al., 2003), and Callichthyidae (Oliveira et al., 1992). Using the Cyprinidae and Salmonidae to illustrate attributes of polyploidy in fishes, Le Comber & Smith concluded that polyploidy may have been of considerable importance in the

evolution of fishes. Since most theories related to the origin and effects of polyploidy come from plant genetics, fish offer diverse alternative modular organisms to test those theories (Le Comber and Smith, 2004).

From a phylogenetic perspective, one large assemblage of fishes (primarily freshwater) referred to as the Otocephala (including Siluriformes, Cypriniformes, Characiformes, Gymnotiformes etc) contain multiple examples of extant polyploids, with some species up to octaploid. Although there still remain many gaps in the data, it is worth noting that the otocephalan polyploid families (Cyprinidae, Catostomidae, Cobitidae, Callichthyidae, Loricaridae, and Curimatidae) are all hyper-diverse lineages of freshwater fish, constituting a large proportion of the known polyploid actinopterygiian families within one massive clade. Of the remaining polyploid fishes, all are either brackish, anadromous, or freshwater (Nelson, 2006). Whether aided by the expansion of their genomes or not, polyploid Otocephala include species capable of: breathing air, adapting to extreme conditions such as low oxygen or salinity gradients, mud burrowing and travelling over land (Nelson, 2006). Size variation, characteristic morphological reductions such as secondary loss of sight, Weberian reduction, vestigial fins, lack of scales, absent jaw teeth, and the retention of ancestral bony plates are among some of the morphological features common amongst polyploid fish (Nelson, 2006).

There are no polyploid actinopterygiians that are purely marine, or it may just be that they await discovery (Yi and Streelman, 2005). There is an interesting deviation known among the salmonids where triploids prefer saltwater (Galbreath and Thorgaard, 1995). With a few exceptions, polyploidy is more common amongst lower teleosts and relict bony fishes rather than higher teleost lineages such as the Perciformes (Leggatt and Iwama, 2003). As the largest order of fishes with over 10,000 species, the Perciformes are primarily marine shore fishes, with about 2,000 distributed strictly in freshwater, and about 2,200 that occur in freshwater for at least part of their life history (anadromous, catadromous etc.) (Nelson, 2006). Of the 156 Perciformes families, only one that we know of is polyploid (Channidae: freshwater; snakeheads), while all the rest are diploid. Thus, polyploidization events seem to have occurred within particular families, while the majority of fish genomes have remained diploid with a mean of 48 chromosomes. Some early work by on fish genome sizes suggested that taxa characterized by larger genomes should display disproportionately

stronger fluctuations in genome size. According to this hypothesis, one would expect a negative correlation between average within-family genome size and its corresponding coefficient of variation (Hinegardner, 1968; Hinegardner and Rosen, 1972). This hypothesis was tested with an extended data set, and analyses failed to confirm the original HR correlation (Pie et al., 2007). A significant skew in the frequency distribution of fish genome sizes suggests that the dynamics underlying genome size evolution are driven by multiplicative phenomena, possibly related to chromosomal rearrangements and the expansion of transposable elements (Pie et al., 2007).

It is now accepted that two rounds of WGD occurred during the early diversification of chordates and vertebrates, with extensive evidence supporting the subsequent teleost fish-specific genome duplication (FSGD) (Dehal and Boore, 2005; Hoegg et al., 2004; Van de Peer et al., 2009). The evolutionary and ecological consequences of these events are likely to have had affected relative rates of diversification and competitive interactions between duplicated and non-duplicated species. Furthermore, if such events are placed within a temporal framework, their consequences can be considered in the light of historical events that have inevitably shaped global biodiversity (i.e. mass extinctions and the availability of new niches) (Fawcett et al., 2009). The ability of a polyploid organism to occupy a new niche is crucial because otherwise competition with the presumably well-adapted diploid progenitor would be particularly pronounced. Moreover, newly formed polyploid populations are likely to be small, potentially allowing drift to fix ecologically relevant traits rapidly (although selection will be weaker). This may explain the prevalence of polyploidy in freshwater species of fish, as population sizes tend to be smaller than marine fishes (Yi and Streelman, 2005), thus allowing for drift to maintain ploidy shifts at a greater rate than in marine species where selection against polyploids will be much stronger. An intriguing hypothesis based on comparative genomics in plants is that genome duplication events tended to be clustered around the Cretaceous-Tertiary boundary (K-T boundary), when many plant species went extinct, suggesting that polyploidy might have increased survival during times of environmental upheaval (Fawcett et al., 2009).

1.7 Genome Size & Diversification

Evidence suggests that polyploid Corydoradinae catfish lineages are more species rich than diploid lineages. The correlation between polyploidy and species richness has received a considerable amount of attention. Here I review the relevant methodology for investigating such correlations within comparative frameworks, the work that has been done on plants and fishes, and subsequently, the plausible mechanisms by which genome duplication can drive diversification rates.

Recent methodological advances in evolutionary biology are allowing researchers to test fundamental questions about patterns of species richness across the Tree of Life that have previously been out of reach (Rabosky and Alfaro, 2010). These advances involve the use of phylogenetic trees in combination with taxonomic information in order to address hypotheses concerning patterns of speciation through time and space at different hierarchal levels (Alfaro et al., 2009b; Bininda-Emonds et al., 2007; McPeek, 2008; Phillimore and Price, 2008; Venditti et al., 2010). In particular, the inference of diversification rates (speciation – extinction) using time calibrated molecular phylogenies to detect acceleration and deceleration (shifts) in diversification (Chan and Moore, 2005; Cusimano and Renner, 2010; Liow et al., 2010; Moore and Donoghue, 2009; Quental et al., 2010; Rabosky, 2006; Rabosky, 2009a; Rabosky, 2009b; Rabosky, 2009c; Rabosky, 2010; Rabosky and Lovette, 2008), how character states affect diversification (FitzJohn, 2010; FitzJohn et al., 2009; Maddison, 2006; Maddison et al., 2007), and detecting signatures of extinction (Purvis, 2008; Rabosky, 2009c; Rabosky, 2010; Rabosky and Lovette, 2008). Challenges persist, primarily in the integration of paleontological diversification with contemporary molecular phylogenies (Rabosky and Alfaro, 2010), however, existing methodological frameworks are currently available to test hypotheses concerning the role character evolution on diversification rates.

A fundamental question arising from studies of WGDs is whether such events accelerate diversification rates by providing a greater repertoire of genetic material for selection to act upon (Kraaijeveld, 2010; Ohno, 1970). Evidence from plants and fishes suggests that WGDs may account for the diversification of relatively small proportions of extant species (Santini et al., 2009; Wood et al., 2009). Ancient rounds of duplications among vertebrate ancestors have been well established, however,

subsequent diversification within certain hyperdiverse lineages (i.e. Perciformes) may ultimately be determined by ecological success and morphological plasticity (Alfaro et al., 2009b; Friedman, 2010; Rabosky, 2009a; Rabosky, 2009b). Positive correlations between increases in chromosome numbers and C-value with rates of diversification/cladogenesis within vertebrate polyploid groups are very rare, and tend to be marginally significant (Mank and Avise, 2006a; Mank and Avise, 2006b; Santini et al., 2009). The lack of a correlation may be due to the highly conserved genome size and chromosome numbers within the most diverse lineage of fishes (Perciformes >10,000 species) that inevitably skew the results of such analyses. New comprehensive phylogenies emerging for vertebrate orders containing polyploids, preferably focusing on those lineages that have undergone multiple rounds of duplication, will open the door for these questions to be re-examined at different scales. Furthermore, there is some evidence to suggest that decreases in genome size drive diversification rates (Kraaijeveld, 2010).

1.8 Mechanisms of Diversification post WGDs

Evidence from remnant duplicate gene pairs has been interpreted as evidence that an ancient genome duplication event of tetraploidization (followed by rediploidization) enabled the diversification of gene functions necessary to promote the explosive speciation in fish (Luo et al., 2007; Volff, 2005). The loss of certain genes, their subdivision, and acquisition of novel functions over evolutionary time seem to be linked with the evolution of fish variability (Lynch, 2007; Meyer and Schartl, 1999; Siegel et al., 2007; Vogel, 1998). Yet, despite the indirect evidence, a link between a specific genome duplication event and an increase in overall complexity and diversity remains to be established (Donoghue and Purnell, 2005; Kraaijeveld, 2010; Otto and Whitton, 2000; Soltis et al., 2010). Correlations between specific duplications and increased diversity are problematic, as the genetic signature of single duplication events tends to be obscured by extensive genomic expansion, contraction, and subsequent gene loss (Blanc and Wolfe, 2004; Crow and Wagner, 2006). Drawing from a large pool of cytogenetic data, some researchers have suggested that the probability of extinction was reduced by a factor of at least 5.5 in lineages following the fish-specific genome duplication (Crow and Wagner, 2006).

A relatively recent theory suggests that duplicate genes arise at a rate of 0.01/gene/million years, evolve in a neutral manner for a brief period, and are subsequently 'silenced' within a few million years (Lynch and Conery, 2000; Lynch and Force, 2000). The few remaining genes that are not silenced post-duplication are likely to undergo strong purifying selection. The authors argue that the silencing may play a significant role in the passive origin of new species, suggesting that genome size and complexity can increase due to decrease (or fluctuations) in effective population size (Lynch and Conery, 2003). In support of this theory, previous research suggested that freshwater fish species that have smaller effective population sizes than marine fish, also have larger genomes (Yi and Streelman, 2005). The authors demonstrate that genome size is negatively correlated with genetic variability, and independent of phylogeny, body size and generation time. However, these conclusions might be limited by gaps in the phylogenetic data and estimation of effective population sizes that are notoriously difficult (Gregory and Witt, 2008). Positive correlates between species richness and evolutionary increases in C-value within actinopterygiian fishes are relatively small, yet significant enough to support

the notion that genomic architecture influences the multiplication of species (Mank and Avise, 2006).

Chromosomal rearrangements are known to occur following WGDs (Jaillon et al., 2004), and have been shown to contribute to reproductive isolation (Rebollo et al., 2010; Rieseberg, 2001). Furthermore, the duplication of pigmentation genes may also be a route to reproductive isolation, as recent evidence suggests that following the fish specific genome duplication, novel pigment cells have arisen and increased in complexity thereby contributing to colour pattern diversity amongst teleost lineages (Braasch et al., 2009). Alternative explanations for increase in organismal complexity and accelerated diversification arises from microRNAs, through the regulation of gene expression (Frith et al., 2005; Lee et al., 2007; Taft et al., 2007). Recent evidence from microRNAs suggests that WGDs account for an increase in the diversity of microRNA family members, but not the origin of novel families (Heimberg et al., 2008). This suggests that the expansion of the microRNA repertoire may be of greater importance to the origin of vertebrate complexity than the increase in protein coding genes (Heimberg et al., 2008).

1.9 Neotropical Freshwater Fishes: Diversity and Distribution

The Neotropics contain unparalleled biotic diversity in both terrestrial and freshwater ecosystems. Within this region, the Corydoradinae are widespread throughout multiple river basins, while *Corydoras* represents one of the oldest and most diverse genera of catfishes. I therefore chose to investigate the spatiotemporal distribution of the group, and review the literature on Neotropical fish diversity and distribution, as well as the relevant hypotheses of historical biogeography (and methods for testing them). Furthermore, I review the relevant literature on the contemporary distribution of Neotropical fish and conservation priorities for this highly threatened region.

With more than 25% of the world's freshwater connected by a massive network of river systems and lakes spread over an entire continent, Neotropical freshwater habitats set the stage for the diversification of unique ichthyofauna (Lundberg et al., 1998). This ecosystem is characterized by the most species rich freshwater fish assemblage on the planet, as estimates suggest more than 8,000 species inhabit the rivers, lakes and streams of South America (Albert and Reis, 2011; Schaefer, 1998). From a taxonomic perspective, the majority of this diversity is contained within the Otophysi superclade, primarily represented by Characiformes, Siluriformes and Gymnotiformes (in order of diversity), while Perciformes (primarily represented by the Cichlidae) are also highly diverse. Fossil evidence has shown that the vast majority of this ichthyofaunal assemblage was well established by the Neogene and even as far back as the Palaeogene (Hoorn et al., 2010), suggesting that the majority of diversity and complexity has accumulated steadily over long time periods as opposed to the recent radiations observed in other areas (Albert and Reis, 2011). Recent diversification within the last couple million years has also contributed to species richness (Lopez-Fernandez et al., 2010), however, the majority of lineages and diversity in morphospace existed long before the Quaternary (Lundberg, 1998).

With over 3,000 species, the order Siluriformes is of the most diverse vertebrate orders currently recognized, as 1 in 20 vertebrates is a catfish (Nelson, 2006). The Loricarioidae is a recognized superfamily of catfish, represented by more than 1,200 species in six different families that are all endemic to the Neotropics: Trichomycteridae, Nematogenyiidae, Callichthyidae, Scoloplacidae, Astroblepidae and Loricariidae (Nelson, 2006). As loricarioids have been traditionally considered a

sister group of African amphliids, their origins may date as far back as the Late Aptian-Albian (>112MYA), predating the separation of South America from other continents. In fact, fossil evidence from the callichthyid *Corydoras revelatus* (Cockerell, 1925) suggests that diversification within the Callichthyidae was well under way early in the Cenozoic. These data, along with fossil evidence from the Characiformes and certain Perciformes lineages support the idea that the diversity of Neotropical fishes is well rooted in the Palaeogene and earlier.

Are the oldest Neotropical fish lineages the most diverse? Is there a link between age, diversity and distribution? What are the main mechanisms that have shaped spatiotemporal patterns of Neotropical fish distribution? Despite many questions remaining unanswered, a number of patterns and hypotheses concerning Neotropical fish diversification and biogeography have emerged and survived rigorous tests using comparative frameworks.

1.10 Neotropical Historical Biogeography

The geological history of the South American continent and its freshwater ecosystems has been shaped by an ancient and dynamic sequence of continental drift, climatic fluctuation, marine incursions, tectonic uplift and west-east compression (Lundberg et al., 1998). This complex history reveals massive marine incursions from the Caribbean and South Atlantic, an eastern and western flow of the paleo-Amazonas system, massive wetlands, the northward flow of the paleo-Amazonas-Orinoco system, and a variety of arches creating barriers between different basins (Lundberg et al., 1998). Throughout this history, multiple episodes of vicariance and subsequent geodispersal (through stream capture events) have invariably affected the biogeography of many fish species (Albert and Reis, 2011), and suggest that much of the observed diversification has occurred in allopatry due to such events.

Given the ancient origins of Neotropical fish diversification, the latter processes have inevitably shaped patterns of faunal distribution observed today. Despite the inherent difficulty of reconciling paleogeography and hydrological history with phylogenetic history and modern patterns of distribution, it remains essential to further our understanding of Neotropical mechanisms of diversification. However, ecology should not be overlooked when considering historical biogeography, and the patterns that have shaped the large-scale distribution of clades (Wiens and Donoghue, 2004). One interesting ecological aspect of Neotropical ichthyofauna is that many species exhibit phylogenetic niche conservatism (Albert and Reis, 2011; Wiens, 2004; Wiens and Donoghue, 2004). As species retain ancestral ecological characteristics, they are ill suited to adapt to intermediate habitats, moving between regions that have similar ecological conditions while going locally extinct under conditions or in areas that are less ecologically suitable (Wiens, 2004). This phenomenon has only recently begun to receive attention by phylogeneticists studying Neotropical ichthyology, and may help explain many of observed patterns of non-adaptive radiation.

Several key hypotheses have been proposed to explain patterns of Neotropical fish biogeography (See Chapter 4, Table 1). Undoubtedly more hypotheses will be formulated, as it is often the case that many independent studies of Neotropical biogeography conclude with the proposition of new hypotheses to account for observed patterns. This is not surprising given the complexity of the region and the

evolutionary timescale involved, however, with the integration of multidisciplinary approaches bridging the gaps between ecology, evolution and biogeography, it is likely that common patterns will emerge for certain lineages.

1.11 Testing Biogeographic Hypotheses

More often than not, biogeographic treatments have been lacking in the sense that they often form a supplement of molecular systematic studies, and involve a significant amount of inductive story telling (Crisp et al., 2011; Morrone, 2009). However, for a number of years, methodological advances are allowing researchers to explicitly test biogeographic hypotheses using comparative frameworks and models based on likelihood and probability (Lemey et al., 2009; Ree et al., 2005; Ree and Smith, 2008; Templeton, 2009). Furthermore, bridging the gaps between ecology, biogeography, molecular phylogeny and paleontology is providing valuable insights, and has only begun recently (Crisp et al., 2011). This multidisciplinary approach may contribute new answers to some old questions (Grandcolas et al., 2008), and should be employed when such data are available.

Two main methods have been traditionally employed to test biogeographic hypotheses using phylogenetic information: parsimony based area cladograms and likelihood based ancestral area reconstruction (Morrone, 2009; Parenti and Ebach, 2009). Both methods have their advantages and disadvantages, yet both provide valuable information and have contributed greatly to understanding spatiotemporal patterns of biotic distribution. However, likelihood based ancestral area reconstruction using dispersal - vicariance models have undoubtedly been most popular amongst researchers testing biogeographic scenarios (Ronquist, 1997). The specific method has been criticized for inflexibility of parameterization and simplistic assumptions (Kodandaramaiah, 2010; Ree et al., 2005), and has subsequently been replaced with a more complex Dispersal – Extinction – Cladogenesis (DEC) model (Ree and Smith, 2008). The advantage of this methodological advance is that the latter model incorporates physical and biological information that can be parameterized in the model, allowing the user to set a number of different conditions regarding a priori information on dispersal probability and limitations (Ree and Smith, 2008). By comparing resulting likelihood values, one is then able to more accurately test predictions concerning relative dispersal, extinction and cladogenesis. More recently, the integration of GIS information with Bayesian statistics and phylogenetics has led to significant conceptual and methodological advances (Kozak et al., 2008; Lemey et al., 2009), and presents a considerable step forward for inter and intraspecific approaches to testing biogeographic hypotheses.

1.12 Current Distribution, Biodiversity hotspots, & Conservation

In the face of globalization, habitat loss and anthropogenic disturbance, freshwater ecosystems are possibly the most endangered habitats on our planet (Millennium Ecosystem Assessment: MEA 2005). The Amazon basin, and Neotropical river systems are key areas that host a massive proportion of freshwater biodiversity while facing numerous problems (Revenga et al., 1998). Major threats currently identified that are causing significant declines in freshwater fish biodiversity include physical alteration, habitat destruction, water withdrawal, overexploitation, pollution and invasive species (Revenga et al., 2005). More specifically, hydroelectric dam construction, commercial navigation waterways, oil and gas extraction and exploration, road development (such as the Trans-Amazonica highway), agriculture, mining and logging are all encroaching on rainforest habitats and biodiversity hotspots (Thieme et al., 2007). Despite calls for an increased effort to gather relevant information for effective management and policy implementation, targets have still not been met and major gaps in our knowledge of the biodiversity of certain key areas (e.g. Amazonas lowlands) remain. Scientists have repeatedly advocated for an ecosystem approach to freshwater management, however, that remains to be implemented on a large scale due to the complexity of managing river systems that cross borders and a multitude of habitats (Dudgeon et al., 2006). Furthermore, proposal to protect remote Amazonian basins (such as the Madre de Dios system) are inevitably problematic as connectivity between different river catchments is being permanently altered via hydroelectric dam construction and diversion of major tributaries in the recent cases of the Rio Madeira and Rio Xingu (Thieme et al., 2007).

Although Marine Protected Areas (MPAs) have received a great deal of attention (while also being currently established worldwide), Freshwater Protected Areas (FPAs) have been almost entirely ignored as a concept (Abell et al., 2007). Within Brazil, there are currently a large number of national parks and protected areas, however, these do not adequately protect freshwater ecosystems (Mittermeier et al., 2005). Arguments for the conservation of freshwaters for their own sake have gained minimal public support, however, evidence suggests that the benefit of maintaining functional freshwaters greatly outweighs the costs of impaired freshwater systems in terms of ecosystem services (Abell et al., 2007). In an effort to promote the conservation of freshwater ecosystems, and as a step towards the identification of

potential suitable areas, a significant effort has been to outline the worlds freshwater 'ecoregions' as units for freshwater biodiversity conservation (Abell et al., 2008). These data are particularly relevant as they have been assembled based on fish biodiversity and endemism, constituting a useful tool for underpinning global and regional conservation planning efforts and conservation strategies (Abell et al., 2008). More similar efforts are required on an even finer scale, which ideally should be updated on regular basis as our knowledge of distribution of species increases, considering that more than 200 new species of freshwater fish are described every year (Albert and Reis, 2011).

The Brazilian Atlantic Forest and the Cerrado (savannah) are two key biodiversity hotspots with a large number of endemic species, while three larger biodiversity wilderness areas have been classified that contain the majority of the taxon load: the Amazon, Pantanal and Caatinga (Mittermeier et al., 2005). In terms of freshwater fish diversity, a large number of critically endangered species belong to the order Cyprinidontiformes (Rivulidae), restricted to the south and south eastern ecoregions of Brazil (Agostinho et al., 2005). More recent efforts have resulted in the delineation of 540 small watershed areas harbouring 819 freshwater fish with restricted ranges within Brazil alone (Nogueira et al., 2010). These areas are highly threatened due to deforestation and hydroelectric dam construction, with a very large proportion of endemic species that will go extinct if measures are not taken to adequately protect them (Nogueira et al., 2010). Furthermore, deforestation and habitat loss is not only leading to loss of ichthyofaunal diversity but also alteration in community structure which may lead to ecosystem effects (Bojsen and Barriga, 2002). These problems also extend to large species that rely on large river stretches for migration as part of their reproductive strategy. These species would be best conserved under the concept of umbrella species, which has yet to be applied to aquatic ecosystems (Roberge and Angelstam, 2004).

Chapter 2: Competition and phylogeny determine community structure in Müllerian co-mimics

2.1 Abstract:

Until recently, the study of negative and antagonistic interactions (e.g. competition and predation) has dominated our understanding of community structure, maintenance and assembly (May, 1981). Nevertheless, a recent theoretical model suggests that positive interactions (e.g. mutualisms) may counterbalance competition, facilitating long-term coexistence even among ecologically undifferentiated species (Gross, 2008). Müllerian mimics are mutualists that share the costs of predator education (Rowland et al., 2007) and are therefore ideally suited for the investigation of positive and negative interactions in community dynamics. The sole empirical test of this model in a Müllerian mimetic community supports the prediction that positive interactions outweigh the negative effects of spatial overlap (without quantifying resource acquisition) (Elias et al., 2008). Understanding the role of trophic niche partitioning in facilitating the evolution and stability of Müllerian mimetic communities is now of critical importance, but has yet to be formally investigated. Here I show that resource partitioning and phylogeny determine community structure and outweigh the positive effects of Müllerian mimicry in a species rich group of neotropical catfishes. From multiple independent reproductively isolated allopatric communities displaying convergently evolved colour patterns, 92% consist of species that do not compete for resources. Significant differences in phylogenetically conserved traits (snout morphology and body size) were consistently linked to trait specific resource acquisition. Thus, I report the first evidence that competition for trophic resources and phylogeny are pivotal factors in the stable evolution of Müllerian mimicry rings. More generally, our work demonstrates that competition for resources is likely to play a dominant role in the structuring of communities that are simultaneously subject to the effects of both positive and negative interactions.

2.2 Introduction:

Positive interactions - such as mutualistic associations - can play important roles in the maintenance of community structure, potentially outweighing the negative effects of competition for niche space (Gross, 2008). The empirical evidence for such phenomena is biased towards plants (Brooker et al., 2008; Callaway, 1995), with a single study on mimetic butterfly communities (Elias et al., 2008) complementing such research. The study convincingly demonstrated that Müllerian mimic butterflies converge spatially, but only considered habitat utilisation, while resource consumption and trophic niche were indirectly inferred as likely correlates of other variables (such as forest structure, topography, and flight height). However, until now the extent of trophic overlap or the evolution of morphological traits associated with resource acquisition has not been directly quantified in Müllerian mimetic communities and this limits our ability to infer the importance of mimicry in determining community structure. Combining ecological, phylogenetic and morphological analyses provides a valuable empirical test of the recent theoretical proposition (Gross, 2008) that positive interactions among competitors can promote multispecies coexistence and the consequences of these interactions in terms of species diversity (Stachowicz, 2001). Tropical freshwater fish offer a novel vertebrate perspective on the evolution of mimetic community structure, as they inhabit discontinuous habitats and are exposed to a multitude of piscivorous predators (Ruxton et al., 2004).

The Corydoradinae (Teleostei: Siluriformes: Callichthyidae) are a species rich group of freshwater catfishes that inhabit streams, rivers and floodplains throughout South America (Fuller and Evers, 2005). The genus *Corydoras* comprises the majority of the Corydoradinae, and is the most species rich genus of catfish with over 150 described species and as many undescribed taxa (Fuller and Evers, 2005; Nelson, 2006). The Corydoradinae are almost all benthic omnivorous detritivores, consuming algae, terrestrial and aquatic insects, annelids and zooplankton (Fuller and Evers, 2005; Nijssen, 1970). At many sites, as many as three almost identically coloured species coexist and aggregate in large mixed shoals (Fuller and Evers, 2005; Nijssen, 1970; Sands, 1994), with each geographic location hosting a different shared colour pattern. These colour patterns include both cryptic and disruptive elements (e.g. spots, countershading and eye bars) and putatively aposematic elements (e.g. strongly

contrasting black and white stripes, orange and black patches and conspicuously colored spines). Interestingly, some colour patterns have also been adopted by species belonging to different families and orders (*Otocinclus* (Axenrot and Kullander, 2003), *Brachyrhamdia* and *Serrapinnis*).

In most cases, coexisting Corydoradinae species differ in snout morphology and body size (Nijssen, 1970). Recorded predators of the Corydoradinae include *Plagioscon squamosissimus* (Perciformes) (Luz-Agostinho et al., 2008) and *Hoplias malabaricus* (Characiformes) (Nijssen, 1970), while kingfishers, egrets and herons are the dominant avian predators of armoured catfishes (Power, 1984). Corydoradinae are protected by sharp, lockable pectoral and dorsal spines, tough scutes covering the side and dorsal surfaces (Nelson, 2006), and toxins secreted from the axillary glands (Greven et al., 2006; Kiehl et al., 2006; Wright, 2009). The widespread distribution of Corydoradinae, propensity to aggregate, shared colour patterns and post-capture defences make them a unique system to study the mechanisms underpinning community structure.

2.3 Methods:

2.3.1 Sample Acquisition & Phylogenetic Analyses

A total of 425 taxa (226 OTUs with multiple representatives when available) were obtained from wild populations collected by the authors, or purchased as wild caught aquarium imports (SI Table 1). Species for which I lack genetic material (*C. mamore*, *C. evelynae*, *C. ourastigma*, *C. crimmeni*, *C.* sp. CW19, *C.* sp. CW26, *C.* sp. C135, *C.* sp. C76, *C.* sp. C77), where identified and assigned to lineages on the basis of geometric morphometric analysis, with which it is possible to differentiate between lineages. Partial sequences of 12S rRNA, 16S rRNA, ND4, $tRNA^{HIS}$, $tRNA^{SER}$, Cytochrome b (Cytb), and Recombination Activating Gene (RAG1) were amplified for 425 taxa, and a nuclear intron from F-Reticulon 4 amplified for 24 taxa, using the polymerase chain reaction (PCR) with the primers detailed in (Table 1). All products were sequenced in both directions using Big Dye terminator technology (Applied Biosystems). The quality of chromatograms was visually inspected and contigs were assembled using Geneious v 4.7 (Drummond AJ, 2009). A total of 50 alternative alignments for the 12s, 16s, and F-Reticulon-4 markers were generated using ProAlign (Loytynoja and Milinkovitch, 2001), discarding unstable positions that differed more than 50% (Gap opening penalty 7-15; gap extension penalty 3-7). The ND4, Cytb, and RAG1 genes were aligned with MUSCLE (Edgar, 2004), and all alignments were checked by eye. Substitution saturation and base compositional biases were tested for each gene and partition. Model selection was performed using the hLRT criterion under a fixed BIONJ-JC topology in JModelTest (Posada, 2008). All data were partitioned by gene and coding position where appropriate. Incongruent Length Difference tests were performed to test for heterogeneity between nuclear and mitochondrial data sets. Subsequent analyses were performed on the following data sets: 1) MIT: all mitochondrial markers (For practical purposes, 12s+16s considered as a single partition, as were $tRNA^{HIS}$+$tRNA^{SER}$); 2) MITNUC: the MIT and RAG1 data combined; 3) MITNUC2: a smaller subset of the MITNUC data combined with the F-Reticulon 4 intron for 25 representative taxa; 4) RAG1: a single nuclear marker independent of mitochondrial data. I rely primarily on the MIT dataset as it represents the largest sample of linked markers, and use the others for comparisons. RAxML (Stamatakis, 2006) using the web server RAxML BlackBox (Stamatakis et al., 2008) was used for maximum likelihood analyses under a mixed partition model for all

analyses. Random starting trees were used for each independent ML tree search and all other parameters were set on default. Topological robustness was investigated using 500 non-parametric bootstrap replicates. Analyses were conducted under both GTR+G and GTR+I+G in order to assess whether implementing P-Invar and Gamma together affected parameter estimation. Bayesian analyses were performed using MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). Metropolis-coupled Markov chain Monte Carlo (MCMC) runs were set with random starting trees, one cold and three heated chains for 30 million generations, sampled every 1,000 generations for all data sets. I used the default uniform Dirichlet distribution for the base frequencies, and default prior distributions for all other parameters. Bayesian posterior probabilities were then calculated from the sample points after MCMC convergence. To ensure that analyses were not trapped in local optima, two independent MCMC runs were performed. Topologies and posterior clade probabilities from different runs were compared for congruence using Tracer 1.4 (Rambaut and Drummond, 2004), to ensure adequate estimated sample sizes and ensure adequate mixing of parameters. All trees estimated prior to convergence were discarded. Trees from different runs were then combined using LogCombiner (Drummond and Rambaut, 2007), and maximum clade credibility trees (mean node heights) were estimated using TreeAnnotator (Drummond and Rambaut, 2007).

Table 1- Primers

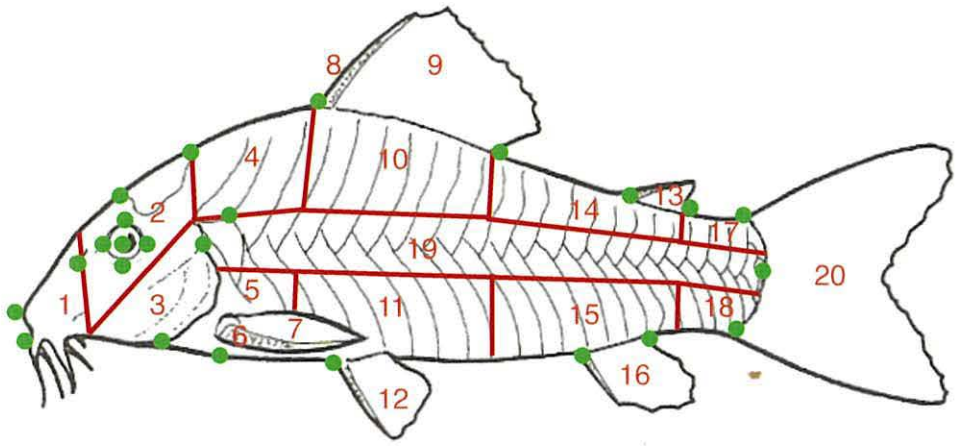
Locus	Primer Sequences	°C
12s	12sA-5 F (5'-AAACTGGGATTAGATACCCCACTAT-3') 12sB-3 R (5'-GAGGGTGACGGGCGGTGTGT-3')	58-60
16s	16sA5 F (5'-CGCCTGTTTATCAAAAACAT-3') 16sBR3 R (5'-CCGGTCTGAACTCAGATCACGT-3')	53-55
ND4	L11935 (5'-CCA AAA GCA CAC GTA GAA GC-3') H12857 (5'-ACC AAG AGT TTT GGT TCC TA-3')	53-55
Cytb	GLU-Cory F (5'-CGACTTGAAAACCCATTGTTG-3') tRNA THr (5'-CCGGCGCTTTATGAGTTAAG-3')	53-55
Rag1	Rag 1 MA F (5'AAGGAGAGGGGTATAGATGATA3') Rag1 MA R (5'-GCAAAACGCTGAGAGTTGAA-3')	55
FR-4	RTN4-DG F (5'-GTYTGGYTGGTYRTGGTRAARCC-3') FRETICUL4-R2 R (5'-AARTCCATCTCACGCAGGA-3')	54

Primer sequences, sequence length and optimal annealing temperatures for the six markers employed in this study. Primers for the FR-4 intronic region were newly developed by Juan Montoya-Burgos, while new RAG1 primers were also developed specifically for the Corydoradinae.

2.3.2 Geometric Morphometrics

A total of 200 preserved individuals, representing over 120 different species (including all mimetic taxa), were photographed and used for digital landmark-based morphometric analysis of body shape (SI Table 1). I defined 24 easily identifiable homologous landmarks (Figure 1), including two for scaling (ruler 1cm). Photographs with landmarks were digitalized (using tpsDig by F.J. Rohlf, available at http://life.bio.sunysb.edu/morph/), and converted to coordinates under Procrustes superimposition (using CoordGen6 by H.D. Sheets, part of the Integrated Morphometrics Package (IMP) at: http://www2.canisius.edu/~sheets/morphsoft.html) in order to describe shape change independent of size (removing potential ontogenetic effects and issues of allometry). I used PCAgen and CVAgen packages from IMP in order to create multivariate plots of Procrustes superimpositions (with axes normalized to lengths of one for all plots and outputs) and vectors of CV coefficients (scaled to a length of one) respectively. I also conducted pairwise comparisons of co-existing lineages to check for statistically significant differences in shape using Goodall's *F*-tests as implemented in TwoGroup6. Deformation plots were generated to identify landmarks contributing to shape changes across lineages and between co-mimics.

Figure 1- Colour Pattern Outline and Landmarks



Areas selected, characters: 1) snout 2) upper posterior head / eye + nuchal plate 3) lower posterior head / gill plate 4) upper anterior body 5) lower anterior body 6) pectoral spine 7) pectoral fin 8) dorsal spine 9) dorsal fin 10) upper mid body 11) lower mid body 12) pelvic fin 13) adipose fin 14) upper posterior body 15) lower posterior body 16) anal fin 17) upper posterior caudal 18) lower posterior caudal 19) lateral section 20) caudal fin. Colour pattern characteristics scored as present (1), absent (0) or variable (0.5) for each of the 20 sections defined above were: a) contrasting bands (single band of colour either horizontally or vertically), b) reticulations, c) spots (small areas of pigmentation or lack of it, less than 5mm in diameter, d) blotches (large areas of dark pigmentation without clearly defined borders of shape, greater than 10mm in diamater), e) bright coloration (areas of bright coloration e.g. orange, yellow etc), f) patches (very dark or black pigmented areas with defined borders and shape, greater than 10mm in diameter, g) stripes, h) uniform brown and i) light coloured surface lacking any dark pigmentation. A total of 24 homologous landmarks shown as green circles. The outline figure was modified from Nelson (2006), Fishes of the World.

2.3.3 Stable Isotope Analysis

Muscle tissue was collected from twenty individuals (where possible) from 18 species belonging to eight different independent mimicry rings each species for each respective site. All samples were dried overnight in an incubator at 60°C to constant weight before storage in 1.5ml centrifuge tubes containing silica gel. On return to the laboratory, the tissue samples were homogenized to a fine powder using a mortar and pestle. Approximately 0.7 mg of homogenized muscle was weighed out and distributed into tin capsules for analysis by continuous-flow isotope ratio mass spectrometry (CF-IRMS), using a Costech (model ECS 4010) elemental analyzer (EA) with a ThermoFinnigan Delta Plus XP mass spectrometer. I used a number of

gelatine and alanine standards (two for every ten samples), in order to obtain a standard deviation of 0.2 ‰ for $\delta^{15}N$ and 0.1 ‰ for $\delta^{13}C$. Samples of ground tryptophan were incorporated into each run as independent isotope standards and to calculate C and N abundance. These internal laboratory standards have been measured against secondary international isotope standards provided by the NIST and IAEA". All data are referenced to the primary international standards AIR ($d^{15}N$) and V-PDB ($d^{13}C$). The effects of lipid extraction were checked on a subset of samples using a 10:5:4 methanol/chloroform/water extraction, repeated 3 times to obtain a clear supernatant (Pinnegar and Polunin, 1999). Isotope values from lipid and non-lipid extracted samples of the same aliquot were the same. Separate aliquots of muscle tissue were used to obtain carbon and nitrogen isotope analyses. All resulting carbon and nitrogen stable isotope ratios were checked for conformity to a normal distribution and analysed with one-way ANOVAs for mimicry rings consisting of >2 species, followed by Tukey-Kramer post-hoc tests. In cases with only two species, I used a two-sample T-test to compare means, followed by Bonferroni post-hoc corrections. Significant differences in stable isotope ratios were then used to determine the degree of dietary differentiation in order to identify communities composed of species that partition trophic resources from those that do not. However, stomach contents analyses were not conducted and therefore these results cannot be extended to quantify of dietary overlap (i.e. the proportion of food items shared between coexisting species.

2.3.4 Colour Pattern Analysis

Pictures of live fishes were obtained from specimens caught in the wild and later kept under standardized aquarium conditions. Colour patterns (not hue) of mimetic species were quantified by dividing the lateral section of each fish into 20 subsections (Figure 1). Subsections were then scored for the presence (0), absence (1), or variability (0.5) of particular patterns (contrasting bands, reticulations, spots, blotches, bright patches, dark patches, stripes, uniform brown and lack of patterns). Using these data I created a matrix describing colour pattern based on 100 characters for 52 mimetic species, and generated a pairwise Euclidian distance matrix using MVSP 3.1 (Kovach, 1999). A second pairwise matrix was created to score the respective members of independent mimicry rings in terms of geographic distribution, where sympatric species were scored 1, while allopatric species were scored 0. The significance of the relationship

between the two matrices was investigated using a Mantel test with 10,000 permutations using the software zt (Bonnet and Van de Peer, 2002).

2.4 Results:

2.4.1 Phylogenetics and Mimicry

A taxonomically comprehensive (80% coverage) phylogeny was constructed from 425 taxa (SI Table 1) using Bayesian and Maximum Likelihood methods to resolve the relationships within the Corydoradinae and determine whether colour patterns are the result of convergence or shared ancestry (Figure 2). Differences in tree topologies were small between mitochondrial and nuclear datasets, and both identified nine major lineages (Figures 4, 5, 6, and 7). Using the resulting phylogeny, I identified 52 species belonging to 24 different mimicry rings, composed of two or three species each (Figure 3). All lineages included taxa that were members of mimicry rings with the exception of Lineage 2. A comparison of topological positions of respective co-mimics shows that 92% of mimetic communities are composed of species belonging to evolutionarily distinct lineages. In the only two mimetic groups with species belonging to the same genetic lineage, these are not sister species and apparent convergence may be due to close genetic affinity rather than convergence. As all other shared patterns are the product of convergence in sympatry, and all Corydoradinae are well protected, I consider Müllerian mimicry (Müller, 1878) to be the most convincing explanation for the pattern convergence. Patterns signalling unprofitability to predators do not have to be conspicuous but should be distinctive (Endler and Mappes, 2004), while cryptic and aposematic elements present in individual prey are not necessarily mutually exclusive (Merilaita and Ruxton, 2007; Wright, 2011; Wuster et al., 2004).

Of the 52 mimetic species, the majority belong to Lineages 1, 8 and 9 (Figure 2), suggesting a non-random frequency of co-occurrence between members of different lineages. In all cases, genetic distance is great enough between co-mimics for them to be considered reproductively isolated (mean pairwise mitochondrial distance = 11.16%±4.4 (STdev)). Furthermore, cytogenetic data indicate that respective members of different lineages have undergone extensive genomic duplications, with chromosome complements ranging from 2n=44-134 and genome sizes ranging from 1.1- 8.75pg (Hinegardner and Rosen, 1972; Oliveira et al., 1992). The majority of this variation occurs between lineages, however, there is also significant variation within lineages and often between sibling species.

Figure 2- Phylogenetic Relationships of Corydoradinae including co-mimics

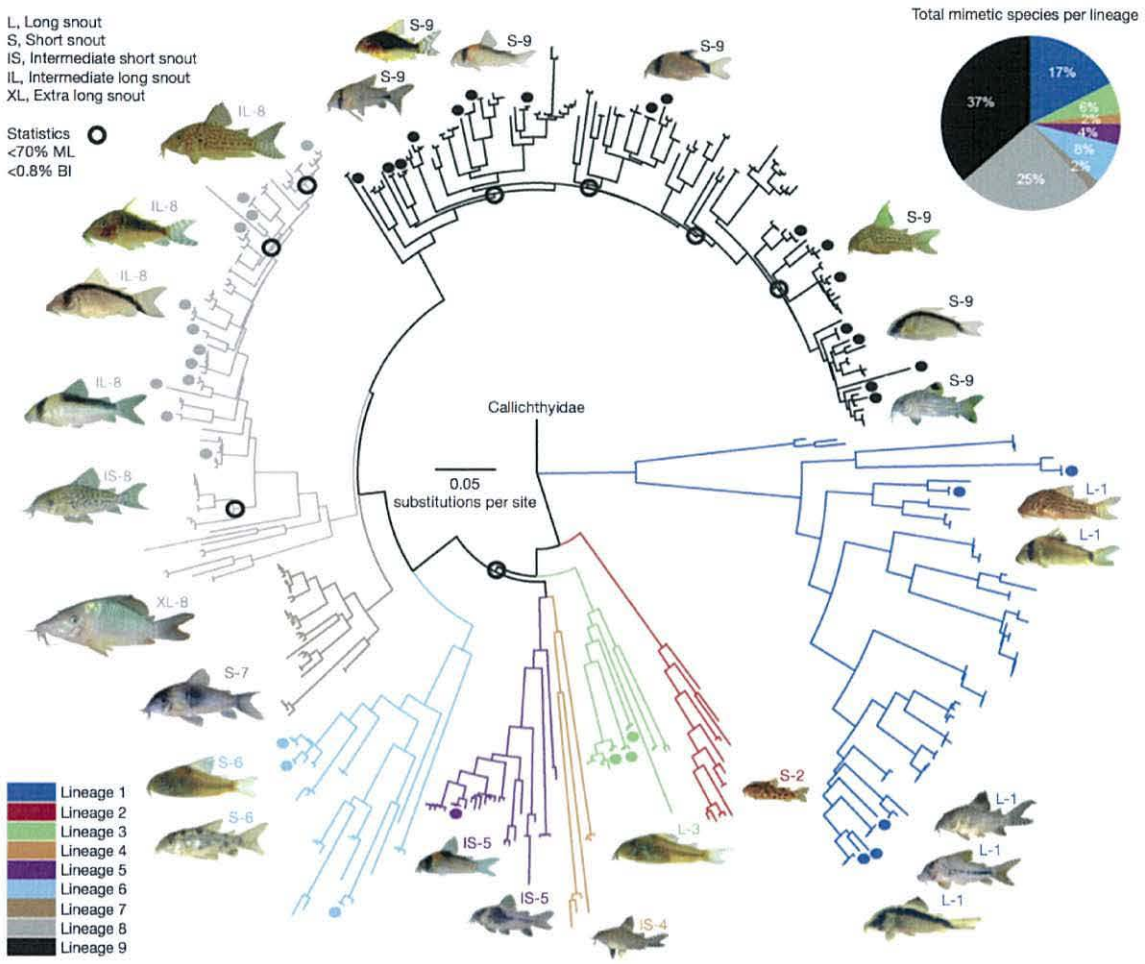


Figure 1 | Phylogenetic relationships of Corydoradinae including co-mimics. The pie chart shows percentage of mimetic species per lineage. Branches with mimetic species at tips are indicated with coloured circles (coded by lineage). Nodes with support below 0.8 (Bayesian inference; BI) probability and 70% (maximum likelihood; ML) are denoted with black open circles. Codes on pictures indicate snout types as determined by morphometrics and genetic lineage (L, long; S, short; IS, intermediate short; XL, extra long; IL, intermediate long). Representative images of morphotypes and colour patterns clockwise from lineage 1: (L-1) *Corydoras maculifer*, (L-1) *C. simulatus*, (L-1) C. sp. C109, (L-1) C. sp. C92, (L-1) *C. narcissus*; (S-2) *A. poecilius**; (L-3) *S. prionotus*; (IS-4) *C. mamore*; (IS-5) C. sp. CW19, (IS-5) *C. nijsseni*; (S-6) *C. paleatus*, (S-6) *C. nattereri*; (S-7) C. sp. CW26; (XL-8) *C. multiradiatus**; (IS-8) *C. sodalis**; (IL-8) *C. imitator*, (IL-8) C. sp. CW6, (IL-8) *C. seussi*, (IL-8) C. sp. C122; (S-9) C. sp. C91, (S-9) *C. gossei*, (S-9) *C. adolfoi*, (S-9) *C. metae*, (S-9) *C. araguaiaensis*, (S-9) *C. arcuatus*, (S-9) *C. julii*. *Non-mimetic taxa.

Figure 3- Geographical Distribution of Mimetic Communities

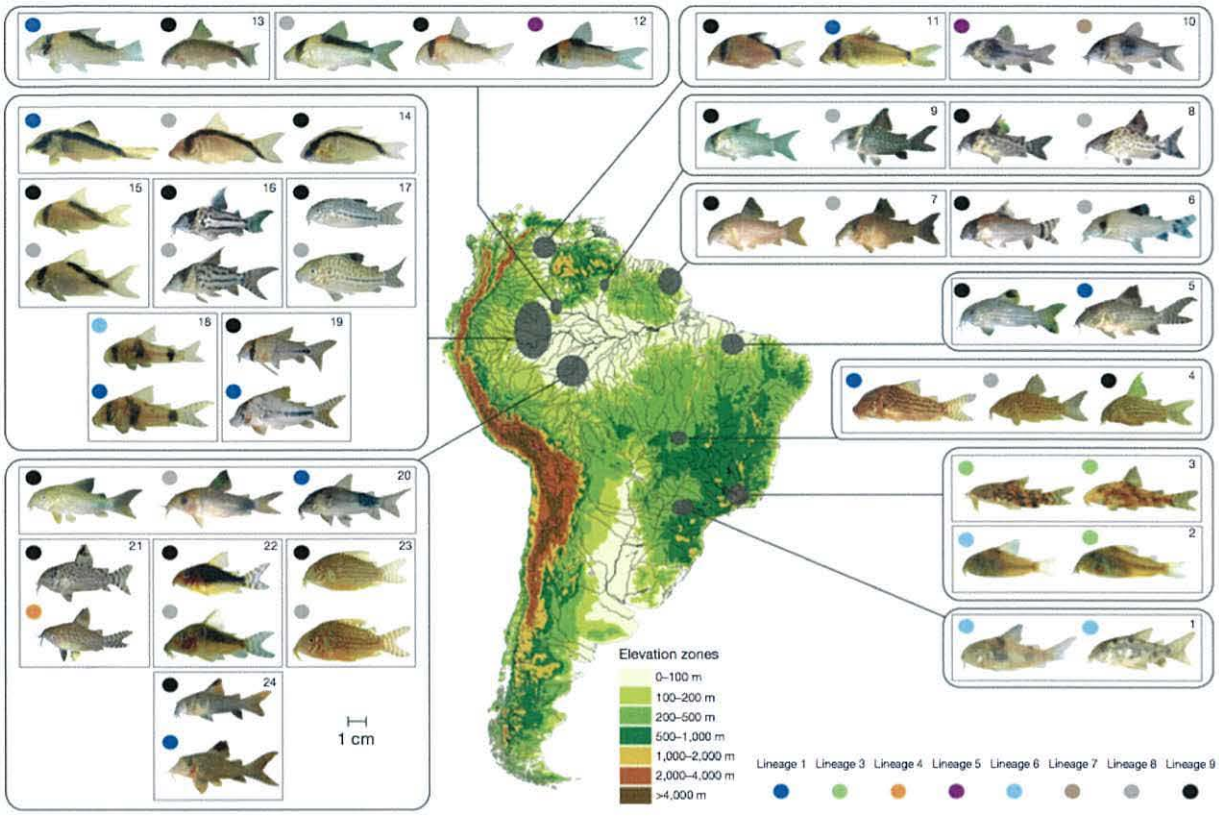
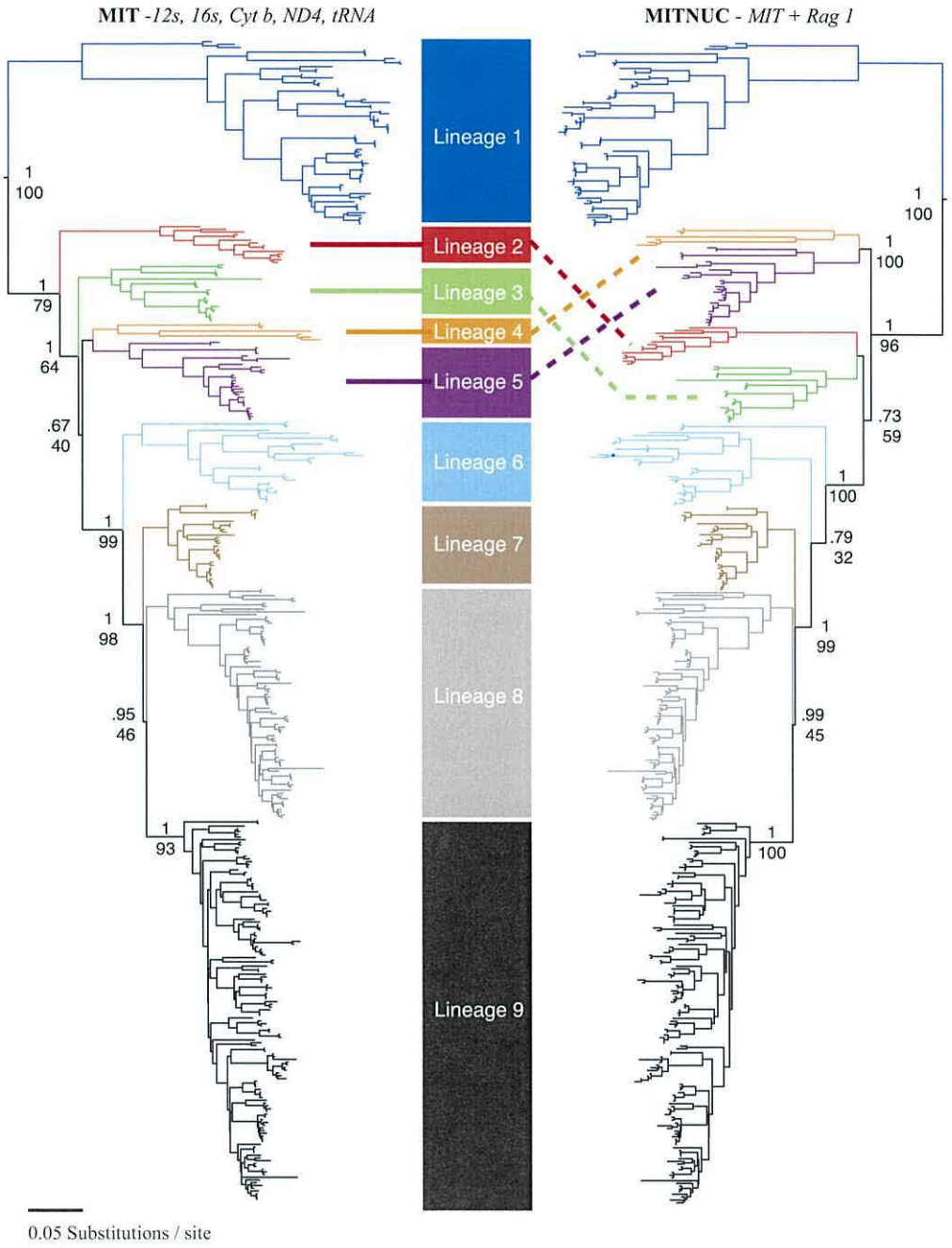


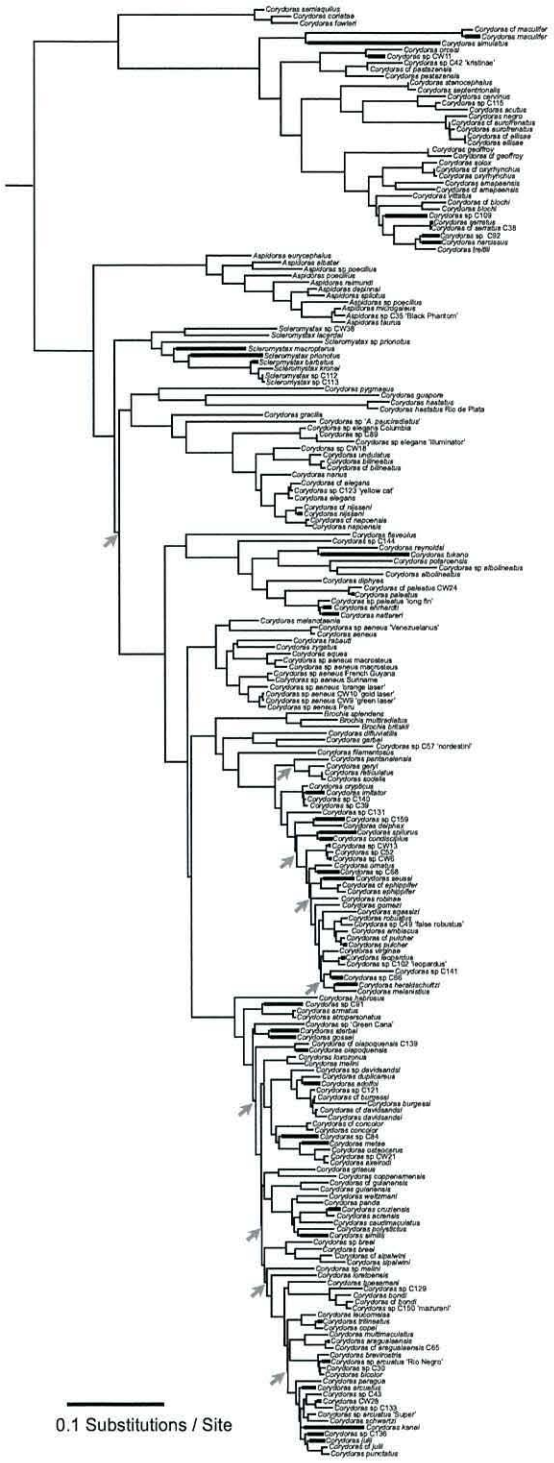
Figure 2 | Geographical distribution of mimetic communities. Genetic lineages are denoted by coloured circles; small grey rectangles represent independent mimetic communities numbered 1–24. Larger black rectangles indicate communities belonging to the same drainage or basin. Grey ellipses indicate approximate geographical distribution. Species images: (1) *C. paleatus*, *C. ehrhardti*; (2) *C. nattereri*, *Scleromystax prionotus*; (3) *S. barbatus*, *S. macropterus*; (4) *C. maculifer*, *C.* sp. C122, *C. araguaiaensis*; (5) *C. julii*, *C.* sp. C109; (6) *C. oiapoquensis*, *C. condisciplus*; (7) *C.* sp. C135, *C.* sp. C136; (8) *C. evelynae*, *C.* sp. CW13; (9) *C. kanei*, *C. crimmeni*; (10) *C.* sp. CW19, *C.* sp. CW26; (11) *C. metae*, *C. simulatus*; (12) *C. imitator*, *C. adolfoi*, *C. nijsseni*; (13) *C. serratus*, *C.* cf. *arcuatus*; (14) *C. narcissus*, *C.* sp. CW6, *C. arcuatus*; (15) *C.* sp. C84, *C.* sp. C156; (16) *C.* sp. CW28, *C. pulcher*; (17) *C. trilineatus*, *C. leopardus*; (18) *C. tukano*, *C.* sp. CW11; (19) *C.* sp. C91, *C.* sp. C92; (20) *C. similis*, *C.* sp. C66, *C. ourastigma*; (21) *C. cruziensis*, *C. mamore*; (22) *C. gossei*, *C. seussi*; (23) *C. sterbai*, *C. haraldshultzi*; (24) *C.* sp. C76, *C.* sp. C77.

Figure 4- Concatenated Topological Comparison



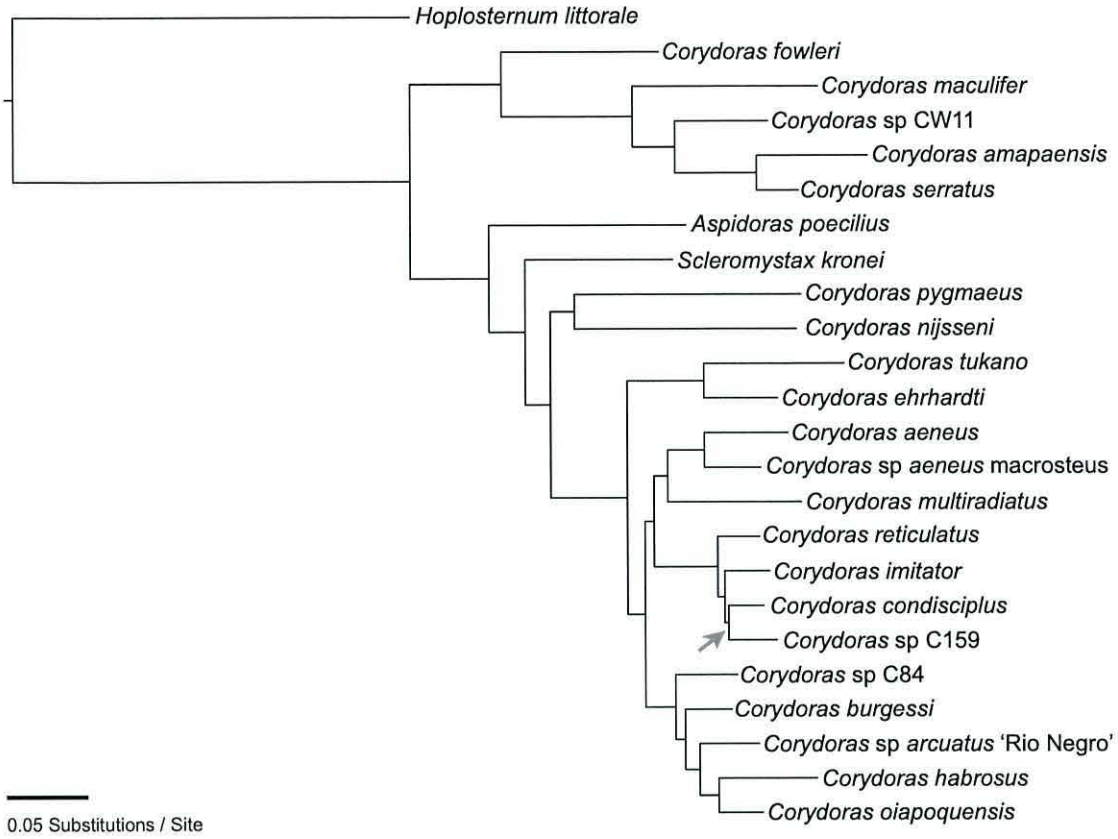
Mitochondrial (MIT) topology on the left from a concatenated dataset combining 12s, 16s, ND4, Cytb, and tRNAs with a total of 2668 bps. Mitochondrial + Nuclear (MITNUC) topology on the right with the combined MIT data and RAG1 totalling 3424 bps. Analyses were fully partitioned with RAxML (500 bootstrap replicates) and MrBayes (30 million generations), for 425 taxa. Support values labelled for major lineage separations (Bayesian probabilities above, ML bootstrap consensus below). Lineages highlighted with respective colours and dotted lines denote topological differences between the two datasets.

Figure 5- Pruned Mitochondrial Tree



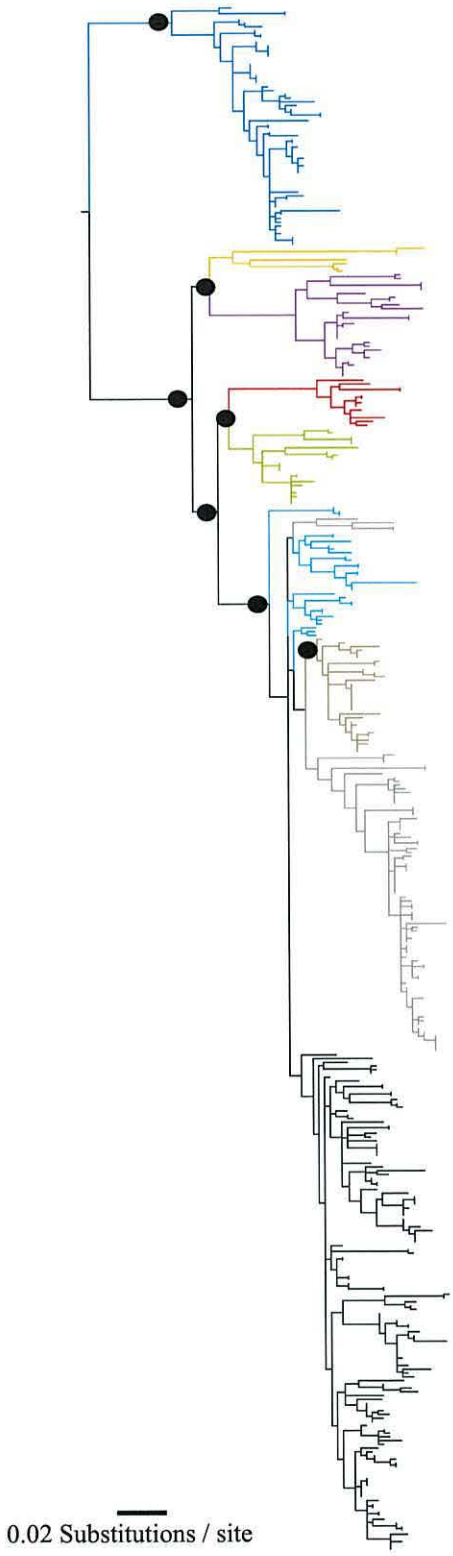
Mitochondrial topology constructed from a fully partitioned RAxML analysis (500 bootstraps replicates) and MrBayes (30 million generations) pruned to include a single representative per species (total 221). Branches with mimetic species are highlighted on the tree in black. Areas of support lower than 0.8 (BI) probability and 70% (ML) are denoted with a slanted grey arrow pointing to the node in question. All other nodes in the tree were well supported.

Figure 6- MITNUC2



Topology constructed with RAxML (500 bootstrap replicates) from concatenated data (combined MITNUC with F-Reticulon 4) of 24 representative taxa and a total of 4095 bps (671bp FR-4 intron). Major areas of support weaker than 0.8 probability and 70% ML denoted with slanted grey arrow pointing to the node in question.

Figure 7- Rag 1 Tree



Topology constructed with RAxML (500 bootstrap replicates) and MrBayes (10 million generations) using 756 bps for 413 taxa. Lineages highlighted with respective colours and major areas of support greater than 0.8 probability and 70% ML denoted with black circles.

2.4.2 Stable Isotopes

Trophic interactions were elucidated using stable isotopes of carbon ($^{13}C/^{12}C$, reported as $d^{13}C$) and nitrogen ($^{15}N/^{14}N$, reported as $d^{15}N$), allowing us to assess the extent of dietary overlap among co-mimics. Nitrogen isotopes are particularly informative, as values increase in a stepwise manner between trophic levels; for example carnivore tissues have higher $d^{15}N$ values than herbivore tissues (Peterson and Fry, 1987). Carbon isotope ratios ($\delta^{13}C$) may change slightly with trophic level, but the major source of variation has been attributed to differences in the sources of primary production, and $\delta^{13}C$ values are typically more useful in deriving foraging locations (Rubenstein and Hobson, 2004; West et al., 2006). Significant differences ($p < 0.001$) following post-hoc corrections were found in mean $\delta^{15}N$ values within six mimicry rings composed of species belonging to different lineages, with different morphology (Table 2 & 3). Niche overlap (no significant differences in mean $\delta^{15}N$) was only observed between two co-mimic pairs (Figure 8). Differences in $\delta^{13}C$ were significant between species in five out of the eight mimicry rings examined (Table 2 & 3). Divergence of isotopic signatures reflects dietary segregation between long snouted species compared to short snouted species. There is a clear relationship between morphology and niche occupation in the Corydoradinae, as larger long snouted species always occupy a lower relative 'trophic level' (consistently lower $\delta^{15}N$) than smaller short snouted species (Table 3). Minor variation in isotopic signatures could also result from physiological differences between species. However, this is unlikely here given the scale of observed differences and the close genetic affinity of Corydoradinae.

Table 2- Isotope Statistics

Table 1 | Isotope statistics

Site	Species comparisons	$\delta^{13}C$ (*P* value)	$\delta^{15}N$ (*P* value)	$\delta^{13}C$ (d.f., *F*, *P*)	$\delta^{15}N$ (d.f., *F*, *P*)	Test type
4	*C. araguaiaensis* versus *C. maculifer*	0.606	0.00002	42, 0.679, 0.513	42, 52.588, 0.00000	One-way ANOVA plus Tukey–Kramer
4	*C. araguaiaensis* versus *C.* sp. C122	0.957	0.00126			
4	*C. maculifer* versus *C.* sp. C122	0.569	0.00029			
12	*C. adolfoi* versus *C. imitator*	0.00002	0.00002	41, 41.02, 0.00000	41, 46.104, 0.00000	One-way ANOVA plus Tukey–Kramer
12	*C. adolfoi* versus *C. nijsseni*	0.00002	0.00004			
12	*C. imitator* versus *C. nijsseni*	0.762	0.295			
13	*C. arcuatus* versus *C. serratus*	0.00000	0.00000	39, 47.062, 0.00000	39, 128.05, 0.00000	*t*-test plus Bonferroni
18	*C. tukano* versus *C.* sp. CW11	0.00000	0.00000	40, 27.878, 0.00000	40, 56.858, 0.00000	*t*-test plus Bonferroni
2	*C. nattereri* versus *S. prionotus*	0.00522	0.00000	35, 8.914, 0.00522	35, 43.837, 0.00000	*t*-test plus Bonferroni
3	*S. barbatus* versus *S. macropterus*	0.00000	0.186	41, 41.823, 0.00000	42, 1.808, 0.186	*t*-test plus Bonferroni
1	*C. paleatus* versus *C. ehrhardti*	0.275	0.842	28, 1.112, 0.275	28, 0.041, 0.842	*t*-test plus Bonferroni

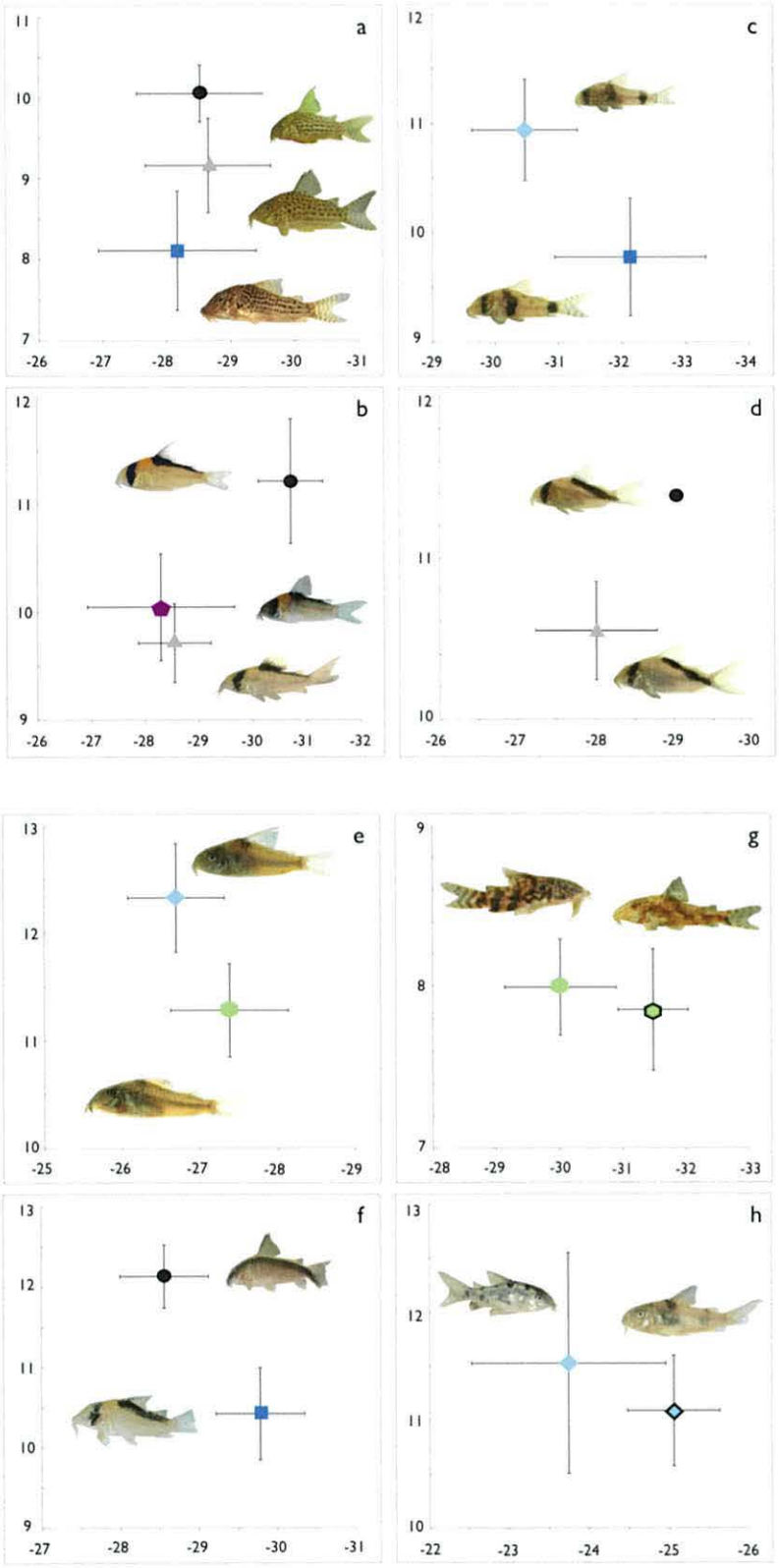
Site 1, Rio Tibaji; site 2, Rio Fau; site 3, east coast; site 4, Rio Araguaia; site 12, Rio Negro; site 13, Rio Negro; site 18, Rio Tiquie. Statistical comparisons are shown between species pairs (*P* values) and mimicry rings within sites for carbon and nitrogen ratios (d.f., degrees of freedom; *F*, *F* test statistic; *P*, *P* value). Standard Bonferroni corrected critical $P = 0.025$.

Table 3- Isotope Means

Locality	Species	Snout/ Lineage	N	$\delta^{13}C$	$\delta^{15}N$
Rio Araguaia/ Amazonas	*C. araguaiaensis*	S-9	20	-28.52±0.99	10.05±0.35
	C. maculifer	L-1	15	-28.17±1.23	8.11±0.74
	C. sp. C122	IL-8	8	-28.65±0.98	9.16±0.58
Upper Rio Negro/ Amazonas	*C. arcuatus*	S-9	23	-28.56±0.57	12.13±0.39
	C. serratus	L-1	18	-29.78±0.56	10.42±0.58
Upper Rio Negro/ Amazonas	*C. adolfoi*	S-9	16	-30.7±0.6	11.22±0.58
	C. imitator	IL-8	20	-28.54±0.67	9.71±0.36
	C. nijsseni	IS-5	6	-28.29±1.36	10.05±0.5
Rio Tiquie/ Amazonas	*C. tukano*	S-6	22	-30.48±0.83	10.94±0.46
	C. sp. CW11	L-1	20	-32.13±1.19	9.78±0.54
Rio Tiquie/ Amazonas	*C.* sp. C84	S-9	1	-29.05	11.39
	C. sp. C156	L-1	20	-28.01±0.77	10.55±0.31
Rio Fau/ Rio de Plata	*C. nattereri*	S-6	19	-26.7±0.62	12.33±0.51
	S. prionotus	L-3	17	-27.38±0.76	11.28±0.43
Sao Paulo/ Rio de Plata	*S. barbatus*	L-3	21	-31.48±0.6	7.9±0.38
	S. macropterus	L-3	22	-30.02±0.88	8±0.3
Rio Tibaji/ Rio de Plata	*C. paleatus*	S-6	10	-23.75±1.33	11.53±1.02
	C. ehrhardti	S-6	20	-25.06±0.58	11.09±0.51

Snout morphology as defined by morphometric groupings; genetic; carbon and nitrogen isotope ratios with respective standard deviations.

Figure 8- Stable Isotope Plots

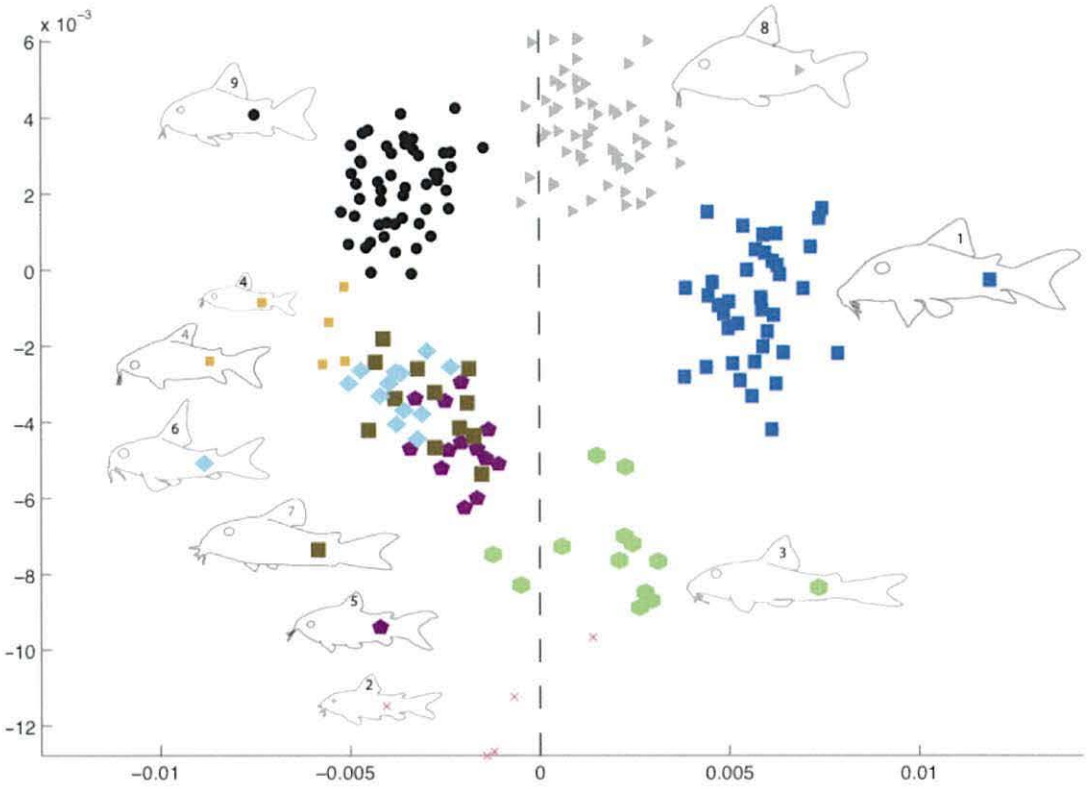


Mean ratios $\delta^{13}C$ on the x-axis and $\delta^{15}N$ on the y-axis. Species from top to bottom: (a) *C. araguaiaensis, C.* sp. C122, *C. maculifer*; (b) *C. adolfoi, C. nijsseni, C. imitator*; (c) *C. tukano, C.* sp. CW11; (d) *C.* sp. C84, *C.* sp. C159; (e) *C. nattereri, S. prionotus*; (f) *C. cf. arcuatus, C. serratus*; (g)*S. barbatus,S. macropterus*; (h) *C. paleatus, C. ehrhardti*

2.4.3 Geometric Morphometrics

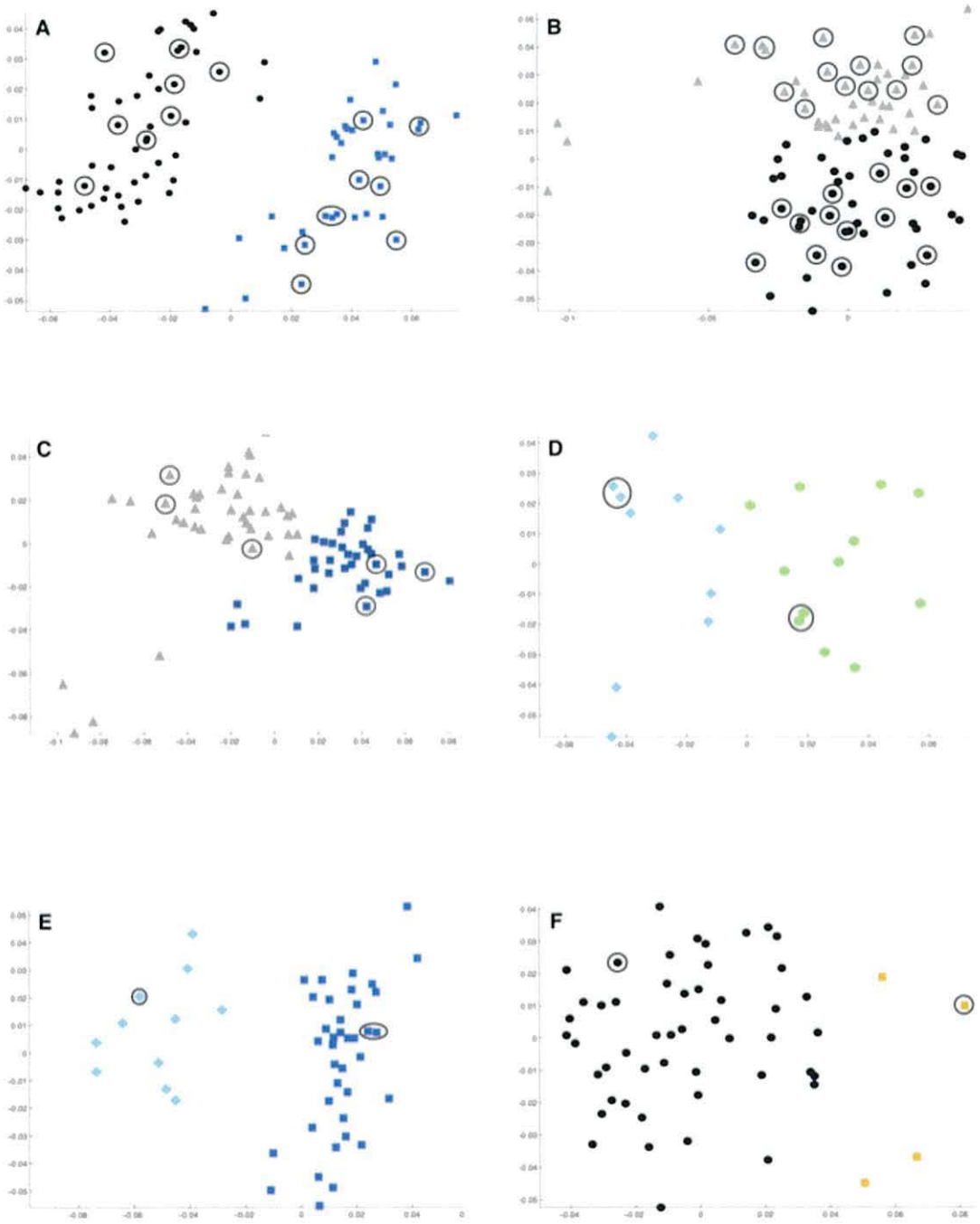
Quantifying traits with adaptive value is valuable in the study of trophic differentiation. Body size and snout or jaw morphology are particularly important traits for food acquisition in fishes (Clabaut et al., 2007; Genner et al., 1999). I used a landmark based geometric morphometric approach, grouping our results in PCA and CVA plots by phylogenetic lineage to assess the extent of morphological conservatism within and between lineages and co-mimics (Figure 9 & 10). In 92% of cases examined, mimicry rings were found to be composed of species with significant morphometric differences, an observation confirmed with pairwise F-tests comparing partial Procrustes distances between respective co-mimic lineages ($p < 0.01$ for all pairwise comparisons) (Table 4). Key characters determining these differences include snout length, eye-position, and body depth, and these are most likely important phenotypic traits facilitating niche differentiation, as they are directly associated with observed differences in niche occupation. Only two mimicry rings consist of species that are members of overlapping morphometric groups. Furthermore, observed morphological differences in snout and body size are phylogenetically conserved, suggesting a role for niche conservatism within lineages. Evidence from stable isotopes for eight mimicry rings and morphological analysis of all known communities suggests that ecologically relevant morphological divergence has resulted in snout specific species assortment within mimetic communities more often than expected at random. Moreover, the majority of observed mimicry rings consist of species that have diverged in terms of resource acquisition, but converged in spatial occupation and colour pattern.

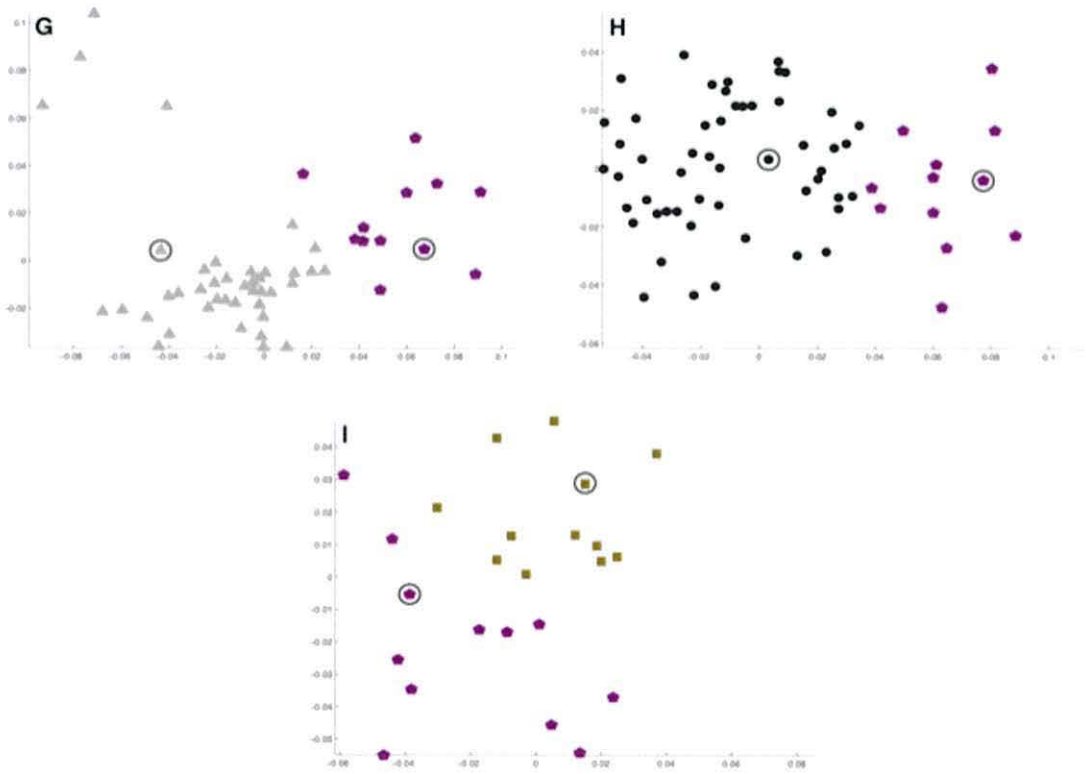
Figure 9- CVA Morphometrics



The CVA plot (vectors of CV coefficients scaled to a length of one) separates short snouts (Lineages 2, 4, 5, 6, 7, 9) and long snouts (Lineages 1, 3, 8) by a vertical axis (dotted line). Representative lineage number and shape marked on the outline of each respective group. Bartlett's test (CVA/MANOVA) revealed six axes of differentiation while an assignment test showed that taxa are grouped in their respective lineage with 99% accuracy.

Figure 10- PCA Morphometrics





Scatter plot of the PCA axes scores between respective co-mimic lineages (Axes are PC1 (x) and PC2 (y) for all plots). Co-mimics are highlighted with black circles. Different colours represent each respective lineage: A) Lineage 1 and 8, B) Lineage 8 and 9, C) Lineage 1 and 8, D) Lineage 3 and 6, E) Lineage 1 and 6, F) Lineage 4 and 9, G) Lineage 5 and 8, H) Lineage 5 and 9, I) Lineage 5 and 7.

Table 4- Morphometric Tests

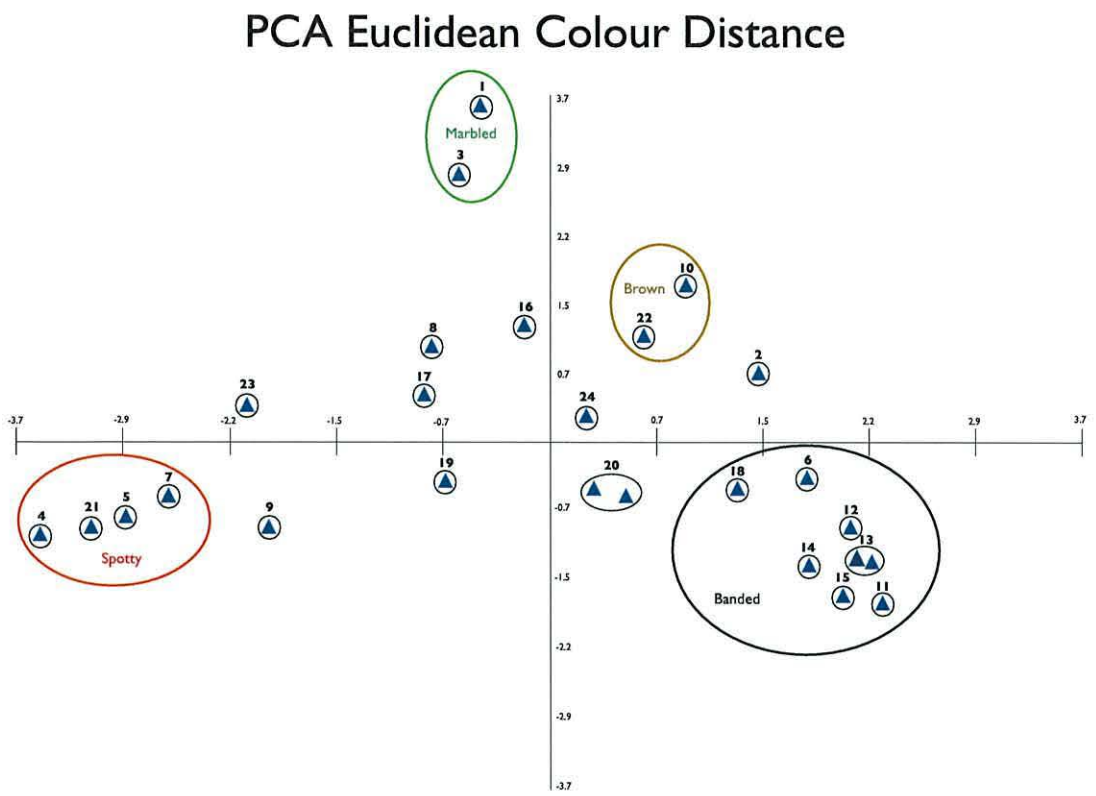
Lineages	F	df	p-values	Dbm*
1vs6	16.49	44, 2068	0	0.0677
1vs8	27.87	44, 3476	0	0.0622
1vs9	42.28	44, 3784	0	0.0722
3vs6	8.34	44, 924	0	0.0629
4vs9	8.56	44, 2288	0	0.0629
5vs7	3.04	44, 1012	3.5472E-10	0.0422
5vs8	21.42	44, 2376	0	0.0837
5vs9	18.71	44, 2684	0	0.074
8vs9	11.66	44, 4004	0	0.0393

Pairwise Goodall's F-test results (2500 bootstraps) comparing Mahalanobis distances between respective co-mimic lineages. *Distance between means.

2.4.4 Colour Patterns

To further investigate the evolution of colour patterns among co-mimics I quantified the geographic distribution of colour patterns and compared them within and between communities. Mimetic Corydoradinae catfishes display a variety of contrasting colour pattern characteristics such as blocks of colour, bright spines, patches, bands, stripes, spots and reticulations. Using 20 different sections of the fish I scored the presence (1), absence (0), or variability (0.5) of different colour pattern characteristics (Figure 1). This allowed a quantification of pattern for each mimetic species that could then be compared to all other respective mimics using Euclidean pairwise distance matrices (Figure 11). A Mantel test revealed that sympatric co-mimics are more similar in coloration than those in allopatry ($r= -0.585$, $p=0.001$), indicating a highly significant relationship between colour pattern and geographic distribution.

Figure 11- PCA Euclidean Colour Distance



PCA plot showing similarity in colour within mimicry rings and differences between them. Each species is represented by a blue triangle, however only one is visible in most cases due to complete overlap of mimetic coloration. Mimetic groups circled with respect to greater pattern similarity (i.e. Marbled, Spotty, Banded, Brown), while intermediate patterns contain a variety of elements and therefore do not necessarily fit into the latter groupings. Species analyzed are listed by respective mimetic communities:

1) *C. paleatus, C. ehrhardti*, 2) *C. nattereri, S. prionotus*, 3) *S. barbatus, S. macropterus*, 4) *C. maculifer, C. sp. C122, C. araguaiaensis*, 5) *C. julii, C.* sp. C109, 6) *C. oiapoquensis, C. condisciplus*, 7) *C.* sp. C135, *C.* sp. C136, 8) *C. evelynae, C.* sp. CW13, 9) *C. kanei, C. crimmeni*, 10) *C.* sp. CW19, *C.* sp. CW26, 11) *C. metae, C. simulatus*, 12) *C. imitator, C. adolfoi, C. nijsseni*, 13) *C. serratus, C. cf. arcuatus*, 14) *C. narcissus, C.* sp. CW6, *C. arcuatus*, 15) C. sp. C84, C. sp. C159, 16) C. sp. CW28, C. pulcher, 17) *C. trilineatus, C. leopardus*, 18) *C. tukano, C.* sp. CW11, 19) *C.* sp. C91, *C.* sp. C92, 20) *C. similis, C.* sp. C66, *C. ourastigma*, 21) *C. cruziensis, C. mamore*, 22) *C. gossei, C. seussi*, 23) *C. sterbai, C. haraldshultzi*, 24) *C.* sp. C76, *C.* sp. C77.

2.5 Discussion:

I report a diverse assemblage of vertebrate Müllerian mimics that inhabit aquatic environments through which they signal to predators foraging in two different optical modalities; water and air. I demonstrate that dietary resource partitioning coupled with morphological and phylogenetic differences determine community assembly despite the positive benefits of Müllerian mimicry. These results suggest that the benefits accrued by Müllerian co-mimics are not sufficient to overcome the need for ecological differentiation for stable long-term coexistence, thereby reinforcing the position that antagonistic interactions set ecological limits on the diversification of mutualistic communities. Despite these limits, Müllerian mimicry may increase diversification rates among allopatric communities if predation driven directional selection simultaneously leads to convergence of coloration among sympatric taxa, but divergence among allopatric taxa. In conclusion, many neotropical habitats are critically threatened by anthropogenic pollution, deforestation and river obstruction and modification. As a result we may lose many of these unique species and communities before we fully appreciate their extraordinary diversity.

SI Table 1 – Species List and Accession Numbers

	Lineage	Genus	Species	Voucher Code	Drainage	ACC 12s	ACC 16s	ACC ND4	ACC Cytb	ACC Rag1	ACC FR4
Callichthyinae	Lineage 0	*Dianema*	*D. longibarbus*	LBP 557-7230	Amazon	GU210442	GU210867	GU210020	GU209684	-	-
Callichthyinae	Lineage 0	*Dianema*	*D. urostriatum*	MT89	Amazon	GU210539	GU210964	GU210114	GU209685	-	-
Callichthyinae	Lineage 0	*Hoplosternum*	*H. littorale*	LBP 210-4134	Amazon	GU210443	GU210868	GU210021	GU209686	GU209261	GU210992
Corydoradinae	Lineage 1	*Corydoras*	*C. acutus* *	MA41	Amazon	GU210339	GU210764	GU209918	GU209605	GU209183	-
Corydoradinae	Lineage 1	*Corydoras*	*C. amapaensis* *	MA154	Guyana coastal rivers	GU210164	GU210589	GU209745	GU209360	GU208946	-
Corydoradinae	Lineage 1	*Corydoras*	*C. amapaensis* *	MA155	Guyana coastal rivers	GU210165	GU210590	GU209746	GU209361	GU208947	-
Corydoradinae	Lineage 1	*Corydoras*	*C. amapaensis*	MA156	Guyana coastal rivers	GU210166	GU210591	GU209747	GU209362	GU208948	GU210979
Corydoradinae	Lineage 1	*Corydoras*	*C. aurofrenatus*	ANSP 182420-1470	Rio de la Plata	GU210327	GU210752	GU209907	GU209332	GU208918	-
Corydoradinae	Lineage 1	*Corydoras*	*C. aurofrenatus*	ANSP 182420-1471	Rio de la Plata	GU210328	GU210753	GU209908	GU209333	GU208919	-
Corydoradinae	Lineage 1	*Corydoras*	*C. blochi*	MHNG 2652.007-GY04-237	Guyana coastal rivers	GU210236	GU210661	GU209816	GU209341	GU208927	-
Corydoradinae	Lineage 1	*Corydoras*	*C. blochi*	ANSP 180648-1462	Guyana coastal rivers	GU210324	GU210749	GU209904	GU209342	GU208928	-
Corydoradinae	Lineage 1	*Corydoras*	*C. cervinus* *	MA125	Amazon	GU210146	GU210571	GU209727	GU209358	GU208944	-
Corydoradinae	Lineage 1	*Corydoras*	*C. cervinus* *	MA126	Amazon	GU210147	GU210572	GU209728	GU209359	GU208945	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *aurofrenatus*	MA324	Rio de la Plata	GU210308	GU210733	GU209888	GU209366	GU208952	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *blochi*	MHNG 2707.015-SU07-624	Guyana coastal rivers	GU210239	GU210664	GU209819	GU209368	GU208954	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *ellisae*	MA37	Rio de la Plata	GU210336	GU210761	GU209915	GU209377	GU208962	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *geoffroy*	MHNG 2683.016-GF06-459	Guyana coastal rivers	GU210249	GU210674	GU209829	GU209379	GU208964	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *geoffroy*	MHNG 2683.016-GF06-460	Guyana coastal rivers	GU210250	GU210675	GU209830	GU209380	GU208965	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *geoffroy*	MHNG SU08-112	Guyana coastal rivers	GU210264	GU210689	GU209844	GU209381	GU208966	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *maculifer*	MA40	Amazon	GU210338	GU210763	GU209917	GU209386	GU208970	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *oxyrhynchus*	MHNG SU08-1112	Guyana coastal rivers	GU210267	GU210692	GU209847	GU209392	GU208976	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *oxyrhynchus*	MHNG SU08-1113	Guyana coastal rivers	GU210268	GU210693	GU209848	GU209393	GU208977	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *pastazensis*	MA299	Amazon	GU210287	GU210712	GU209867	GU209395	GU208979	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *serratus* 'C38'	MA151	Amazon	GU210161	GU210586	GU209742	GU209397	GU208980	-
Corydoradinae	Lineage 1	*Corydoras*	*C. coriatae*	MT14	Amazon	GU210428	GU210853	GU210006	GU209408	GU208991	-

Corydoradinae	Lineage 1	*Corydoras*	*C. ellisae* *	MT24	Rio de la Plata	GU210469	GU210894	GU210047	GU209430	GU209012	-
Corydoradinae	Lineage 1	*Corydoras*	*C. ellisae* *	MT25	Rio de la Plata	GU210470	GU210895	GU210048	GU209431	GU209013	-
Corydoradinae	Lineage 1	*Corydoras*	*C. ellisae*	MT26	Rio de la Plata	GU210471	GU210896	GU210049	GU209432	GU209014	-
Corydoradinae	Lineage 1	*Corydoras*	*C. fowleri* *	MA108	Amazon	GU210133	GU210558	GU209715	GU209441	GU209023	-
Corydoradinae	Lineage 1	*Corydoras*	*C. geoffroy* *	MHNG 2700.007-GF07-120	Guyana coastal rivers	GU210226	GU210651	GU209806	GU209443	GU209025	-
Corydoradinae	Lineage 1	*Corydoras*	*C. geoffroy* *	MHNG 2700.007-GF07-102	Guyana coastal rivers	GU210227	GU210652	GU209807	GU209444	GU209026	-
Corydoradinae	Lineage 1	*Corydoras*	*C. maculifer* *	LBP 7213-32890	Amazon	GU210210	GU210635	GU209790	GU209481	GU209063	GU210991
Corydoradinae	Lineage 1	*Corydoras*	*C. maculifer* *	MA95-27707	Amazon	GU210378	GU210803	GU209956	GU209482	GU209064	-
Corydoradinae	Lineage 1	*Corydoras*	*C. narcissus* *	MA210	Amazon	GU210212	GU210637	GU209792	GU209500	GU209082	-
Corydoradinae	Lineage 1	*Corydoras*	*C. negro*	MA52	Rio de la Plata	GU210348	GU210773	GU209927	GU209504	-	-
Corydoradinae	Lineage 1	*Corydoras*	*C. negro*	MA62	Rio de la Plata	GU210355	GU210780	GU209934	GU209505	GU209086	-
Corydoradinae	Lineage 1	*Corydoras*	*C. orcesi*	MA304	Amazon	GU210293	GU210718	GU209873	GU209512	GU209093	-
Corydoradinae	Lineage 1	*Corydoras*	*C. ourastigma* *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 1	*Corydoras*	*C. oxyrhynchus*	MHNG SU08-1191	Guyana coastal rivers	GU210284	GU210709	GU209864	GU209516	GU209097	-
Corydoradinae	Lineage 1	*Corydoras*	*C. oxyrhynchus*	MHNG SU08-1192	Guyana coastal rivers	GU210285	GU210710	GU209865	GU209517	GU209098	-
Corydoradinae	Lineage 1	*Corydoras*	*C. pastazensis* *	MT42	Amazon	GU210488	GU210913	GU210066	GU209527	-	-
Corydoradinae	Lineage 1	*Corydoras*	*C. pastazensis* *	MT43	Amazon	GU210489	GU210914	GU210067	GU209528	-	-
Corydoradinae	Lineage 1	*Corydoras*	*C. semiaquilus*	ANSP 178613-1459	Amazon	GU210321	GU210746	GU209901	GU209553	GU209132	-
Corydoradinae	Lineage 1	*Corydoras*	*C. septentrionalis*	MA114	Orinoco	GU210139	GU210564	GU209721	GU209659	GU209236	-
Corydoradinae	Lineage 1	*Corydoras*	*C. septentrionalis*	MA213	Orinoco	GU210214	GU210639	GU209794	GU209660	GU209237	-
Corydoradinae	Lineage 1	*Corydoras*	*C. serratus* *	MA309	Amazon	GU210298	GU210723	GU209878	GU209557	GU209136	-
Corydoradinae	Lineage 1	*Corydoras*	*C. serratus* *	LBP 6869-32562	Amazon	GU210193	GU210618	GU209774	GU209556	GU209135	GU210981
Corydoradinae	Lineage 1	*Corydoras*	*C. simulatus* *	MT62	Orinoco	GU210510	GU210935	-	GU209564	GU209143	-
Corydoradinae	Lineage 1	*Corydoras*	*C. simulatus* *	MT63	Orinoco	GU210511	GU210936	-	GU209565	GU209144	-
Corydoradinae	Lineage 1	*Corydoras*	*C. simulatus* *	MT64	Orinoco	GU210512	GU210937	-	GU209566	GU209145	-
Corydoradinae	Lineage 1	*Corydoras*	*C. solox*	MHNG 2666.036-GF03-093	Guyana coastal rivers	GU210251	GU210676	GU209831	GU209572	GU209151	-
Corydoradinae	Lineage 1	*Corydoras*	*C. solox*	MHNG 2666.036-GF03-099	Guyana coastal rivers	GU210252	GU210677	GU209832	GU209573	GU209152	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C115' *	MA183	Amazon	GU210186	GU210611	GU209767	GU209604	GU209182	-

Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C42'	MA27	Amazon	GU210263	GU210688	GU209843	GU209625	GU209203	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C53'	MA30	Amazon	GU210288	GU210713	GU209868	GU209630	GU209208	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C77' *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'CW11 long nose' *	LBP 7712-32694	Amazon	GU210201	GU210626	GU209781	GU209639	GU209216	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'CW11 long nose' *	LBP 7712-32724	Amazon	GU210203	GU210628	GU209783	GU209640	GU209217	GU210980
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2682.025-GF06-204	Guyana coastal rivers	GU210253	GU210678	GU209833	GU209315	GU208901	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2682.025-GF06-201	Guyana coastal rivers	GU210254	GU210679	GU209834	GU209316	GU208902	GU210982
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2681.064-GF06-129	Guyana coastal rivers	GU210255	GU210680	GU209835	GU209317	GU208903	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2681.018-GF06-071	Guyana coastal rivers	GU210256	GU210681	GU209836	GU209318	GU208904	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C109' *	LBP 5549-27241	Maranhao	GU210130	GU210555	GU209712	GU209653	GU209230	-
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C92' *	MA211	Amazon	GU210213	GU210638	GU209793	GU209304	GU208890	-
Corydoradinae	Lineage 1	*Corydoras*	*C. stenocephalus*	MHNG PE08-910	Amazon	GU210279	GU210704	GU209859	GU209554	GU209133	-
Corydoradinae	Lineage 1	*Corydoras*	*C. stenocephalus*	MHNG PE08-911	Amazon	GU210280	GU210705	GU209860	GU209555	GU209134	-
Corydoradinae	Lineage 1	*Corydoras*	*C. treitlii* *	MT76	Maranhao	GU210525	GU210950	GU210100	GU209663	GU209240	-
Corydoradinae	Lineage 1	*Corydoras*	*C. treitlii* *	MT77	Maranhao	GU210526	GU210951	GU210101	GU209664	GU209241	-
Corydoradinae	Lineage 1	*Corydoras*	*C. treitlii* *	MT78	Maranhao	GU210527	GU210952	GU210102	GU209665	GU209242	-
Corydoradinae	Lineage 1	*Corydoras*	*C. vittatus* *	MT84	Maranhao	GU210534	GU210959	GU210109	GU209677	GU209254	-
Corydoradinae	Lineage 1	*Corydoras*	*C. vittatus*	MT85	Maranhao	GU210535	GU210960	GU210110	GU209678	GU209255	-
Corydoradinae	Lineage 1	*Corydoras*	*C. vittatus*	MT86	Maranhao	GU210536	GU210961	GU210111	GU209679	GU209256	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. albater*	MA329	Amazon	GU210313	GU210738	GU209893	GU209283	GU208870	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. depinnai*	MA307	Sao Francisco	GU210296	GU210721	GU209876	GU209285	-	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. eurycephalus* *	MA176	Amazon	GU210180	GU210605	GU209761	GU209286	GU208872	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. microgaleus*	MA153	Amazon	GU210163	GU210588	GU209744	GU209287	GU208873	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. poecilus*	LBP 1272-11098	Amazon	GU210542	GU210967	GU210117	GU209288	GU208874	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. poecilus* *	LBP 1272-11100	Amazon	GU210543	GU210968	GU210118	GU209289	GU208875	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. raimundi*	LBP 5568	Maranhao	GU210131	GU210556	GU209713	GU209290	GU208876	-
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. 'C35 Black Phantom'	MA177	Amazon	GU210181	GU210606	GU209762	GU209291	GU208877	-
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus* *	MA96-27708	Amazon	GU210379	GU210804	GU209957	GU209284	GU208871	GU211001

Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus* *	LBP 1437-12304	Amazon	GU210544	GU210969	GU210119	GU209294	GU208880	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. spilotus*	MA325	Maranhao	GU210309	GU210734	GU209889	GU209292	GU208878	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. spilotus*	MA327	Maranhao	GU210311	GU210736	GU209891	GU209293	GU208879	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. taurus*	MA326	Upper Paraguay river	GU210310	GU210735	GU209890	GU209295	GU208881	-
Corydoradinae	Lineage 2	*Aspidoras*	*A. taurus*	MA328	Upper Paraguay river	GU210312	GU210737	GU209892	GU209296	GU208882	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. barbatus* *	LBP 2077-14417	East Coastal Rivers of Brazil	GU210444	GU210869	GU210022	GU209687	GU209262	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. barbatus* *	LBP 2083-14429	East Coastal Rivers of Brazil	GU210445	GU210870	GU210023	GU209688	GU209263	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. barbatus* *	LBP 2083-14430	East Coastal Rivers of Brazil	GU210446	GU210871	GU210024	GU209689	GU209264	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. kronei* *	LBP 7716-32796	East Coastal Rivers of Brazil Rio de la Plata	GU210208	GU210633	GU209788	GU209692	GU209267	GU211000
Corydoradinae	Lineage 3	*Scleromystax*	*S. kronei* *	LBP 2658-17416	East Coastal Rivers of Brazil Rio de la Plata	GU210447	GU210872	GU210025	GU209690	GU209265	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. kronei* *	LBP 2658-17418	East Coastal Rivers of Brazil Rio de la Plata	GU210448	GU210873	GU210026	GU209691	GU209266	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. lacardai*	LBP 1966-13676	East Coastal Rivers of Brazil	GU210449	GU210874	GU210027	GU209693	GU209268	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. lacerdai*	LBP 1966-13677	East Coastal Rivers of Brazil	GU210451	GU210876	GU210029	GU209694	GU209269	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. lacerdai*	LBP 1966-13705	East Coastal Rivers of Brazil	GU210452	GU210877	GU210030	GU209695	GU209270	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. macropterus* *	LBP 461-5639	East Coastal Rivers of Brazil	GU210453	GU210878	GU210031	GU209696	GU209271	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. macropterus* *	LBP 461-5640	East Coastal Rivers of Brazil	GU210454	GU210879	GU210032	GU209697	GU209272	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. macropterus* *	LBP 461-5642	East Coastal Rivers of Brazil	GU210455	GU210880	GU210033	GU209698	GU209273	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. prionotus* *	LBP 1267-11105	East Coastal Rivers of Brazil	GU210456	GU210881	GU210034	GU209699	GU209274	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. prionotus* *	LBP 1267-11106	East Coastal Rivers of Brazil Rio de la Plata	GU210457	GU210882	GU210035	GU209700	GU209275	-
Corydoradinae	Lineage 3	*Scleromystax*	*S. prionotus*	LBP 1267-11107	East Coastal Rivers of Brazil Rio de la Plata	GU210458	GU210883	GU210036	GU209701	GU209276	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'C112'	MA323	East Coastal Rivers of Brazil Rio de la Plata	GU210307	GU210732	GU209887	GU209702	GU209277	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'C113'	LBP 1237-11125	Sao Fransicso	GU210459	GU210884	GU210037	GU209704	GU209279	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'CW42'	MA112	Sao Fransicso	GU210137	GU210562	GU209719	GU209705	GU209280	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'CW42'	MA50	Sao Fransicso	GU210346	GU210771	GU209925	GU209706	GU209281	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'C113' *	MT90	Sao Fransicso	GU210541	GU210966	GU210116	GU209703	GU209278	-
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. *prionotus*	LBP 2575-15724	East Coastal Rivers of Brazil	GU210460	GU210885	GU210038	GU209707	GU209282	-
Corydoradinae	Lineage 4	*Corydoras*	*C.* cf. *hastatus*	MT104-13615	Rio de la Plata	GU210389	GU210814	GU209967	GU209462	GU209044	-
Corydoradinae	Lineage 4	*Corydoras*	*C. guapore* *	MA73	Amazon	GU210364	GU210789	GU209943	GU209453	GU209035	-

Corydoradinae	Lineage 4	*Corydoras*	*C. hastatus*	MA136	Rio de la Plata	GU210153	GU210578	GU209734	GU209463	GU209045	-
Corydoradinae	Lineage 4	*Corydoras*	*C. hastatus*	LBP 1709-12815	Amazon	GU210405	GU210830	GU209983	GU209461	GU209043	-
Corydoradinae	Lineage 4	*Corydoras*	*C. mamore* *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 4	*Corydoras*	*C. pygmaeus* *	MT51	Amazon	GU210498	GU210923	GU210076	GU209538	GU209117	-
Corydoradinae	Lineage 4	*Corydoras*	*C. pygmaeus* *	MT52	Amazon	GU210499	GU210924	GU210077	GU209539	GU209118	GU210993
Corydoradinae	Lineage 4	*Corydoras*	*C. pygmaeus* *	MT53	Amazon	GU210500	GU210925	GU210078	GU209540	GU209119	GU210994
Corydoradinae	Lineage 5	*Corydoras*	*C. bilineatus*	MA68	Amazon	GU210359	GU210784	GU209938	GU209340	GU208926	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *bilineatus*	LBP 1959-13647	Rio de la Plata	GU210386	GU210811	GU209964	GU209367	GU208953	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *elegans*	MHNG 2602.19-BR98-161	Amazon	GU210231	GU210656	GU209811	GU209376	-	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *elegans*	MT8	Amazon	GU210529	GU210954	GU210104	GU209375	GU208961	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *napoensis*	LBP 1962-13660	Amazon	GU210390	GU210815	GU209968	GU209388	GU208972	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *napoensis*	LBP 1962-13644	Amazon	GU210391	GU210816	GU209969	GU209387	GU208971	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* cf. *nijsenni*	LBP 419-5180	Amazon	GU210392	GU210817	GU209970	GU209389	GU208973	-
Corydoradinae	Lineage 5	*Corydoras*	*C. elegans* *	MA116	Amazon	GU210141	GU210566	GU209722	GU209429	GU209011	-
Corydoradinae	Lineage 5	*Corydoras*	*C. elegans* *	MT22	Amazon	GU210467	GU210892	GU210045	GU209427	GU209009	-
Corydoradinae	Lineage 5	*Corydoras*	*C. elegans* *	MT23	Amazon	GU210468	GU210893	GU210046	GU209428	GU209010	-
Corydoradinae	Lineage 5	*Corydoras*	*C. gracilis* *	MA301	Amazon	GU210290	GU210715	GU209870	GU209451	GU209033	-
Corydoradinae	Lineage 5	*Corydoras*	*C. gracilis*	MA88	Amazon	GU210376	GU210801	GU209954	GU209452	GU209034	-
Corydoradinae	Lineage 5	*Corydoras*	*C. nanus*	MHNG SU08-575	Guyana coastal rivers	GU210270	GU210695	GU209850	GU209495	GU209077	-
Corydoradinae	Lineage 5	*Corydoras*	*C. nanus*	MHNG SU08-576	Guyana coastal rivers	GU210271	GU210696	GU209851	GU209496	GU209078	-
Corydoradinae	Lineage 5	*Corydoras*	*C. napoensis* *	LBP 556-7226	Amazon	GU210408	GU210833	GU209986	GU209498	GU209080	-
Corydoradinae	Lineage 5	*Corydoras*	*C. napoensis* *	LBP 556-7225	Amazon	GU210409	GU210834	GU209987	GU209497	GU209079	-
Corydoradinae	Lineage 5	*Corydoras*	*C. napoensis*	LBP 556-7227	Amazon	GU210410	GU210835	GU209988	GU209499	GU209081	-
Corydoradinae	Lineage 5	*Corydoras*	*C. nijsenni* *	MA322	Amazon	GU210306	GU210731	GU209886	GU209507	GU209088	-
Corydoradinae	Lineage 5	*Corydoras*	*C. nijsenni* *	MT125-5191	Amazon	GU210412	GU210837	GU209990	GU209506	GU209087	-
Corydoradinae	Lineage 5	*Corydoras*	*C. nijsseni* *	LBP 6861-32532	Amazon	GU210190	GU210615	GU209771	GU209508	GU209089	GU210998
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *'A pauciradiatus'* *	LBP 548-7187	Amazon	GU210430	GU210855	GU210008	GU209594	GU209173	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp.*'A pauciradiatus'* *	LBP 4308-23982	Amazon	GU210382	GU210807	GU209960	GU209596	GU209175	-

Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *'A pauciradiatus'* *	LBP 548-7188	Amazon	GU210431	GU210856	GU210009	GU209595	GU209174	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C123 yellow cat' *	MA84	Amazon	GU210373	GU210798	GU209951	GU209608	GU209186	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C89'	MA82	Amazon	GU210372	GU210797	GU209950	GU209637	GU209215	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'CW18'	MA308	Amazon	GU210297	GU210722	GU209877	GU209645	GU209222	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'CW19' *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *'elegans* Columbia'	MA74	Orinoco	GU210365	GU210790	GU209944	GU209650	GU209227	-
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *'elegans* illuminator'	MA331	Amazon	GU210315	GU210740	GU209895	GU209651	GU209228	-
Corydoradinae	Lineage 5	*Corydoras*	*C. undulatus*	LBP 566-7386	East Coastal Rivers of Brazil Rio de la Plata	GU210441	GU210866	GU210019	GU209672	GU209249	-
Corydoradinae	Lineage 6	*Corydoras*	*C. albolineatus*	MA321	Amazon	GU210305	GU210730	GU209885	GU209314	GU208900	-
Corydoradinae	Lineage 6	*Corydoras*	*C. albolineatus*	MT1	Amazon	GU210383	GU210808	GU209961	GU209312	GU208898	-
Corydoradinae	Lineage 6	*Corydoras*	*C. albolineatus*	MT2	Amazon	GU210464	GU210889	GU210042	GU209313	GU208899	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* cf. *paleatus* 'CW24'	MA61	Uruguay basin	GU210354	GU210779	GU209933	GU209394	GU208978	-
Corydoradinae	Lineage 6	*Corydoras*	*C. diphyes* *	MA157	Rio de la Plata	GU210167	GU210592	GU209748	GU209421	GU209004	-
Corydoradinae	Lineage 6	*Corydoras*	*C. diphyes* *	MA158	Rio de la Plata	GU210168	GU210593	GU209749	GU209422	GU209005	-
Corydoradinae	Lineage 6	*Corydoras*	*C. diphyes*	MT21	Rio de la Plata	GU210466	GU210891	GU210044	GU209420	GU209003	-
Corydoradinae	Lineage 6	*Corydoras*	*C. ehrhardti* *	LBP 7713-32752	Rio de la Plata	GU210206	GU210631	GU209786	GU209426	GU209008	GU210983
Corydoradinae	Lineage 6	*Corydoras*	*C. ehrhardti* *	LBP 741-7990	East Coastal Rivers of Brazil Rio de la Plata	GU210399	GU210824	GU209977	GU209424	GU209006	-
Corydoradinae	Lineage 6	*Corydoras*	*C. ehrhardti*	LBP 741-8893	East Coastal Rivers of Brazil Rio de la Plata	GU210400	GU210825	GU209978	GU209425	GU209007	-
Corydoradinae	Lineage 6	*Corydoras*	*C. flaveolus* *	MA109	Rio de la Plata	GU210134	GU210559	GU209716	GU209439	GU209021	-
Corydoradinae	Lineage 6	*Corydoras*	*C. flaveolus* *	MA97-27837	Rio de la Plata	GU210380	GU210805	GU209958	GU209440	GU209022	-
Corydoradinae	Lineage 6	*Corydoras*	*C. flaveolus*	MT115-12321	Rio de la Plata	GU210401	GU210826	GU209979	GU209438	GU209020	-
Corydoradinae	Lineage 6	*Corydoras*	*C. nattereri* *	MA218-32825	Rio de la Plata	GU210218	GU210643	GU209798	GU209502	GU209084	-
Corydoradinae	Lineage 6	*Corydoras*	*C. nattereri* *	MA219-32826	Rio de la Plata	GU210219	GU210644	GU209799	GU209503	GU209085	-
Corydoradinae	Lineage 6	*Corydoras*	*C. nattereri*	LBP 903-9697	East Coastal Rivers of Brazil Rio de la Plata	GU210411	GU210836	GU209989	GU209501	GU209083	-
Corydoradinae	Lineage 6	*Corydoras*	*C. paleatus* *	LBP 568-7374	East Coastal Rivers of Brazil Rio de la Plata	GU210413	GU210838	GU209991	GU209518	GU209099	-
Corydoradinae	Lineage 6	*Corydoras*	*C. paleatus* *	LBP 567-7416	East Coastal Rivers of Brazil Rio de la Plata	GU210414	GU210839	GU209992	GU209519	GU209100	-
Corydoradinae	Lineage 6	*Corydoras*	*C. potaroensis* *	MT46	Guyana coastal rivers	GU210492	GU210917	GU210070	GU209622	GU209200	-
Corydoradinae	Lineage 6	*Corydoras*	*C. potaroensis* *	MT47	Guyana coastal rivers	GU210493	GU210918	GU210071	GU209623	GU209201	-

Corydoradinae	Lineage 6	*Corydoras*	*C. potaroensis*	MT48	Guyana coastal rivers	GU210494	GU210919	GU210072	GU209624	GU209202	-
Corydoradinae	Lineage 6	*Corydoras*	*C. reynoldsi*	MA335	Amazon	GU210318	GU210743	GU209898	GU209545	GU209124	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. 'C144'	MA86	Amazon	GU210374	GU210799	GU209952	GU209614	GU209192	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. '*paleatus* long fin'	MA128	Aquarium Bred	GU210148	GU210573	GU209729	GU209657	GU209234	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. '*paleatus* long fin'	MA129	Aquarium Bred	GU210149	GU210574	GU209730	GU209658	GU209235	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. *albolineatus* *	LBP 5153-26290	Rio de la Plata	GU210127	GU210552	GU209709	GU209593	GU209172	-
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. *albolineatus* *	LBP 1957-13560	Rio de la Plata	GU210437	GU210862	GU210015	GU209592	GU209171	-
Corydoradinae	Lineage 6	*Corydoras*	*C. tukano* *	LBP 6872-32672	Amazon	GU210200	GU210625	GU209780	GU209671	GU209248	GU210987
Corydoradinae	Lineage 6	*Corydoras*	*C. tukano* *	LBP 549-7194	Amazon	GU210438	GU210863	GU210016	GU209669	GU209246	-
Corydoradinae	Lineage 6	*Corydoras*	*C. tukano* *	LBP 549-7195	Amazon	GU210440	GU210865	GU210018	GU209670	GU209247	-
Corydoradinae	Lineage 7	*Corydoras*	*C. aeneus* *	MA118	Orinoco	GU210142	GU210567	GU209723	GU209308	GU208894	GU210995
Corydoradinae	Lineage 7	*Corydoras*	*C. aeneus* *	MA120	Orinoco	GU210143	GU210568	GU209724	GU209309	GU208895	-
Corydoradinae	Lineage 7	*Corydoras*	*C. aeneus*	MA144	Orinoco	GU210156	GU210581	GU209737	GU209310	GU208896	-
Corydoradinae	Lineage 7	*Corydoras*	*C. eques* *	MA318	Amazon	GU210302	GU210727	GU209882	GU209436	GU209018	-
Corydoradinae	Lineage 7	*Corydoras*	*C. melanotaenia* *	MT34	Magdalena	GU210480	GU210905	GU210058	GU209487	GU209069	-
Corydoradinae	Lineage 7	*Corydoras*	*C. melanotaenia* *	MT35	Magdalena	GU210481	GU210906	GU210059	GU209486	GU209068	-
Corydoradinae	Lineage 7	*Corydoras*	*C. rabauti* *	MT54	Amazon	GU210501	GU210926	GU210079	GU209541	GU209120	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW10 Gold Laser'	MA169	Amazon	GU210175	GU210600	GU209756	GU209577	GU209156	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW10 Gold Laser'	MA42	Amazon	GU210340	GU210765	GU209919	GU209578	GU209157	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW9 Green Laser'	MA159	Amazon	GU210169	GU210594	GU209750	GU209579	GU209158	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW9 Green Laser'	MA160	Amazon	GU210170	GU210595	GU209751	GU209580	GU209159	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW26' *	-	Orinoco	-	-	-	-	-	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'F Guyana'	MHNG 2666.037-GF03-097	Guyana coastal rivers	GU210246	GU210671	GU209826	GU209576	GU209155	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru orange'	MHNG PE08-915	Amazon	GU210282	GU210707	GU209862	GU209585	GU209164	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru orange'	MA59	Amazon	GU210353	GU210778	GU209932	GU209586	GU209165	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	MHNG PE08-920	Amazon	GU210281	GU210706	GU209861	GU209584	GU209163	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	LBP 1350-11447	Amazon	GU210432	GU210857	GU210010	GU209581	GU209160	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	LBP 1350-11448	Amazon	GU210433	GU210858	GU210011	GU209582	GU209161	-

Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	LBP 1350-11449	Amazon	GU210434	GU210859	GU210012	GU209583	GU209162	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG 2671.014-SU05-575	Guyana coastal rivers	GU210229	GU210654	GU209809	GU209587	GU209166	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG 2671.014-SU05-576	Guyana coastal rivers	GU210230	GU210655	GU209810	GU209588	GU209167	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG 2673.028-SU05-267	Guyana coastal rivers	GU210232	GU210657	GU209812	GU209589	GU209168	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG SU08-1194	Guyana coastal rivers	GU210274	GU210699	GU209854	GU209590	GU209169	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG SU08-1195	Guyana coastal rivers	GU210275	GU210700	GU209855	GU209591	GU209170	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'venezuelanus'* *	MA181	Orinoco	GU210185	GU210610	GU209766	GU209673	GU209250	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'* *	MA98-27845	Rio de la Plata	GU210381	GU210806	GU209959	GU209480	GU209062	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	LBP 2699-10980	Rio de la Plata	GU210435	GU210860	GU210013	GU209477	GU209059	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	LBP 2863-18564	Rio de la Plata	GU210436	GU210861	GU210014	GU209478	GU209060	-
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	MA204-32760	Rio de la Plata	GU210207	GU210632	GU209787	GU209479	GU209061	GU210978
Corydoradinae	Lineage 7	*Corydoras*	*C. zygatus*	MA51	Amazon	GU210347	GU210772	GU209926	GU209683	GU209260	-
Corydoradinae	Lineage 7	*Corydoras*	*C. zygatus*	MT87	Amazon	GU210537	GU210962	GU210112	GU209681	GU209258	-
Corydoradinae	Lineage 7	*Corydoras*	*C. zygatus*	MT88	Amazon	GU210538	GU210963	GU210113	GU209682	GU209259	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ambiacus* *	MA47	Amazon	GU210345	GU210770	GU209924	GU209321	GU208907	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ambiacus* *	MA46	Amazon	GU210344	GU210769	GU209923	GU209320	GU208906	-
Corydoradinae	Lineage 8	*Corydoras*	*C. britski* *	LBP 15-3602	Rio de la Plata	GU210545	GU210970	GU210120	GU209297	GU208883	-
Corydoradinae	Lineage 8	*Corydoras*	*C. britski* *	LBP 688-8112	Rio de la Plata	GU210546	GU210971	GU210121	GU209298	GU208884	-
Corydoradinae	Lineage 8	*Corydoras*	*C. multiradiatus* *	MA146	Amazon	GU210157	GU210582	GU209738	GU209299	GU208885	GU210984
Corydoradinae	Lineage 8	*Corydoras*	*C. splendens* *	LBP 2017-14215	Amazon	GU210547	GU210972	GU210122	GU209300	GU208886	-
Corydoradinae	Lineage 8	*Corydoras*	*C. splendens* *	LBP 2017-14216	Amazon	GU210548	GU210973	GU210123	GU209301	GU208887	-
Corydoradinae	Lineage 8	*Corydoras*	*C. agassizi*	MA334	Amazon	GU210317	GU210742	GU209897	GU209311	GU208897	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ambiacus* *	MHNG 2602.12-BR98-143	Amazon	GU210228	GU210653	GU209808	GU209319	GU208905	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *crypticus* 'C140'	MA306	Amazon	GU210295	GU210720	GU209875	GU209613	GU209191	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *ephippifer*	MA336	Amazonas	GU210319	GU210744	GU209899	GU209378	GU208963	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *imitator* 'C39' *	LBP 6868-32582	Amazon	GU210194	GU210619	GU209775	GU209621	GU209199	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *leopardus* 'C102' *	MA113	Amazon	GU210138	GU210563	GU209720	GU209383	GU208968	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *leopardus* 'C102'	MA87	Amazon	GU210375	GU210800	GU209953	GU209384	-	-

Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *pulcher*	MT9	Amazon	GU210540	GU210965	GU210115	GU209574	GU209153	-
Corydoradinae	Lineage 8	*Corydoras*	*C. condisciplus* *	MHNG 2681.046-GF06-131	Guyana coastal rivers	GU210257	GU210682	GU209837	GU209403	GU208986	GU210985
Corydoradinae	Lineage 8	*Corydoras*	*C. condisciplus* *	MHNG 2682.024-GF06-208	Guyana coastal rivers	GU210258	GU210683	GU209838	GU209404	GU208987	-
Corydoradinae	Lineage 8	*Corydoras*	*C. condisciplus* *	MA53	Guyana coastal rivers	GU210349	GU210774	GU209928	GU209405	GU208988	-
Corydoradinae	Lineage 8	*Corydoras*	*C. crimmeni* *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 8	*Corydoras*	*C. crypticus* *	MT15	Amazon	GU210439	GU210864	GU210017	GU209409	GU208992	-
Corydoradinae	Lineage 8	*Corydoras*	*C. crypticus* *	MT16	Amazon	GU210450	GU210875	GU210028	GU209410	GU208993	-
Corydoradinae	Lineage 8	*Corydoras*	*C. crypticus*	MT17	Amazon	GU210461	GU210886	GU210039	GU209411	GU208994	-
Corydoradinae	Lineage 8	*Corydoras*	*C. delphax* *	MT18	Amazon	GU210462	GU210887	GU210040	GU209415	GU208998	-
Corydoradinae	Lineage 8	*Corydoras*	*C. delphax* *	MT19	Amazon	GU210463	GU210888	GU210041	GU209416	GU208999	-
Corydoradinae	Lineage 8	*Corydoras*	*C. delphax*	MT20	Amazon	GU210465	GU210890	GU210043	GU209417	GU209000	-
Corydoradinae	Lineage 8	*Corydoras*	*C. difluviatilis*	LBP 382-4624	Rio de la Plata	GU210397	GU210822	GU209975	GU209419	GU209002	-
Corydoradinae	Lineage 8	*Corydoras*	*C. difluviatilis*	LBP 382-4608	Rio de la Plata	GU210398	GU210823	GU209976	GU209418	GU209001	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ephippifer*	MA220	Amazon	GU210220	GU210645	GU209800	GU209433	GU209015	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ephippifer*	MA221	Amazon	GU210221	GU210646	GU209801	GU209434	GU209016	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ephippifer*	MA58	Amazon	GU210352	GU210777	GU209931	GU209435	GU209017	-
Corydoradinae	Lineage 8	*Corydoras*	*C. filamentosus*	MHNG 2707.015-SU07-625	Guyana coastal rivers	GU210240	GU210665	GU209820	GU209437	GU209019	-
Corydoradinae	Lineage 8	*Corydoras*	*C. garbei*	LBP 330-3920	Sao Francisco	GU210402	GU210827	GU209980	GU209442	GU209024	-
Corydoradinae	Lineage 8	*Corydoras*	*C. geryi*	MA172	Amazon	GU210176	GU210601	GU209757	GU209445	GU209027	-
Corydoradinae	Lineage 8	*Corydoras*	*C. geryi*	MA173	Amazon	GU210177	GU210602	GU209758	GU209446	GU209028	-
Corydoradinae	Lineage 8	*Corydoras*	*C. gomezi*	MT27	Amazon	GU210472	GU210897	GU210050	GU209363	GU208949	-
Corydoradinae	Lineage 8	*Corydoras*	*C. gomezi*	MT28	Amazon	GU210473	GU210898	GU210051	GU209364	GU208950	-
Corydoradinae	Lineage 8	*Corydoras*	*C. haraldshultzei* *	MT31	Amazon	GU210477	GU210902	GU210055	GU209458	GU209040	-
Corydoradinae	Lineage 8	*Corydoras*	*C. haraldshultzei* *	MT32	Amazon	GU210478	GU210903	GU210056	GU209459	GU209041	-
Corydoradinae	Lineage 8	*Corydoras*	*C. haraldshultzei*	MT33	Amazon	GU210479	GU210904	GU210057	GU209460	GU209042	-
Corydoradinae	Lineage 8	*Corydoras*	*C. imitator* *	LBP 6862-32502	Amazon	GU210188	GU210613	GU209769	GU209464	GU209046	GU210986
Corydoradinae	Lineage 8	*Corydoras*	*C. imitator* *	MA189-32557	Amazon	GU210192	GU210617	GU209773	GU209465	GU209047	-
Corydoradinae	Lineage 8	*Corydoras*	*C. leopardus* *	MA337	Amazon	GU210320	GU210745	GU209900	GU209470	GU209052	-

Corydoradinae	Lineage 8	*Corydoras*	*C. leopardus* *	MA39	Amazon	GU210337	GU210762	GU209916	GU209447	GU209029	-
Corydoradinae	Lineage 8	*Corydoras*	*C. melanistius*	ANSP 180693-1460	Guyana coastal rivers	GU210330	GU210755	GU209910	GU209484	GU209066	-
Corydoradinae	Lineage 8	*Corydoras*	*C. melanistius*	ANSP 180693-1461	Guyana coastal rivers	GU210331	GU210756	GU209911	GU209485	GU209067	-
Corydoradinae	Lineage 8	*Corydoras*	*C. melanistius*	MA217	Orinoco	GU210217	GU210642	GU209797	GU209483	GU209065	-
Corydoradinae	Lineage 8	*Corydoras*	*C. ornatus*	MA64	Amazon	GU210356	GU210781	GU209935	GU209513	GU209094	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pantanalensis* *	MHNG MUS-562	Rio de la Plata	GU210261	GU210686	GU209841	GU209525	GU209106	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pantanalensis* *	LBP 691-8125	Rio de la Plata	GU210415	GU210840	GU209993	GU209523	GU209104	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pantanalensis*	LBP 691-8126	Rio de la Plata	GU210416	GU210841	GU209994	GU209524	GU209105	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pulcher* *	LBP 909-8957	Amazon	GU210419	GU210844	GU209997	GU209534	GU209113	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pulcher* *	MT49	Amazon	GU210495	GU210920	GU210073	GU209532	GU209111	-
Corydoradinae	Lineage 8	*Corydoras*	*C. pulcher*	MT50	Amazon	GU210497	GU210922	GU210075	GU209533	GU209112	-
Corydoradinae	Lineage 8	*Corydoras*	*C. reticulatus* *	MA130	Amazon	GU210150	GU210575	GU209731	GU209543	GU209122	GU210977
Corydoradinae	Lineage 8	*Corydoras*	*C. reticulatus* *	MA131	Amazon	GU210151	GU210576	GU209732	GU209544	GU209123	-
Corydoradinae	Lineage 8	*Corydoras*	*C. reticulatus*	LBP 553-7214	Amazon	GU210420	GU210845	GU209998	GU209542	GU209121	-
Corydoradinae	Lineage 8	*Corydoras*	*C. robinae* *	MT55	Amazon	GU210502	GU210927	GU210080	GU209546	GU209125	-
Corydoradinae	Lineage 8	*Corydoras*	*C. robinae* *	MT56	Amazon	GU210503	GU210928	GU210081	GU209547	GU209126	-
Corydoradinae	Lineage 8	*Corydoras*	*C. robinae*	MT57	Amazon	GU210504	GU210929	GU210082	GU209548	GU209127	-
Corydoradinae	Lineage 8	*Corydoras*	*C. robustus* *	MA143	Amazon	GU210155	GU210580	GU209736	GU209549	GU209128	-
Corydoradinae	Lineage 8	*Corydoras*	*C. seussi* *	LBP 545-7123	Amazon	GU210423	GU210848	GU210001	GU209560	GU209139	-
Corydoradinae	Lineage 8	*Corydoras*	*C. seussi* *	MT59	Amazon	GU210506	GU210931	GU210084	GU209558	GU209137	-
Corydoradinae	Lineage 8	*Corydoras*	*C. seussi*	MT60	Amazon	GU210508	GU210933	GU210086	GU209559	GU209138	-
Corydoradinae	Lineage 8	*Corydoras*	*C. sodalis* *	LBP 530-7126	Amazon	GU210426	GU210851	GU210004	GU209571	GU209150	-
Corydoradinae	Lineage 8	*Corydoras*	*C. sodalis*	LBP 530-7124	Amazon	GU210427	GU210852	GU210005	GU209569	GU209148	-
Corydoradinae	Lineage 8	*Corydoras*	*C. sodalis* *	LBP 530-7125	Amazon	GU210429	GU210854	GU210007	GU209570	GU209149	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C52'	MA78	Amazon	GU210369	GU210794	GU209947	GU209629	GU209207	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW13' *	MA20	Amazon	GU210202	GU210627	GU209782	GU209641	GU209218	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C131 *leopardus*'	MA72	Amazon	GU210363	GU210788	GU209942	GU209385	GU208969	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C141 *pulcher*'	MA175	Amazon	GU210179	GU210604	GU209760	GU209396	-	-

Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C49 false *robustus'*	MT69	Amazon	GU210517	GU210942	GU210092	GU209627	GU209205	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C49 false *robustus'*	MT70	Amazon	GU210519	GU210944	GU210094	GU209628	GU209206	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C57 nordestini' *	MA184-32501	Sao Francisco	GU210187	GU210612	GU209768	GU209631	GU209209	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C66 *similis'* *	MA56	Amazon	GU210350	GU210775	GU209929	GU209632	GU209210	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C122' *	LBP 7214-32901	Amazon	GU210211	GU210636	GU209791	GU209633	GU209211	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C122 *	LBP 7214-32927	Amazon	GU210301	GU210726	GU209881	GU209634	GU209212	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW6 *narcissus'* *	MT71	Amazon	GU210520	GU210945	GU210095	GU209646	GU209223	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW6 *narcissus'* *	MT72	Amazon	GU210521	GU210946	GU210096	GU209647	GU209224	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW6 *narcissus'*	MT73	Amazon	GU210522	GU210947	GU210097	GU209648	GU209225	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C135' *	-	Guyana coastal rivers	-	-	-	-	-	-
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C159' *	LBP 7711-32652	Amazon	GU210197	GU210622	GU209778	GU209655	GU209232	GU210990
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C159' *	LBP 7711-32670	Amazon	GU210198	GU210623	GU209779	GU209656	GU209233	-
Corydoradinae	Lineage 8	*Corydoras*	*C. spilurus*	MA76	Guyana coastal rivers	GU210367	GU210792	GU209946	GU209652	GU209229	-
Corydoradinae	Lineage 8	*Corydoras*	*C. virginae*	MT81	Amazon	GU210531	GU210956	GU210106	GU209674	GU209251	-
Corydoradinae	Lineage 8	*Corydoras*	*C. virginae*	MT82	Amazon	GU210532	GU210957	GU210107	GU209675	GU209252	-
Corydoradinae	Lineage 8	*Corydoras*	*C. virginae*	MT83	Amazon	GU210533	GU210958	GU210108	GU209676	GU209253	-
Corydoradinae	Lineage 9	*Corydoras*	*C. acrensis*	MA179	Amazon	GU210183	GU210608	GU209764	GU209302	GU208888	-
Corydoradinae	Lineage 9	*Corydoras*	*C. acrensis*	MA69	Amazon	GU210360	GU210785	GU209939	GU209303	GU208889	-
Corydoradinae	Lineage 9	*Corydoras*	*C. adolfoi* *	LBP 6863-32527	Amazon	GU210189	GU210614	GU209770	GU209305	GU208891	-
Corydoradinae	Lineage 9	*Corydoras*	*C. adolfoi* *	LBP 6864-32543	Amazon	GU210191	GU210616	GU209772	GU209306	GU208892	-
Corydoradinae	Lineage 9	*Corydoras*	*C. adolfoi* *	LBP 6871-32727	Amazon	GU210204	GU210629	GU209784	GU209307	GU208893	-
Corydoradinae	Lineage 9	*Corydoras*	*C. araguaiaensis* *	MA94-27706	Amazon	GU210377	GU210802	GU209955	GU209324	GU208910	-
Corydoradinae	Lineage 9	*Corydoras*	*C. araguaiaensis* *	MA162-27799	Amazon	GU210171	GU210596	GU209752	GU209322	GU208908	-
Corydoradinae	Lineage 9	*Corydoras*	*C. araguaiaensis* *	LBP 7212-32879	Amazon	GU210209	GU210634	GU209789	GU209323	GU208909	-
Corydoradinae	Lineage 9	*Corydoras*	*C. arcuatus* *	MT3	Amazon	GU210475	GU210900	GU210053	GU209325	GU208911	-
Corydoradinae	Lineage 9	*Corydoras*	*C. arcuatus* *	MT4	Amazon	GU210485	GU210910	GU210063	GU209326	GU208912	-
Corydoradinae	Lineage 9	*Corydoras*	*C. arcuatus*	MT5	Amazon	GU210496	GU210921	GU210074	GU209327	GU208913	-
Corydoradinae	Lineage 9	*Corydoras*	*C. armatus*	MA107	Amazon	GU210132	GU210557	GU209714	GU209328	GU208914	-

Corydoradinae	Lineage 9	*Corydoras*	*C. armatus*	MA111	Amazon	GU210136	GU210561	GU209718	GU209329	GU208915	-
Corydoradinae	Lineage 9	*Corydoras*	*C. atropersonatus*	MA303	Amazon	GU210292	GU210717	GU209872	GU209330	GU208916	-
Corydoradinae	Lineage 9	*Corydoras*	*C. atropersonatus*	ANSP 182532-1472	Amazon	GU210326	GU210751	GU209906	GU209331	GU208917	-
Corydoradinae	Lineage 9	*Corydoras*	*C. axelrodi* *	MA43	Orinoco	GU210341	GU210766	GU209920	GU209334	GU208920	-
Corydoradinae	Lineage 9	*Corydoras*	*C. axelrodi* *	MA44	Orinoco	GU210342	GU210767	GU209921	GU209335	GU208921	-
Corydoradinae	Lineage 9	*Corydoras*	*C. axelrodi* *	MA45	Orinoco	GU210343	GU210768	GU209922	GU209336	GU208922	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bicolor*	MHNG 2651.078-GY04-424	Guyana coastal rivers	GU210245	GU210670	GU209825	GU209339	GU208925	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bicolor*	MHNG 2671.095-SU05-429	Guyana coastal rivers	GU210237	GU210662	GU209817	GU209337	GU208923	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bicolor*	MHNG 2671.095-SU05-430	Guyana coastal rivers	GU210238	GU210663	GU209818	GU209338	GU208924	-
Corydoradinae	Lineage 9	*Corydoras*	*C. boesemani*	MHNG 2673.074-SU05-127	Guyana coastal rivers	GU210222	GU210647	GU209802	GU209343	GU208929	-
Corydoradinae	Lineage 9	*Corydoras*	*C. boesemani*	MHNG 2673.074-SU05-129	Guyana coastal rivers	GU210223	GU210648	GU209803	GU209344	GU208930	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bondi*	MHNG 2651.040-GY04-122	Guyana coastal rivers	GU210243	GU210668	GU209823	GU209345	GU208931	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bondi*	MHNG 2651.040-GY04-123	Guyana coastal rivers	GU210244	GU210669	GU209824	GU209346	GU208932	-
Corydoradinae	Lineage 9	*Corydoras*	*C. bondi*	ANSP 182982-1467	Orinoco	GU210329	GU210754	GU209909	GU209347	GU208933	-
Corydoradinae	Lineage 9	*Corydoras*	*C. breei*	MHNG SU08-190	Guyana coastal rivers	GU210265	GU210690	GU209845	GU209348	GU208934	-
Corydoradinae	Lineage 9	*Corydoras*	*C. breei*	MHNG SU08-568	Guyana coastal rivers	GU210277	GU210702	GU209857	GU209350	GU208936	-
Corydoradinae	Lineage 9	*Corydoras*	*C. breei*	MHNG SU08-191	Guyana coastal rivers	GU210266	GU210691	GU209846	GU209349	GU208935	-
Corydoradinae	Lineage 9	*Corydoras*	*C. brevirostris*	MT6	Orinoco	GU210507	GU210932	GU210085	GU209351	GU208937	-
Corydoradinae	Lineage 9	*Corydoras*	*C. brevirostris*	MT7	Orinoco	GU210518	GU210943	GU210093	GU209352	GU208938	-
Corydoradinae	Lineage 9	*Corydoras*	*C. burgessi* *	MA310-6867	Amazon	GU210299	GU210724	GU209879	GU209354	GU208940	-
Corydoradinae	Lineage 9	*Corydoras*	*C. burgessi* *	LBP 6867-32741	Amazon	GU210205	GU210630	GU209785	GU209353	GU208939	GU210996
Corydoradinae	Lineage 9	*Corydoras*	*C. caudimaculatus* *	LBP 562-7255	Amazon	GU210385	GU210810	GU209963	GU209357	GU208943	-
Corydoradinae	Lineage 9	*Corydoras*	*C. caudimaculatus*	LBP 562-7253	Amazon	GU210549	GU210974	GU210124	GU209355	GU208941	-
Corydoradinae	Lineage 9	*Corydoras*	*C. caudimaculatus*	LBP 562-7254	Amazon	GU210550	GU210975	GU210125	GU209356	GU208942	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *araguaiaensis* 'C65'	MA57	Amazon	GU210351	GU210776	GU209930	GU209365	GU208951	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *bondi*	MA165	Orinoco	GU210172	GU210597	GU209753	GU209369	GU208955	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *bondi*	MA166	Orinoco	GU210173	GU210598	GU209754	GU209370	GU208956	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *concolor*	LBP 2306-15843	Orinoco	GU210387	GU210812	GU209965	GU209371	GU208957	-

Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *concolor*	LBP 2306-15844	Orinoco	GU210388	GU210813	GU209966	GU209372	GU208958	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *davidsandsi*	MA311	Amazon	GU210300	GU210725	GU209880	GU209373	GU208959	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *davidsandsi*	MA319	Amazon	GU210303	GU210728	GU209883	GU209374	GU208960	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *guianensis*	LBP 5395-27091	Amazon	GU210128	GU210553	GU209710	GU209382	GU208967	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *punctatus*	MA350	Guyana coastal rivers	GU210334	GU210759	GU209914	GU209469	GU209051	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *sipalwini*	MHNG 2671.094-SU05-427	Guyana coastal rivers	GU210224	GU210649	GU209804	GU209398	GU208981	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *sipalwini*	MHNG 2671.094-SU05-428	Guyana coastal rivers	GU210225	GU210650	GU209805	GU209399	GU208982	-
Corydoradinae	Lineage 9	*Corydoras*	*C. concolor* *	MT10	Orinoco	GU210384	GU210809	GU209962	GU209400	GU208983	-
Corydoradinae	Lineage 9	*Corydoras*	*C. concolor* *	MT11	Orinoco	GU210395	GU210820	GU209973	GU209401	GU208984	-
Corydoradinae	Lineage 9	*Corydoras*	*C. concolor* *	MT12	Orinoco	GU210406	GU210831	GU209984	GU209402	GU208985	-
Corydoradinae	Lineage 9	*Corydoras*	*C. copei*	MT13	Amazon	GU210417	GU210842	GU209995	GU209575	GU209154	-
Corydoradinae	Lineage 9	*Corydoras*	*C. coppenamensis*	MHNG 2690.017-SU01-463	Guyana coastal rivers	GU210234	GU210659	GU209814	GU209406	GU208989	-
Corydoradinae	Lineage 9	*Corydoras*	*C. coppenamensis*	MHNG 2690.017-SU01-466	Guyana coastal rivers	GU210235	GU210660	GU209815	GU209407	GU208990	-
Corydoradinae	Lineage 9	*Corydoras*	*C. cruziensis* *	MA152	Amazon	GU210162	GU210587	GU209743	GU209643	GU209220	-
Corydoradinae	Lineage 9	*Corydoras*	*C. davidsandsi* *	LBP 551-7201	Amazon	GU210393	GU210818	GU209971	GU209412	GU208995	-
Corydoradinae	Lineage 9	*Corydoras*	*C. davidsandsi*	LBP 551-7202	Amazon	GU210394	GU210819	GU209972	GU209413	GU208996	-
Corydoradinae	Lineage 9	*Corydoras*	*C. davidsandsi*	LBP 551-7203	Amazon	GU210396	GU210821	GU209974	GU209414	GU208997	-
Corydoradinae	Lineage 9	*Corydoras*	*C. duplicareus* *	MA167	Amazon	GU210174	GU210599	GU209755	GU209423	-	-
Corydoradinae	Lineage 9	*Corydoras*	*C. evelynae* *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 9	*Corydoras*	*C. gossei* *	LBP 544-7168	Amazon	GU210403	GU210828	GU209981	GU209449	GU209031	-
Corydoradinae	Lineage 9	*Corydoras*	*C. gossei* *	LBP 544-7169	Amazon	GU210404	GU210829	GU209982	GU209450	GU209032	-
Corydoradinae	Lineage 9	*Corydoras*	*C. gossei* *	MT29	Amazon	GU210474	GU210899	GU210052	GU209448	GU209030	-
Corydoradinae	Lineage 9	*Corydoras*	*C. griseus*	MA71	Guyana coastal rivers	GU210362	GU210787	GU209941	GU209635	GU209213	-
Corydoradinae	Lineage 9	*Corydoras*	*C. guianensis*	MHNG 2683.055-GF06-574	Guyana coastal rivers	GU210247	GU210672	GU209827	GU209454	GU209036	-
Corydoradinae	Lineage 9	*Corydoras*	*C. guianensis*	MHNG 2683.055-GF06-575	Guyana coastal rivers	GU210248	GU210673	GU209828	GU209455	GU209037	-
Corydoradinae	Lineage 9	*Corydoras*	*C. habrosus*	MA142	Orinoco	GU210154	GU210579	GU209735	GU209457	GU209039	GU210989
Corydoradinae	Lineage 9	*Corydoras*	*C. habrosus*	MT30	Orinoco	GU210476	GU210901	GU210054	GU209456	GU209038	-
Corydoradinae	Lineage 9	*Corydoras*	*C. julii* *	MA147	Maranhao	GU210158	GU210583	GU209739	GU209468	GU209050	-

Corydoradinae	Lineage 9	*Corydoras*	*C. julii* *	LBP 5548-27240	Maranhao	GU210129	GU210554	GU209711	GU209467	GU209049	-
Corydoradinae	Lineage 9	*Corydoras*	*C. julli*	LBP 1359-11429	Amazon	GU210407	GU210832	GU209985	GU209466	GU209048	-
Corydoradinae	Lineage 9	*Corydoras*	*C. kanei* *	MA349	Amazon	GU210332	GU210757	GU209912	GU209654	GU209231	-
Corydoradinae	Lineage 9	*Corydoras*	*C. leucomelas* *	MA122	Amazon	GU210144	GU210569	GU209725	GU209471	GU209053	-
Corydoradinae	Lineage 9	*Corydoras*	*C. leucomelas*	MA123	Amazon	GU210145	GU210570	GU209726	GU209472	GU209054	-
Corydoradinae	Lineage 9	*Corydoras*	*C. leucomelas*	MHNG PE08-933	Amazon	GU210272	GU210697	GU209852	GU209473	GU209055	-
Corydoradinae	Lineage 9	*Corydoras*	*C. loretoensis*	MA298	Amazon	GU210286	GU210711	GU209866	GU209474	GU209056	-
Corydoradinae	Lineage 9	*Corydoras*	*C. loretoensis*	ANSP 181122-1475	Amazon	GU210325	GU210750	GU209905	GU209475	GU209057	-
Corydoradinae	Lineage 9	*Corydoras*	*C. loxozonus*	MA351	Orinoco	GU210335	GU210760	-	GU209476	GU209058	-
Corydoradinae	Lineage 9	*Corydoras*	*C. melini* *	MA115	Orinoco	GU210140	GU210565	-	GU209488	GU209070	-
Corydoradinae	Lineage 9	*Corydoras*	*C. melini*	MA77	Orinoco	GU210368	GU210793	-	GU209489	GU209071	-
Corydoradinae	Lineage 9	*Corydoras*	*C. metae* *	MT36	Orinoco	GU210482	GU210907	GU210060	GU209491	GU209073	-
Corydoradinae	Lineage 9	*Corydoras*	*C. metae* *	MT37	Orinoco	GU210483	GU210908	GU210061	GU209492	GU209074	-
Corydoradinae	Lineage 9	*Corydoras*	*C. metae* *	MT38	Orinoco	GU210484	GU210909	GU210062	GU209493	GU209075	-
Corydoradinae	Lineage 9	*Corydoras*	*C. multimaculatus*	MA302	Oriental Costal Rivers	GU210291	GU210716	GU209871	GU209494	GU209076	-
Corydoradinae	Lineage 9	*Corydoras*	*C. oiapoquensis* *	MA180	Guyana coastal rivers	GU210184	GU210609	GU209765	GU209509	GU209090	GU210997
Corydoradinae	Lineage 9	*Corydoras*	*C. oiapoquensis* *	MHNG 2682.023-GF06-185	Guyana coastal rivers	GU210259	GU210684	GU209839	GU209510	GU209091	-
Corydoradinae	Lineage 9	*Corydoras*	*C. oiapoquensis* *	MHNG 2682.023-GF06-186	Guyana coastal rivers	GU210260	GU210685	GU209840	GU209511	GU209092	-
Corydoradinae	Lineage 9	*Corydoras*	*C. osteocarus*	ANSP 185052-1476	Orinoco	GU210322	GU210747	GU209902	GU209514	GU209095	-
Corydoradinae	Lineage 9	*Corydoras*	*C. osteocarus*	ANSP 185052-1477	Orinoco	GU210323	GU210748	GU209903	GU209515	GU209096	-
Corydoradinae	Lineage 9	*Corydoras*	*C. panda* *	MHNG PE08-918	Amazon	GU210283	GU210708	GU209863	GU209522	GU209103	-
Corydoradinae	Lineage 9	*Corydoras*	*C. panda* *	MT40	Amazon	GU210486	GU210911	GU210064	GU209520	GU209101	-
Corydoradinae	Lineage 9	*Corydoras*	*C. panda* *	MT41	Amazon	GU210487	GU210912	GU210065	GU209521	GU209102	-
Corydoradinae	Lineage 9	*Corydoras*	*C. paragua*	MA67	Amazon	GU210358	GU210783	GU209937	GU209526	GU209107	-
Corydoradinae	Lineage 9	*Corydoras*	*C. polystictus*	LBP 1957-13648	Rio de la Plata	GU210418	GU210843	GU209996	GU209530	GU209109	-
Corydoradinae	Lineage 9	*Corydoras*	*C. polystictus*	MT44	Rio de la Plata	GU210490	GU210915	GU210068	GU209529	GU209108	-
Corydoradinae	Lineage 9	*Corydoras*	*C. polystictus*	MT45	Rio de la Plata	GU210491	GU210916	GU210069	GU209531	GU209110	-
Corydoradinae	Lineage 9	*Corydoras*	*C. punctatus*	MHNG SU08-110	Guyana coastal rivers	GU210262	GU210687	GU209842	GU209535	GU209114	-

Corydoradinae	Lineage 9	*Corydoras*	*C. punctatus*	MHNG SU08-1115	Guyana coastal rivers	GU210269	GU210694	GU209849	GU209536	GU209115	-
Corydoradinae	Lineage 9	*Corydoras*	*C. punctatus*	MHNG SU08-1200	Guyana coastal rivers	GU210276	GU210701	GU209856	GU209537	GU209116	-
Corydoradinae	Lineage 9	*Corydoras*	*C. schwartzi* *	LBP 1783-7120	Amazon	GU210421	GU210846	GU209999	GU209551	GU209130	-
Corydoradinae	Lineage 9	*Corydoras*	*C. schwartzi* *	LBP 1783-7121	Amazon	GU210422	GU210847	GU210000	GU209552	GU209131	-
Corydoradinae	Lineage 9	*Corydoras*	*C. schwartzi*	MT58	Amazon	GU210505	GU210930	GU210083	GU209550	GU209129	-
Corydoradinae	Lineage 9	*Corydoras*	*C. similis* *	LBP 547-7184	Amazon	GU210424	GU210849	GU210002	GU209562	GU209141	-
Corydoradinae	Lineage 9	*Corydoras*	*C. similis* *	LBP 547-7185	Amazon	GU210425	GU210850	GU210003	GU209563	GU209142	-
Corydoradinae	Lineage 9	*Corydoras*	*C. similis* *	MT61	Amazon	GU210509	GU210934	GU210087	GU209561	GU209140	-
Corydoradinae	Lineage 9	*Corydoras*	*C. sipaliwini*	MHNG 2707.017-SU07-287	Guyana coastal rivers	GU210241	GU210666	GU209821	GU209567	GU209146	-
Corydoradinae	Lineage 9	*Corydoras*	*C. sipaliwini*	MHNG 2707.017-SU07-288	Guyana coastal rivers	GU210242	GU210667	GU209822	GU209568	GU209147	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *'arcuatus* super' *	MT65	Amazon	GU210513	GU210938	GU210088	GU209600	GU209179	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *'arcuatus* super' *	MT66	Amazon	GU210514	GU210939	GU210089	GU209601	GU209180	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C121 *burgessi'*	MA178	Amazon	GU210182	GU210607	GU209763	GU209607	GU209185	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C121 *burgessi'*	MT67	Amazon	GU210515	GU210940	GU210090	GU209606	GU209184	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C129' *	MA149	Guyana coastal rivers	GU210159	GU210584	GU209740	GU209609	GU209187	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C129' *	MA150	Guyana coastal rivers	GU210160	GU210585	GU209741	GU209610	GU209188	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C129' *	MHNG 2651.049-GY04-156	Guyana coastal rivers	GU210233	GU210658	GU209813	GU209611	GU209189	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C133 ornatus short snout'	MT68	Amazon	GU210516	GU210941	GU210091	GU209617	GU209195	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C136' *	MA305	Guyana coastal rivers	GU210294	GU210719	GU209874	GU209644	GU209221	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C139 *oiapoquensis'*	MA174	Guyana coastal rivers	GU210178	GU210603	GU209759	GU209390	GU208974	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C139 *oiapoquensis'*	MA79	Guyana coastal rivers	GU210370	GU210795	GU209948	GU209391	GU208975	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C150 mazurani'	MA70	Guyana coastal rivers	GU210361	GU210786	GU209940	GU209615	GU209193	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C150 mazurani'	MA75	Guyana coastal rivers	GU210366	GU210791	GU209945	GU209616	GU209194	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C30'	MA215	Guyana coastal rivers	GU210215	GU210640	GU209795	GU209618	GU209196	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C30'	MA216	Guyana coastal rivers	GU210216	GU210641	GU209796	GU209619	GU209197	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C30'	MA66	Guyana coastal rivers	GU210357	GU210782	GU209936	GU209620	GU209198	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C43'	MA80	Amazon	GU210371	GU210796	GU209949	GU209626	GU209204	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C91 Peru *bondi'* *	MA300	Amazon	GU210289	GU210714	GU209869	GU209638	-	-

Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW21 *axelrodi*'	MA320	Orinoco	GU210304	GU210729	GU209884	GU209642	GU209219	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *arcuatus* 'Rio Negro' *	LBP 4348-24081	Amazon	GU210126	GU210551	GU209708	GU209597	GU209176	GU210999
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *arcuatus* 'Rio Negro' *	LBP 7709-32587	Amazon	GU210195	GU210620	GU209776	GU209598	GU209177	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *arcuatus* 'Rio Negro' *	LBP 7709-32609	Amazon	GU210196	GU210621	GU209777	GU209599	GU209178	GU210988
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *armatus* 'Green cana'	MA330	Amazon	GU210314	GU210739	GU209894	GU209602	-	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *breei*	MHNG SU08-583	Guyana coastal rivers	GU210278	GU210703	GU209858	GU209603	GU209181	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C84' *	MA197-32671	Amazon	GU210199	GU210624	-	GU209490	GU209072	GU210976
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW28' *	MA110	Amazon	GU210135	GU210560	GU209717	GU209612	GU209190	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C76' *	-	Amazon	-	-	-	-	-	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *davidsandsi*	MA134	Amazon	GU210152	GU210577	GU209733	GU209649	GU209226	-
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *melini*	MA333	Amazon	GU210316	GU210741	GU209896	GU209636	GU209214	-
Corydoradinae	Lineage 9	*Corydoras*	*C. sterbai* *	MT74	Amazon	GU210523	GU210948	GU210098	GU209661	GU209238	-
Corydoradinae	Lineage 9	*Corydoras*	*C. sterbai* *	MT75	Amazon	GU210524	GU210949	GU210099	GU209662	GU209239	-
Corydoradinae	Lineage 9	*Corydoras*	*C. trilineatus* *	MHNG PE08-922	Amazon	GU210273	GU210698	GU209853	GU209668	GU209245	-
Corydoradinae	Lineage 9	*Corydoras*	*C. trilineatus* *	MT79	Amazon	GU210528	GU210953	GU210103	GU209666	GU209243	-
Corydoradinae	Lineage 9	*Corydoras*	*C. trilineatus* *	MT80	Amazon	GU210530	GU210955	GU210105	GU209667	GU209244	-
Corydoradinae	Lineage 9	*Corydoras*	*C. weitzmani*	MA35	Amazon	GU210333	GU210758	GU209913	GU209680	GU209257	-

Species selected for morphometric analyses are denoted by asterisk. Species names of undescribed taxa have been labelled based on their respective C-Numbers (Datz) and CorydorasWorld numbers when available, otherwise the trade name alias or 'sp' was used.

Chapter 3: Whole genome duplication accelerates net diversification rates in Neotropical catfishes

3.1 Abstract:

Whole genome duplication (WGD) has been hypothesized to play a major role in evolution, as multiple rounds of WGD at the base of vertebrate and plant lineages are thought to have contributed to their subsequent evolutionary success. Nevertheless, the idea that WGD can act as a driver of speciation remains understudied due to a lack of comprehensive phylogenetic, genomic, and taxonomic data. Here I provide the first robust evidence of accelerated diversification rates associated with WGD, using a comprehensive time calibrated molecular phylogeny of the teleost subfamily Corydoradinae combined with genome size data. My results suggest that WGDs accelerate diversification rates, and that a likely mechanism driving this process may be the observed increase in complexity of colour patterns post genome duplication.

3.2 Introduction:

Over the last 40 years, gene and genome duplications have been recognised as playing important roles in the mode and tempo of evolution in a variety of organisms (Lynch and Conery, 2000; Ohno, 1970). For example, multiple rounds of whole genome duplication (WGDs) at the base of vertebrate and plant lineages have been implicated in their subsequent evolutionary success (Ohno, 1970; Wood et al., 2009), and WGDs have been implicated in the diversification of 15% of angiosperms and 31% of ferns (Wood et al., 2009). WGD can lead to profound genomic changes, including duplicated genes acquiring novel adaptive functions (neo and subfunctionalization) (Force et al., 1999; Lynch, 2007; Lynch and Conery, 2000; Taylor et al., 2001), accumulation of transposable elements, increases in the diversity of miRNA family members(Heimberg et al., 2008), the rearrangement of chromosomes, and differential silencing of protein coding genes (Kraaijeveld, 2010). These processes have been theoretically linked to increases in diversification rates through the reciprocal silencing of duplicated genes in allopatric populations leading to postzygotic isolation on secondary contact (divergent resolution) (Lynch and Force, 2000; Van de Peer et al., 2009) and increased genetic material leading to greater potential for adaptation (Lynch and Conery, 2000; Ohno, 1970). Preferential retention of pigmentation genes following WGDs has been suggested to play a role in teleost fish diversification (Braasch et al., 2009), with teleosts having 30% more pigmentation genes compared to tetrapods and that large parts of the melanocyte regulatory network have been retained in duplicate post WGD. Thus it seems that following Fish-Specific WGDs, novel pigment cell types have evolved, while existing cell types have been subfunctionalized. These data suggest that WGDs can lead to an increase in phenotypic complexity which is likely to accelerate diversification rates via assortative mating based on colour pattern.

Recent investigations testing the relationship between WGD and increased diversification rates in vertebrates and angiosperms have produced equivocal results (Hoegg et al., 2004; Kraaijeveld, 2010; Santini et al., 2009; Wood et al., 2009). In teleost fishes, WGDs appear to have played some role in 10% of extant teleost diversity (Santini et al., 2009) and there is an overall weak positive correlation between species richness and genome size (Mank and Avise, 2006). In plants, WGDs have been implicated in the diversification of 15% of angiosperms and 31% of ferns

(Wood et al., 2009), but while WGDs have been hypothesized to lead to accelerated rates of cladogenesis in some angiosperms and ferns, no detectable increase in net diversification post WGD has yet been identified (Soltis et al., 2010; Wood et al., 2009). The lack of a clear relationship between WGDs and increased diversification rate may be dependent on the timescale and the subsequent diploidization process, whereby the effects of duplication become less pronounced over time (Wolfe, 2001). The pivotal questions on whether WGDs do accelerate diversification rates and the mechanisms through which it occurs therefore remain open (Otto, 2007).

Corydoradinae catfishes are found throughout the neotropics and are ideally suited for a comparative analysis of genome size and diversification rate as the subfamily is composed of multiple lineages that differ in taxon diversity, nuclear genomic content, and colour pattern complexity (Dunham et al., 1980; Oliveira et al., 1992). Here I test the hypothesis that increases in genome size driven by genome duplication lead to accelerated diversification rates within Corydoradinae lineages. Firstly, I estimated haploid nuclear DNA content (referred to as the C-value throughout) for representatives of all known Corydoradinae lineages using Feulgen Image Densitometry, secondly, I constructed a comprehensive time calibrated molecular phylogeny using an uncorrelated relaxed clock, thirdly, I estimated rates and shifts of temporal and topological diversification using the phylogeny to test whether genome duplications affect diversification rates, and fourthly, I quantified colour patterns for each lineage to test whether speciation rate is correlated with the rate of colour pattern change. I also consider body size as an alternative trait that may account for patterns of accelerated diversification within the Corydoradinae.

3.3 Methods:

3.3.1 Taxonomic Sampling & Phylogenetic Analyses

A total of 206 representative taxa were used for the analysis, including three outgroups from the Callichthyinae (Genera: *Hoplosternum* and *Dianema*), and ingroup taxa covering all known lineages of the Corydoradinae (Genera: *Aspidoras*, *Scleromystax*, and *Corydoras*). I estimate that these representatives cover approximately 80% of the described taxonomic diversity of the subfamily Corydoradinae. Voucher information and GenBank accession numbers are provided **(SI Table 2)**, and all sequences and alignments were used from published data (Alexandrou et al., 2011). I used available measurements from the CorydorasWorld website (www.corydorasworld.com) to quantify mean body size for each species included within the analysis. To account for dimorphism in body size within the Corydoradinae, as observed within the genus *Scleromystax*, and the larger females than males within the rest of the group, we used mean maximum size between males and females.

In order to construct an ultrametric tree, I used a mitochondrial dataset of 2668 bps (containing partial sequences of 12S rRNA, 16S rRNA, ND4, $tRNA^{HIS}$, $tRNA^{SER}$, and Cytochrome b) and 203 Corydoradinae taxa (Alexandrou et al., 2011). These were subsequently used to compare topology to the output obtained from BEAST v1.5.4 (Drummond and Rambaut, 2007). I used the software package Path-O-Gen v1.2 (Drummond and Rambaut, 2007) to investigate the temporal signal and 'clock-likeness' of our non-calibrated ML and BI phylogenies. The callichthyid data was found to have a mean root-tip distance= 0.398 and coefficient of variation= 0.1127 (with a Stdev=4.4852E-2 and Variance=2.0117E-3) strongly supporting the non-clocklike nature of the dataset. I also used a likelihood-ratio test (LRT) to examine rate heterogeneity by calculating likelihood scores with and without an enforced molecular clock. I found significant rate heterogeneity among the branches of the callichthyid tree (LRT= 1128.676, df=204, $p<0.001$), suggesting a relaxed clock method would be most appropriate.

The best scoring tree from the RAxML v7.2.8 (Stamatakis, 2006) analysis was used as input in r8s v1.7 (Sanderson, 2003) and analysed using penalized likelihood (PL) with the truncated Newton algorithm and non-parametric rate smoothing (NPRS) with

the Powell algorithm using a single fixed calibration point of 58.5MYA at the Corydoradinae root. However, as BEAST re-estimates dates and topology simultaneously, the purpose of the r8s tree was limited to providing a framework that satisfied node age prior calibration points used in BEAST, serving as a starting point for subsequent UCLN analyses. I used the uncorrelated lognormal (UCLN) relaxed clock method implemented in BEAST v1.5.4 (Drummond and Rambaut, 2007) to estimate divergence times. BEAST runs were conducted under birth death and pure birth (Yule) priors, the latter yielding younger estimates. As the Yule prior may have been influenced by a pull towards the present in my analyses, I relied on birth-death priors for all results presented herein. All analyses in BEAST were partitioned by genic region and codons where appropriate, and independent MCMC chains were run for 20 million generations, sampling every 1000 generations. To ensure adequate mixing of parameters and that effective sample sizes exceeded 200, all results were inspected using Tracer v1.4 (Rambaut and Drummond, 2004). I built maximum clade credibility trees with mean node heights using TreeAnnotator v1.5.4 (Drummond and Rambaut, 2007; Hardie et al., 2002). I relied on 95% highest posterior density (HPD) estimates for divergence times, yielding a series of dates with upper and lower bounds.

The callichthyid tree was calibrated using the fossil *C. revelatus*† described from the Maiz Gordo Formation in Sunchal, Juyuy Province, Argentina dated to the late Paleocene at approximately 58.2-58.5 million years before present (Reis, 1998b). As the fossil was well preserved in its entirety, morphological features could be critically examined and compared to extant material. Four diagnosing features have helped identify the phylogenetic placement of *C. revelatus*† according to Reis (Reis, 1998b): "1) first infraorbital bone possesses an anterior expansion forming a large articular facet with the external border of the lateral ethmoid; 2) exposed nuchal plate; 3) forked caudal fin; 4) contact between the posterior supraoccipital process (elongated) and the nuchal plate". Thus, multiple morphological characters support the assignment of the fossil to the genus *Corydoras*. As the genus *Corydoras* represents the most basal lineage (Lineage 1) within the Corydoradinae, I assigned the fossil as the common ancestor of the subfamily Corydoradinae, as opposed to common ancestor of the family Callichthyidae. Other fossils of pectoral spines exist for various callichthyid species, however, due to their incomplete nature I did not use them for

this analysis. The *C. revelatus*† date served as a minimum age for calibration under a lognormal distribution (offset 58.5; mean 1.0; stdev 1.0). I used this age to fix the Corydoradinae root in order to provide a starting tree for the BEAST analysis that satisfied the prior calibration parameters. The age root of the callichthyid tree (connecting the Callichthyinae and Corydoradinae) was estimated based on the calibration provided by the *C. revelatus*† fossil, and after multiple independent analyses was determined to have a minimum age of approximately 90 million years before present. I therefore used this age to calibrate the root of our tree in BEAST under a lognormal distribution (offset 90; mean 2.0; stdev 1.2). Furthermore, two well-documented vicariant events were used for further calibration under normal distributions in BEAST: 1) Separation of Amazon and Rio de la Plata plates (mean 10; stdev 1.5); 2) Separation of Amazon and Orinoco plates (mean 6.5; stdev 1.0).

As the calibration point of the *C. revelatus* fossil differs from previous published analyses, inevitably the estimated dates recovered herein differ as well. Notably, Peng et al (Peng et al., 2006) estimated the basal age of Siluriformes at 173 MYA using *C. revelatus* as a fossil and *C. rabauti* as the representative extant taxon. I consider this calibration to be incorrect for two reasons: 1) Corydoradinae catfishes are not the most ancestral extant representatives of Siluriformes (trichomycterids more suitable); 2) Many representative taxa are missing from the analysis that would influence estimated dates. Subsequently, Lundberg & Sullivan (Lundberg et al., 2007) presented a calibrated phylogenetic framework with the most comprehensive siluriform taxonomic coverage to date. However, the calibration of Callichthyidae – Corydoradinae node using *C. revelatus* was problematic in their analysis as the fossil should be placed at the root of all Corydoradinae and not as a common ancestor between the Corydoradinae and Callichthyinae. More recently, Santini et al (Santini et al., 2009) published a calibrated phylogenetic framework for all Actinopterygii that included *C. revelatus* in the same position as proposed by Lundberg & Sullivan (Lundberg et al., 2007). Differences in estimated dates between these phylogenies are likely to result from the incorrect taxonomic placement of fossils for calibration, and the use of highly conserved (Rag 1 & Rag 2) or rapidly mutating (COI, Cyt B, ND 4) phylogenetic markers.

3.3.2 Feulgen Image Densitometry

C-values were estimated from erythrocyte nuclei for 65 species across most callichthyid lineages. Air-dried blood smears were prepared and stained according to the standard vertebrate protocol discussed in previous work (Hardie et al., 2002). A total of 7 species were used as standards: *Gallus domesticus*, *Equus ferus caballus*, *Betta splendens, Poecilia reticulata, Chromobotia macracanthus, Danio rerio,* and *Polypterus birchir*. All samples were processed in one batch at the same time the minimize variation in estimates. Measurements of nuclear area and IOD (Integrated Optical Density) were made using a PriorLux microscope at 100x magnification mounted with a Retiga 2000R CCD camera, and the Image-Pro plus 7 software. C-values were estimated for approximately 100 non-overlapping nuclei from up to 5 different fields per slide. A standard curve of IOD vs known c-values was generated to check the accuracy of the stain across the range of erythrocytes employed as standards, consistently yielding highly significant linear regressions ($r^2 > 0.95$, $P < 0.0001$). Hydrolysis and fixation time were also tested to identify optimal timing for IOD measurements. C-value calculations of unknowns were calculated based on the mean IOD of the standards. As most estimates are based on *Gallus domesticus* as a standard, I used this to calculate C-values and checked all results with the remaining standards that have accurately estimated c-values. I assembled genome size estimates for all other available species within the Callichthyidae from the Genome Size Database (www.animalgenomesize.com). Mean values per lineage were calculated from the available data and assigned to taxa for which C-value estimates were not available.

3.3.3 Ancestral Reconstruction & Character Associated Diversification

I used a parsimony model as implemented in Mesquite v2.74 (Maddison and Maddison, 2010) to reconstruct ancestral C-values for all nodes across the Corydoradinae phylogeny. This reconstruction was used as an approximation to identify ancestral nodes where duplications are likely to have occurred. To test whether C-values were associated with diversification rates, I used the Binary State Speciation and Extinction method (Maddison et al., 2007). Taxa were coded in binary as diploid (0) or polyploid (1), where diploids have a C-value ranging between 0.5 – 1pg, while taxa with C-values greater than 1.5pg were coded as polyploid. I compared

likelihoods of a constrained and unconstrained model using an LRT to obtain a p-value for the hypothesis that C-values are associated with increased diversification rate. Analyses were performed with diversitree v0.6.1 (FitzJohn, 2011), run via the Mesquite.R package in Mesquite v2.74 (Maddison and Maddison, 2010).

To further investigate whether C-values were associated with diversification rates, we used the QuaSSE method (FitzJohn, 2010), as implemented in the R package diversitree v0.6-3 (FitzJohn, 2011). This method computes the likelihood of the observed data (the tree plus character states) assuming that the trait evolves under Brownian motion and influences speciation through some function. I used maximum likelihood to fit models where speciation rates were constant, or where they varied linearly or sigmoidally with C-value, followed by a likelihood ratio test to compute the statistical improvement of trait-dependent models over the constant rates models. Non-nested models were compared using AIC. Not all species are present in the phylogeny, but all known clades are represented. I corrected for sampling on a clade-by-clade basis by assigning different clades appropriate sampling fractions (varying from 0.48 to 1.00), following (FitzJohn, 2010). We repeated these tests using body size as an alternative variable associated with diversification rate.

3.3.4 Topological & Temporal Diversification Rate Analyses

With 1000 trees sampled from the posterior distribution of the UCLN analysis, I used SymmeTREE v1.1 (Chan and Moore, 2005) to test for significant shifts in topological diversification, against simulated null distributions. I performed a series of whole-tree surveys to detect whether there was significant variation in diversification across the entire phylogeny, and subsequently performed tests to identify the nodes at which the shifts in diversification occur. I also conducted the relative cladogenesis test as implemented in the R package Geiger v1.3 (Harmon et al., 2008). Both the relative cladogenesis and SymmeTREE tests are considered topological tests, rather than temporal tests employed using MEDUSA and LASER. Given the extreme diversities present within certain lineages, and the "trickle down" effect of significance values with this test, we took a parsimonious approach and considered only those nodes closest to the tips of the tree as being most significant.

I extracted branching times estimated from the BEAST analysis and used these to calculate the γ statistic for the empirical tree in the R package Laser v2.0 (Rabosky,

2006) with the gamStat function. I then simulated 10,000 phylogenies to the full clade size (total extant clade diversity) under a null hypothesis of a rate constant pure-birth process using a Monte Carlo constant rates (MCCR) test as implemented. This allows taxa to be randomly pruned from the simulated trees, thus recreating the effects of incomplete taxonomic sampling. Given the observed γ value tabulated from the empirical, the MCCR test yields a null distribution of the γ statistic for the simulated phylogenies and a p-value (alpha=0.05) to assess the significance of the observed γ statistic. I tested rate constant and rate variable models and compared AIC (Aikake Information Criterion) values in order to determine a best-fit model. I then used Ape v2.6.2 (Paradis et al., 2004) to construct lineage through time plots from trees recovered from the stationary distribution of the BEAST analysis.

Mean extant diversities per lineage were estimated by incorporating undescribed and described species on the basis of genetic distance, colour patterns, morphology and geographic distribution. I first calculated the total number of described species per lineage, assigning missing species to genetic lineages based on morphological and morphometric synapomorphies (Alexandrou et al., 2011). To increase the accuracy of our taxonomic estimates, I obtained all available data on undescribed taxa from Fuller & Evers (Fuller and Evers, 2005) and estimated the total number of known taxa that have yet to be described (C-Numbers DATZ), isolated from closest known relatives by river basin, colour pattern and morphology. As many of these undescribed species may not constitute biological species *sensu* Mayr, I discarded taxa that were likely to be varieties of described species (in cases of closely related species with identical colour pattern and overlapping geographic ranges) and made a conservative mean estimate from the total number described and unique undescribed taxa (Table 5). The tree recovered from the BEAST analysis was then pruned down to a single representative branch per major lineage, resulting in a total of 10 branches. The pruning is a necessary component of temporal diversification analyses, in order to assign taxonomic richness to the tips of a tree, thereby using the pruned phylogeny as a backbone to test hypotheses.

I assigned taxonomic information (estimated mean species richness per lineage) to each branch and tested shifts in diversification rates using R. Net diversification rates were calculated using the extant age of lineages from the UCLN analysis and extant

estimated diversities per lineage using the Magellon and Sanderson method (Magallon and Sanderson, 2001) implemented in the R package Geiger v1.3 (Harmon et al., 2008). I performed tests with both upper and lower bounds obtained from the 95% HPD intervals determined in BEAST, and high and low background extinction rates (ε=0-0.95). Using Laser I tested one rate (fitNDR_1rate) and two rate (fitNDR_2rate) models with high and low background extinction levels and compared resulting log-likelihood values using a likelihood ratio test (LRT). To further explore shifts in diversification rate, I used MEDUSA (Alfaro et al., 2009) as implemented in Geiger. This method uses a stepwise approach by adding rate shifts to a tree until AIC scores stabilize. I also tested for rate variation using the delta-AICrc function that fits a series of rate-variable and rate-constant variants of the birth-death model to the resulting branching times recovered from the BEAST analysis. The resulting statistic (dAICrc) is derived from the difference in AIC scores between the best-fit rate-constant and rate-variable models. I simulated 5000 rate-constant (Yule pure-birth) phylogenies with 206 taxa to show the null distribution of the dAICrc test statistic and compare it to the one obtained for the Corydoradinae phylogeny.

To test whether the trait-diversification associations are robust to lineage-specific shifts in diversification, we conducted additional analyses where the QuaSSE parameters were allowed to differ in different parts of the tree. I used the split identified by MEDUSA, grouping lineages 7-9 (the "foreground" clade), with the rest of the tree as the "background" group. I then proceeded as above to compute maximum likelihood and AIC values for models where speciation rates varied linearly or sigmoidally with respect to C-values or body size, but now allowing for the parameters for these functions to vary between the two sections of the tree.

I quantified total numbers of colour patterns for each lineage within the phylogeny using the method described in (Alexandrou et al., 2011). Briefly, this involved separating the lateral side of each fish into 20 different sections, then scoring the presence (1), absence (0), or variability of different colour pattern characteristics (such as blocks of colour, bright spines, patches, bands, stripes, spots and reticulations). These data were then used to plot colour patterns using Principal Coordinates based on a Euclidean pairwise distance matrix. Colour patterns were then grouped by similarity based on distances in order to allow for an approximate quantification of different patterns within each lineage. I then used the crown age per

lineage and calculated a rate of colour pattern change per MY. I did the same for taxa within the phylogeny to get a speciation rate per lineage (relying solely on the taxa included within the phylogeny), and correlated speciation rate against the rate of colour pattern change using a linear regression.

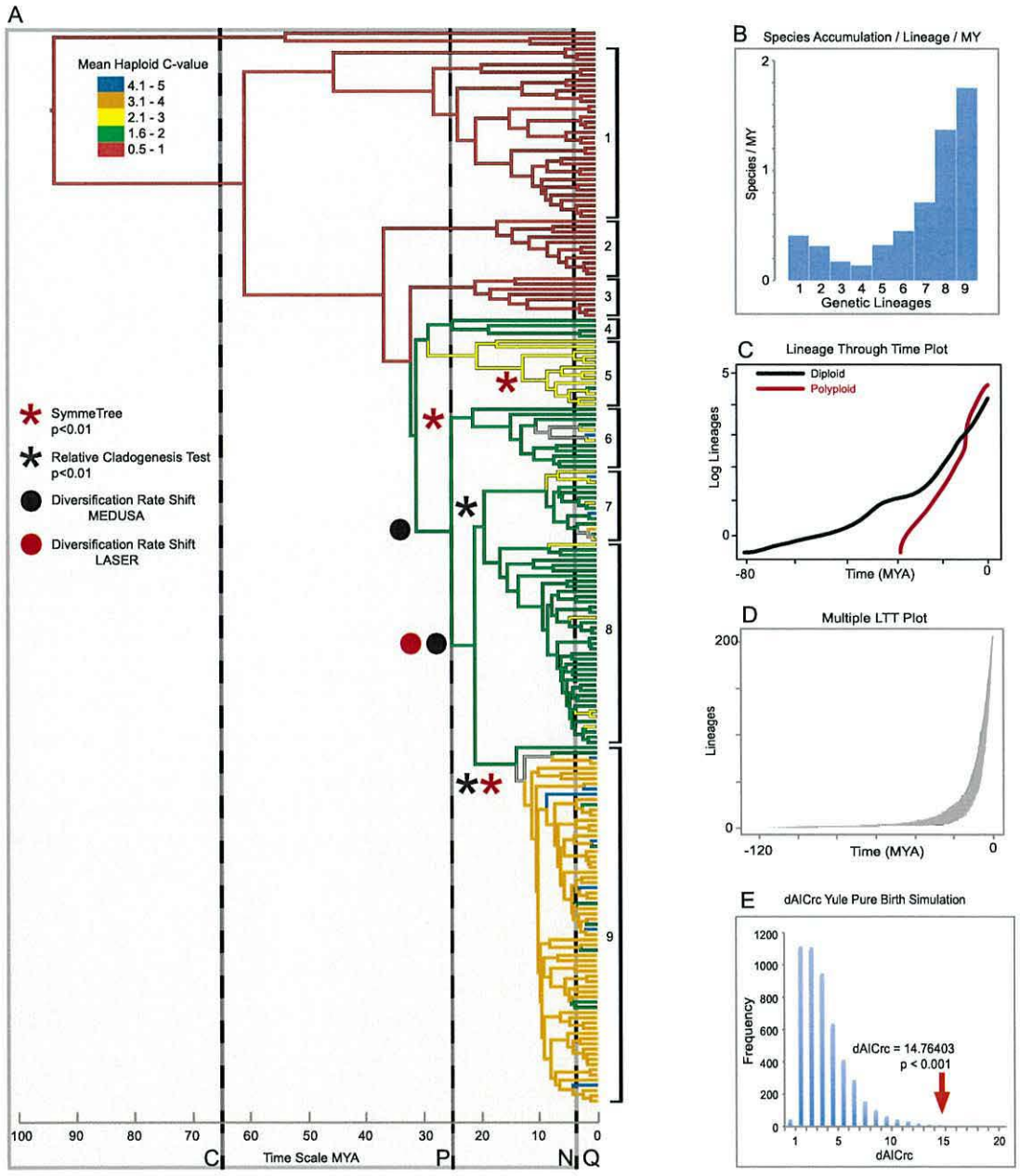
3.4 Results:

3.4.1 Phylogenetics

The phylogeny recovered from the BEAST analysis (Figure 12) was nearly identical to the mitochondrial tree presented in Alexandrou et al (Alexandrou et al., 2011), which was expected given that the same matrix was used (even though BEAST re-estimates the phylogeny). Of the nine monophyletic lineages recovered in our analysis, all were well supported with posterior probabilities greater than 0.9. When compared to the published mitochondrial phylogeny (Alexandrou et al., 2011), probabilities for nodes in the phylogeny recovered herein are slightly higher, a result that may be explained by differences in prior choice between BEAST and MrBayes. The root of the Corydoradinae tree was estimated as 59 – 64 MY based on the 95% highest posterior density (HPD) confidence intervals.

Our results (Figure 12) support the occurrence of major lineage separation throughout the Paleogene and early Neogene. During this ancient divergence of lineages, it is likely that the major ecologically relevant morphological differences were established, notably in body size and snout morphology (Alexandrou et al., 2011). These differences have been shown to contribute to niche partitioning in terms of resource acquisition, and therefore constitute significant ancient evolutionary transitions that have subsequently affected the ecological stability of co-existing Corydoradinae species (Alexandrou et al., 2011). However, these transitions alone do not adequately explain the observed patterns of accelerated net diversification, as most speciation events observed occur during the Miocene (mean = 7.79MYA).

Figure 12- Phylogeny, Genome Sizes, and Diversification



(A) Corydoradinae species tree with ancestral reconstruction of genome sizes as denoted by colored branches. Shifts in topological and temporal diversification rate denoted with asterisks and circles respectively. (B) Species accumulation per million years per genetic lineage based on upper estimates. (C) Lineage through time plot with diploid and polyploid species. (D) Multiple lineage through time plot based on 18,000 trees from the posterior distribution of the BEAST analysis. (E) Histogram of rate constant phylogenies simulated under the yule model compared to observed phylogeny, with a difference in AIC scores and associate p-value.

Table 5 – Diversification Rates

Lineage	Richness	HPD	stDEV HPD	Mean e0 r	stDEV e0 r	Mean e95 r	stDEV e95 r
Callichthyidae	272	94.7	6.51	0.059	0.004	0.028	0.002
Callichthyinae	17	54.75	31.47	0.062	0.036	0.013	0.007
Corydoradinae	255	61.35	3.89	0.091	0.006	0.043	0.003
Lineage 1	42	44.85	12.37	0.071	0.02	0.025	0.007
Lineage 2	23	37.35	12.09	0.069	0.022	0.020	0.007
Lineage 3	9	20.95	8.56	0.065	0.027	0.012	0.005
Lineage 4	7	25	9.48	0.054	0.021	0.010	0.004
Lineage 5	13	20.65	8.98	0.100	0.044	0.024	0.010
Lineage 6	18	21.15	7.28	0.110	0.038	0.03	0.010
Lineage 7	14	8.3	1.98	0.199	0.048	0.043	0.010
Lineage 8	63	16.45	5.16	0.221	0.069	0.089	0.028
Lineage 9	72	13.75	4.6	0.276	0.092	0.115	0.038

Mean species richness per lineage, net diversification rates (r) *sensu* Magellon & Sanderson, under high (e95) and low (e0) extinction values, and Highest Posterior Density (HPD) estimates based on BEAST analyses.

3.4.2 Feulgen Image Densitometry

The distribution of estimated Corydoradinae C-values ranged between 0.51pg – 4.8pg. To test the accuracy of the Fuelgen densitometry method, I correlated IOD against C-value for the standards employed, resulting in a highly significant coefficient of determination (R^2 = 0.986). I then plotted IOD against all newly estimated C-values for the Corydoradinae, which also resulted in a highly significant relationship (R^2 = 0.974). I then separated resulting C-values by lineage (SI Table 2) in order to compare ranges and means between and within different lineages. Within basal Corydoradinae lineages (1, 2, & 3), C-values ranged between 0.51- 0.94pg, while derived lineages (4 – 9) exhibited much greater variation ranging between 1.42 – 4.8pg. The largest C-values were recovered from lineages 6, 7 and 9, at 4.12, 4.4 and 4.8pg respectively, suggesting that multiple independent duplications have occurred along different branches of the Corydoradinae phylogeny.

3.4.3 Ancestral Reconstruction

The ancestral reconstruction of C-values revealed a conserved pattern of genome size evolution within basal lineages (lineages 1, 2 and 3 including Callichthyinae outgroups), while the more derived polyploid lineages (lineages 4-9) exhibit significant genomic expansion. Ancestral character states were highly supported in nearly all cases (Equivocal reconstruction was denoted in grey – see Figure 12). Basal diploid lineages have inherited conserved C-values ranging 0.5-1pg of nuclear DNA, and lack the significant variation observed in derived lineages. Our results suggest that an initial WGD event (leading to a C-value increase from the basal range of 0.5-1pg to 2.1-3pg) is likely to have occurred in the common ancestor of lineages 4-9 between 25 – 38 MYA. This ancestral event was followed by multiple independent duplications within different Corydoradinae lineages. The common ancestor of lineage 5 was reconstructed to have a mean C-value ranging between 1.1-2pg. All other derived polyploid lineages were reconstructed with a common ancestral C-value ranging between 2.1-3pg. However, results revealed that within lineage fluctuation in C-value was particularly pronounced in lineages 7 and 9. Notably, lineage 9 contains multiple taxa with C-values greater than 4.1pg, others ranging between 2.1-3pg, while the majority of reconstructed ancestral nodes vary between 3.1-4pg.

3.4.4 Topological & Temporal Diversification Rates

The relative cladogenesis test yielded significant p-values ($p < 0.01$) for a shift at the node of common ancestry for all polyploid lineages (Figure 12). Three more nodes associated with significant ($p < 0.01$) shifts in diversification rate were identified using SymmeTREE (Figure 12). No significant shifts in diversification were found within any of the diploid lineages (1-3). The estimated gamma statistic for the Corydoradinae phylogeny was positive ($\gamma = 2.35$), however, MCCR null gamma simulations yielded a critical value (0.05 percentile of the null distribution, corresponding to alpha = 0.05) of -2.6, while the 0.975 percentile of the null distribution confirmed the significance of the observed positive gamma value. The test for rate variation using the delta-AICrc test statistic revealed the birth-death model as the best constant rate model, and the yule2rate as the best rate variable model, with a difference in AIC scores of 14.76403. Furthermore, I fit time-varying speciation and extinction (SPVAR & EXVAR) models to branching times derived from the Corydoradinae phylogeny, resulting in a better AIC score for time-varying extinction (AIC = -498.6816) rather than the time-varying speciation (AIC = -496.7855).

Net diversification rates per lineage were initially estimated using Magellon and Sanderson method (Table 5) and yielded consistently higher rates for polyploid lineages as compared to diploid lineages. Under a relative extinction rate ($\varepsilon = 0.95$), mean diversification rate for all diploid lineages was $r = 0.018 \pm 0.006$, while polyploid lineages averaged at $r = 0.052 \pm 0.04$, whereas a low relative extinction rate ($\varepsilon = 0$) resulted in mean diversification of $r = 0.067 \pm 0.003$ for diploids and $r = 0.16 \pm 0.08$ for polyploids. Lineage 9 resulted in the highest diversification rate for the Corydoradinae ($r = 0.276$ for $\varepsilon = 0$, $r = 0.115$ for $\varepsilon = 0.95$), while lineage 4 resulted in the lowest values ($r = 0.054$ for $\varepsilon = 0$, $r = 0.01$ for $\varepsilon = 0.95$). Overall, lineages having undergone multiple duplications have significantly higher diversification rates than those that inherit a conserved c-value and/or those that are diploid as revealed by a t-test ($p=0.00083$, $df=5$).

The results of the combined taxonomic and phylogenetic analysis consistently resulted in higher likelihoods under a two-rate model (fitNDR_2rate) with high extinction rates ($\varepsilon= 0.95$). The best shift point identified by the two-rate model was

the node of common ancestry for lineages 7, 8 and 9 (AIC = 144.6108, λ_1 = 0.334, λ_2 = 1.29), supporting a 3.9x increase in speciation. The same node was identified under high and low background extinction values (ε = 0-0.95), suggesting that these results are robust to assumptions about extinction. A decrease in diversification rate was identified at the node of common ancestry for the Callichthyinae outgroups (AIC = 149.4245, λ_1 = 0.706, λ_2 = 0.126). I repeated calculations using the taxonomic info separate from phylogenetic data and found the same shifts identified above. Using MEDUSA we identified a significant shift in temporal diversification rate associated with lineages 6, 7, 8 and 9. Results show that net diversification increased from r = 0.027 to r = 0.184, while extinction rates decreased from ε = 0.87 to ε = 0.000015, with an improvement in likelihood of 14.87 with the partition added. These likelihood scores are not directly comparable to those obtained by LASER, however, similar shifts in temporal diversification were identified.

3.4.5 Character Associated Diversification

I analysed speciation (λ) and extinction (μ) as associated with the states of a character (in this case C-values coded as diploid$_0$ or polyploid$_1$) using BiSSE. The models I compared were equal λ, equal μ, with unequal q values and the resulting likelihood difference was highly significant (Log-Likelihood Difference = 16.856084, p-value = 0.0002, df=2). The unconstrained model yielded values associated with the diploid state of λ_0 = 0.099 and μ_0 = 0.049, while the polyploid state resulted in λ_1 = 0.226 and μ_1 = 0.182. The unconstrained character transition rate was q01 = 0.001 (diploid to polyploid) and q10 = 0.0000007 (polyploid to diploid). With the constrained model ($\mu_1 = \mu_0$ and q10 = q01) values associated with the diploid state were λ_0 = 0.087 and μ_0 = 0.041, while the polyploid state resulted in λ_1 = 0.18, and the character transition rate was q01 = 0.00005. These data support a relationship between higher C-values and accelerated diversification rates and suggest that it is more likely for Corydoradinae species to undergo genomic expansion rather contraction.

Both genome size and body size were positively significantly correlated with speciation rates using sigmoidal models (C-value: χ_3^2 = 23.5, p <0.001, mass: χ_3^2= 14.3, p < 0.001), and this pattern was strongest for c-values (Table 6). The largest C-values are concentrated in the most diverse part of the tree, and any trait that is concentrated in this section of the tree could be found to be statistically associated

with elevated rates of diversification. Indeed, once I allowed the more diverse "foreground" clade (lineages 7-9) to have different patterns of diversification to the "background group, the C-values were no longer significantly correlated with diversification (compared with the separate constant rates model $\chi_6^2 = 6.2$, $p = 0.40$). In contrast, the body size result remained significant, with speciation rates being positively correlated with body size in both foreground and background species (vs. separate constant rates model X_6^2 = 16.1, p = 0.013). C-values were found to be associated with the split identified by MEDUSA and Laser, whereas body size was not. Furthermore, no alternative synapomorphies could be identified for the clade forming lineages 7, 8 and 9 (the node identified by MEDUSA and Laser), suggesting that the association with C-values best explains this transition.

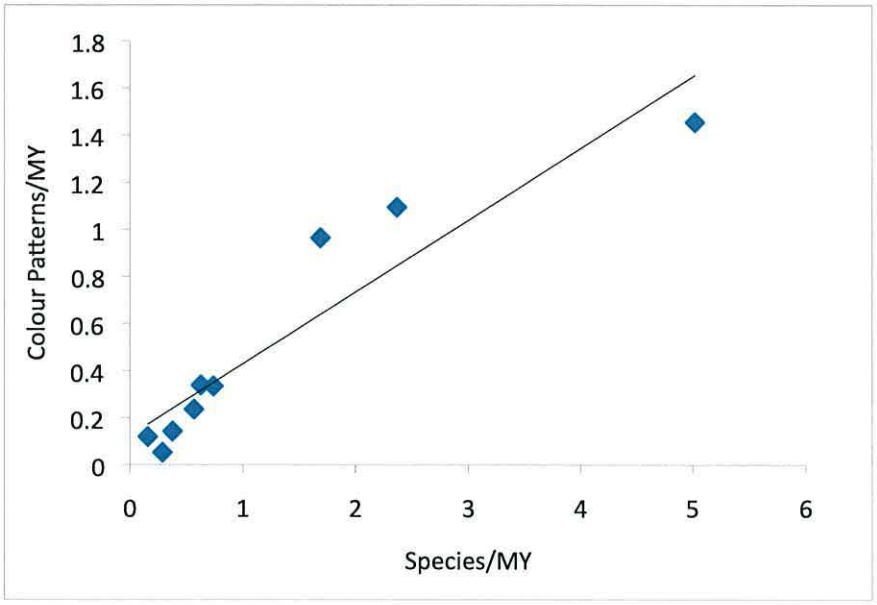
The rate of colour pattern change was significantly positively correlated with speciation rate ($R^2 = 0.877$, $p = 0.0006$). Notably, lineages 7, 8 and 9 were found to have the highest rates of colour pattern change, suggesting a relationship between increase in genome size, colour pattern change and accelerated diversification rate (Figure 13).

Table 6- QuaSSE Results

Models	Df	Body Size lnLikelihood	C-value lnLikelihood	Body Size AIC	C-value AIC	Body Size ChiSq	C-value ChiSq	Body Size Pr(>|Chi|)	C-value Pr(>|Chi|)
Base model	3	-471.5	-568.74	949	1143.5				
Linear speciation	4	-468.84	-560.96	945.69	1129.9	5.313	15.558	0.021172	8.00E-05
Sigmoidal speciation	6	-464.36	-557.01	940.72	1126	14.284	23.461	0.002543	3.24E-05
Clade-specific constant speciation	4	-456.74	**-553.98**	921.48	**1116**	29.522	**29.522**	5.53E-08	**5.53E-08**
Clade-specific linear speciation	6	-455.2	-553.9	922.4	1119.8	32.599	29.672	3.91E-07	1.62E-06
Clade-specific sigmoidal speciation	10	**-448.71**	-550.88	**917.43**	1121.8	**45.574**	35.725	**1.06E-07**	8.17E-06

Likelihood values, AIC scores, degrees of freedom and Chi-Squared test results based on different models implemented in QuaSSE.

Figure 13- Rate of Colour Pattern Change vs. Speciation



Scatter plot with trendline showing positive relationship between rate of colour pattern change and speciation ($R^2 = 0.877$, $p = 0.0006$).

3.5 Discussion:

Increased diversification rates following WGDs have not been conclusively demonstrated for any animal or plant group prior to this study. Here I show that a shift in diversification rate was associated with ancestral WGD within the Corydoradinae, while lineages within the subfamily having undergone subsequent WGDs are more species rich than those that have not (It remains unclear whether subsequent duplications are accelerating diversification further). Furthermore, WGDs linked to the increase in diversification occur in derived lineages (in Lineages 6, 7, 8, 9), whereas more basal lineages experience slower diversification in the absence of WGDs. Lineages with C-values ranging between 0.5-1pg experience slower net diversification rates than those that undergo subsequent within lineage duplications and exhibit higher C-values. Furthermore, species in lineages 7, 8 and 9 have more chromosomes than species in other lineages, adding further support to our conclusion that a WGD event accelerated diversification rates (Oliveira et al., 1992; Oliveira et al., 1993a; Scheel et al., 1972; Shimabukuro-Dias et al., 2004a). Body size was also correlated with diversification rate, with our analyses suggesting that larger species diversify more rapidly than smaller species, however, the difference between large and small species is minimal. Despite the correlation, this relationship does not explain the shift in diversification rate and is considered a complementary association (as it is less important than the association between WGD and diversification shift), due to body size variation across the phylogeny and any trait associated with species rich lineages could be shown to be statistically associated with diversification rate.

I demonstrate that lineages having undergone genome duplications are more species rich than those that have not, but have not thus far addressed the mechanisms that may lead to these increases. The role of divergent resolution (reciprocal silencing) in increasing diversification rates of groups having undergone WGDs has received much attention (Taylor et al., 2001), and while theoretically appealing, empirical support is lacking and the expected patterns and distribution of diversity under the model poorly explored. Firstly, divergent resolution can only increase diversification rates if allopatric populations in which genes are reciprocally silenced are re-united (divergent resolution increases sympatric taxon diversity). If divergent resolution has led to increased diversification rates in the Corydoradinae I expect polyploid taxa from the *same lineage* to be living in sympatry. This is not the case, and in all

communities investigated thus far, only a single polyploid species from each lineage is found in sympatry. This may result from reunited taxa hybridising (indicating a incomplete reproductive isolation) on reunification leaving a single hybrid taxon, or a lack of ecological differentiation among reunited taxa leading to competitive exclusion (Alexandrou et al., 2011), again resulting in only a single taxon per lineage at any one site.

However, that does not exclude silencing and neo/sub-functionalisation playing a role in increasing diversification rates. Colour pattern change is higher in lineages associated with the shift in diversification rate, and this is what I would expect if genes associated with colour patterns that have been duplicated are reciprocally silenced in different isolated populations. Colour pattern has strong adaptive value in the Corydoradinae with Müllerian mimetic relationships occurring among sympatric coexisting species and with different allopatric communities differing in colour pattern. Accelerated colour pattern change associated with cladogenesis has already been shown for *Ithomia* butterflies, and these patterns are implicated in multiple mimicry rings (Jiggins et al, 2006). Thus, reciprocal silencing of duplicated genes may increase the rate of colour pattern change in allopatric polyploid populations that then leads to an increase in taxon diversity. In addition, when diversification rate increases within a lineage, the radiation may be constrained over time by the availability of niche space, thereby effectively reducing diversification and maintaining species richness at a specific threshold or carrying capacity (Rabosky, 2009a). However, WGDs could alter this pattern, extending ecological limits on diversification by the addition of extra genetic material for adaptation, which may also explain why polyploids tend to be successful during times of environmental disturbance (Fawcett et al., 2009; Van de Peer et al., 2009) (See Chapter 4).

In conclusion I find no evidence that divergent resolution has increased the species richness of polyploid lineages. Given the observed relationship between accelerated diversification rate, large C-values and faster colour pattern transitions, it seems more likely that there is an increase in complexity of pigmentation genes following genome duplication, and that this might accelerate diversification as a consequence of assortative mating. Despite the appeal of this explanation, it remains speculative, and in need of a causal link through direct comparison of pigmentation genes pre and post genome duplication across lineages to confirm or reject this hypothesis. Nevertheless,

previous research has shown that WGDs can lead to increased complexity of pigment cells and their subfunctionalization in fishes (Braasch et al., 2009), thereby providing further support for the hypothesis.

SI Table 2 – Accession Numbers, Species List, C-Values, Body Size

	Lineage	Genus	Species	Voucher Code	Hap C-value (pg)	Body Size (mm)	ACC 12s	ACC 16s	ACC ND4	ACC Cytb
Callichthyinae	Lineage 0	*Dianema*	*D. longibarbus*	LBP 557-7230	**0.94**	100	GU210442	GU210867	GU210020	GU209684
Callichthyinae	Lineage 0	*Dianema*	*D. urostriatum*	MT89	0.99	125	GU210539	GU210964	GU210114	GU209685
Callichthyinae	Lineage 0	*Hoplosternum*	*H. littorale*	LBP 210-4134	0.85	210	GU210443	GU210868	GU210021	GU209686
Corydoradinae	Lineage 1	*Corydoras*	*C. acutus*	MA41	**0.65**	67.5	GU210339	GU210764	GU209918	GU209605
Corydoradinae	Lineage 1	*Corydoras*	*C. amapaensis*	MA154	**0.65**	65	GU210164	GU210589	GU209745	GU209360
Corydoradinae	Lineage 1	*Corydoras*	*C. aurofrenatus*	ANSP 182420-1470	**0.65**	52.5	GU210327	GU210752	GU209907	GU209332
Corydoradinae	Lineage 1	*Corydoras*	*C. blochi*	MHNG 2652.007-GY04-237	**0.65**	62.5	GU210236	GU210661	GU209816	GU209341
Corydoradinae	Lineage 1	*Corydoras*	*C. cervinus*	MA125	0.6	65	GU210146	GU210571	GU209727	GU209358
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *blochi*	MHNG 2707.015-SU07-624	**0.65**	62.5	GU210239	GU210664	GU209819	GU209368
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *geoffroy*	MHNG 2683.016-GF06-459	**0.65**	67.5	GU210249	GU210674	GU209829	GU209379
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *maculifer*	MA40	**0.65**	65	GU210338	GU210763	GU209917	GU209386
Corydoradinae	Lineage 1	*Corydoras*	*C. coriatae*	MT14	0.81	62.5	GU210428	GU210853	GU210006	GU209408
Corydoradinae	Lineage 1	*Corydoras*	*C. ellisae*	MT24	**0.65**	62.5	GU210469	GU210894	GU210047	GU209430
Corydoradinae	Lineage 1	*Corydoras*	*C. fowleri*	MA108	0.65	67.5	GU210133	GU210558	GU209715	GU209441
Corydoradinae	Lineage 1	*Corydoras*	*C. geoffroy*	MHNG 2700.007-GF07-120	**0.65**	67.5	GU210226	GU210651	GU209806	GU209443
Corydoradinae	Lineage 1	*Corydoras*	*C. maculifer*	LBP 7213-32890	**0.65**	65	GU210210	GU210635	GU209790	GU209481
Corydoradinae	Lineage 1	*Corydoras*	*C. negro*	MA52	**0.65**	62.5	GU210348	GU210773	GU209927	GU209504
Corydoradinae	Lineage 1	*Corydoras*	*C. orcesi*	MA304	**0.65**	67.5	GU210293	GU210718	GU209873	GU209512
Corydoradinae	Lineage 1	*Corydoras*	*C. oxyrhynchus*	MHNG SU08-1191	**0.65**	67.5	GU210284	GU210709	GU209864	GU209516
Corydoradinae	Lineage 1	*Corydoras*	*C. pastazensis*	MT42	0.78	67.5	GU210488	GU210913	GU210066	GU209527
Corydoradinae	Lineage 1	*Corydoras*	*C. semiaquilus*	ANSP 178613-1459	0.51	77.5	GU210321	GU210746	GU209901	GU209553
Corydoradinae	Lineage 1	*Corydoras*	*C. septentrionalis*	MA114	**0.65**	55	GU210139	GU210564	GU209721	GU209659
Corydoradinae	Lineage 1	*Corydoras*	*C. serratus*	MA309	0.7	62.5	GU210298	GU210723	GU209878	GU209557
Corydoradinae	Lineage 1	*Corydoras*	*C. simulatus*	MT64	0.52	67.5	GU210512	GU210937	-	GU209566
Corydoradinae	Lineage 1	*Corydoras*	*C. solox*	MHNG 2666.036-GF03-099	**0.65**	67.5	GU210252	GU210677	GU209832	GU209573
						62.5				

Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C42'	MA27	**0.65**	77.5	GU210263	GU210688	GU209843	GU209625
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C53'	MA30	**0.65**	62.5	GU210288	GU210713	GU209868	GU209630
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'CW11 long nose *reynoldsi*'	LBP 7712-32724	**0.65**	62.5	GU210203	GU210628	GU209783	GU209640
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2681.018-GF06-071	**0.65**	65	GU210256	GU210681	GU209836	GU209318
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C109'	LBP 5549-27241	**0.65**	62.5	GU210130	GU210555	GU209712	GU209653
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C92'	MA211	**0.65**	62.5	GU210213	GU210638	GU209793	GU209304
Corydoradinae	Lineage 1	*Corydoras*	*C. stenocephalus*	MHNG PE08-910	**0.65**	67.5	GU210279	GU210704	GU209859	GU209554
Corydoradinae	Lineage 1	*Corydoras*	*C. treitlii*	MT76	**0.65**	67.5	GU210525	GU210950	GU210100	GU209663
Corydoradinae	Lineage 1	*Corydoras*	*C. vittatus*	MT84	**0.65**	57.5	GU210534	GU210959	GU210109	GU209677
Corydoradinae	Lineage 2	*Aspidoras*	*A. albater*	MA329	**0.76**	37.5	GU210313	GU210738	GU209893	GU209283
Corydoradinae	Lineage 2	*Aspidoras*	*A. depinnai*	MA307	**0.76**	37.5	GU210296	GU210721	GU209876	GU209285
Corydoradinae	Lineage 2	*Aspidoras*	*A. eurycephalus*	MA176	**0.76**	45	GU210180	GU210605	GU209761	GU209286
Corydoradinae	Lineage 2	*Aspidoras*	*A. microgaleus*	MA153	**0.76**	37.5	GU210163	GU210588	GU209744	GU209287
Corydoradinae	Lineage 2	*Aspidoras*	*A. poecilus*	LBP 1272-11098	**0.76**	42.5	GU210542	GU210967	GU210117	GU209288
Corydoradinae	Lineage 2	*Aspidoras*	*A. raimundi*	LBP 5568	**0.76**	42.5	GU210131	GU210556	GU209713	GU209290
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. 'C35 Black Phantom'	MA177	**0.76**	45	GU210181	GU210606	GU209762	GU209291
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus*	MA96-27708	**0.76**	42.5	GU210379	GU210804	GU209957	GU209284
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus*	LBP 1437-12304	**0.76**	42.5	GU210544	GU210969	GU210119	GU209294
Corydoradinae	Lineage 2	*Aspidoras*	*A. spilotus*	MA325	**0.76**	42.5	GU210309	GU210734	GU209889	GU209292
Corydoradinae	Lineage 2	*Aspidoras*	*A. taurus*	MA328	**0.76**	52.5	GU210312	GU210737	GU209892	GU209296
Corydoradinae	Lineage 3	*Scleromystax*	*S. barbatus*	LBP 2083-14430	0.94	97.5	GU210446	GU210871	GU210024	GU209689
Corydoradinae	Lineage 3	*Scleromystax*	*S. kronei*	LBP 2658-17418	**0.8**	82.5	GU210448	GU210873	GU210026	GU209691
Corydoradinae	Lineage 3	*Scleromystax*	*S. lacerdai*	LBP 1966-13705	**0.8**	52.5	GU210452	GU210877	GU210030	GU209695
Corydoradinae	Lineage 3	*Scleromystax*	*S. macropterus*	LBP 461-5642	0.82	67.5	GU210455	GU210880	GU210033	GU209698
Corydoradinae	Lineage 3	*Scleromystax*	*S. prionotus*	LBP 1267-11106	0.6	62.5	GU210457	GU210882	GU210035	GU209700
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'C113'	LBP 1237-11125	**0.8**	62.5	GU210459	GU210884	GU210037	GU209704
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'CW42'	MA112	**0.8**	67.5	GU210137	GU210562	GU209719	GU209705
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. *prionotus*	LBP 2575-15724	0.82	62.5	GU210460	GU210885	GU210038	GU209707

Corydoradinae	Lineage 4	*Corydoras*	*C.* cf. *hastatus*	MT104-13615	2.2	27	GU210389	GU210814	GU209967	GU209462
Corydoradinae	Lineage 4	*Corydoras*	*C. guapore*	MA73	**2.44**	42.5	GU210364	GU210789	GU209943	GU209453
Corydoradinae	Lineage 4	*Corydoras*	*C. hastatus*	LBP 1709-12815	2.2	27	GU210405	GU210830	GU209983	GU209461
Corydoradinae	Lineage 4	*Corydoras*	*C. pygmaeus*	MT51	2.68	27	GU210498	GU210923	GU210076	GU209538
Corydoradinae	Lineage 5	*Corydoras*	*C. bilineatus*	MA68	**2.02**	52.5	GU210359	GU210784	GU209938	GU209340
Corydoradinae	Lineage 5	*Corydoras*	*C. elegans*	MT23	2.24	52.5	GU210468	GU210893	GU210046	GU209428
Corydoradinae	Lineage 5	*Corydoras*	*C. gracilis*	MA301	**2.02**	37.5	GU210290	GU210715	GU209870	GU209451
Corydoradinae	Lineage 5	*Corydoras*	*C. nanus*	MHNG SU08-575	**2.02**	47.5	GU210270	GU210695	GU209850	GU209495
Corydoradinae	Lineage 5	*Corydoras*	*C. napoensis*	LBP 556-7227	1.96	47.5	GU210410	GU210835	GU209988	GU209499
Corydoradinae	Lineage 5	*Corydoras*	*C. nijsseni*	LBP 6861-32532	**2.02**	47.5	GU210190	GU210615	GU209771	GU209508
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *'A pauciradiatus'*	LBP 548-7187	1.86	27.5	GU210430	GU210855	GU210008	GU209594
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C123 yellow cat'	MA84	**2.02**	42.5	GU210373	GU210798	GU209951	GU209608
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C89'	MA82	**2.02**	45	GU210372	GU210797	GU209950	GU209637
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'CW18'	MA308	**2.02**	47.5	GU210297	GU210722	GU209877	GU209645
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. '*elegans* Columbia'	MA74	**2.02**	47.5	GU210365	GU210790	GU209944	GU209650
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. '*elegans* illuminator'	MA331	**2.02**	47.5	GU210315	GU210740	GU209895	GU209651
Corydoradinae	Lineage 5	*Corydoras*	*C. undulatus*	LBP 566-7386	2	52.5	GU210441	GU210866	GU210019	GU209672
Corydoradinae	Lineage 6	*Corydoras*	*C. albolineatus*	MA321	2.6	47.5	GU210305	GU210730	GU209885	GU209314
Corydoradinae	Lineage 6	*Corydoras*	*C.* cf. *paleatus* 'CW24'	MA61	4.12	67.5	GU210354	GU210779	GU209933	GU209394
Corydoradinae	Lineage 6	*Corydoras*	*C. diphyes*	MT21	**2.48**	47.5	GU210466	GU210891	GU210044	GU209420
Corydoradinae	Lineage 6	*Corydoras*	*C. ehrhardti*	LBP 741-8893	**2.48**	52.5	GU210400	GU210825	GU209978	GU209425
Corydoradinae	Lineage 6	*Corydoras*	*C. flaveolus*	MT115-12321	2.46	52.5	GU210401	GU210826	GU209979	GU209438
Corydoradinae	Lineage 6	*Corydoras*	*C. nattereri*	LBP 903-9697	1.79	62.5	GU210411	GU210836	GU209989	GU209501
Corydoradinae	Lineage 6	*Corydoras*	*C. paleatus*	LBP 567-7416	1.62	67.5	GU210414	GU210839	GU209992	GU209519
Corydoradinae	Lineage 6	*Corydoras*	*C. potaroensis*	MT48	**2.48**	42.5	GU210494	GU210919	GU210072	GU209624
Corydoradinae	Lineage 6	*Corydoras*	*C. reynoldsi*	MA335	**2.48**	47.5	GU210318	GU210743	GU209898	GU209545
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. 'C144'	MA86	**2.48**	27	GU210374	GU210799	GU209952	GU209614
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. *albolineatus*	LBP 1957-13560	**2.48**	47.5	GU210437	GU210862	GU210015	GU209592

Corydoradinae	Lineage 6	*Corydoras*	*C. tukano*	LBP 549-7195	**2.48**	42.5	GU210440	GU210865	GU210018	GU209670
Corydoradinae	Lineage 7	*Corydoras*	*C. aeneus*	MA144	4.4	70	GU210156	GU210581	GU209737	GU209310
Corydoradinae	Lineage 7	*Corydoras*	*C. eques*	MA318	**2.42**	52.5	GU210302	GU210727	GU209882	GU209436
Corydoradinae	Lineage 7	*Corydoras*	*C. melanotaenia*	MT35	1.64	57.5	GU210481	GU210906	GU210059	GU209486
Corydoradinae	Lineage 7	*Corydoras*	*C. rabauti*	MT54	2.05	52.5	GU210501	GU210926	GU210079	GU209541
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW10 Gold Laser'	MA42	2.15	57.5	GU210340	GU210765	GU209919	GU209578
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW9 Green Laser'	MA160	1.84	57.5	GU210170	GU210595	GU209751	GU209580
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'F Guyana'	MHNG 2666.037-GF03-097	4.12	52.5	GU210246	GU210671	GU209826	GU209576
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru orange'	MA59	**2.42**	62.5	GU210353	GU210778	GU209932	GU209586
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	LBP 1350-11447	3.3	52.5	GU210432	GU210857	GU210010	GU209581
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG 2671.014-SU05-575	**2.42**	52.5	GU210229	GU210654	GU209809	GU209587
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'venezuelanus'*	MA181	1.79	52.5	GU210185	GU210610	GU209766	GU209673
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	MA98-27845	2.39	57.5	GU210381	GU210806	GU209959	GU209480
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	MA204-32760	1.39	57.5	GU210207	GU210632	GU209787	GU209479
Corydoradinae	Lineage 7	*Corydoras*	*C. zygatus*	MA51	**2.42**	67.5	GU210347	GU210772	GU209926	GU209683
Corydoradinae	Lineage 8	*Corydoras*	*C. ambiacus*	MA46	1.97	62.5	GU210344	GU210769	GU209923	GU209320
Corydoradinae	Lineage 8	*Corydoras*	*C. britski*	LBP 688-8112	1.67	85	GU210546	GU210971	GU210121	GU209298
Corydoradinae	Lineage 8	*Corydoras*	*C. multiradiatus*	MA146	**2.16**	95	GU210157	GU210582	GU209738	GU209299
Corydoradinae	Lineage 8	*Corydoras*	*C. splendens*	LBP 2017-14216	**2.16**	77.5	GU210548	GU210973	GU210123	GU209301
Corydoradinae	Lineage 8	*Corydoras*	*C. agassizii*	MA334	2.05	67.5	GU210317	GU210742	GU209897	GU209311
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *leopardus* 'C102'	MA87	1.94	62.5	GU210375	GU210800	GU209953	GU209384
Corydoradinae	Lineage 8	*Corydoras*	*C. condisciplus*	MA53	**2.16**	62.5	GU210349	GU210774	GU209928	GU209405
Corydoradinae	Lineage 8	*Corydoras*	*C. crypticus*	MT17	**2.16**	62.5	GU210461	GU210886	GU210039	GU209411
Corydoradinae	Lineage 8	*Corydoras*	*C. delphax*	MT20	2.59	67.5	GU210465	GU210890	GU210043	GU209417
Corydoradinae	Lineage 8	*Corydoras*	*C. difluviatilis*	LBP 382-4608	1.94	47.5	GU210398	GU210823	GU209976	GU209418
Corydoradinae	Lineage 8	*Corydoras*	*C. ephippifer*	MA58	2.09	62.5	GU210352	GU210777	GU209931	GU209435
Corydoradinae	Lineage 8	*Corydoras*	*C. filamentosus*	MHNG 2707.015-SU07-625	**2.16**	47.5	GU210240	GU210665	GU209820	GU209437
Corydoradinae	Lineage 8	*Corydoras*	*C. garbei*	LBP 330-3920	**2.16**	47.5	GU210402	GU210827	GU209980	GU209442

Corydoradinae	Lineage 8	*Corydoras*	*C. geryi*	MA173	**2.16**	67.5	GU210177	GU210602	GU209758	GU209446
Corydoradinae	Lineage 8	*Corydoras*	*C. gomezi*	MT27	**2.16**	57.5	GU210472	GU210897	GU210050	GU209363
Corydoradinae	Lineage 8	*Corydoras*	*C. haraldshultzei*	MT31	2.42	82.5	GU210477	GU210902	GU210055	GU209458
Corydoradinae	Lineage 8	*Corydoras*	*C. imitator*	LBP 6862-32502	2.3	67.5	GU210188	GU210613	GU209769	GU209464
Corydoradinae	Lineage 8	*Corydoras*	*C. leopardus*	MA337	1.96	72.5	GU210320	GU210745	GU209900	GU209470
Corydoradinae	Lineage 8	*Corydoras*	*C. melanistius*	ANSP 180693-1460	2.28	55	GU210330	GU210755	GU209910	GU209484
Corydoradinae	Lineage 8	*Corydoras*	*C. ornatus*	MA64	**2.16**	67.5	GU210356	GU210781	GU209935	GU209513
Corydoradinae	Lineage 8	*Corydoras*	*C. pantanalensis*	LBP 691-8126	1.81	77.5	GU210416	GU210841	GU209994	GU209524
Corydoradinae	Lineage 8	*Corydoras*	*C. pulcher*	MT50	2.54	62.5	GU210497	GU210922	GU210075	GU209533
Corydoradinae	Lineage 8	*Corydoras*	*C. reticulatus*	LBP 553-7214	2.16	62.5	GU210420	GU210845	GU209998	GU209542
Corydoradinae	Lineage 8	*Corydoras*	*C. robinae*	MT57	2.27	67.5	GU210504	GU210929	GU210082	GU209548
Corydoradinae	Lineage 8	*Corydoras*	*C. robustus*	MA143	**2.16**	87.5	GU210155	GU210580	GU209736	GU209549
Corydoradinae	Lineage 8	*Corydoras*	*C. seussi*	MT60	**2.16**	67.5	GU210508	GU210933	GU210086	GU209559
Corydoradinae	Lineage 8	*Corydoras*	*C. sodalis*	LBP 530-7125	2.11	57.5	GU210429	GU210854	GU210007	GU209570
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C52'	MA78	**2.16**	72.5	GU210369	GU210794	GU209947	GU209629
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW13'	MA20	**2.16**	67.5	GU210202	GU210627	GU209782	GU209641
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C131 *leopardus'*	MA72	**2.16**	85	GU210363	GU210788	GU209942	GU209385
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C141 *pulcher'*	MA175	**2.16**	57.5	GU210179	GU210604	GU209760	GU209396
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C49 false *robustus'*	MT70	**2.16**	62.5	GU210519	GU210944	GU210094	GU209628
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C57 nordestini'	MA184-32501	**2.16**	55	GU210187	GU210612	GU209768	GU209631
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C66 *similis'*	MA56	**2.16**	60	GU210350	GU210775	GU209929	GU209632
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C122	LBP 7214-32927	**2.16**	67.5	GU210301	GU210726	GU209881	GU209634
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW6 *narcissus'*	MT73	2.68	57.5	GU210522	GU210947	GU210097	GU209648
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C159'	LBP 7711-32652	**2.16**	67.5	GU210197	GU210622	GU209778	GU209655
Corydoradinae	Lineage 8	*Corydoras*	*C. spilurus*	MA76	**2.16**	52.5	GU210367	GU210792	GU209946	GU209652
Corydoradinae	Lineage 8	*Corydoras*	*C. virginae*	MT83	**2.16**	57.5	GU210533	GU210958	GU210108	GU209676
Corydoradinae	Lineage 9	*Corydoras*	*C. acrensis*	MA179	**3.42**	52.5	GU210183	GU210608	GU209764	GU209302
Corydoradinae	Lineage 9	*Corydoras*	*C. adolfoi*	LBP 6863-32527	3.77	57.5	GU210189	GU210614	GU209770	GU209305

Corydoradinae	Lineage 9	*Corydoras*	*C. araguaiaensis*	MA94-27706	4.36	57.5	GU210377	GU210802	GU209955	GU209324
Corydoradinae	Lineage 9	*Corydoras*	*C. arcuatus*	MT3	2.28	52.5	GU210475	GU210900	GU210053	GU209325
Corydoradinae	Lineage 9	*Corydoras*	*C. armatus*	MA111	4.3	45	GU210136	GU210561	GU209718	GU209329
Corydoradinae	Lineage 9	*Corydoras*	*C. atropersonatus*	MA303	**3.42**	47.5	GU210292	GU210717	GU209872	GU209330
Corydoradinae	Lineage 9	*Corydoras*	*C. axelrodi*	MA43	3.2	47.5	GU210341	GU210766	GU209920	GU209334
Corydoradinae	Lineage 9	*Corydoras*	*C. bicolor*	MHNG 2651.078-GY04-424	**3.42**	47.5	GU210245	GU210670	GU209825	GU209339
Corydoradinae	Lineage 9	*Corydoras*	*C. boesemani*	MHNG 2673.074-SU05-129	**3.42**	47.5	GU210223	GU210648	GU209803	GU209344
Corydoradinae	Lineage 9	*Corydoras*	*C. bondi*	MHNG 2651.040-GY04-123	2.65	45	GU210244	GU210669	GU209824	GU209346
Corydoradinae	Lineage 9	*Corydoras*	*C. breei*	MHNG SU08-191	**3.42**	42.5	GU210266	GU210691	GU209846	GU209349
Corydoradinae	Lineage 9	*Corydoras*	*C. brevirostris*	MT7	3.28	57.5	GU210518	GU210943	GU210093	GU209352
Corydoradinae	Lineage 9	*Corydoras*	*C. burgessi*	LBP 6867-32741	**3.42**	52.5	GU210205	GU210630	GU209785	GU209353
Corydoradinae	Lineage 9	*Corydoras*	*C. caudimaculatus*	LBP 562-7255	**3.42**	47.5	GU210385	GU210810	GU209963	GU209357
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *araguaiaensis* 'C65'	MA57	2.64	57.5	GU210351	GU210776	GU209930	GU209365
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *bondi*	MA165	**3.42**	45	GU210172	GU210597	GU209753	GU209369
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *concolor*	LBP 2306-15843	**3.42**	57.5	GU210387	GU210812	GU209965	GU209371
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *davidsandsi*	MA319	**3.42**	57.5	GU210303	GU210728	GU209883	GU209374
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *guianensis*	LBP 5395-27091	**3.42**	47.5	GU210128	GU210553	GU209710	GU209382
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *punctatus*	MA350	**3.42**	47.5	GU210334	GU210759	GU209914	GU209469
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *sipalwini*	MHNG 2671.094-SU05-427	**3.42**	47.5	GU210224	GU210649	GU209804	GU209398
Corydoradinae	Lineage 9	*Corydoras*	*C. concolor*	MT12	**3.42**	57.5	GU210406	GU210831	GU209984	GU209402
Corydoradinae	Lineage 9	*Corydoras*	*C. copei*	MT13	**3.42**	47.5	GU210417	GU210842	GU209995	GU209575
Corydoradinae	Lineage 9	*Corydoras*	*C. coppenamensis*	MHNG 2690.017-SU01-466	**3.42**	47.5	GU210235	GU210660	GU209815	GU209407
Corydoradinae	Lineage 9	*Corydoras*	*C. cruziensis*	MA152	**3.42**	45	GU210162	GU210587	GU209743	GU209643
Corydoradinae	Lineage 9	*Corydoras*	*C. davidsandsi*	LBP 551-7203	3.57	57.5	GU210396	GU210821	GU209974	GU209414
Corydoradinae	Lineage 9	*Corydoras*	*C. duplicareus*	MA167	3.62	52.5	GU210174	GU210599	GU209755	GU209423
Corydoradinae	Lineage 9	*Corydoras*	*C. gossei*	MT29	**3.42**	57.5	GU210474	GU210899	GU210052	GU209448
Corydoradinae	Lineage 9	*Corydoras*	*C. griseus*	MA71	**3.42**	47.5	GU210362	GU210787	GU209941	GU209635
Corydoradinae	Lineage 9	*Corydoras*	*C. guianensis*	MHNG 2683.055-GF06-574	**3.42**	47.5	GU210247	GU210672	GU209827	GU209454

Corydoradinae	Lineage 9	*Corydoras*	*C. habrosus*	MA142	2.53	32	GU210154	GU210579	GU209735	GU209457
Corydoradinae	Lineage 9	*Corydoras*	*C. julii*	MA147	4.2	52.5	GU210158	GU210583	GU209739	GU209468
Corydoradinae	Lineage 9	*Corydoras*	*C. kanei*	MA349	3.17	52.5	GU210332	GU210757	GU209912	GU209654
Corydoradinae	Lineage 9	*Corydoras*	*C. leucomelas*	MA122	**3.42**	52.5	GU210144	GU210569	GU209725	GU209471
Corydoradinae	Lineage 9	*Corydoras*	*C. loretoensis*	MA298	4.3	47.5	GU210286	GU210711	GU209866	GU209474
Corydoradinae	Lineage 9	*Corydoras*	*C. loxozonus*	MA351	**3.42**	52.5	GU210335	GU210760	-	GU209476
Corydoradinae	Lineage 9	*Corydoras*	*C. melini*	MA77	2.98	42.5	GU210368	GU210793	-	GU209489
Corydoradinae	Lineage 9	*Corydoras*	*C. metae*	MT38	4.15	47.5	GU210484	GU210909	GU210062	GU209493
Corydoradinae	Lineage 9	*Corydoras*	*C. multimaculatus*	MA302	**3.42**	37.5	GU210291	GU210716	GU209871	GU209494
Corydoradinae	Lineage 9	*Corydoras*	*C. oiapoquensis*	MHNG 2682.023-GF06-186	4.5	45	GU210260	GU210685	GU209840	GU209511
Corydoradinae	Lineage 9	*Corydoras*	*C. osteocarus*	ANSP 185052-1477	**3.42**	44	GU210323	GU210748	GU209903	GU209515
Corydoradinae	Lineage 9	*Corydoras*	*C. panda*	MT41	2.96	47.5	GU210487	GU210912	GU210065	GU209521
Corydoradinae	Lineage 9	*Corydoras*	*C. paragua*	MA67	**3.42**	37.5	GU210358	GU210783	GU209937	GU209526
Corydoradinae	Lineage 9	*Corydoras*	*C. polystictus*	MT45	**3.42**	47.5	GU210491	GU210916	GU210069	GU209531
Corydoradinae	Lineage 9	*Corydoras*	*C. punctatus*	MHNG SU08-110	2.9	47.5	GU210262	GU210687	GU209842	GU209535
Corydoradinae	Lineage 9	*Corydoras*	*C. schwartzi*	LBP 1783-7120	**3.42**	57.5	GU210421	GU210846	GU209999	GU209551
Corydoradinae	Lineage 9	*Corydoras*	*C. similis*	LBP 547-7184	3.39	57.5	GU210424	GU210849	GU210002	GU209562
Corydoradinae	Lineage 9	*Corydoras*	*C. sipaliwini*	MHNG 2707.017-SU07-287	**3.42**	47.5	GU210241	GU210666	GU209821	GU209567
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *'arcuatus* super'	MT65	**3.42**	57.5	GU210513	GU210938	GU210088	GU209600
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C121 *burgessi'*	MA178	**3.42**	57.5	GU210182	GU210607	GU209763	GU209607
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C129'	MA149	**3.42**	52.5	GU210159	GU210584	GU209740	GU209609
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C133 ornatus short snout'	MT68	**3.42**	42.5	GU210516	GU210941	GU210091	GU209617
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C136'	MA305	**3.42**	52.5	GU210294	GU210719	GU209874	GU209644
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C139 *oiapoquensis'*	MA174	**3.42**	45	GU210178	GU210603	GU209759	GU209390
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C150 mazurani'	MA70	**3.42**	47.5	GU210361	GU210786	GU209940	GU209615
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C30'	MA215	**3.42**	52.5	GU210215	GU210640	GU209795	GU209618
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C43'	MA80	4.8	52.5	GU210371	GU210796	GU209949	GU209626
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C91 Peru *bondi'*	MA300	2.78	52.5	GU210289	GU210714	GU209869	GU209638

Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW21 *axelrodi*'	MA320	**3.42**	47.5	GU210304	GU210729	GU209884	GU209642
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *arcuatus* 'Rio Negro'	LBP 7709-32609	**3.42**	52.5	GU210196	GU210621	GU209777	GU209599
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *armatus* 'Green cana'	MA330	**3.42**	52.5	GU210314	GU210739	GU209894	GU209602
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *breei*	MHNG SU08-583	**3.42**	42.5	GU210278	GU210703	GU209858	GU209603
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C84'	MA197-32671	**3.42**	47.5	GU210199	GU210624	-	GU209490
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW28'	MA110	**3.42**	62.5	GU210135	GU210560	GU209717	GU209612
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *davidsandsi*	MA134	**3.42**	57.5	GU210152	GU210577	GU209733	GU209649
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *melini*	MA333	**3.42**	42.5	GU210316	GU210741	GU209896	GU209636
Corydoradinae	Lineage 9	*Corydoras*	*C. sterbai*	MT75	3.16	62.5	GU210524	GU210949	GU210099	GU209662
Corydoradinae	Lineage 9	*Corydoras*	*C. trilineatus*	MT80	3.4	52.5	GU210530	GU210955	GU210105	GU209667
Corydoradinae	Lineage 9	*Corydoras*	*C. weitzmani*	MA35	2.56	52.5	GU210333	GU210758	GU209913	GU209680

Species names of undescribed taxa have been labelled based on their respective C-Numbers (Datz) and CorydorasWorld numbers when available, otherwise the trade name alias or 'sp' was used. C-values in bold are mean estimates per lineage in cases where samples were not available for direct measurement.

Chapter 4: Historical biogeography, paleoclimate and present distribution of Neotropical armoured catfishes (Siluriformes: Corydoradinae)

4.1 Abstract:

Neotropical freshwater fish comprise the most species rich assemblage of vertebrates on the planet. Corydoradinae catfishes are widely distributed throughout the Neotropics, making them ideal candidates for an investigation of historical biogeography. Here I use a time calibrated molecular phylogeny to test biogeographic scenarios using the dispersal-extinction-cladogeneis (DEC) model. I compare cladogenetic events with the paleoclimatic record to infer whether speciation and climatic fluctuation events coincide. Furthermore, I assigned species diversity into different ecoregions in order to identify current basins with high levels of endemism, and discuss conservation implications. Historical biogeographic analyses support the Taxon Pulse, Paleogeography/Hydrogeology and Phylogenetic Niche Conservatism hypotheses. The majority of cladogenetic events occurred within basins, rather than as a result of large-scale vicariant events, such as the separations of the paleo-basins. Although climatic fluctuations of the Oligocene and Miocene seem to coincide with the time period of major cladogenetic events, there is no apparent causal link between climatic change and speciation as the evidence is anecdotal. Finally, I identified multiple major ecoregions with high levels of species richness and genetic diversity throughout the known range of the Corydoradinae that are currently under threat from anthropogenic disturbance.

4.2 Introduction:

The exceptional diversity of Neotropical freshwater fishes has been estimated to exceed 8,000 species (Schaefer, 1998), making it one of the most diverse assemblages of vertebrates on the planet. Moreover, new species are being discovered and described almost daily from poorly sampled river basins, suggesting current estimates of species diversity are gross underestimates of the true diversity. This extraordinary diversity has accumulated over vast periods of time, as South America's plate tectonic setting was established in the Early Cretaceous, following the separation from Africa and the birth of the South Atlantic (Lundberg et al., 1998). The subsequent uplift of the Andes, resulting from continental compression, which occurred in several phases throughout its 90MY history and has had dramatic effects on the geography and hydrology of the continent (Lundberg et al., 1998). Modern patterns of Neotropical fish distribution have been shaped as a consequence of the Andean uplift, and foreland basin subsistence that formed an extensive network of lacustrine habitats (Lundberg et al., 1998). Despite fossil evidence suggesting that generic-level lineages were already differentiated by the Paleogene, subsequent separation of basins within the Neogene (such as the Parana, Amazon, Orinoco, Guyanas, Magdalena, Sao Francisco, and East Coastal Brazil) has led to significant allopatric isolation of the modern Neotropical freshwater ichthyofauna and contributed greatly to their diversification (Lundberg et al., 1998). However, current patterns of fish distribution within the Neotropical region, areas of endemism, and the historical processes that have shaped their diversity remain understudied (Hubert and Renno, 2006). Furthermore, there is still much debate concerning the timing and importance of various paleogeographic, hydrological and climatic processes on the Neotropical fauna (Hubert and Renno, 2006).

To date, a number of hypotheses have been proposed to account for the current distributions and patterns of diversity of Neotropical fishes and other vertebrates (Table 7). Many of these hypotheses are being tested using phylogenetic comparative frameworks and relaxed molecular clock dating which incorporates the fossil record and vicariant events (Drummond and Rambaut, 2007; Lemey et al., 2009; Magallon and Sanderson, 2001; Sanderson, 2003). Several models have been developed that allow rates of dispersal and local extinction to be estimated, while also applying predetermined constraints. Such approaches have refined the Dispersal-Vicariance

model (Ronquist, 1997), leading to the Dispersal-Extinction-Cladogenesis model, which allows explicit tests of biogeographic scenarios using maximum likelihood methods (Kodandaramaiah, 2010; Ree and Smith, 2008). Despite the progress achieved using these new methods, many caveats remain, and interspecific tests of historical biogeography still lack the statistical rigor currently employed in intraspecific studies.

The processes governing historical biogeographic patterns are highly complex and have occurred over long time scales, therefore multidisciplinary approaches combining phylogenetics with paleoclimatic records are important to better understand the spatiotemporal distribution of Neotropical fishes. Paleoclimatic events clearly play significant roles in many of the biogeographic hypotheses detailed above (Table 7). Notably, Pleistocene glacial cycles and marine incursions resulting from sea level fluctuations during the Miocene and Pleistocene are of direct relevance to a number of hypotheses. However, specific tests that investigate the role of paleoclimatic fluctuations and that include paleoclimatic data (such as paleo-isotope records, Foraminifera and stromatolite deposits) have thus far not been conducted. In addition to multidisciplinary approaches incorporating historical datasets (paleoclimate, phylogenetics, geology, paleontology and hydrology), a firm understanding of current patterns of distribution is essential for the study of historical biogeography. Quantifying present distributions of species requires a combination taxonomic data with detailed information on known occurrences (based on type localities, museum collections and scientific expeditions). Despite the fact that such records are difficult to assemble and are patchy, they are necessary in order to analyze historical processes. This is because the more detail we have about current distribution and diversity, the better we can document and reconstruct ancestral dispersal events. Moreover, as anthropogenic disturbance of Neotropical freshwater habitats continues to intensify, the conservation and management of existing ecosystems and their biota is becoming a priority (Abell et al., 2008).

In this Chapter, I focus on the Corydoradinae subfamily of armoured catfishes (Siluriformes; Callichthyidae), as they are highly diverse and very widely distributed throughout Neotropics, have dated fossils (*Corydoras revelatus*) and occur in environments where vicariant events suitable for testing biogeographic hypotheses have occurred. Here I use a time-calibrated molecular phylogeny for the subfamily

Corydoradinae to reconstruct the ancestral distribution of the group and test specific hypotheses concerning their spatiotemporal distribution. I assess the role of paleoclimate change, and how this may have affected diversification and historical distribution. I also separate all known species into currently defined ecoregions and discuss present distributions and implications for conservation. Using these data, I consider our results in the light of currently proposed biogeographic hypotheses for the diversification of neotropical fishes.

Table 7- Biogeographic Hypotheses

Hypothesis	Cause	Effect	Epoch	Habitat	Citations
Paleogeography & Hydrogeology	Geomorphology changes river courses and captures	Allopatric speciation between species occupying different basins	Tertiary	Terrestrial; Freshwater	(Lundberg et al., 1998; Montoya-Burgos, 2003)
Museum	Marine incursions	Vicariance and basal trichotomy	Miocene	Terrestrial; Freshwater	(Antonelli et al., 2010; Hoorn, 1993)
River	River width	Different species on opposite sides of the river bank	Late Miocene	Terrestrial; Freshwater	(Hall and Harvey, 2002; Wallace, 1852)
Refuge	Climatic fluctuations	Species accumulation in isolated refugia	Cenozoic (Pleistocene)	Terrestrial	(Haffer, 1969)
River-Refuge	Climatic fluctuations	Refuges enhancing allopatric speciation across rivers	Post-Miocene	Terrestrial	(Ayres and Cluttonbrock, 1992; Haffer, 1997)
Sea Level Fluctuations	Climatic fluctuations	Enhanced dispersal during low sea level, isolation populations during high sea level	Pleistocene	Terrestrial; Freshwater	(Bermingham and Martin, 1998; Cardoso and Montoya-Burgos, 2009)
Taxon Pulses	Barrier formation and breakdown	Vicariance and range expansion centered on stable core region	N/A	Terrestrial	(Erwin, 1979; Halas et al., 2005)
Disturbance – Vicariance	Climatic fluctuations and forest/riverine heterogeneity	Interspecific competition and species isolation due to invasions	Pleistocene	Terrestrial	(Colinvaux, 1993)
Gradient	Steep environmental gradients	Parapatric speciation	N/A	Terrestrial; Freshwater	(Endler, 1982a)
Phylogenetic Niche Conservatism	Clades retain niches and ecological traits over time	Expansion to areas with suitable niches; local extinction from maladaptation	N/A	Terrestrial; Freshwater	(Wiens, 2004)

4.3 Methods:

4.3.1 Taxon Sampling & Phylogenetic Analysis

Briefly, a time-calibrated phylogeny was constructed using a relaxed molecular clock and multiple fossil and paleogeographic calibration points (See Chapters 2 and 3 Methods for detailed description of phylogenetic methods). The phylogeny consisted of 206 taxa (including 3 Callichthyinae outgroups) and 2664 bps of mitochondrial DNA (Alexandrou et al., 2011). Taxa included in the analysis, along with their distributions and genbank IDs are all listed as supplementary material (SI Table 3). To investigate past and present distributions of species I used several different methods.

4.3.2 Biogeographic Analyses: Past and Present

Data on the distribution of described Corydoradinae species was assembled from primary taxonomic literature (Ferraris, 2007; Isbrucker, 2001), and for undescribed species based on collection sites and personal field observations. Species were then assigned to eight different paleobiogeographic regions defined in previous studies on Neotropical fishes (Vari, 1988), with the addition of the North-Eastern Brazilian basin. I excluded the Uruguay basin, as no species in the current study were endemic to that area.

Firstly to investigate the distributions of extant species I grouped all species into Ecoregions. Ecoregions are defined as a large area encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species (Abell et al., 2008; Dinerstein et al., 1995; Groves et al., 2002). Ecoregions that encompass areas with similar ichthyofauna have previously been defined for Neotropical freshwater fish based on the distributions and composition of freshwater endemic species (Abell et al., 2008). I grouped all species of Corydoradinae (both described and undescribed) with known distributions and/or type localities by river basin. We then mapped the species diversity per ecoregion, and compared the number of species belonging to different genetic lineages in each basin. In contrast with the analysis of paleo-biogeographic regions, grouping species into ecoregions allows a fine scale examination of the present distribution of the Corydoradinae. The results of this investigation allow inference of current Corydoradinae biodiversity hotspots (in

terms of species richness and genetic diversity) with implications for management and conservation.

In order to reconstruct ancestral distributions and geographic ranges I used a model-based inference method as implemented in Lagrange v 2.0 (Ree et al., 2005; Ree and Smith, 2008). I chose this method as opposed to area cladogram approaches because it allows the user to directly test a number of biogeographic scenarios using a likelihood framework. This technique is based on the dispersal-extinction-cladogenesis (DEC) model, which simultaneously specifies transition rates between discrete states (coded as different biogeographic areas) and estimates likelihoods of ancestral states (in this case range inheritance scenarios) across the internal nodes of a phylogeny. I applied two different DEC models to estimate distribution ranges and associated likelihood values for the whole phylogeny (all Corydoradinae), one unconstrained (M0) and one constrained (M1). The M0 model allows for dispersal between all ancestral distribution ranges, whereas the M1 model constrains dispersal between non-adjacent ranges (separated by one intercalated area) to 1/10 the rate of adjacent ranges, and excludes the possibility of dispersal between ranges separated by two or more intercalated areas (Chiachio et al., 2008). I then performed the same tests on each of the nine genetic lineages, to estimate dispersal and extinction rates for each lineage independently. Dates used to infer particular cladogenetic events were based on the 95% highest posterior density obtained from the BEAST analysis (Drummond and Rambaut, 2007).

4.3.3 Paleoclimate and Diversification

To investigate the role of climate fluctuations on distribution I used previously a published dataset of δ^{18} oxygen ($\delta^{18}O$) isotope records as a proxy for historical temperature change (Zachos et al., 2001; Zachos et al., 2008). These data span a time period of approximately 65 MY and have been repeatedly used to infer the effect of extrinsic factors (historical temperature fluctuations) on the evolution of a variety of different organisms (Hardman and Hardman, 2008; Harzhauser et al., 2007; Mittelbach et al., 2007). I extracted speciation times from the time calibrated phylogeny using the R package Ape (Paradis et al., 2004). Speciation frequency and $\delta^{18}O$ values were plotted as a function of time on the same graph, to infer whether major paleoclimatic events coincided with cladogenetic events within and/or between

lineages. As direct correlations between $\delta^{18}O$ data and speciation frequency may yield relationships that are too general for a causal link to be established, the purpose of the comparison between paleoclimate and speciation in this instance was to infer whether climate change may have influenced shifts in diversification (See Chapter 3 Methods for diversification rate shifts). This allowed us to assess the general role of paleoclimate throughout the history of diversification of the Corydoradinae, but also to check whether speciation was highest in frequency during specific epochs and time periods of climatic fluctuation. Furthermore, by plotting speciation frequency against time, I was able to check whether cladogenetic events were concentrated in the Pleistocene, thereby allowing us to accept or reject the Refuge, Sea Level Fluctuations and Disturbance- Vicariance hypotheses (as speciation in the latter cases is expected to be occurring primarily during the Pleistocene).

4.4 Results:

4.4.1 Phylogenetics & Dispersal-Extinction-Cladogenesis

See (Alexandrou et al., 2011) & Chapter 3 for full results of phylogenetics and the relaxed molecular clock analyses.

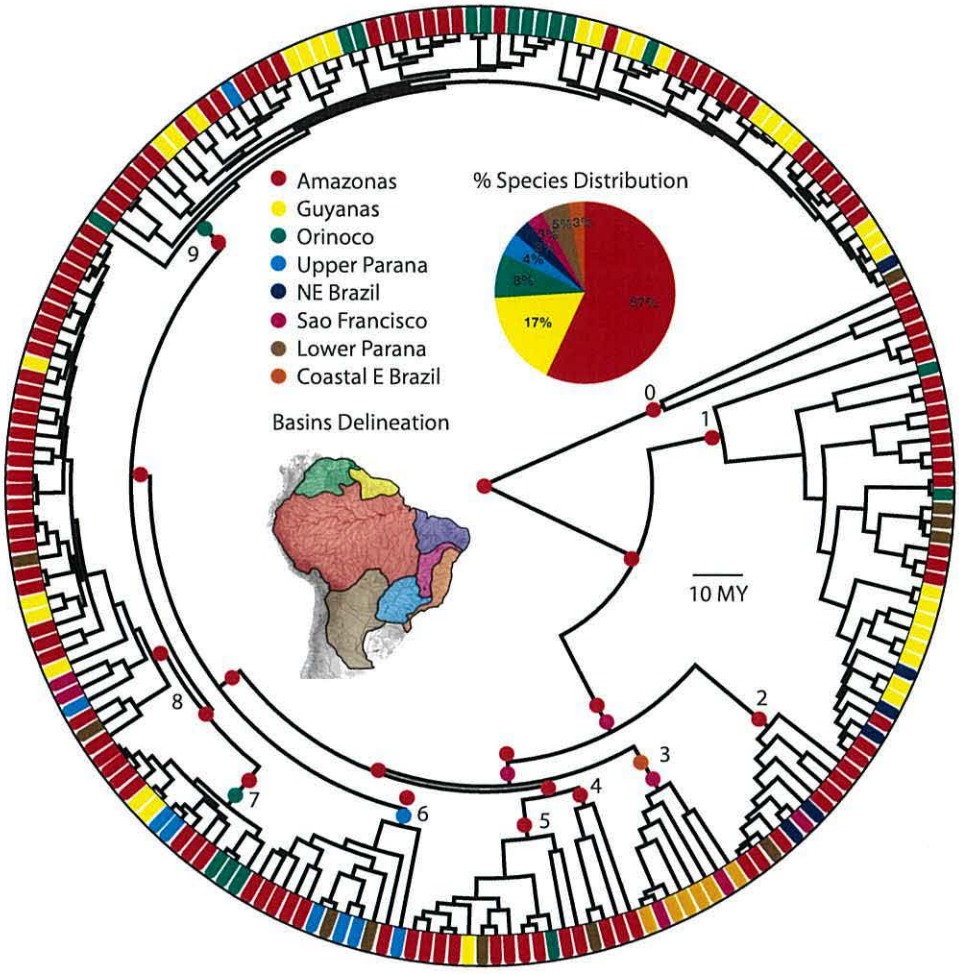
The M1 model performed marginally better than M0, for the global analyses of all Corydoradinae using the DEC model. Under the constrained scenario, dispersal and extinction values were also higher (0.006343 and 0.005726 respectively). When analyzing each lineage as a separate dataset, the M1 model performed better for lineages 2, 3, 4, 5 and 7 while the M0 model performed best for lineages 1, 6, 8, and 9 (Table 8). Differences in likelihood scores between M0 and M1 were relatively small, while differences in dispersal and extinction values under the different models were much greater. Thus, for the whole phylogeny, constraining dispersal under the M1 model fits the dataset better than an unconstrained scenario of dispersal. I relied on results from the M1 model as it provided a better fit to our data, and mapped the ancestral range reconstruction from the latter model onto our phylogenetic tree (Figure 14). The most likely ancestral range for all Corydoradinae was reconstructed as the Amazon basin, which was also identified as the most likely ancestral range for all Callichthyidae (Callichthyinae + Corydoradinae). Our results show that the majority of cladogenetic events are occurring within basins (86%), as opposed to between basins due to vicariance (14%). Moreover, 54% of the total cladogenesis occurred within the Amazon basin alone, while 14% occurred in the Guyanas.

Table 8- Lagrange DEC values

	M0 - Unconstrained			M1 - Constrained		
	LH	Dispersal	Extinction	LH	Dispersal	Extinction
All	-315.6	0.003859	0.002383	**-315**	**0.006343**	**0.005726**
Lineage 1	**-41.89**	**0.004735**	**0.0001754**	-46.51	0.00737	0.006825
Lineages 2 & 3	-35.64	0.006188	3.14E-09	**-33.84**	**0.01204**	**2.34E-09**
Lineages 4 & 5	-22.79	0.007903	4.29E-09	**-22.64**	**0.008243**	**4.29E-09**
Lineage 6	-15.1	0.008419	4.29E-09	-15.1	0.008419	4.29E-09
Lineage 7	-13.99	0.01256	4.29E-09	**-13.59**	**0.01539**	**4.29E-09**
Lineage 8	**-42.34**	**0.008203**	**0.01232**	-42.67	0.0102	0.01334
Lineage 9	**-97.72**	**0.007086**	**0.003747**	-99.9	0.01066	0.01019

Global and lineage specific likelihood, dispersal and extinction values under constrained and unconstrained scenarios.

Figure 14- Ancestral Reconstruction and Dispersal



Results from the Lagrange analysis of ancestral dispersal events. The pie chart shows the percentage of species distributed in different paleobasins. Lineages are denoted with a number next to the most recent common ancestral node for each.

Within lineage 1, dispersal events occurred from the Amazonas basin to all other adjacent basins with the exception of the Upper Parana and Sao Francisco. In particular, two vicariant events between the Amazonas and Orinoco basins occurred during very different time periods: 19.4MYA for *C. simulatus* (Orinoco) + *C. maculifer* (Amazonas) and 0.7MYA for *C. septentrionalis* (Orinoco) + *C. stenocephalus* (Amazonas). This result suggests that dispersal may be an ongoing process between the Amazonas and Orinoco basins, potentially through the Casiquiare river corridor (Winemiller et al., 2008). A dispersal event also occurred from the Amazonas to the Lower Parana basin, and the vicariant event is estimated to have occurred 11.3MYA: *C. negro*, *C. ellisae*, *C. aurofrenatus* (Lower Parana) and *C. cervinus*, *C.* sp. C115, *C. acutus* (Amazonas). Furthermore, a dispersal event occurred from the Guyanas to NE Brazil 8.9MYA, and from the Amazonas to North East Brazil 3MYA.

The ancestral range for lineage 2 (genus: *Aspidoras*) was also reconstructed as Amazonas, with species subsequently dispersing into the NE Brazil, Sao Francisco and Lower Parana basins. Vicariant events were reconstructed between Amazonas and NE Brazil 8MYA, Sao Francisco and NE Brazil 2.2MYA, and between Amazonas and Lower Parana 1.7MYA.

The ancestral reconstruction for lineage 3 (genus: *Scleromystax*) supports dispersal from the East coast of Brazil to the Sao Francisco, with a dominant presence in the former basin. Two vicariant events between the East Coast of Brazil and Sao Francisco basins occurred at 13.6MYA and 1.4MYA, respectively. This is the only lineage with such high levels of endemicity along the East Coast of Brazil and remarkably no known representatives in the Amazonian region, thereby making it the most geographically restricted Corydoradinae lineage. These results support the hypothesis that dispersing out of the East Coast of Brazil is a difficult process and rarely occurs with non-adjacent basins (Chiachio et al., 2008). Considering that most internal nodes of the Corydoradinae phylogeny indicate Amazonian origins, it is most likely that the common ancestor of *Scleromystax* became isolated in coastal regions between 30 - 35MYA, as evidenced by relaxed clock estimates. Ancestral range reconstructions for the most recent common ancestor of *Aspidoras* and *Scleromystax* suggest that dispersal events occurred from the Amazon towards Sao Francisco, with subsequent isolation of *Scleromystax* species within the East Coast of Brazil.

Furthermore, evidence suggests that once species are isolated in the East Coast of Brazil, they remain restricted and can only disperse between the latter basin and Sao Francisco.

Results for lineages 4 and 5 support an ancestral range in the Amazonas basin, followed by dispersal to the Lower Parana, Orinoco and Guyanas basins. A vicariant event occurred 2.5MYA between two species in lineage 4: *C. hastatus* (Lower Parana) + *C.* cf. *hastatus* (Amazonas). Within lineage 5 most species are presently distributed in the Amazonas basin, with a few notable vicariant events occurring between *C.* sp. 'elegans Columbia' (Orinoco) + *C.* sp. C89 (Amazonas) 3.5MYA, and *C. bilineatus* (Amazonas) + *C. undulatus* (Lower Parana) 1.9MYA. Moreover, dispersal occurred between the Amazonas towards the Guyanas basin 6.6MYA, as evidenced by the presence of *C. nanus* within the Guyanas.

The reconstruction for the ancestral range of lineage 6 supports ancient dispersal from Amazonas into the Upper Parana 21MYA, while some species also dispersed more recently from the Upper Parana to the Lower Parana basin. A notable vicariant event separating species inhabiting the Amazonas (*C. reynoldsi*, *C. tukano*, *C.* sp. C40, *C.* sp. *albolineatus*, *C. albolineatus*) and Parana basins (*C. diphyes*, *C. ehrhardti*, *C. nattereri*, *C.* cf. *paleatus* CW24, *C. paleatus*) occurred 12.6MYA, while dispersal between the Upper and Lower Parana basins seems to be an ongoing process. Multiple dispersal events were reconstructed for lineage 7, with an initial ancestral migration from Amazonas into the Orinoco basin 8.2MYA (with three ancestral species currently distributed in the Orinoco: *C. melanotaenia*, *C. aeneus*, and *C.* sp. *'aeneus venezuelanus'*), followed by subsequent dispersal throughout Amazonas, Upper Parana and Guyanas basins. A vicariant event between *C. eques* (Amazonas) and *C.* sp. *'aeneus macrosteus'* (Upper Parana) occurred 2.7MYA, while *C.* sp. *aeneus* 'French Guyana' and *C.* sp. *aeneus* 'Suriname' from the Guyanas basin were separated from their closest Amazonian ancestor (*C.* sp. *aeneus* 'Peru orange') 4.6MYA.

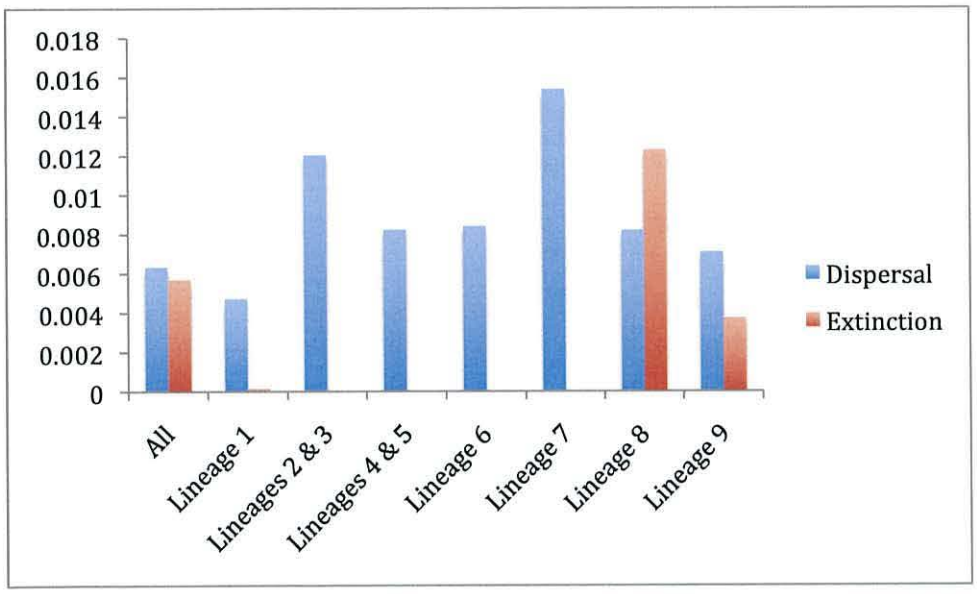
The majority of species belonging to lineage 8 occur in the Amazonas basin, and as such the ancestral reconstruction supports common ancestry in the latter basin. Species within lineage 8 have dispersed into adjacent basins of Upper and Lower Parana, Sao Francisco and the Guyanas, and while some species are known to occur

in the Orinoco, none were available for the current study. Vicariant events occurred between *C. britski* (Lower Parana) + *C. multiradiatus* (Amazonas) 5.8MYA, *C.* sp. C131 *'leopardus'* (Amazonas) + *C. spilurus*, *C. condisciplus* (Guyanas) 6.3MYA, *C. haraldschultzi* (Amazonas) + *C. melanistius* (Guyanas) 2.5MYA, and *C. garbei*, *C.* sp. C57 'nordestini' (Sao Francisco) + *C. difluviatilis* (Upper Parana) 10.5MYA. However, most of the cladogenesis within lineage 8 seems to have occurred within the Amazonas basin rather than as a result of allopatric isolation into adjacent basins.

Lineage 9 is by far the most species rich group of the Corydoradinae, widely distributed across all basins with the exception of the East coast of Brazil and Sao Francisco. An initial dispersal event was reconstructed for the common ancestor supporting movement from Amazonas towards the Orinoco basin 13.6MYA. Notable vicariant events within lineage 9 occurred between: C. sp. *arcuatus* 'Rio Negro' (Amazonas) + *C. bicolor*, *C. brevirostris*, *C.* sp. C30 (Guyanas) 1.5MYA; *C. similis* (Amazonas) + *C. polystictus* (Upper Parana) 4.1MYA; *C.* sp. *davidsandsi*, *C. adolfoi*, *C. duplicareus*, *C. burgessi*, *C.* cf. *davidsandsi*, *C. davidsandsi*, *C.* sp. C121 (Amazonas) + *C.* cf. *concolor*, *C. concolor* (Orinoco) 7.7MYA; *C. guianensis* (Guyanas) + *C.* cf. *guianensis* (Amazonas) 3.5MYA; *C.* cf. *bondi* (Orinoco) + *C.* sp. C150 'mazurani' (Guyanas) 0.8MYA; *C. julii* (NE Brazil) + *C. paragua* (Lower Parana) 1.7MYA. Multiple dispersal events within lineage 9 occurred in the Northern River systems from the Amazonas towards the Orinoco basin 7.7MYA and 6.1MYA, from the Orinoco to the Guyanas 7.8MYA, and from the Amazonas to the Guyanas 3.7MYA and 5.1MYA. In some cases the Orinoco basin seems to have served as a stepping-stone between the Amazonas and Guyanas, whereas in others dispersal occurred directly between Amazonas and Guyanas. Some of these patterns may be confounded by taxonomic coverage, ancestors occupying the Orinoco may have gone locally extinct, or reduced sampling effort in the Orinoco as compared to Guyanas and Amazonas. Despite widespread dispersal, species from lineage 9 are entirely absent from East coastal Brazil and Sao Francisco, while there are only two occurrences in the Parana basins (Upper and Lower respectively) and one occurrence in NE Brazil. Thereby the majority of diversity is concentrated in the Amazonas, Orinoco and Guyana basins respectively, with multiple dispersal events occurring between these adjacent areas.

For most lineages (2-5 and 7), when tested independently, likelihood scores were higher under the constrained model than the unconstrained. However, the most diverse lineages (1, 8 and 9) performed better under the unconstrained model, while lineage 6 performed equally under both models. Overall, lineage 7 had the highest constrained dispersal value (0.01539), while lineage 8 had the highest unconstrained dispersal value (0.008203). In the case of the most diverse lineages (8 & 9), lineage 8 had the highest extinction value overall (0.01232), exceeding dispersal, while lineage 9 also had a high extinction value (0.003747). All other lineages retained very low background extinction levels, suggesting that local extinction within lineages 8 and 9 may explain the relatively high extinction value for the whole phylogeny (Figure 15). Overall, dispersal and cladogenesis do not seem to be clustered in time, as migration between adjacent basins is likely to be an ongoing process.

Figure 15- Dispersal & Extinction



Global and lineage specific dispersal and exinction values from the Lagrange analysis.

4.4.2 Present Distribution in Ecoregions

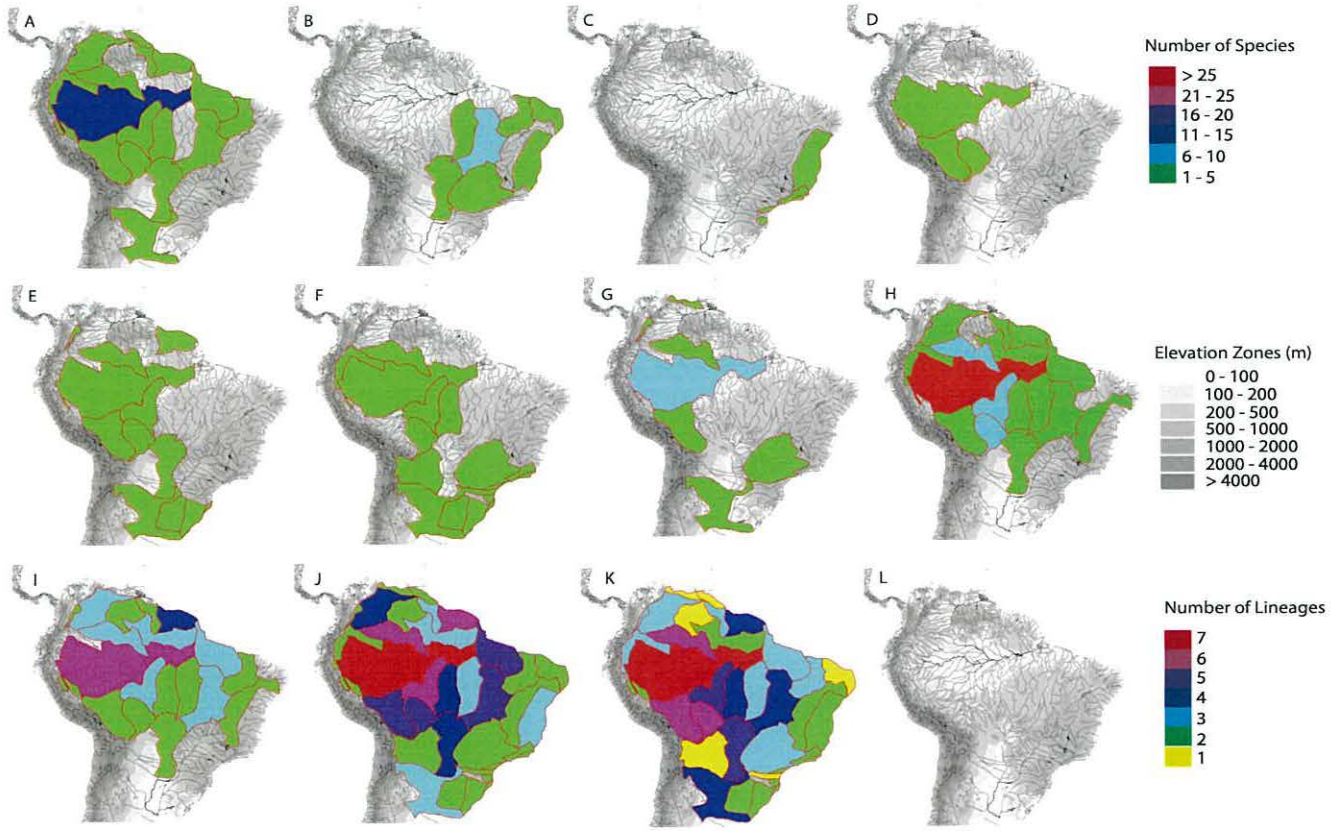
Plotting the species diversity of a total of 348 Corydoradinae taxa, revealed that the distribution varied across the 25 different ecoregions (Table 9 & Figures 16a-j). The area defined as the Amazonian lowlands has the highest level of diversity with nearly 80 taxa, while the Rio Negro, Guyanas, Madeira, Tocantins – Araguaia, Amazonas estuary, Guapore – Itenez, Mamore – Madre de Dios and Orinoco Llanos ecoregions (following in order of species richness) are also inhabited by significant numbers of endemic species. The great diversity found within the Amazonian lowlands may be simply an artifact of the size of this ecoregion being the largest in terms of area, or it may serve as an ancestral hotspot capable of maintaining and generating diversity. Thus, there is an imbalance in the distribution of the Corydoradinae, with the vast majority of species occupying habitats in the Northern River Systems (NRS= Amazon + Orinoco + Guyanas) as opposed to the Southern River Systems (SRS= Parana-Paraguay + Uruguay + Sao Francisco + coastal rivers of eastern Brazil). This division of diversity has been shown before at intrageneric and intraspecific levels, but not necessarily at the inter-generic/subfamily level (Lovejoy and de Araujo, 2000; Montoya-Burgos, 2003). However, when these results are broken down by genetic lineage, finer scale patterns of endemism emerge. While species from Lineage 1 are widely distributed across multiple ecoregions from the Guyanas in the north to the Lower Parana in the south (with the greatest number of species in the Amazonian Lowlands), Lineages 2 and 3 are distributed across comparatively few ecoregions in the east and north east of Brazil. The latter lineages were found to have the most restricted distributions of all Corydoradinae, with Lineage 3 exclusively occupying the fewest ecoregions within the most threatened area in Brazil (the coastal Atlantic forest).

Table 9- Diversity per Ecoregion

Ecoregion	Total	Undescribed	Described	Lineages
Caribbean Drainages - Trinidad	3	0	3	7
Orinoco Piedmont	2	1	1	5, 7, 9
Orinoco Llanos	13	4	9	1, 8, 9
Orinoco Guiana Shield	3	1	2	9
Orinoco Delta & Coastal Drainages	1	0	1	1
Essequibo	6	1	5	1, 8, 9
Guianas	22	2	20	1, 5, 8, 9
Western Amazon Piedmont	5	1	4	1, 6, 8
Rio Negro	25	10	15	1, 5, 6-9
Amazonas Guiana Shield	8	5	3	8, 9
Amazonas Lowlands	79	43	36	1, 4-9
Ucayali - Urubamba Piedmont	2	0	2	1, 9
Mamore - Madre de Dios Piedmont	16	10	6	1, 4, 5, 7-9
Guapore - Itenez	17	2	15	1, 4-6, 8, 9
Tapajos - Juruena	12	10	2	1, 6, 8, 9
Madeira Brazilian Shield	22	15	7	1, 5, 6, 8, 9
Xingu	6	3	3	2, 8, 9
Amazonas Estuary & Coastal	17	14	3	1, 8, 9
Tocantins - Araguaia	19	10	9	1, 2, 8, 9
Parnaiba	3	0	3	1, 2, 9
Northeastern Caatinga & Coastal	5	0	5	2
S. Francisco	5	2	3	8, 9
Northeastern Mata Atlantica	7	2	5	2, 3
Paraiba do Sul	3	1	2	3, 6
Southeastern Mata Atlantica	5	0	5	3, 6
Chaco	1	0	1	6
Paraguay	11	4	7	1, 2, 5, 8, 9
Upper Parana	4	0	4	2, 6, 7
Lower Parana	9	2	7	1, 5-7
Iguassu	4	1	3	6
Ribeira de Iguape	4	0	4	3, 6
Lower Uruguay	3	1	2	5, 6
Laguna dos Patos	2	0	2	5, 6
Fluminense	2	0	2	3, 6
Tramandai - Mampituba	2	0	2	5, 6

Total species diversity of described and undescribed Corydoradinae in different Ecoregions, along with Lineage Diversity (LD

Figure 16- Species and Lineage Distribution in Ecoregions



Panels A-I depict number of species per lineage (Lineages 1-9 respectively. Panel J depicts total diversity for all lineages, while panel K depicts Lineage Diversity (LD), and panel L shows the default map.

Comparatively species poor lineages (Lineage 3 & 4) appear to have more restricted distributions than species rich lineages. However, Lineages 5, 6 and 7 are not particularly species rich yet they are distributed throughout multiple relatively distant ecoregions. Lineage 9 is by far the most diverse, and also the most widely distributed (occupying the greatest number of different ecoregions), while Lineage 8 being the second most diverse is less widely distributed than lineages 1 and 6 (which consist comparatively fewer species). I tested available data on mean egg sizes and egg numbers produced by species within different lineages in order to try and account for differences in dispersal based on a biological explanation, but found no discernable relationships. However, observed differences in distribution may be attributed to the extent of larval dispersal or the timing of ancestral dispersal in relation to historical connectivity of basins (i.e. if the ancestor originates in the Amazon prior to the separation of Amazonas-Orinoco, Amazonas-Rio de Plata etc, then species have a greater chance of dispersing further into various basins). As information on the dispersal potential of larval Corydoradinae is currently unavailable, and the detailed inter-relationships of many taxa used for the ecoregion analysis remain unknown, it is not possible to test either hypothesis in further detail at this time.

In terms of diversity of lineages (LD) per ecoregion, quantified as the number of genetic lineages present in each region, (Table 9 & Figure 16k) a slightly different pattern emerges when compared to species diversity. The greatest LD was found in the Amazonian lowlands (7 lineages), which is also inhabited by the highest number of species (79). However, the Rio Negro, Guapore – Itenez, and Mamore – Madre de Dios basins are inhabited by significantly fewer species compared to the Amazonian lowlands area, while maintaining high levels of LD (6 lineages). Also, many ecoregions appear to be depauperate in terms of LD as they are inhabited by only one or two lineages. Thereby, there is some indication that ecoregions with many species also have the highest LD, yet it seems that this signal is confounded by the fact that certain ecoregions with high levels of species diversity do not have equally high levels of LD (Guyanas, Madeira, Amazonas Estuary and Tocantins – Araguaia).

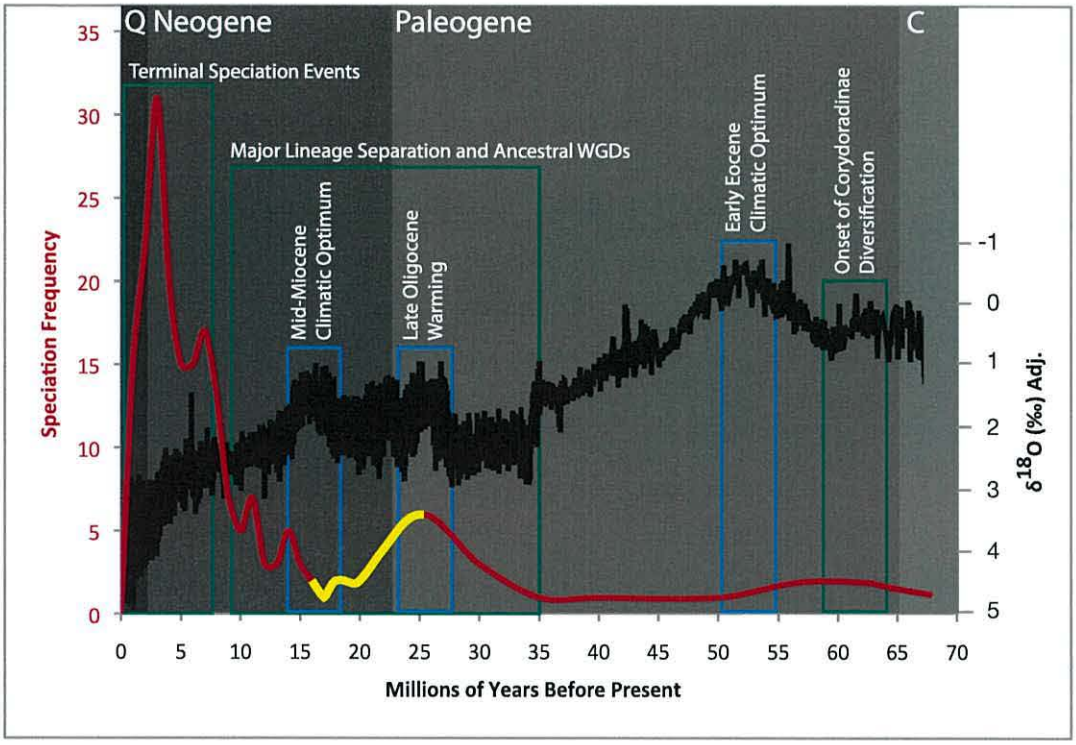
Furthermore, our survey of the available taxonomic data indicates that certain ecoregions are inhabited by more undescribed taxa than others. In total, our survey included 348 taxa, of which 203 have been described and 145 remain undescribed (Table 9). Areas that are inhabited by a greater proportion of described to undescribed

species are likely to be the result of increased sampling and expedition efforts, such as the Guyanas which were extensively surveyed during early expeditions (Nijssen, 1970). Up to 43 taxa have yet to be formally described from the Amazonas lowlands ecoregion (54% of the total diversity), while other ecoregions with high levels of diversity have even higher percentages of undescribed taxa. Notably, diverse ecoregions that also have the highest levels of undescribed taxa include the Tapajos – Juruena, Amazonas Estuary, Madeira, Mamore –Madre de Dios, Tocantins – Araguaia, and Rio Negro (83%, 82%, 68%, 63%, 53%, 40% respectively). These data indicate that the latter regions should be sampled more intensely in an effort to formally describe and catalogue the endemic taxa found within these biodiversity hotspots.

4.4.3 Paleoclimate and Diversification

Overall, I found that climatic fluctuations during the late Oligocene and Mid Miocene coincide with major lineage partitioning (35-8 MYA), which may have contributed to the subsequent diversification of the Corydoradinae (Figure 17). The extent to which these fluctuations actually influenced particular speciation events is almost impossible to tease apart using these data. Nevertheless, there is an indication that paleoclimatic events such as the Mid-Miocene climatic optimum and Late Oligocene warming are likely to have affected major patterns of diversification leading to the separation of Corydoradinae lineages. The shift in diversification during late Neogene and throughout the Pliocene may be attributed to intrinsic rather than extrinsic factors, such as Whole Genome Duplication events (WGDs), as the lineages with the majority of species have been shown to have duplicate genomes and that this can drive diversification rate (Chapter 3 results).

Figure 17- Paleoclimate and Speciation



Paleoclimatic fluctuations based on Zachos et al. (2001) data plotted with speciation frequency of the Corydoradinae. Yellow line on speciation frequency curve denotes shift in diversification rate (identified by MEDUSA and LASER in Chapter 3) within 95% HPD boundaries determined by BEAST.

4.5 Discussion:

4.5.1 Biogeographic Hypotheses

From the previously developed hypotheses accounting for historical patterns of neotropical fish distribution, I identified two as the most likely to explain some of the observed cladogenetic patterns for the Corydoradinae. Firstly, I found some support for the Taxon Pulse hypothesis, assuming that species arise in continuously occupied stable centers of diversification from which they periodically disperse into adjacent areas (Halas et al., 2005). The Amazonian lowlands appear to be a stable core region with high levels of diversity from which taxa have dispersed in such a manner (towards the Orinoco, Guyanas, Parana etc). This hypothesis is supported by the ancestral reconstruction of basins, indicating that in most cases species disperse from the Amazon to adjacent basins, while maintaining high levels of diversity within the Amazon (although *Scleromystax* is an exception as it is not present in the Amazon). Our data also indicate that distributional ranges of taxa periodically fluctuate around the continuously occupied Amazonas basin, while dispersal has still been historically interrupted by paleogeographic and hydrological barriers which potentially contribute to subsequent episodes of vicariant speciation (Halas et al., 2005). Furthermore, the absence of particular lineages in certain areas may be explained by a lack of participation in certain dispersal/expansion events within a given lineage, rather than by local extinction events. Despite the fact that the Taxon Pulse hypothesis has traditionally been used for terrestrial vertebrates (Erwin, 1979; Halas et al., 2005), our evidence suggests that it is highly relevant in the case of the spatiotemporal distribution of the Corydoradinae.

Secondly, I found support for the Paleogeography & Hydrogeology hypothesis, as the boundary displacements between the paleo Amazonas - Orinoco and Parana (11.8 – 10MYA) systems have contributed to some of the observed vicariant events (Montoya-Burgos, 2003). However, division between species occupying northern and southern river systems varied greatly temporally, suggesting that although these barriers prevent dispersal in some cases, they remain semipermeable (Lovejoy et al., 2010), and dispersal has occurred until relatively recently. My data also support the hypothesis that dispersal occurred between the middle Orinoco and Amazon via the Guyanas (Lovejoy and de Araujo, 2000). The Paleogeography & Hydrogeology and

Taxon Pulse hypotheses are not mutually exclusive, and could potentially be unified under a framework relevant to freshwater vertebrates. Thirdly, phylogenetic niche conservatism has been shown for many vertebrates (Kozak and Wiens, 2010; Wiens et al., 2010; Wiens and Donoghue, 2004; Wiens and Graham, 2005) and is likely to play an important role in the allopatric divergence of Corydoradinae lineages, as they retain ancestral morphological traits associated with resource acquisition and habitat occupancy (Alexandrou et al., 2011). Niche conservatism inevitably restricts dispersal into or via areas with unsuitable ecological characteristics (leading to local extinction) and can thereby shape biogeographic patterns of Corydoradinae catfishes. Furthermore, the majority of cladogenetic events seem to be occurring primarily within the Amazon (and other basins), and not necessarily as a result of allopatric isolation due to basin separations.

In the case of mimetic Corydoradinae species, diversification in geographic terms can also be explained by colour pattern convergence. As co-mimics become allopatrically isolated by invading new sites, evidence from multiple mimicry rings suggests that new phenotypic optima (in terms of colour patterns) arise at each new site. Thus geographic isolation combined with colour pattern convergence can have a strong effect on diversification and reproductive isolation, influencing biogeographic patterns within and between basins. As competition and phylogeny have already been shown to be important determinants of community structure (combined with mimetic mutualistic relationships), it is very likely that mimicry and competition accelerate diversification within certain regions while leading to extinction in others, thereby contributing to pattern of spatiotemporal distribution (Alexandrou et al., 2011; Elias et al., 2008; Mallet and Dasmahapatra, 2011).

I identified a number of hypotheses that poorly fit the observed patterns of Corydoradinae distribution in time and space. The Refuge hypothesis can be rejected as cladogenesis is not concentrated in this time period (most speciation events are pre-Quaternary), and species are not found in postulated refugia. I reject the Museum hypothesis, as there is no phylogenetic evidence to support a basal trichotomy for taxa from Guianan, Napo/Inambari and Belem/Para regions. I reject the River hypothesis, as we do not find different species on opposite sides of a given river, nor evidence to suggest that river width leads to allopatric isolation. I reject the Disturbance - Vicariance hypothesis as the majority of speciation events predate climatic

fluctuations of the Pleistocene. I reject the Gradient hypothesis, as representative Corydoradinae populations seem to be isolated from one another without evidence of speciation across steep environmental gradients (despite the fact that parapatric differentiation may still account for some of the observed patterns of distribution). I find the Sea Level Fluctuation hypothesis does not account for major patterns of biogeographic distribution of the Corydoradinae, as Pleistocene sea level fluctuation does not explain allopatric differentiation between species (Miller et al., 2005). However, the Sea Level Fluctuation hypothesis may still account for some patterns of isolation within the Guyanas coastal region (Cardoso and Montoya-Burgos, 2009). Finally, I did not find support for the River-Refuge, as there is no indication of allopatric speciation between species occupying adjacent intra-riverine corridors (Ayres and Cluttonbrock, 1992).

Throughout the evolution of a group that is widely distributed over 60MY, no single hypothesis is likely to account for all observed patterns of spatiotemporal distribution. Different hypothesis are relevant during different time periods for different lineages. Thus, one must consider these ideas within the context of time period, the depth of the phylogeny (basal splits of lineages vs diversification within lineages; i.e. inter vs intra-generic patterns), and the geological and climatic history of the area. The implications for hypotheses explaining the biotic enrichment of neotropical fishes is that existing ideas may account for some of the observed patterns, yet much of the biogeographic diversification process remains unaccounted for. Multidisciplinary approaches combining the use of phylogenetic comparative methods, fossils, paleoclimatic data, geological/hydrological information, ecological niche requirements and detailed records on current distribution are necessary to further refine existing theories. Furthermore, interspecific investigations of historical biogeography currently lack the rigorous statistical framework currently under development for intraspecific analyses, such as the use of Bayesian model testing (Lemey et al., 2009; Templeton, 2009). The lack of explicit models and statistical frameworks may be due to the complexity of events over extended time periods, however, more efforts to develop such models for hypothesis testing of interspecific historical biogeography would greatly reduce inductive narrative approach that has been common to such studies until now. The field of higher level (taxonomically) historical biogeography could be advanced by simulating the effect of different

biogeographic scenarios on tree topologies and the clustering of species within different regions. Simulations could then be compared to observed data to derive differences in likelihood scores using an AIC criterion. Despite these issues, a critical examination of existing hypotheses and proposals of new methodology to test them is not the aim of our current work.

4.5.2 Comparison to Previous Work on Neotropical Fish Biogeography

Prior to the use of molecular phylogenetic frameworks for testing hypotheses of historical biogeography, morphological studies on various groups of Characiformes were quick to refute the Refuge hypothesis as data suggested that diversification predated the Pleistocene (Vari, 1988; Weitzman, 1982). These ideas were subsequently confirmed from a paleontological perspective, supporting the fact that Paleogene fossils reveal diversification of a variety of freshwater catfishes, characins and cichlids now prominent in Amazonian waters (Lundberg, 1998; Lundberg et al., 1998; Malabarba et al., 2010). In fact, the fossil *Corydoras revelatus* used to calibrate our molecular phylogeny was found in the Maíz Gordo Formation in Salta, Argentina, and has been dated to 58.2-58.5MYA (Cockerell, 1925), suggesting that the genus *Corydoras* was well established by that time (Reis, 1998b). The area of its discovery was analogous to the current pantanal, a wetland lacustrine environment located in the headwaters of a massive river system known as the paleo Amazon-Orinoco (Lundberg et al., 1998). This system acted as a northern flowing freshwater corridor effectively connecting modern day western Amazonian lowlands with the southern basins, and is likely to have had a profound effect on the distribution of Corydoradinae.

The Museum hypothesis has been supported by work conducted on a number of marine derived taxa (Myliobatiformes, Sciaenidae, Engraulididae, and Belonidae) that originate during the Miocene (Lovejoy et al., 2006; Lovejoy and de Araujo, 2000). The Museum hypothesis has also been supported by a study focusing on piranha genera, however, the latter work also supports the paleogeography and hydrogeology hypothesis (Hubert et al., 2007). The Hydrogeological hypothesis was proposed based on phylogenetic analysis of the catfish genus *Hypostomus*, and is likely to account for certain large scale patterns of neotropical fish diversification during the late Tertiary (Montoya-Burgos, 2003). The River hypothesis has been proposed to play an important role in the distribution of the Hypoptomatinae and Neoplecostominae

catfishes, where species are adapted to specific riverbank habitats and dispersal across rivers is restricted by eco-morphological adaptations (Chiachio et al., 2008). Furthermore, a recent population level analysis of the catfish *Pseudancistrus brevispinis* proposed the Sea Level Fluctuation hypothesis, suggesting that during high sea level intervals, isolated populations diverge leading to allopatric groups (Cardoso and Montoya-Burgos, 2009). In the latter case, as populations endemic to the Guyanas originate from ancestral colonization from the Amazonas basin, it might be the case that the Taxon pulse hypothesis (with the Amazonas as a stable core of diversity) may also explain observed patterns of diversification. In conclusion, most work on different fish and other vertebrate supports a variety of different hypotheses, but all support the idea that spatiotemporal patterns of distribution were well established by the Quaternary, and the Andean uplift was crucial for the evolution of the Amazonian landscapes and ecosystems (Hoorn et al., 2010; Lundberg et al., 1998).

4.5.3 Paleoclimate, Diversification and Genome Duplication

Climatic fluctuations potentially contributed to early major cladogenetic events separating the majority of Corydoradinae lineages. The timing of these fluctuations also coincide with ancestral WGDs, which may or may not have been triggered by the climatic fluctuations. Nevertheless, they are likely to have contributed the subsequent success and accelerated diversification of newly forming lineages (within lineage diversification as opposed to lineage separations) (Chapter 3 Discussion). A recent hypothesis for plants suggests that genome duplications in Angiosperm lineages are clustered in time and coincide with the Cretaceous-Tertiary (KT) mass extinction event (Fawcett et al., 2009). The authors propose that these duplications potentially contribute to the diversification of some Angiosperm lineages during times of climatic fluctuation and perturbation, as species with duplicated sets of genes are better able to adapt given the increased amount of genetic material available for selection to act upon. However, I find that the evidence in favour of this hypothesis for the Corydoradinae is anecdotal, despite the timing of duplications and major lineage separation coinciding with Oligocene and Miocene climatic fluctuations. Furthermore, there is no evidence to suggest that vertebrates having undergone whole genome duplication are better suited (than their diploid ancestors) to adapt to new niches (Van de Peer et al., 2009).

Although a causal link is lacking, it is possible that genome duplications have played a role in biogeographic distribution as lineages with largest genome sizes are the most widely distributed. Ancestral genome duplication events (reconstructed in Chapter 3) appear to have occurred within the Amazon basin between the Late Paleogene and Early Neogene with subsequent duplications in a variety of adjacent basins, as illustrated by the geographic range reconstruction. Moreover, species with different genome sizes are more likely to be able to coexist due to reproductive barriers and genomic incompatibility, whereas species with more similar genomic composition may be more prone to hybridization. In fact, it is very rare to find species coexisting in the wild with the same chromosome complement and genome size, which indicates that differences in ploidy may play a role in community composition thereby affecting biogeographic patterns.

4.5.4 Ecoregions & Conservation

The high levels of species richness (and LD) within the Amazon and adjacent regions offers further support for the Latitudinal Diversity Gradient, suggesting that species richness increases towards the equator (Mittelbach et al., 2007). The Amazonas lowlands, Rio Negro, Guyanas, Madeira, Tocantins – Araguaia, Amazonas estuary, Guapore – Itenez, Mamore – Madre de Dios and Orinoco Llanos ecoregions were all found to have high levels of Corydoradinae species richness. From the latter, the Amazonas lowlands, Rio Negro, Guapore – Itenez, and Mamore – Madre de Dios ecoregions were found to have the highest levels of LD, while the Amazonas lowlands, Tapajos – Juruena, Amazonas Estuary, Madeira, Mamore –Madre de Dios, Tocantins – Araguaia, and Rio Negro ecoregions were also inhabited by large numbers of species that have yet to be described. Furthermore, the Guyanas, Sao Francisco and east coastal Brazilian ecoregions are inhabited by more endemic species than other areas, as these basins are very isolated and limit dispersal to other adjacent areas. Along with rapid deforestation and drought, the building of hydroelectric dams along major tributaries of the Amazon River is now a critical issue affecting current patterns of distribution, migration and continuity of habitats (Barthem et al., 1991; Esguicero and Arcifa, 2010; Fearnside, 2006a; Fearnside, 2006b; Godinho and Kynard, 2009; Nogueira et al., 2010). This increase of anthropogenic disturbance and habitat destruction has made the Amazon and its satellite basins a priority for conservation of freshwater biodiversity hotspots, and the

current diversity and distribution of armoured catfishes with restricted geographical ranges should be considered and incorporated into managerial decisions concerning the protection of these habitats and their biota.

SI Table 3- Species List and Accession Numbers

	Lineage	Genus	Species	Voucher Code	Basin	ACC 12s	ACC 16s	ACC ND4	ACC Cytb
Callichthyinae	Lineage 0	*Dianema*	*D. longibarbus*	LBP 557-7230	Amazonas	GU210442	GU210867	GU210020	GU209684
Callichthyinae	Lineage 0	*Dianema*	*D. urostriatum*	MT89	Amazonas	GU210539	GU210964	GU210114	GU209685
Callichthyinae	Lineage 0	*Hoplosternum*	*H. littorale*	LBP 210-4134	Amazonas	GU210443	GU210868	GU210021	GU209686
Corydoradinae	Lineage 1	*Corydoras*	*C. acutus*	MA41	Amazonas	GU210339	GU210764	GU209918	GU209605
Corydoradinae	Lineage 1	*Corydoras*	*C. amapaensis*	MA154	Guyana	GU210164	GU210589	GU209745	GU209360
Corydoradinae	Lineage 1	*Corydoras*	*C. aurofrenatus*	ANSP 182420-1470	Lower Parana	GU210327	GU210752	GU209907	GU209332
Corydoradinae	Lineage 1	*Corydoras*	*C. blochi*	MHNG 2652.007-GY04-237	Guyana	GU210236	GU210661	GU209816	GU209341
Corydoradinae	Lineage 1	*Corydoras*	*C. cervinus*	MA125	Amazonas	GU210146	GU210571	GU209727	GU209358
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *blochi*	MHNG 2707.015-SU07-624	Guyana	GU210239	GU210664	GU209819	GU209368
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *geoffroy*	MHNG 2683.016-GF06-459	Guyana	GU210249	GU210674	GU209829	GU209379
Corydoradinae	Lineage 1	*Corydoras*	*C.* cf. *maculifer*	MA40	Amazonas	GU210338	GU210763	GU209917	GU209386
Corydoradinae	Lineage 1	*Corydoras*	*C. coriatae*	MT14	Amazonas	GU210428	GU210853	GU210006	GU209408
Corydoradinae	Lineage 1	*Corydoras*	*C. ellisae*	MT24	Lower Parana	GU210469	GU210894	GU210047	GU209430
Corydoradinae	Lineage 1	*Corydoras*	*C. fowleri*	MA108	Amazonas	GU210133	GU210558	GU209715	GU209441
Corydoradinae	Lineage 1	*Corydoras*	*C. geoffroy*	MHNG 2700.007-GF07-120	Guyana	GU210226	GU210651	GU209806	GU209443
Corydoradinae	Lineage 1	*Corydoras*	*C. maculifer*	LBP 7213-32890	Amazonas	GU210210	GU210635	GU209790	GU209481
Corydoradinae	Lineage 1	*Corydoras*	*C. negro*	MA52	Lower Parana	GU210348	GU210773	GU209927	GU209504
Corydoradinae	Lineage 1	*Corydoras*	*C. orcesi*	MA304	Amazonas	GU210293	GU210718	GU209873	GU209512
Corydoradinae	Lineage 1	*Corydoras*	*C. oxyrhynchus*	MHNG SU08-1191	Guyana	GU210284	GU210709	GU209864	GU209516
Corydoradinae	Lineage 1	*Corydoras*	*C. pastazensis*	MT42	Amazonas	GU210488	GU210913	GU210066	GU209527
Corydoradinae	Lineage 1	*Corydoras*	*C. semiaquilus*	ANSP 178613-1459	Amazonas	GU210321	GU210746	GU209901	GU209553
Corydoradinae	Lineage 1	*Corydoras*	*C. septentrionalis*	MA114	Orinoco	GU210139	GU210564	GU209721	GU209659
Corydoradinae	Lineage 1	*Corydoras*	*C. serratus*	MA309	Amazonas	GU210298	GU210723	GU209878	GU209557
Corydoradinae	Lineage 1	*Corydoras*	*C. simulatus*	MT64	Orinoco	GU210512	GU210937	-	GU209566
Corydoradinae	Lineage 1	*Corydoras*	*C. solox*	MHNG 2666.036-GF03-099	Guyana	GU210252	GU210677	GU209832	GU209573
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C115'	MA183	Amazonas	GU210186	GU210611	GU209767	GU209604
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C42'	MA27	Amazonas	GU210263	GU210688	GU209843	GU209625
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C53'	MA30	Amazonas	GU210288	GU210713	GU209868	GU209630
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'CW11 long nose *reynoldsi*'	LBP 7712-32724	Amazonas	GU210203	GU210628	GU209783	GU209640
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. *amapaensis*	MHNG 2681.018-GF06-071	Guyana	GU210256	GU210681	GU209836	GU209318
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C109'	LBP 5549-27241	NE Brazil	GU210130	GU210555	GU209712	GU209653
Corydoradinae	Lineage 1	*Corydoras*	*C.* sp. 'C92'	MA211	Amazonas	GU210213	GU210638	GU209793	GU209304
Corydoradinae	Lineage 1	*Corydoras*	*C. stenocephalus*	MHNG PE08-910	Amazonas	GU210279	GU210704	GU209859	GU209554
Corydoradinae	Lineage 1	*Corydoras*	*C. treitlii*	MT76	NE Brazil	GU210525	GU210950	GU210100	GU209663

Corydoradinae	Lineage 2	*Aspidoras*	*A. albater*	MA329	Amazonas	GU210313	GU210738	GU209893	GU209283
Corydoradinae	Lineage 2	*Aspidoras*	*A. depinnai*	MA307	São Francisco	GU210296	GU210721	GU209876	GU209285
Corydoradinae	Lineage 2	*Aspidoras*	*A. eurycephalus*	MA176	Amazonas	GU210180	GU210605	GU209761	GU209286
Corydoradinae	Lineage 2	*Aspidoras*	*A. microgaleus*	MA153	Amazonas	GU210163	GU210588	GU209744	GU209287
Corydoradinae	Lineage 2	*Aspidoras*	*A. poecilus*	LBP 1272-11098	Amazonas	GU210542	GU210967	GU210117	GU209288
Corydoradinae	Lineage 2	*Aspidoras*	*A. raimundi*	LBP 5568	NE Brazil	GU210131	GU210556	GU209713	GU209290
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. 'C35 Black Phantom'	MA177	Amazonas	GU210181	GU210606	GU209762	GU209291
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus*	MA96-27708	Amazonas	GU210379	GU210804	GU209957	GU209284
Corydoradinae	Lineage 2	*Aspidoras*	*A.* sp. *poecilus*	LBP 1437-12304	Amazonas	GU210544	GU210969	GU210119	GU209294
Corydoradinae	Lineage 2	*Aspidoras*	*A. spilotus*	MA325	NE Brazil	GU210309	GU210734	GU209889	GU209292
Corydoradinae	Lineage 2	*Aspidoras*	*A. taurus*	MA328	Lower Parana	GU210312	GU210737	GU209892	GU209296
Corydoradinae	Lineage 3	*Scleromystax*	*S. barbatus*	LBP 2083-14430	Coastal E Brazil	GU210446	GU210871	GU210024	GU209689
Corydoradinae	Lineage 3	*Scleromystax*	*S. kronei*	LBP 2658-17418	Coastal E Brazil	GU210448	GU210873	GU210026	GU209691
Corydoradinae	Lineage 3	*Scleromystax*	*S. lacerdai*	LBP 1966-13705	Coastal E Brazil	GU210452	GU210877	GU210030	GU209695
Corydoradinae	Lineage 3	*Scleromystax*	*S. macropterus*	LBP 461-5642	Coastal E Brazil	GU210455	GU210880	GU210033	GU209698
Corydoradinae	Lineage 3	*Scleromystax*	*S. prionotus*	LBP 1267-11106	Coastal E Brazil	GU210457	GU210882	GU210035	GU209700
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'C113'	LBP 1237-11125	São Francisco	GU210459	GU210884	GU210037	GU209704
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. 'CW42'	MA112	São Francisco	GU210137	GU210562	GU209719	GU209705
Corydoradinae	Lineage 3	*Scleromystax*	*S.* sp. *prionotus*	LBP 2575-15724	Coastal E Brazil	GU210460	GU210885	GU210038	GU209707
Corydoradinae	Lineage 4	*Corydoras*	*C.* cf. *hastatus*	MT104-13615	Lower Parana	GU210389	GU210814	GU209967	GU209462
Corydoradinae	Lineage 4	*Corydoras*	*C. guapore*	MA73	Amazonas	GU210364	GU210789	GU209943	GU209453
Corydoradinae	Lineage 4	*Corydoras*	*C. hastatus*	LBP 1709-12815	Amazonas	GU210405	GU210830	GU209983	GU209461
Corydoradinae	Lineage 4	*Corydoras*	*C. pygmaeus*	MT51	Amazonas	GU210498	GU210923	GU210076	GU209538
Corydoradinae	Lineage 5	*Corydoras*	*C. bilineatus*	MA68	Amazonas	GU210359	GU210784	GU209938	GU209340
Corydoradinae	Lineage 5	*Corydoras*	*C. elegans*	MT23	Amazonas	GU210468	GU210893	GU210046	GU209428
Corydoradinae	Lineage 5	*Corydoras*	*C. gracilis*	MA301	Amazonas	GU210290	GU210715	GU209870	GU209451
Corydoradinae	Lineage 5	*Corydoras*	*C. nanus*	MHNG SU08-575	Guyana	GU210270	GU210695	GU209850	GU209495
Corydoradinae	Lineage 5	*Corydoras*	*C. napoensis*	LBP 556-7227	Amazonas	GU210410	GU210835	GU209988	GU209499
Corydoradinae	Lineage 5	*Corydoras*	*C. nijsseni*	LBP 6861-32532	Amazonas	GU210190	GU210615	GU209771	GU209508
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. *' A pauciradiatus'*	LBP 548-7187	Amazonas	GU210430	GU210855	GU210008	GU209594
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C123 yellow cat'	MA84	Amazonas	GU210373	GU210798	GU209951	GU209608
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'C89'	MA82	Amazonas	GU210372	GU210797	GU209950	GU209637
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. 'CW18'	MA308	Amazonas	GU210297	GU210722	GU209877	GU209645
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. '*elegans* Columbia'	MA74	Orinoco	GU210365	GU210790	GU209944	GU209650
Corydoradinae	Lineage 5	*Corydoras*	*C.* sp. '*elegans* illuminator'	MA331	Amazonas	GU210315	GU210740	GU209895	GU209651
Corydoradinae	Lineage 5	*Corydoras*	*C. undulatus*	LBP 566-7386	Lower Parana	GU210441	GU210866	GU210019	GU209672
Corydoradinae	Lineage 6	*Corydoras*	*C. albolineatus*	MA321	Amazonas	GU210305	GU210730	GU209885	GU209314

Corydoradinae	Lineage 6	*Corydoras*	*C. diphyes*	MT21	Lower Parana	GU210466	GU210891	GU210044	GU209420
Corydoradinae	Lineage 6	*Corydoras*	*C. ehrhardti*	LBP 741-8893	Upper Parana	GU210400	GU210825	GU209978	GU209425
Corydoradinae	Lineage 6	*Corydoras*	*C. flaveolus*	MT115-12321	Upper Parana	GU210401	GU210826	GU209979	GU209438
Corydoradinae	Lineage 6	*Corydoras*	*C. nattereri*	LBP 903-9697	Upper Parana	GU210411	GU210836	GU209989	GU209501
Corydoradinae	Lineage 6	*Corydoras*	*C. paleatus*	LBP 567-7416	Upper Parana	GU210414	GU210839	GU209992	GU209519
Corydoradinae	Lineage 6	*Corydoras*	*C. potaroensis*	MT48	Guyana	GU210494	GU210919	GU210072	GU209624
Corydoradinae	Lineage 6	*Corydoras*	*C. reynoldsi*	MA335	Amazonas	GU210318	GU210743	GU209898	GU209545
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. 'C144'	MA86	Amazonas	GU210374	GU210799	GU209952	GU209614
Corydoradinae	Lineage 6	*Corydoras*	*C.* sp. *albolineatus*	LBP 1957-13560	Amazonas	GU210437	GU210862	GU210015	GU209592
Corydoradinae	Lineage 6	*Corydoras*	*C. tukano*	LBP 549-7195	Amazonas	GU210440	GU210865	GU210018	GU209670
Corydoradinae	Lineage 7	*Corydoras*	*C. aeneus*	MA144	Orinoco	GU210156	GU210581	GU209737	GU209310
Corydoradinae	Lineage 7	*Corydoras*	*C. eques*	MA318	Amazonas	GU210302	GU210727	GU209882	GU209436
Corydoradinae	Lineage 7	*Corydoras*	*C. melanotaenia*	MT35	Orinoco	GU210481	GU210906	GU210059	GU209486
Corydoradinae	Lineage 7	*Corydoras*	*C. rabauti*	MT54	Amazonas	GU210501	GU210926	GU210079	GU209541
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW10 Gold Laser'	MA42	Amazonas	GU210340	GU210765	GU209919	GU209578
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'CW9 Green Laser'	MA160	Amazonas	GU210170	GU210595	GU209751	GU209580
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'F Guyana'	MHNG 2666.037-GF03-097	Guyana	GU210246	GU210671	GU209826	GU209576
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru orange'	MA59	Amazonas	GU210353	GU210778	GU209932	GU209586
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Peru'	LBP 1350-11447	Amazonas	GU210432	GU210857	GU210010	GU209581
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus* 'Suriname'	MHNG 2671.014-SU05-575	Guyana	GU210229	GU210654	GU209809	GU209587
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'venezuelanus'*	MA181	Orinoco	GU210185	GU210610	GU209766	GU209673
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	MA98-27845	Upper Parana	GU210381	GU210806	GU209959	GU209480
Corydoradinae	Lineage 7	*Corydoras*	*C.* sp. *aeneus 'macrosteus'*	MA204-32760	Upper Parana	GU210207	GU210632	GU209787	GU209479
Corydoradinae	Lineage 7	*Corydoras*	*C. zygatus*	MA51	Amazonas	GU210347	GU210772	GU209926	GU209683
Corydoradinae	Lineage 8	*Corydoras*	*C. ambiacus*	MA46	Amazonas	GU210344	GU210769	GU209923	GU209320
Corydoradinae	Lineage 8	*Corydoras*	*C. britski*	LBP 688-8112	Upper Parana	GU210546	GU210971	GU210121	GU209298
Corydoradinae	Lineage 8	*Corydoras*	*C. multiradiatus*	MA146	Amazonas	GU210157	GU210582	GU209738	GU209299
Corydoradinae	Lineage 8	*Corydoras*	*C. splendens*	LBP 2017-14216	Amazonas	GU210548	GU210973	GU210123	GU209301
Corydoradinae	Lineage 8	*Corydoras*	*C. agassizii*	MA334	Amazonas	GU210317	GU210742	GU209897	GU209311
Corydoradinae	Lineage 8	*Corydoras*	*C.* cf. *leopardus* 'C102'	MA87	Amazonas	GU210375	GU210800	GU209953	GU209384
Corydoradinae	Lineage 8	*Corydoras*	*C. condisciplus*	MA53	Guyana	GU210349	GU210774	GU209928	GU209405
Corydoradinae	Lineage 8	*Corydoras*	*C. crypticus*	MT17	Amazonas	GU210461	GU210886	GU210039	GU209411
Corydoradinae	Lineage 8	*Corydoras*	*C. delphax*	MT20	Amazonas	GU210465	GU210890	GU210043	GU209417
Corydoradinae	Lineage 8	*Corydoras*	*C. difluviatilis*	LBP 382-4608	Sao Francisco	GU210398	GU210823	GU209976	GU209418
Corydoradinae	Lineage 8	*Corydoras*	*C. ephippifer*	MA58	Amazonas	GU210352	GU210777	GU209931	GU209435
Corydoradinae	Lineage 8	*Corydoras*	*C. filamentosus*	MHNG 2707.015-SU07-625	Guyana	GU210240	GU210665	GU209820	GU209437
Corydoradinae	Lineage 8	*Corydoras*	*C. garbei*	LBP 330-3920	Sao Francisco	GU210402	GU210827	GU209980	GU209442

Corydoradinae	Lineage 8	*Corydoras*	*C. gomezi*	MT27	Amazonas	GU210472	GU210897	GU210050	GU209363
Corydoradinae	Lineage 8	*Corydoras*	*C. haraldshultzei*	MT31	Amazonas	GU210477	GU210902	GU210055	GU209458
Corydoradinae	Lineage 8	*Corydoras*	*C. imitator*	LBP 6862-32502	Amazonas	GU210188	GU210613	GU209769	GU209464
Corydoradinae	Lineage 8	*Corydoras*	*C. leopardus*	MA337	Amazonas	GU210320	GU210745	GU209900	GU209470
Corydoradinae	Lineage 8	*Corydoras*	*C. melanistius*	ANSP 180693-1460	Guyana	GU210330	GU210755	GU209910	GU209484
Corydoradinae	Lineage 8	*Corydoras*	*C. ornatus*	MA64	Amazonas	GU210356	GU210781	GU209935	GU209513
Corydoradinae	Lineage 8	*Corydoras*	*C. pantanalensis*	LBP 691-8126	Lower Parana	GU210416	GU210841	GU209994	GU209524
Corydoradinae	Lineage 8	*Corydoras*	*C. pulcher*	MT50	Amazonas	GU210497	GU210922	GU210075	GU209533
Corydoradinae	Lineage 8	*Corydoras*	*C. reticulatus*	LBP 553-7214	Amazonas	GU210420	GU210845	GU209998	GU209542
Corydoradinae	Lineage 8	*Corydoras*	*C. robinae*	MT57	Amazonas	GU210504	GU210929	GU210082	GU209548
Corydoradinae	Lineage 8	*Corydoras*	*C. robustus*	MA143	Amazonas	GU210155	GU210580	GU209736	GU209549
Corydoradinae	Lineage 8	*Corydoras*	*C. seussi*	MT60	Amazonas	GU210508	GU210933	GU210086	GU209559
Corydoradinae	Lineage 8	*Corydoras*	*C. sodalis*	LBP 530-7125	Amazonas	GU210429	GU210854	GU210007	GU209570
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C52'	MA78	Amazonas	GU210369	GU210794	GU209947	GU209629
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW13'	MA20	Amazonas	GU210202	GU210627	GU209782	GU209641
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C131 *leopardus*'	MA72	Amazonas	GU210363	GU210788	GU209942	GU209385
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C141 *pulcher*'	MA175	Amazonas	GU210179	GU210604	GU209760	GU209396
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C49 false *robustus*'	MT70	Amazonas	GU210519	GU210944	GU210094	GU209628
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C57 nordestini'	MA184-32501	Sao Francisco	GU210187	GU210612	GU209768	GU209631
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C66 *similis*'	MA56	Amazonas	GU210350	GU210775	GU209929	GU209632
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C122	LBP 7214-32927	Amazonas	GU210301	GU210726	GU209881	GU209634
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'CW6 *narcissus*'	MT73	Amazonas	GU210522	GU210947	GU210097	GU209648
Corydoradinae	Lineage 8	*Corydoras*	*C.* sp. 'C159'	LBP 7711-32652	Amazonas	GU210197	GU210622	GU209778	GU209655
Corydoradinae	Lineage 8	*Corydoras*	*C. spilurus*	MA76	Guyana	GU210367	GU210792	GU209946	GU209652
Corydoradinae	Lineage 8	*Corydoras*	*C. virginae*	MT83	Amazonas	GU210533	GU210958	GU210108	GU209676
Corydoradinae	Lineage 9	*Corydoras*	*C. acrensis*	MA179	Amazonas	GU210183	GU210608	GU209764	GU209302
Corydoradinae	Lineage 9	*Corydoras*	*C. adolfoi*	LBP 6863-32527	Amazonas	GU210189	GU210614	GU209770	GU209305
Corydoradinae	Lineage 9	*Corydoras*	*C. araguaiaensis*	MA94-27706	Amazonas	GU210377	GU210802	GU209955	GU209324
Corydoradinae	Lineage 9	*Corydoras*	*C. arcuatus*	MT3	Amazonas	GU210475	GU210900	GU210053	GU209325
Corydoradinae	Lineage 9	*Corydoras*	*C. armatus*	MA111	Amazonas	GU210136	GU210561	GU209718	GU209329
Corydoradinae	Lineage 9	*Corydoras*	*C. atropersonatus*	MA303	Amazonas	GU210292	GU210717	GU209872	GU209330
Corydoradinae	Lineage 9	*Corydoras*	*C. axelrodi*	MA43	Orinoco	GU210341	GU210766	GU209920	GU209334
Corydoradinae	Lineage 9	*Corydoras*	*C. bicolor*	MHNG 2651.078-GY04-424	Guyana	GU210245	GU210670	GU209825	GU209339
Corydoradinae	Lineage 9	*Corydoras*	*C. boesemani*	MHNG 2673.074-SU05-129	Guyana	GU210223	GU210648	GU209803	GU209344
Corydoradinae	Lineage 9	*Corydoras*	*C. bondi*	MHNG 2651.040-GY04-123	Guyana	GU210244	GU210669	GU209824	GU209346
Corydoradinae	Lineage 9	*Corydoras*	*C. breei*	MHNG SU08-191	Guyana	GU210266	GU210691	GU209846	GU209349
Corydoradinae	Lineage 9	*Corydoras*	*C. brevirostris*	MT7	Guyana	GU210518	GU210943	GU210093	GU209352

Corydoradinae	Lineage 9	*Corydoras*	*C. caudimaculatus*	LBP 562-7255	Amazonas	GU210385	GU210810	GU209963	GU209357
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *araguaiaensis* 'C65'	MA57	Amazonas	GU210351	GU210776	GU209930	GU209365
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *bondi*	MA165	Orinoco	GU210172	GU210597	GU209753	GU209369
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *concolor*	LBP 2306-15843	Orinoco	GU210387	GU210812	GU209965	GU209371
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *davidsandsi*	MA319	Amazonas	GU210303	GU210728	GU209883	GU209374
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *guianensis*	LBP 5395-27091	Amazonas	GU210128	GU210553	GU209710	GU209382
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *punctatus*	MA350	Guyana	GU210334	GU210759	GU209914	GU209469
Corydoradinae	Lineage 9	*Corydoras*	*C.* cf. *sipalwini*	MHNG 2671.094-SU05-427	Guyana	GU210224	GU210649	GU209804	GU209398
Corydoradinae	Lineage 9	*Corydoras*	*C. concolor*	MT12	Orinoco	GU210406	GU210831	GU209984	GU209402
Corydoradinae	Lineage 9	*Corydoras*	*C. copei*	MT13	Amazonas	GU210417	GU210842	GU209995	GU209575
Corydoradinae	Lineage 9	*Corydoras*	*C. coppenamensis*	MHNG 2690.017-SU01-466	Guyana	GU210235	GU210660	GU209815	GU209407
Corydoradinae	Lineage 9	*Corydoras*	*C. cruziensis*	MA152	Amazonas	GU210162	GU210587	GU209743	GU209643
Corydoradinae	Lineage 9	*Corydoras*	*C. davidsandsi*	LBP 551-7203	Amazonas	GU210396	GU210821	GU209974	GU209414
Corydoradinae	Lineage 9	*Corydoras*	*C. duplicareus*	MA167	Amazonas	GU210174	GU210599	GU209755	GU209423
Corydoradinae	Lineage 9	*Corydoras*	*C. gossei*	MT29	Amazonas	GU210474	GU210899	GU210052	GU209448
Corydoradinae	Lineage 9	*Corydoras*	*C. griseus*	MA71	Guyana	GU210362	GU210787	GU209941	GU209635
Corydoradinae	Lineage 9	*Corydoras*	*C. guianensis*	MHNG 2683.055-GF06-574	Guyana	GU210247	GU210672	GU209827	GU209454
Corydoradinae	Lineage 9	*Corydoras*	*C. habrosus*	MA142	Orinoco	GU210154	GU210579	GU209735	GU209457
Corydoradinae	Lineage 9	*Corydoras*	*C. julii*	MA147	NE Brazil	GU210158	GU210583	GU209739	GU209468
Corydoradinae	Lineage 9	*Corydoras*	*C. kanei*	MA349	Amazonas	GU210332	GU210757	GU209912	GU209654
Corydoradinae	Lineage 9	*Corydoras*	*C. leucomelas*	MA122	Amazonas	GU210144	GU210569	GU209725	GU209471
Corydoradinae	Lineage 9	*Corydoras*	*C. loretoensis*	MA298	Amazonas	GU210286	GU210711	GU209866	GU209474
Corydoradinae	Lineage 9	*Corydoras*	*C. loxozonus*	MA351	Orinoco	GU210335	GU210760	-	GU209476
Corydoradinae	Lineage 9	*Corydoras*	*C. melini*	MA77	Orinoco	GU210368	GU210793	-	GU209489
Corydoradinae	Lineage 9	*Corydoras*	*C. metae*	MT38	Orinoco	GU210484	GU210909	GU210062	GU209493
Corydoradinae	Lineage 9	*Corydoras*	*C. multimaculatus*	MA302	Amazonas	GU210291	GU210716	GU209871	GU209494
Corydoradinae	Lineage 9	*Corydoras*	*C. oiapoquensis*	MHNG 2682.023-GF06-186	Guyana	GU210260	GU210685	GU209840	GU209511
Corydoradinae	Lineage 9	*Corydoras*	*C. osteocarus*	ANSP 185052-1477	Orinoco	GU210323	GU210748	GU209903	GU209515
Corydoradinae	Lineage 9	*Corydoras*	*C. panda*	MT41	Amazonas	GU210487	GU210912	GU210065	GU209521
Corydoradinae	Lineage 9	*Corydoras*	*C. paragua*	MA67	Lower Parana	GU210358	GU210783	GU209937	GU209526
Corydoradinae	Lineage 9	*Corydoras*	*C. polystictus*	MT45	Upper Parana	GU210491	GU210916	GU210069	GU209531
Corydoradinae	Lineage 9	*Corydoras*	*C. punctatus*	MHNG SU08-110	Guyana	GU210262	GU210687	GU209842	GU209535
Corydoradinae	Lineage 9	*Corydoras*	*C. schwartzi*	LBP 1783-7120	Amazonas	GU210421	GU210846	GU209999	GU209551
Corydoradinae	Lineage 9	*Corydoras*	*C. similis*	LBP 547-7184	Amazonas	GU210424	GU210849	GU210002	GU209562
Corydoradinae	Lineage 9	*Corydoras*	*C. sipaliwini*	MHNG 2707.017-SU07-287	Guyana	GU210241	GU210666	GU209821	GU209567
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *'arcuatus* super'	MT65	Amazonas	GU210513	GU210938	GU210088	GU209600
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C121 *burgessi'*	MA178	Amazonas	GU210182	GU210607	GU209763	GU209607

Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C133 ornatus short snout'	MT68	Amazonas	GU210516	GU210941	GU210091	GU209617
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C136'	MA305	Guyana	GU210294	GU210719	GU209874	GU209644
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C139 *oiapoquensis'*	MA174	Guyana	GU210178	GU210603	GU209759	GU209390
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C150 mazurani'	MA70	Guyana	GU210361	GU210786	GU209940	GU209615
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C30'	MA215	Guyana	GU210215	GU210640	GU209795	GU209618
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C43'	MA80	Amazonas	GU210371	GU210796	GU209949	GU209626
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C91 Peru *bondi'*	MA300	Amazonas	GU210289	GU210714	GU209869	GU209638
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW21 *axelrodi'*	MA320	Orinoco	GU210304	GU210729	GU209884	GU209642
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *arcuatus* 'Rio Negro'	LBP 7709-32609	Amazonas	GU210196	GU210621	GU209777	GU209599
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *armatus* 'Green cana'	MA330	Amazonas	GU210314	GU210739	GU209894	GU209602
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *breei*	MHNG SU08-583	Guyana	GU210278	GU210703	GU209858	GU209603
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'C84'	MA197-32671	Amazonas	GU210199	GU210624	-	GU209490
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. 'CW28'	MA110	Amazonas	GU210135	GU210560	GU209717	GU209612
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *davidsandsi*	MA134	Amazonas	GU210152	GU210577	GU209733	GU209649
Corydoradinae	Lineage 9	*Corydoras*	*C.* sp. *melini*	MA333	Orinoco	GU210316	GU210741	GU209896	GU209636
Corydoradinae	Lineage 9	*Corydoras*	*C. sterbai*	MT75	Amazonas	GU210524	GU210949	GU210099	GU209662
Corydoradinae	Lineage 9	*Corydoras*	*C. trilineatus*	MT80	Amazonas	GU210530	GU210955	GU210105	GU209667
Corydoradinae	Lineage 9	*Corydoras*	*C. weitzmani*	MA35	Amazonas	GU210333	GU210758	GU209913	GU209680

Species names of undescribed taxa have been labeled based on their respective C-Numbers (Datz) and CorydorasWorld numbers when available, otherwise the trade name alias or 'sp' was used.

Chapter 5: Discussion

5.1 Evolutionary Ecology: The comparative multidisciplinary approach

It is rare to have the opportunity of addressing a set of questions with a suite of complementary techniques, however, more often than not, this approach provides more comprehensive answers than would a more specialized approach. In this thesis, I have employed molecular phylogenetics (using mitochondrial and nuclear markers), diversification analyses, biogeographic model testing, stable isotope analyses (of carbon and nitrogen), geometric morphometrics, colour pattern analyses, and DNA content quantification. These data have been combined and analyzed using a comparative framework in order to address hypotheses focusing on evolutionary ecology, genome evolution and historical biogeography. This combination of techniques offered great insight, and has helped elucidate observed patterns of species richness, coexistence and spatiotemporal distribution. Nevertheless, testing causality in many of these cases remains a complex endeavor, because links are difficult to establish, as there are many potential alternative explanations that may account for diversity, complexity and adaptation.

A remaining challenge for comparative hypothesis testing is distinguishing among two or more variables that contribute significantly to a pattern, especially when mutually exclusive competing hypotheses are supported. Despite these difficulties, multiple variables underpin almost all patterns in evolutionary ecology. Given the levels of complexity, using an inductive approach of 'pattern before process' often leads to speculative inference attempting to account for observations outside the framework of testable hypotheses. There are no clear-cut answers to questions at the interface of ecology and evolution, and the simplest explanation is not always the most likely. It is therefore necessary to consider all available evidence and negative results, while remembering that no phylogenetic comparative framework is ever complete.

5.2 Aims of the Thesis

Chapter 1 aims to review the literature and provide background information relevant to Chapters 2, 3, and 4.

Chapter 2, aims to (a) construct a multi-locus molecular phylogenetic framework for the Corydoradinae subfamily, (b) establish the evolutionary origins of sympatric species that share colour patterns; (c) investigate resource partitioning between sympatric species using stable isotopes; (d) use geometric mophometrics to investigate morphological differences among genetic lineages; (e) quantify the diversity of colour patterns in observed mimicry rings and test whether sympatric species are more similar in colour than allopatric species. These data were then used to test the idea that positive interactions among mimetic species may outweigh the negative effects of competiton allowing species rich communities to evolve even without trophic divergence.

Chapter 3, aims to (a) construct a fossil calibrated phylogeny using a Bayesian relaxed molecular clock; (b) quantify total nuclear DNA content of multiple species across all Corydoradinae lineages; (c) reconstruct ancestral DNA content across the phylogeny; (d) test for topological and temporal shifts in net diversification rate, combining taxonomic with phylogenetic information; (e) quantify colour patterns and body size across the phylogeny; and finally to test the respective roles of genome size, colour pattern and body size in accelerating net diversification rate.

Chapter 4, aims to: (a) assign each species within the fossil calibrated phylogeny to an ancestral neotropical basin; (b) perform an ancestral area reconstruction analysis using the dispersal-extinction-cladogenesis model under constrained and unconstrained dispersal scenarios; (c) assess the role major paleoclimatic events on speciation frequency; (d) identify the best fit historical biogeographic hypothesis accounting for the spatiotemporal distribution of Corydoradinae species; (e) assign all existing species (described and undescribed) to their respective ecoregions and identify areas containing the highest levels of diversity (species richness and genetic diversity); and (f) to discuss potential conservation implications based on Corydoradinae diversity hotspots.

Chapter 5 aims to discuss: (a) Corydoradinae mimicry compared to other known Müllerian systems; (b) how mimicry affects speciation; (c) how polyploidy affects speciation; (d) biogeography and vicariance; (e) stable coexistence and extinction; (d) the definition of a Corydoradinae species; (e) caveats associated with my conclusions; (f) future work in genomics, mimicry, behaviour, speciation, systematics, morphology, and biogeography; and (g) conclusions of the thesis, with a final synthesis.

5.3 Evolutionary Ecology of Corydoradinae catfishes

5.3.1 Corydoradinae compared to other mimetic systems

Although Corydoradinae catfishes are unique, in that they are the most diverse freshwater fish Müllerian mimics known to date (and probably the most diverse aquatic mimics across phyla), there are many well-documented terrestrial examples of Müllerian mimicry. For the purpose of this discussion, I will compare and contrast Corydoradinae mimicry rings with mimicry rings in other other groups such as butterflies, millipedes, bumblebees, frogs and snakes, thereby providing both invertebrate and vertebrate examples. The most thoroughly investigated groups of mimetic species are the subfamilies Heliconiinae and Ithomiinae butterflies of the family Nymphalidae. They are extremely diverse at the generic and species level (6000 species in 542 genera), are exclusively Neotropical in distribution and therefore offer interesting parallels with the Corydoradinae. Within the Nymphalidae, research has focused on the genus *Heliconius*, where *Heliconius melpomene* and *Heliconius erato* have been shown to be Müllerian co-mimics (Bates, 1862; Turner, 1976). Bates observed geographical patterns for different mimicry rings of *Heliconius*, and documented the variation in colour patterns in different parts of the Amazon (Bates, 1862). Distantly related *Heliconius* mimic each other, while closely related species differ in colour pattern (Turner, 1976), and this has become a typical pattern amongst Müllerian co-mimics observed repeatedly in different organisms.

A striking difference between *Heliconius* and other mimetic groups is the number of sub-species defined as geographic races that differ in habitat, host plants and mimetic coloration (Mallet et al., 1998). Research suggests that these 'races' are likely to be reproductively isolated as colour patterns are important in mate choice leading to greater assortative mating in sympatry (Jiggins et al., 2001). There is also strong selection against hybrids with intermediate colour patterns that are non-mimetic yet hybridization still readily occurs in the wild, and can also result in reproductive isolation from parental species (Mallet et al., 2007; Mavarez et al., 2006). Comparing mimicry rings in Nymphalidae and Corydoradinae, both *Heliconius* and *Corydoras* co-mimics are usually distantly related and closely related sister taxa frequently differ in colour pattern. Differences in host plant association amongst *Heliconius* co-mimics and stable isotope signatures of *Corydoras* suggest that these species partition

resources in sympatry, which is likely to help maintain these communities over long time periods. More parallels between *Heliconius* and Corydoradinae mimicry rings may arise with genomic investigations, where it is already known that homologous genomic regions are responsible for the convergent evolution of wing patterns in *Heliconius melpomene* and *Heliconius erato* (Baxter et al., 2008; Counterman et al., 2010; Joron et al., 2006), while nothing is currently known about the genetics of colour pattern convergence among Corydoradinae co-mimics.

Butterflies of the genus *Ithomia* are widespread throughout the Neotropics, exhibiting significant geographic variation in colour patterns (Beccaloni, 1997). Colour pattern change is associated with cladogenesis within the genus *Ithomia* and speciation events predate the Pleistocene (Jiggins et al., 2006). This group offers an interesting comparison with Corydoradinae patterns of speciation and biogeography (See Chapter 4). Müllerian co-mimics in the Ithomiinae occur between different tribes, genera and species, while they also act as models for other more distantly related Lepidopterans, and often use different host plants thereby potentially partitioning resources (Willmott and Mallet, 2004). These mimicry rings are more species rich than those observed for *Heliconius* and *Corydoras*, yet research suggests that diverse and stable mimetic communities of ithomiines may evolve and coexist as a result of mutualistic interactions (Elias et al., 2008). In *Corydoras*, mutualistic interactions alone are not sufficient to maintain diverse communities and resource competition determines community structure in most cases investigated (See Chapter 2). Further investigation may reveal that the majority of ithomiinae co-mimics partition resources in terms of host plant use despite spatial overlap, yet these relationships remain poorly understood (Elias et al., 2008). Moreover, future research on Corydoradinae mimicry may show that there are multiple sub-species with different patterns that vary across geographic clines, as seems to be the case with butterflies.

Aphelerione millipedes of the Appalachians in North America are known to produce hydrogen cyanide and have recently been shown to form multiple mimicry rings across their range, involving up to 5 species in each community (Marek and Bond, 2009). These species are blind, and thereby exhibit no dichromatism based on sexual selection, making them unique amongst the known Müllerian mimics. Variability in their colour patterns in different geographic regions appears to have arisen through genetic drift, as predicted by the shifting balance hypothesis (Joron and Mallet, 1998).

Like other Müllerian mimetic groups, millipede co-mimics are distantly related, while the consequences of resource partitioning and/or competition for community structure have not been examined. Bumblebees have been suggested as another example of Müllerian mimicry, and while little is known about genetic relationships between different species, colour pattern evolution across geographic ranges has been thoroughly investigated (Williams, 2007). Results from this analysis suggest that dark colored species are associated with the tropics, while pale colored species with temperate clines and banded species are more widespread (Williams, 2007). There appears to be a thermoregulatory explanation for the geographic variation of colour in bumblebees (Williams, 2007), yet it is interesting to note that large scale variations in patterns across the Corydoradinae are also latitudinal (Chapter 2).

In vertebrates, one of the most celebrated examples of mimicry rings involves Neotropical poison dart frogs, particularly within the family Dendrobatidae. *Ranitomeya imitator* has been shown to mimic three different species in different regions, and is known for its high phenotypic plasticity and low genetic divergence (Symula et al., 2001). However, the low genetic divergence between *R. imitator* and its mimics suggests that these may be the same species, as there is incomplete sorting of mitochondrial and nuclear haplotypes (Chouteau et al., 2011). Recent work also challenges the idea of colour pattern advergence within *Ranitomeya* considering that mimics and models have been shown to be equally phenotypically variable (Chouteau et al., 2011). Compared to Corydoradinae mimicry rings, *Ranitomeya* mimicry involves fewer more closely related species within a much more restricted geographic range. South East Asian pitvipers offer a slightly more species rich example of Müllerian mimicry in vertebrates, where conspicuous colour patterns are associated with distantly related sympatric and parapatric species (Sanders et al., 2006). Interestingly, differences in behaviour have led to evolution of sexual dichromatism, where active males are predated on more readily and are seemingly aposematic, whereas females are sedentary and remain cryptic in coloration (Sanders et al., 2006). There are a few *Corydoras* examples in which females are mimics of another *Corydoras* species, but the males retain their ancestral coloration. However, overall, there seem to be more parallels between the Corydoradinae and invertebrate examples of Müllerian mimicry, rather than with vertebrates.

5.3.2 Mimicry & Speciation

Having compared the Corydoradinae to other mimetic groups, here I discuss the importance of mimicry and colour patterns for speciation within the Corydoradinae. The complexity of Corydoradinae mimicry is multifaceted, as mimetic relationships occur between species of different Corydoradinae, while they also extend outside the subfamily and thereby are likely to affect the evolution of other distantly related species (Alexandrou et al., 2011). This extension involves other families of catfishes, such as the observed coexistence of Corydoradinae mimicry rings with various species from the catfish genus *Brachyrhamdia.* These relationships may be considered Müllerian given that both *Corydoras* and *Brachyrhamdia* produce toxins. Furthermore, field observations indicate that tadpoles also share colour patterns with groups of mimetic *Corydoras* and *Brachyrhamdia,* notably in the upper Rio Negro. This suggests that catfish mimicry rings are likely to be more complex than presented herein (Chapter 2), and relationships with other putative mimics need to be investigated more thoroughly. These observations also raise the question of whether other species drive convergence of colour patterns within *Corydoras*, or whether *Corydoras* drive convergence of *Brachyrhamdia* and tadpoles. These issues remain to be investigated, however, our data and field observations support the conclusion that there are more mimetic *Corydoras* than there are *Brachyrhamdia* and it seems that the latter species are only associated with a few Corydoradinae mimicry rings. This suggests that *Corydoras* drive the convergence of colour patterns in other species, as has been shown in the Batesian relationship between *Corydoras diphyes* and *Otocinclus mimulus* (Axenrot and Kullander, 2003). Furthermore, *Corydoras hastatus* and a variety of sympatric non-toxic Characiformes have converged in colour pattern in the Pantanal, adding further support to the hypothesis that *Corydoras* drive convergence in patterns of other species.

It seems paradoxical that multiple mimicry rings with unique patterns should overlap in their geographic range. However, this might be the case with at least some Corydoradinae mimicry rings. This is odd because one might expect that if different mimicry rings overlap, their predators will also do so, potentially leading to single mimetic pattern rather than a variety of patterns. Most mimicry rings have been sampled in different non-overlapping rivers, yet within similar areas in the same larger basin. This suggests that spatial overlap between Corydoradinae mimicry rings

is likely. The extent to which this overlap occurs locally remains unknown and yet might occur in the Rio Tiquie (*C.* sp. C84/*C.* sp. C159 with *C. tukano/C.* sp. CW11) and the upper Rio Negro (*C.* cf. *arcuatus/C. serratus* with *C. adolfoi/C. imitator/C. nijsseni*) based on field observations. Spatial overlap of mimicry rings within larger areas seems to be common, with up to six mimicry rings common to a specific network of rivers. The maintenance of colour pattern diversity in these scenarios may be accounted for by habitat and resource partitioning of different predators. Field observations indicate that some *Corydoras* migrate seasonally, driven by water level fluctuations from small streams to bigger rivers.

Predation events are likely to be highest during the dry season, when water levels are low, visibility is good and *Corydoras* occur in higher densities (during the wet season fish occur in lower densities and frequently in turbid water). Predator assemblages (birds and fish) also fluctuate seasonally, and migrate between river systems. Thereby it is likely that seasonal differences in the abundance of *Corydoras*, availability to predators given water levels, and seasonal changes in predation frequency and predator composition may also contribute to the maintenance of complexity in mimetic patterns amongst spatially overlapping mimicry rings. Furthermore, if the predators of *Corydoras* (such as birds) also feed on other aposematic species (such as butterflies and frogs), they are likely to be aware of multiple warning signals across phyla. This may drive colour pattern convergence in aposematic species that are not part of the same mimicry rings, or lead to divergence of colour patterns if in fact different predators act as selective agents for different mimetic species (i.e. resource partitioning of mimetic prey, with some predators feeding exclusively on butterflies, others on frogs and others on fish). The predation effect and the evolution of colour mimetic patterns are undoubtedly frequency dependent issues, directly influenced by the relative abundance of mimics and predators (an aspect which was not investigated herein). Furthermore, competition for resources within mimicry rings will also be depend on the relative abundance of resources within a given niche. The effects of frequency dependence are likely to be greatest in cases where populations are relatively small and more prone to extinction, and the effects of genetic drift.

The predation effect described above may act locally, but also across wider geographic ranges. Within the Corydoradinae, certain colour patterns are geographically restricted to particular basins: eye bands and dark dorsal stripes or

patches, are only present in the Amazonian, Orinoco and coastal Guyanese basins, while many marbled patterns appear isolated in the southern Rio de Plata basin (e.g. *C. paleatus*, *Aspidoras* spp., *C. ellisae*). The geographic variation of putatively cryptic, disruptive and aposematic patterns may be explained by habitat heterogeneity of rainforests compared to the savannah and more temperate southern regions (or even river chemistry, sediment load, forest cover and substrate), but it is also likely that the predation effect is more intense in rainforests and tropical latitudes than it is in the Rio de Plata. This may affect speciation likelihood (supported by the latitudinal diversity gradient: more species closer to the equator), with more selection occurring in heavily predated rainforests leading to greater variation (although genome duplication, competition, isolation and drift are also contributors to species richness). As different colour patterns may offer different degrees of protection, does the evolution of cryptic coloration in Corydoradinae protect species to a greater extent than aposematism and mimicry? A classification of different colour patterns across the phylogeny combined with an investigation of their respective protective role may yield an answer to this question, however, our evidence indicates that crypsis and aposematism are not mutually exclusive with many species having evolved both cryptic and aposematic colour pattern elements. This suggests that the combination of cryptic, disruptive and aposematic patterns may offer more protection for a given species rather than the evolution of only one.

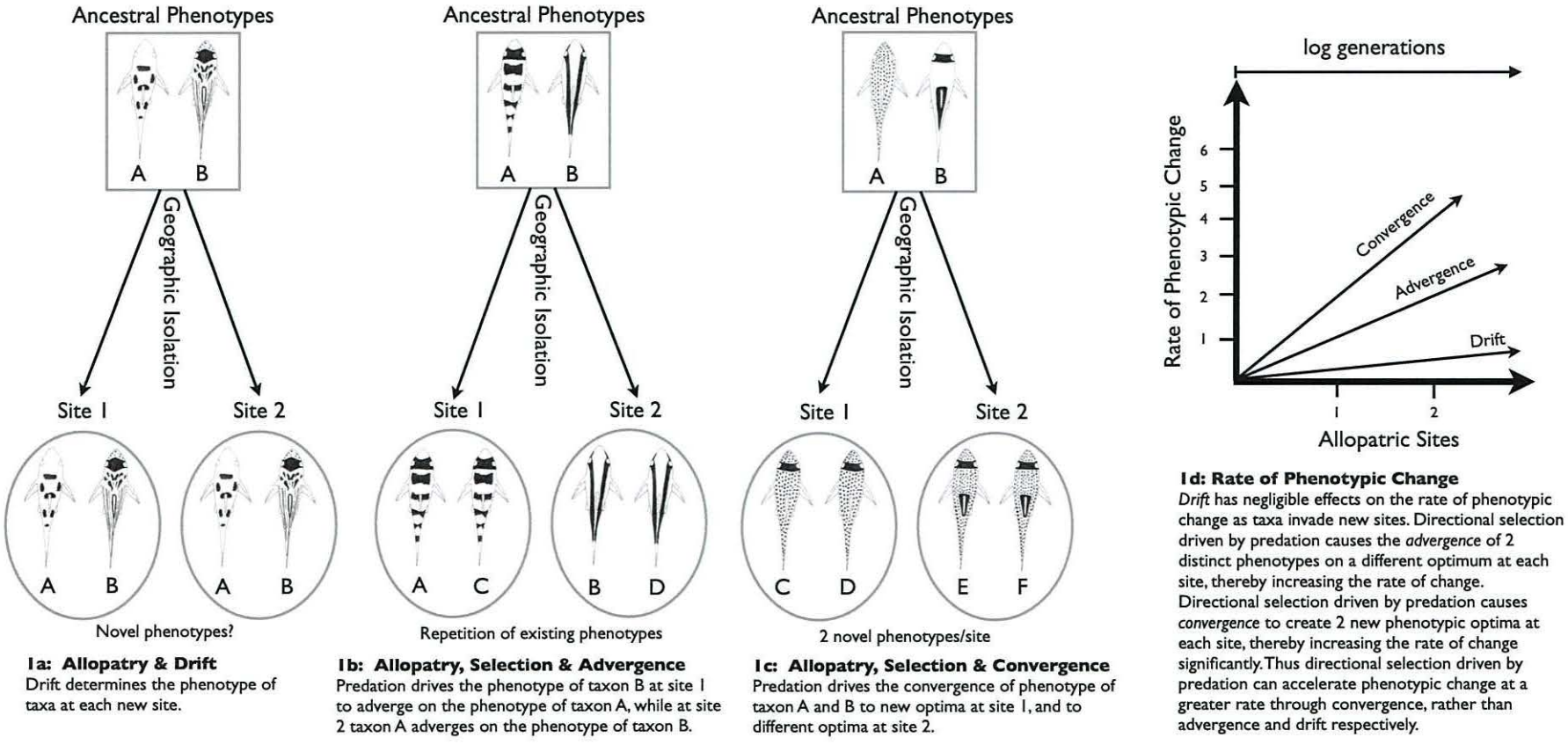
The mechanisms leading to the adoption of almost identical coloration in sympatric species is likely to be complex. Different selection pressures are likely to be found in different geographic localities in addition to differences in selection on different elements of colour patterns within individuals. Cryptic and disruptive colour patterns may be non-signal based with coexisting species evolving identical cryptic or disruptive coloration to escape detection or confuse predators. Many species display patterns that are likely to be cryptic, for example the density of dark pigment spots is related to substrate colour in *C. punctatus* (Nijssen, 1970). Other colour patterns appear to have disruptive elements; blocks of colours may be used by animals to make the detection of edges and boundaries more difficult or to disguise particular body parts (Cuthill et al 2005; Fraser et al, 2007; Schaefer & Stobbe, 2006). Black and white striped animals may be conspicuous when viewed in close proximity, but blend into their surroundings at greater distances (Cott, 1940). Disruptive coloration

appears to reduce predation irrespective of the background the prey is on, and it is likely that the contrasting colour patterns common to some of the Corydoradinae are disruptive in nature. Sympatric species may theoretically converge in colour pattern as a result of directional selection both in cryptic and disruptive colour patterns if they live in mixed species aggregations through 'social mimicry', and/or a combination of the confusion and oddity effect (Alevizon, 1976; Almany et al., 2007; Armbruster and Page, 1996; Krakauer, 1995; Landeau and Terborgh, 1986).

Directional selection by predation acting on sympatric species has the potential to drive the convergence of different patterns at different allopatric sites (as species become isolated or invade new sites), leading to further reproductive isolation within lineages. Thus, directional selection on colour pattern may increase the rate of phenotypic change among allopatric mimicry rings when compared with taxa not involved in mimetic relationships. I propose simplistic verbal/visual models (Figure 18) to illustrate the potential relative effects of drift, advergence, and convergence on the rate of colour pattern change among allopatric populations of Corydoradinae. All models assume: (i) a starting point with two reproductively isolated ancestral species (Taxa A and B respectively) which invade two new geographically isolated sites (Sites 1 and 2 respectively); (ii) that each taxon pair aggregates by actively shoaling at each new site invaded; (iii) that phenotype refers to colour pattern of Corydoradinae catfishes; (iv) that reproductive isolation occurs through assortative mating amongst taxa whose phenotype has reached a new optimum; (v) that advergence and convergence are not mutually exclusive, as drift can be followed by advergence, and subsequently convergence; (vi) and that drift is less powerful than directional selection driven by predation with respect to the rate of phenotypic change at allopatric sites. For these models, I define: (a) *convergence* as the extent to which phenotypes of distantly related taxa converge to new optima under co-evolutionary selection pressure; (b) *advergence* as the extent to which the phenotype of one taxon adverges to resemble another taxon as a result of selection pressure; and (c) *drift* as the random change in allele frequencies underpinning phenotype leading to phenotypic change. These models demonstrate the effects of drift, advergence and convergence on phenotypic change, irrespective of frequency or level of protection, although both of the latter parameters can be applied to the models. I propose that convergence of coloration within a mimicry ring will lead to greater allopatric

diversity among rings than advergence, and both will be greater than drift. As these mechanisms are not mutually exclusive, this implies that the extant phenotype is the product of a variety of mechanisms acting at different temporal stages. I also argue that directional selection driven by predation may result in convergent evolution of coloration through social mimicry and the oddity effect, irrespective of the prey's relative palatability and that this may also drive speciation.

Figure 18- Drift, Advergence and Convergence



1a: Allopatry & Drift
Drift determines the phenotype of taxa at each new site.

1b: Allopatry, Selection & Advergence
Predation drives the phenotype of taxon B at site 1 to adverge on the phenotype of taxon A, while at site 2 taxon A adverges on the phenotype of taxon B.

1c: Allopatry, Selection & Convergence
Predation drives the convergence of phenotype of taxon A and B to new optima at site 1, and to different optima at site 2.

1d: Rate of Phenotypic Change
Drift has negligible effects on the rate of phenotypic change as taxa invade new sites. Directional selection driven by predation causes the *advergence* of 2 distinct phenotypes on a different optimum at each site, thereby increasing the rate of change. Directional selection driven by predation causes *convergence* to create 2 new phenotypic optima at each site, thereby increasing the rate of change significantly. Thus directional selection driven by predation can accelerate phenotypic change at a greater rate through convergence, rather than advergence and drift respectively.

The relative contributions of drift, Advergence, and convergence to the rate of phenotypic change.

5.3.3 Polyploidy & Speciation (See Polyploidy review for further discussion)

Why are some species polyploids while others remain diploid? Recent research suggests that this may be due to mechanisms regulating genome integrity (i.e. mitotic/meiotic dysfunction, genome rearrangement, fusions and fissions, incompatibility between genome and cytoplasm), while unreduced gamete formation and propensity to hybridize are also important. If pre-existing mechanisms regulate genome integrity (Hufton and Panopoulou, 2009), and are phylogenetically conserved amongst different groups at various taxonomic levels (orders, families, subfamilies), could these mechanisms constrain some taxa from becoming polyploids? Are there genomic constraints preventing polyploid formation? If so, they must be phylogenetically conserved, otherwise polyploidy would be more randomly observed across lineages. Once polyploidy is established, species need mechanisms to cope with genomic changes. Many species have evolved such mechanisms to cope because of natural variation in genome copy number associated with mitotic and meiotic cell cycles. Those species with regular alteration of generations, with mitoses in both haploid and diploid phases, are predicted to be especially tolerant of shifts in ploidy (Otto, 2007).

A study examining the historical constraints on gene expression suggests that the number of cell types in existence at the time of a gene's appearance (through duplication or de novo origination) determines its level of tissue specificity for millions of years (Milinkovitch et al., 2010). When duplicate genes are retained they provide raw material for selection (i.e. selection will exploit redundant duplicated genes). Genes duplicated via polyploidization are retained for longer time periods than those that arise via individual gene duplication (Lynch, 2007), and may therefore play significant roles in evolutionary and ecological success. Recent work on yeast has shown that genes essential for the viability of polyploid cells can be classified into three functional groups: those encoding the mitotic spindle (spindle pole body and microtubule organizing centre), those involved in chromosome cohesion, and those essential for homologous recombination (Storchova et al., 2006). It is likely that these essential genes are present in some groups of organisms and absent from others. Some organisms/lineages posses the genes necessary

to cope with polyploidization while others do not, possibly leading to the pattern of phylogenetic clustering of polyploidy across the Tree of Life.

Constraints on the cellular and genomic level are likely to limit polyploidy in some species while allowing others to persist, but what are the ecological and evolutionary consequences of polyploidy within the Corydoradinae? It seems likely and even intuitive that genome duplications would have ecological consequences, as has been shown in plant polyploids that are able to adapt to new niches (Bennett, 1987). However, in the case of the Corydoradinae, ecological benefits associated with genome duplications are difficult to determine and remain unexplored. Overall, there is a tendency for species with larger genomes to occupy higher relative trophic levels, suggesting that perhaps genome duplications do have ecological consequences. However, this observation is confounded as species of *Aspidoras* (a diploid Corydoradinae genus) occupy the same trophic level as polyploid species in lineages 6 and 9. Nevertheless, variation in body size and colour patterns is much greater within polyploid lineages when compared to diploids, both having ecological consequences in terms of resource acquisition and interspecific communication, respectively. It is also possible that polyploid species have a higher metabolism, given that their cells are much larger than diploids, and this may be linked to some extent with resource acquisition at higher relative trophic levels. Body size, which has been shown to be ecologically important in terms of resource acquisition, may also have had evolutionary consequences (Chapter 3), however, species richness cannot be accounted for with this variable alone.

The evolutionary consequences of polyploidy in the Corydoradinae are more evident than ecological consequences. Firstly, there are more species of polyploids than diploids, and ancestral genome duplications coincide with accelerated diversification rates (Chapter 3). Duplications have led to extensive karyotypic variation within polyploid lineages, with multiple chromosomal fissions and fusions that may contribute to reproductive isolation (Oliveira et al., 1992). Furthermore, karyotypic variation is present at the intraspecific level, where allopatric populations of *Corydoras nattereri* have different chromosome complements (Oliveira et al., 1990). Interestingly, a degree of karyotypic variation is also present in diploid lineages, which suggests that chromosomal changes are not restricted

to species with duplicated genomes (Oliveira et al., 1992). Nevertheless, karyotypic variability is much greater within polyploid lineages and has undoubtedly played a significant role in their evolution. Moreover, evidence presented herein suggests that an important causal link between genome duplication and accelerated diversification may be related to the expansion of the pigmentation gene repertoire within polyploids (Chapter 3).

5.3.4 Biogeography & Vicariance

In the case of lineages that have diversified within the Neotropics during the Paleogene and Miocene, such as most Siluriformes and Characiformes, paleogeographic and hydrological transitions during that time period have inevitably shaped some patterns of biogeographic distribution (Lundberg, 1998). However, recent research is revealing that such major transitions cannot account for all observed patterns of distribution, and much of the diversification within the Neotropics may be the result of finer scale processes (Albert and Reis, 2011). This seems to be the case for Corydoradinae catfishes, as our evidence shows that major paleogeographic vicariant processes (such as the separation of the Amazonas basin from the Rio de Plata, Orinoco, and Guyanas) account for a small percentage of the observed speciation events (Chapter 4). Despite the importance of major paleogeographic transitions, Neotropical diversification is highly complex, as illustrated by the evolutionary history of the Corydoradinae, and it is therefore likely that different processes have influenced diversification and distribution in different time periods (ranging from ancient events to very recent ones). Moreover, isolation between basins is rarely complete, as contemporary connections between river systems (particularly during extended rainy seasons) provide opportunities for species to disperse between basins (Winemiller et al., 2008).

Paleoclimatic fluctuations have been invoked to account for many biogeographic patterns within the Neotropics, with particular focus on the effect of glacial cycles and marine incursions (Lundberg et al., 1998). Evidence provided herein suggests that such events did not shape major patterns of Corydoradinae spatiotemporal distribution (Chapter 4). Temporal diversification of Corydoradinae lineages and their distribution into different river systems largely predate Pleistocene glacial events, while there is no evidence to

suggest a significant role for Miocene marine incursions shaping distribution. Furthermore, there is no evidence to support the Fawcett hypothesis (polyploid species have a better chance of surviving major paleoclimatic disturbance via their ability to occupy new niches). Although direct evidence is lacking (as is often the case when trying to establish a causal link between speciation and paleoclimatic events), there may be a link if genome duplications happened during or following a very specific climatic event and if polyploid species could be shown have shifted into new niches created as a result of such an event. However, the morphological divergence in ecologically relevant traits allowing species to occupy separate niches is a transition that is likely to have occurred gradually throughout the evolution of the group, and therefore cannot necessarily be linked to either a specific genome duplication or climatic event.

As discussed above, speciation occurs primarily within basins, and the highest levels of species diversity have been maintained within the Amazon basin from the onset of Corydoradinae diversification (Chapter 4). However, species have dispersed and become isolated within the numerous tributaries of the Amazon river, as well as the Orinoco, Guyanas, Parana, Sao Francisco and East coast of Brazil. The maintenance of ancestral diversity within the Amazon, followed by periodic dispersal into its numerous tributaries and adjacent basins provides support for the Taxon Pulse hypothesis (Erwin, 1979). This general model of historical biogeography involves micro and macro allopatric isolation from an ancestral stable core of diversity, driven by repeated range contractions and expansions (Erwin, 1979). In the context of Taxon pulses, a variety of mechanisms are likely to be driving allopatric and parapatric speciation within Neotropical basins on a fine scale. In the case of the Corydoradinae, field observations combined with molecular phylogenetic results suggest that unique species can be found in different river and stream systems, indicating that isolation occurs between species that occupy very similar and geographically contiguous areas. A recent population genetic analysis of a large migratory catfish (*Pseudoplatystoma corruscans*) has provided evidence of homing behaviour within the La Plata basin in Brazil that contributes to significant population structure (Pereira et al., 2009). Although the life history and geographic range of *Pseudoplatystoma* is very different from *Corydoras*, the latter species also migrate from big rivers to smaller streams for reproduction and thus may represent another case of

catfish homing behaviour. If homing occurs within the Corydoradinae, it might explain why different species are found in adjacent streams of the same larger river system during the dry season (when species reproduce), provided that the microchemistry of different streams is different enough to be detectable.

The dependence of many Corydoradinae species on specific habitats, such as small moderately flowing streams, and the patterns of phylogenetic niche conservatism (where closely related species have the same ecologically relevant morphological traits) observed across lineages suggests that these characteristics are likely to restrict dispersal potential. Restricted dispersal may result from maladaptation to intermediate habitats and niches that the Corydoradinae would have to traverse during a migration, as a result of displacement from their ancestral habitat. Consider a specific population that is well adapted to live in a particular stream and river system, with specific chemistry, resources and predators. If that habitat changes, and the population is displaced (due to extrinsic factors such as climate or changes in hydrology), it will be forced to seek an alternative suitable habitat. In doing so, the population is likely to have to cross deep river channels, fast flowing rapids, dramatic changes in water chemistry, new predators, lack of suitable resources and/or a myriad of hostile conditions to which Corydoradinae catfishes would be maladapted. It may be the case that some populations survive such transitions and are able to colonize new suitable habitats. If not, these conditions could drive local extinction. Therefore, when a population of Corydoradinae is highly adapted to given niche, and such adaptation is conserved within a lineage, the dependence on specific environmental conditions can determine patterns of distribution for that lineage.

Moving outside the context of Corydoradinae biogeography, many fundamental questions remain, primarily concerning large-scale patterns of distribution and diversification in Siluriformes and Characiformes, but also comparing Neotropical fish diversity to other continental freshwater systems. Are there more freshwater fish species in the Neotropics compared to other continents because there is more water volume? Why are the Cypriniformes absent from the Neotropics? Did the ancestral Characiformes outcompete Cypriniformes, thereby restricting them from the South American continent? If so, did this occur before or after the separation of Gondwana? Furthermore, why are

Siluriformes and Characiformes the most diverse and widely distributed freshwater fishes in the Neotropics?

5.3.5 Stable Coexistence & Extinction

The mechanisms of speciation discussed above are primarily focused on allopatric processes. However, as shown in mimetic communities, it is often the case that multiple species of Corydoradinae coexist, and that these communities are ecologically stable over long time periods (Chapter 2). I have already shown and discussed how multiple species share colour patterns in sympatry (through Müllerian mimicry), as well as the mutualistic benefits of co-mimics that share the cost of predator education, but have yet to elaborate on the morphological process of niche differentiation. Ecologically relevant morphological traits, such as snout length and body size, enable different species to partition resources in sympatry, but how and when did they arise? Given that these traits are highly conserved within Corydoradinae lineages, they are most likely features that were present in the most recent common ancestor of each lineage. Therefore, it is very likely that differences in these morphological traits between lineages arose amongst ancestors that are now extinct. Furthermore, these morphological traits (i.e. being short snouted or long snouted) are not habitat specific, as species with both features are sympatric in many cases. Thus, it is unlikely that differences in snout morphology have arisen as a result of adaptation to different environments.

Given that differences in snout morphology appear to enable species to coexist, the origin of these trait differences is interesting. Differences in snout morphology could be explained through ecological speciation in sympatry (Schluter, 2009), or character displacement following multiple colonization of a habitat as has been suggested in the benthic and limnetic forms of sticklebacks (Schluter, 2000). Ecological character displacement results from competition for resources between closely related species where divergent selection leads to morphological differences that enable resource partitioning, and is based on the principle of competitive exclusion (Schluter, 2000). Specifically, ecologically relevant morphological differences between closely related species are pronounced in sympatry, and dissipate in allopatry (Schluter, 2000). As an alternative, ecological speciation is much broader, involving reproductive isolation

between (or within) populations via adaptation to different niches (Rundle and Nosil, 2005; Schluter, 2009). In the case of Corydoradinae ancestors, a number of different scenarios may account for the origins of snout differences. For example: 1.) Ecological character displacement (*sensu* Schluter) occurred between different species of ancestral Corydoradinae multiple times, leading to multiple variations in snout morphology that were subsequently conserved within lineages 2.) Ecological speciation occurred multiple times amongst sympatric ancestors, by divergent natural selection between niches 3.) Trait divergence occurred in allopatry as a result of adaptation to different environments, allowing niche partitioning when allopatric species re-united. Scenario 1 would fit under the classic interpretation of character displacement, whereas scenarios 2 and 3 differ, as they do not require differences in traits between sympatric species that do not occur in allopatry. Displacement of characters between different species may be likely, however, snout morphology of Corydoradinae catfishes does not change in allopatry when compared to sympatric assemblages (which one would expect under a traditional interpretation of character displacement). Furthermore, if two species have the same morphology and compete for resources in sympatry, competitive exclusion might be more likely to occur than trait divergence. Scenario 2 (ecological speciation) is most likely, given that the observed divergence in ecologically relevant morphological traits allows species to coexist by partitioning resources, and can occur in sympatry or allopatry. Scenarios 3 is a parsimonious explanation, because trait divergence can occur in allopatry (where competitive exclusion will be irrelevant), and subsequently contribute to niche partitioning when species are reunited. However, traits such as snout length and body size are not habitat specific, and therefore do not seem to be a product of adaptation to different environments.

The Corydoradinae and Callichthyinae (Callichthyidae: Silurifomres) are sister subfamilies sharing a common ancestor earlier than 100MYA, as estimated using a relaxed molecular clock (Chapter 3). The Callichthyinae are much larger in body size and very different morphologically compared to the Corydoradinae, occupying a higher relative trophic level (as inferred using stable isotope measurements). Fossil evidence of *Corydoras revelatus* (Cockerell, 1925), the presumed ancestor of all extant Corydoradinae, indicates that it was a small bodied species. Thus, the fossil of *C.*

revelatus suggests that major morphological transitions in ecologically relevant morphological traits had already occurred within the Callichthyidae by the Paleogene. Furthermore, an uncatalogued fossil shoal of *C. revelatus* reveals that these species lived in groups very early in their evolution (Reis, 1998b). It seems plausible that Corydoradinae ancestors (such as *C. revelatus*) also competed for resources in the same manner that extant species do. Thus, the separation and subsequent onset of diversification within different lineages may have depended on some form of divergent selection leading to morphological differentiation that would allow niche differentiation and stable coexistence. This would also change the ancestral ecological limits on diversification as species with differences in snout morphology could utilize novel trophic niches (Rabosky, 2009a; Rabosky, 2009b).

Moving back from ancestral to contemporary processes, the vast majority of extant Corydoradinae communities are composed of distantly related species (phylogenetically overdispersed) that differ in morphology and resource acquisition (Chapter 2). From a morphological perspective, sister taxa and/or closely related species (from the same lineage) compete for resources and thereby are likely to suffer from competitive exclusion. The competition for resources between closely related species occurs due to phylogenetic niche conservatism within lineages. Furthermore, species are able to coexist due to genomic and chromosomal differences between lineages maintaining reproductive isolation despite convergence in colour patterns.

5.3.6 What is a Corydoras species?

To some extent, defining a species of Corydoradinae is a philosophical debate that will depend on the choice of species concept. For the purposes of this discussion, I have chosen to use the Biological Species Concept *sensu* Mayr, as groups of interbreeding natural populations that are reproductively isolated from other such groups (Mayr, 1942). However, the boundaries between such groups are not always clear-cut, due to the effects of hybridization and in the case of the Corydoradinae genome duplications and genomic changes. Fundamental questions that would shed light on the origin of Corydoradinae species remain unanswered: Are closely related allopatric species with different colour patterns reproductively isolated via assortative mating? To what extent does genome

duplication, chromosomal rearrangement (fissions and fusions) and reciprocal gene loss contribute to reproductive isolation? Are distantly related species able to hybridize and produce viable offspring? Despite these open questions, much can still be said about the nature of the Corydoradinae radiation and the unique and/or repeated features common to the group.

One striking aspect of colour pattern evolution within the Corydoradinae is the multiple and independent occurrence of colour patterns that are not associated with mimicry rings. Certain colour patterns (both aposematic and cryptic) seem to have independently evolved multiple times in cases where mimicry is not directly implicated, suggesting parallel evolution. In particular, the aposematic and disruptive patterns common to *C. arcuatus*, *C. melini* and *C. brevirostris* have arisen independently in unrelated species of the same and different lineages. Moreover, cryptic patterns found in *C. paleatus, C. diphyes, C. garbei, Aspidoras* spp. and *C. aurofrenatus* have also evolved multiple times. The repeated evolution of colour patterns that are aposematic could be driven by genetic constraints on pigmentation genes or common predators that have learned to avoid patterns of mimetic species. If predators migrate (as both birds and fish do), and retain memory of aposematic mimetic species throughout their geographic range, they are likely to predate more readily on species that are not aposematic. On the other hand, it may be that some these species were once members of mimicry rings that have subsequently been separated from their mimetic congeners are now occupying different river systems. This concords with the hypothesis proposed by Mallet and Dasmahapatra, suggesting that superior competitors associated with multiple mimetic mutualists could benefit if they escape from their ancestral mimetic community (Mallet and Dasmahapatra, 2011). Regardless of the origins of parallel colour pattern evolution, the occurrence of this phenomenon in itself presents a problem for Corydoradinae species descriptions. Traditionally, species have been described based on colour patterns, as DNA sequencing technology has not always been available and morphological differences between species are subtle. The result has been a 'lumping' of species with similar colour, where in actuality they are genetically, morphologically and geographically distinct, and also 'splitting' of geographic variants of single species. Taxonomic issues arise when dealing with co-mimics, where type and paratype material belong to different lineages and have

been placed together solely due to their similarity in colour. Ultimately, this suggests that there are many more Corydoradinae species than current estimates indicate.

Parallel evolution does not only occur with colour patterns in the Corydoradinae, as dwarf species have evolved multiple times in different lineages (*C. pygmaeus*, *C. hastatus*, *C.* sp. '*A. pauciradiatus*', *C.* sp. C144, and *C. habrosus*). Furthermore, similar snout morphology has also evolved multiple times independently, where short snouts are present in Lineages 2, 4, 5, 6, 7, 8 and 9 while long snouts can be found in Lineages 1, 3, and 8. Lineage 8 is of particular interest in this respect, as the most basal sub-clade is composed of long snouted species (*Corydoras* '*Brochis*'), two derived sub-clades of short snouted species (*C. garbei, C. nordestini, C. difluviatilis, C. filamentosus* + *C. pantanalensis, C. sodalis, C. reticulatus, C. geryi*), and the rest of the species (comprising the majority of diversity) have long snouts. This is the only case of within lineage variation in snouts, and despite this variation there are still no cases of sister species which differ in snout morphology. This suggests that phylogenetic niche conservatism is still relevant within Lineage 8, given that ecologically relevant morphological traits are conserved within the different sub-clades. Perhaps due to these examples of parallel evolution in morphology, it has been particularly challenging to identify osteological synapomorphies that could be used to delineate different genetic lineages of Corydoradinae (Britto, 2003). However, sister species never differ in snout morphology, and there is conserved morphological inheritance in snouts within lineages (and even sub-clades of lineage 8). My results indicate that potential synapomorphic features for proposed generic delineation (resulting from the molecular phylogeny) should include snout morphology, eye position, body size and body depth. Colour patterns on their own are uninformative and unsuitable for species descriptions (due to the complexity of Corydoradinae colour pattern evolution), and should only be used for comparison to other closely related species within a molecular phylogenetic framework. Furthermore, geographic ranges need to be accounted for in descriptions (when possible) and taxonomists should try to compare their data with other closely related species that may potential overlap in their distribution.

5.4 Caveat lector

There are various caveats associated with the results and their interpretation within my thesis. I to bring these to the readers attention and discuss them in order of data chapters.

The work conducted on the mimetic relationships of Corydoradinae lacks predation experiments showing these catfish to be truly aposematic. Such experiments were difficult to conduct given the number of different mimetic communities analysed, the number of different colour patterns, and the diversity of predators feeding on these communities in the wild. Although toxicity levels have been quantified for some Corydoradinae species, future work may show that some species are more toxic than others, which may indicate that these mimetic relationships are slightly parasitic (quasi-Batesian) rather than truly mutualistic. Despite such evidence, the fact remains that all species involved in mimetic relationships are well defended, and as such they have been appropriately defined as Müllerian mimics. Colour patterns of Müllerian mimics tend to be conspicuous in order to catch the attention of predators, and yet a variety of patterns common to Corydoradinae co-mimics are seemingly cryptic or disruptive. This issue bring us back to the previous question: are these species truly aposematic? The definition of aposematic coloration is not restricted to bright coloration and may also encompass non-conspicuous patterns that signal unprofitability. Moreover, camouflage and warning coloration are not necessarily mutually exclusive as these mechanisms can act differentially depending on the distance from which a predator attacks. Nevertheless, a quantification of colour patterns using spectral analyses is lacking in this study in order to show the degree of contrast and more accurately describe the diversity of patterns observed. Furthermore, the stable isotope analyses still require calibration using known prey items in order to more accurately describe differences in trophic levels between co-mimics. This should be complemented by stomach contents analyses, which would be useful despite being extremely laborious and time consuming, while offering a temporally limited picture of resource acquisition. However, it is highly unlikely that the conclusions presented herein would be altered significantly in light of stomach contents analyses and environmental isotope data.

The study of diversification rates and genome sizes could benefit greatly from increased taxonomic sampling, as comprehensive phylogenetic coverage is necessary for the accurate estimation of diversification rates. This issue has been dealt with by combining known species richness per lineage with the available phylogenetic framework, however, as new species are being discovered at an alarming rate, and our estimates of net diversification are likely to change. However, as long as species discovery is not biased towards ancestral diploid species at the base of the phylogeny, our findings should not change significantly. Furthermore, because genome sizes were not available for all species (despite our effort to sample as many species as possible) included within the phylogeny, mean values per lineage were used in many cases. As a result, reconstructed values of ancestral genome size are intended as estimates, and are likely to change with further estimations (although again I do not anticipate that this will change overall patterns). In so far as the phylogeny is comprehensive yet still incomplete (as all phylogenies inevitably are), the addition of more taxa may alter inferred patterns of genome size inheritance if additional polyploidy species are discovered. Finally, the lack of a statistical framework capable of distinguishing between two variables that are both significantly related to diversification rate (genome size and body size) makes it difficult to draw concrete conclusions and infer causality. Observed shifts and accelerated diversification rates therefore may be the result of a number of variables. Most importantly, the results clearly support that the observed shift in diversification rate is correlated with increase in genome size, and this in itself has never been demonstrated before.

The analysis of historical biogeography and ancestral area reconstructions of species included within the molecular phylogeny may be confounded by taxonomic sampling. Splits identified between sister species seemingly occupying different basins may end up changing as taxonomic coverage increases. This is because true sister species relationships cannot necessarily be inferred accurately until all extant taxa are included in the phylogeny. However, full taxon coverage is never realistically achievable in a group as species rich as the Corydoradinae, and therefore the results in terms of historical biogeography need to be considered within this context. Furthermore, the use of the dispersal-extinction-cladogenesis model is only one of the many available analytical

methods for the analysis of historical biogeography. It would be useful to compare results between ancestral area reconstruction and area cladograms, as the two different methods may provide support for different hypotheses. Despite the fact that many of the species collected for the phylogeny were from known localities with GPS coordinates, other species used were obtained from the aquarium trade. In latter cases, it was only possible to assign species to specific basins, without detailed information on distribution. Given the scale of basin delineation for the biogeographic analysis, more detailed information on distribution is unlikely to change current results, however, a more detailed analysis based on specific river basins would require such a level of detail.

5.5 Future Work

This thesis has made some small steps forward in answering important questions concerning the origins and maintenance of Corydoradinae biodiversity. However, as patterns emerge, more questions arise. Many issues remain to be investigated, as Corydoradinae catfishes are a new system, providing an excellent opportunity to address some core questions in evolution and ecology. Here I discuss some of these future research directions and methods that could be employed to shed more light on the mechanisms that shape patterns of diversity, complexity and adaptation. The following questions are not listed in order of interest or relative importance, I have simply organized them under relevant categories.

5.5.1 Genomics & Genome Duplication

The advent of next generation sequencing (NGS) has opened new avenues for the investigation of many biological questions, but specifically of interest to the Corydoradinae, the study of genome duplication and pigmentation.

A study on genes associated with pigmentation patterning using a next generation sequencing approach would be of great interest in order to test whether the colour patterns of diploid and polyploid co-mimics have evolved via parallel evolution (i.e. different pigmentation genes leading to the same colour pattern). This approach could be extended to identify paralogous genes (pigment, toxins, armour, MHC etc) post duplication events, their rate of retention or silencing, potential adaptive significance, and their phylogenetic signal. Furthermore, as I have already calibrated the Corydoradinae phylogeny with fossil and paleogeographic data, substitution rates based on relaxed molecular clock estimates could be used to identify the approximate timing of gene duplications, to test the potential adaptive consequences of neo and/or sub-functionalization. Given that complexity in pigmentation (and other morphological and phenotypic traits) has limits, investigating the developmental constraints of colour patterns and how they are controlled by pigmentation pathways may reveal that some lineages are more constrained than others. Moreover, using an evo-devo approach, one could examine the role of the neural crest in ontogenetic changes of colour pattern

throughout larval, juvenile and adult life stages (and the adaptive significance of these changes from an ecological perspective).

As new evidence emerges suggesting that duplications of protein coding genes may contribute less to organismal complexity than previous hypothesized, a comparative analysis of the microRNA repertoire using Northern blotting and genome sequences across Corydoradinae lineages may reveal alternative routes to increased complexity. If such genomic information becomes available, it would also be of great relevance to quantify and classify the percentage of transposable elements and non-coding regions within Corydoradinae genomes, in an attempt to tease apart their potential evolutionary role and fate post duplication. In addition, it would be very useful to estimate genome sizes for more species within the subfamily, potentially making a comparison between flow cytometry and fuelgen image densitometry as alternative methodologies. Another salient question that emerges is whether polyploid species are more resistant to parasites (or whether they make better hosts), an investigation that would start by counting parasite loads in a population and potentially linking resistance or susceptibility to MHC gene families. Comparisons between diploids and polyploids could be interesting from physiological perspective as well, in order to examine whether polyploids have a higher metabolism due to larger cells and what effects this may have on resource partitioning and niche differentiation.

More broadly, it would be worthwhile to investigate whether genome duplication is a by-product of heritable functional plasticity in genes related to spindle fiber formation, chromosome cohesion and homologous recombination, using a comparative framework including diploids and polyploids. As in the case of most potential future investigations proposed above, this too would require the development of extensive genomic resources for multiple species of Corydoradinae.

5.5.2 Behaviour, Mimicry & Speciation

Behavioural experiments were not conducted in this thesis yet would complement existing results and allow further investigation of reproductive isolation among allopatric taxa. Predation experiments in laboratory and field settings using different co-mimics and

a variety of predators (birds and fish) would be of particular interest. This would involve experiments on species with different colour patterns (seemingly cryptic, disruptive and aposematic), and could incorporate an investigation of predator perception at different distances (as patterns that may appear cryptic from a distance could still be aposematic up close). These experiments would shed light on questions such as - are crypsis and aposematism mutually exclusive? Do predators react differently to these patterns based on distance of attack? To what extent are disruptive colours aposematic? Is degree of aposematism related to boldness? Complementing experiments related to mimicry, mate choice trials could be conducted to establish the extent to which changes in colour pattern lead to reproductive isolation through assortative mating, and/or whether species with different patterns (and from different lineages) successfully hybridize.

Müllerian mimicry is not the only route by which sympatric species share colour patterns in the Corydoradinae. Probable Batesian relationships have been identified between Corydoradinae and Loricariidae catfishes (Axenrot & Kullander, 2003) and in mixed shoals of *Corydoras hastatus* and *Serrapinnus kriegi* (along with various other distantly related Characiformes). Furthermore, *Brachyrhamdia* catfishes also share almost identical colour patterns with the Corydoradinae in a number of different sites, and it is not known whether these are Müllerian or Batesian relationships. As Batesian mimicry is considered a parasitic relationship (where the mimic benefits from the defenses of the model), one question that arises is whether Batesian mimics partition resources and how this may affect coexistence. Information is lacking on co-existing species that do not share colour patterns, and how this may be accounted for (imperfect mimicry, convergence in progress, recent contact?).

Since the publication of this research on Müllerian mimicry, three new mimetic groups have come to our attention: *C. guapore, C. caudimaculatus, C. spectabilis* + *C. albolineatus, C. cervinus* both from the Rio Guapore, and *C.* sp. CW51, *C.* sp. CW51 longnose from Columbia. The rate at which new species are being discovered suggests that there are many more Corydoradinae mimicry rings in the neotropics that have yet to be documented. More research is necessary to assemble both taxonomic and distribution information to identify new mimicry rings, and importantly the precise geographic range

of existing ones and where different mimicry rings overlap. If different mimicry rings overlap, why do different mimicry rings retain different patterns as opposed to them all converging on the same pattern? Observations from the field, when sampling the same populations during different seasons, have shown that some Corydoradinae populations are highly mobile and seasonal migrations are related to reproductive behaviour and water level fluctuations. Tracking these migrations in the wild, relative egg and sperm maturation times between different co-existing species and monitoring environmental conditions (water level, temperature, precipitation, pH, sediment load, dissolved oxygen etc) may help reveal fine scale population dynamics and connectivity between large rivers and small streams within the neotropics. Moreover, many catfish are known to be nocturnal, yet the majority of Corydoradinae collected were caught during the day. It is therefore possible that niche differentiation between some co-existing species may be occurring along a temporal axis (diurnal), and not only in terms of resource partitioning. Furthermore, it would be of great relevance to quantify the relative frequency of different co-mimic populations in relation to their predators and resources in the wild, in order to further investigate the relative importance of mimicry and competition. This type of direct field observation would be complemented by a thorough analysis of preferred prey items and stomach contents analyses to identify the basis for trophic differentiation.

Almost all speciation in Corydoradinae catfishes is allopatric or parapatric. Could sympatric speciation have also contributed to some of the observed diversity within the subfamily? One obvious route to sympatric speciation would be via genome duplication, but I have yet to identify sister species in sympatry with different genome sizes and chromosome compliments. However, there is a case of two species coexisting that are most likely sister species yet they differ subtly in snout morphology (which has been identified as an important morphological trait associated with resource acquisition). This case, the two species of Corydoras (*C.* sp. CW34 and *C.* sp. CW35 '*Brochis*') from the Rio Blanco (Rio Negro drainage) in eastern Bolivia may have diverged in sympatry via character displacement (in terms of snout morphologies) and resource partitioning. Furthermore, there is an absence of population level genetic investigations of Corydoradinae catfishes, as all current investigations involved interspecific comparisons. Population dynamics may be very different across lineages due to differences in

population size. For example, species from lineage 1 appear to have smaller population sizes than species from lineage 9. Effective population size (Ne) has profound implications for many processes such as the relative strength of drift and selection, and extinction risk. Moreover, the responses of diploid and polyploid taxa to fluctuations in population size are likely to be very different. A study investigating population dynamics should also consider the frequency dependent relationship between co-mimics, predators and resources, as this remains understudied in Corydoradinae communities.

5.5.3 Systematics, Morphology, & Biogeography

The subfamily Corydoradinae is in need of extensive revision. Currently, molecular phylogenetic evidence confirms that the genus *Corydoras* is not monophyletic, with the Aspidoradini tribe nested between *Corydoras* lineages. The phylogeny presented in this thesis identified seven independent *Corydoras* lineages with consistent morphological differences that should be split into distinct genera. Geometric morphometric measurements confirm that species belonging to different lineages have unique body shape, however, synapomorphies would be beneficial and make assigning species to genera easier for working field biologists. A number of disused generic names are available in the taxonomic literature that could be resurrected for such a revision (see appendix). Identifying osteological synapomorphies in the Corydoradinae is not trivial and has been attempted by several authors (Britto, 2003; Nijssen, 1970; Reis, 1998a) with little success. From the molecular systematic perspective, increasing taxon coverage, and sequencing more nuclear genetic markers will prove very valuable for further resolution of interspecific relationships.

It may be fruitful to deviate from traditional techniques involving laborious dissections and staining, and employ the use of x-ray imaging and 3D models of the skeleton to identify potential synapomorphies. Despite the cost of obtaining the necessary equipment (x-ray micro-CT scanner and 3D imaging software), sample processing would be rapid, allowing the analysis of large numbers of specimens in a shorter time than osteological staining requires. Such information could be mapped onto a molecular phylogenetic framework for comparison, and then used to reconstruct ancestral body shape across internal nodes of the phylogeny. This analysis could be taken further by analyzing fossil

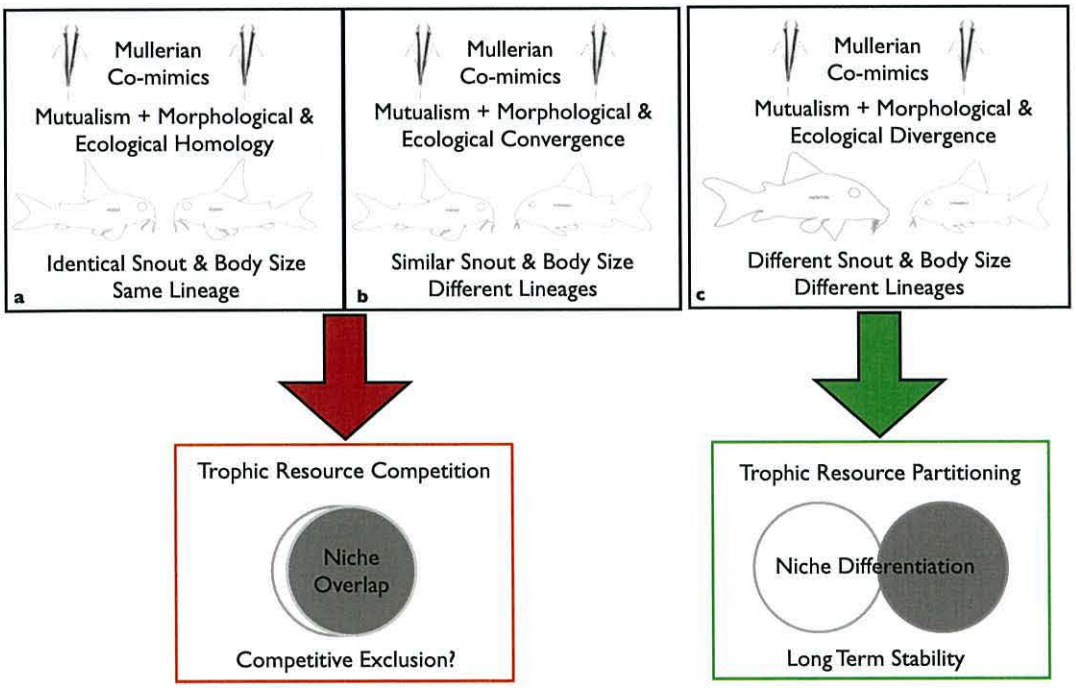
material in a similar manner (or using landmark based techniques) and comparing ancestral reconstructions with actual extinct taxa, potential providing a 'best fit' in order to more accurately identify the phylogenetic position of the fossil along internal phylogenetic nodes. This would greatly improve the accuracy of molecular clock estimations, given the usual uncertainty involved in the placement of fossil calibration points. Many Neotropical river basins remain very poorly sampled if not entirely unexplored. This suggests the diversity of the Corydoradinae may be higher than current estimates. Notably, the Rio Xingu, Rio Tocantins, Rio Tapajos and the inaccessible borders of Brazil and central Peru have barely been sampled. Furthermore, vast stretches of the Ecuadorian Amazon and the Colombian and Venezuelan regions within the Orinoco, undoubtedly also harbor considerable diversity. Despite the difficulty of mounting expeditions in these regions (due to inaccessibility and security concerns), there is a necessity to do so as these regions are under threat and will most certainly yield many new species of *Corydoras*, contributing greatly to our current understanding of patterns of biogeography.

From a biogeographic perspective, more attention should be given to the phenomenon of phylogenetic niche conservatism, as it is likely to have played a major role in the spatiotemporal distribution of many neotropical fish groups, by limiting dispersal between adjacent areas with contrasting environmental conditions, while simultaneously contributing to local extinction events. Further questions arise when one considers patterns of Neotropical diversity in relation to other continents. For example, why is the freshwater ichthyofaunal diversity of the Neotropics greater than southeast Asia and Africa? Why are there more mimetic species in the Neotropics than in other areas? Are allopatric processes capable of generating more diversity than sympatric processes? If more species coexist in given communities, does this lead to more diversity through niche development and interspecific interaction, or less diversity as a result of competition and extinction through competitive exclusion? Furthermore, many interesting parallels exist between the loaches of South East Asia and the armored catfishes of neotropics (in terms of ecological specialization, morphology, and genetics) that have yet to be thoroughly investigated.

5.6 Conclusions

This study has demonstrated that dietary resource partitioning coupled with morphological and phylogenetic differences are more important in determining community structure and assembly than the positive benefits of Müllerian mimicry. Phylogenetically conserved ecologically relevant trait differences, along with mutualistic Müllerian associations, allow stable communities to develop and be maintained (Figure 19). This enables long-term coexistence, as co-mimics are trophically differentiated, reproductively isolated, and able to benefit from the mutual protection of the shared colour pattern. As communities undergo assortment of ecologically and morphologically compatible species, lineages with species able to coexist in this stable manner may experience accelerated rates of diversification, resulting from further reproductive isolation by colour pattern mimicry as new populations become fragmented from their ancestral range. Finally, resource competition is likely to be more important than spatial overlap as a negative competitive force structuring animal communities in cases that do not involve aggressive interspecific territorial defence.

Figure 19- Community Structure



Undoubtedly, the balance between positive and negative interactions in mimetic communities will be affected by the relative frequencies and unprofitability of co-mimics, predator and resource abundance, the degree of habitat heterogeneity and dispersal capacities of the co-mimics. While not explicitly quantified, it is unlikely that these parameters are constant in all communities investigated herein, reinforcing the role of antagonism as the principal determinant of community assembly in Müllerian mimics. Using one of the most celebrated examples of facultative mutualism in animal biology, our results suggest that the long-term ecological sustainability of co-existing mutualists will ultimately depend on their ability to partition resources, and that these dietary differences can be significant on the finest of scales.

Whole genome duplication (WGD) has been hypothesized to play a major role in evolution, as multiple rounds of WGD at the base of vertebrate and plant lineages are thought to have contributed to their subsequent evolutionary success. Nevertheless, the idea that WGD can act as a driver of speciation remains understudied due to a lack of comprehensive phylogenetic, genomic, and taxonomic data. Here I provide the first robust evidence of accelerated diversification rates associated with WGD, using a comprehensive time calibrated molecular phylogeny of the teleost subfamily Corydoradinae combined with genome size data. The results suggest that WGDs accelerate diversification rates, and that a likely mechanism driving this process may be the observed increase in complexity of colour patterns post genome duplication. Evidence also suggests that larger body size is associated with accelerated diversification, however, the distinction between large and small body size in terms of foreground and background lineages is minimal. I also find no evidence to support that divergent resolution has led to an increase in diversification, yet I do not reject the potential evolutionary role that neo and subfunctionalization of duplicated genes may have played.

The analyses of historical biogeography and ancestral area reconstruction have revealed three likely hypotheses accounting for the complex patterns of Corydoradinae spatiotemporal distribution. Notably, results support the Taxon Pulse, Paleogeography/Hydrogeology and Phylogenetic Niche Conservatism hypotheses. Taxon pulses are supported, as the Amazonas lowlands appear to be a stable core biogeographic

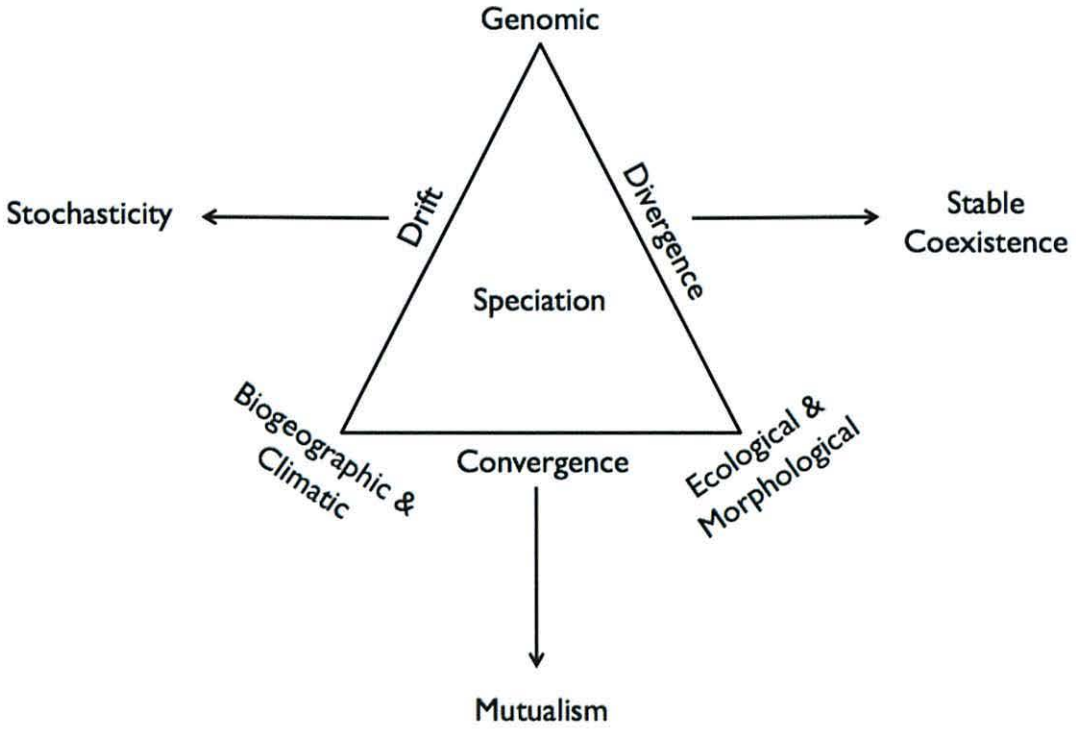
core from which species have dispersed into multiple adjacent and non-adjacent basins. To my knowledge, this is the first time the Taxon Pulse hypothesis has been invoked to account for the historical biogeography of any freshwater vertebrate. Evidence suggests that the Paleogeography/Hydrogeology hypothesis also accounts for some of the observed vicariant events due to boundary displacements between the Amazonas, Parana, Orinoco and other basins. Furthermore, I find that the Phylogenetic Niche Conservatism hypothesis is likely to play an important biogeographic role, as species dispersal is restricted by adaptation to specific niches, and Corydoradinae lineages have been shown to retain conserved ancestral morphological traits associated with resource acquisition and habitat occupancy. Although climatic fluctuations of the Oligocene and Miocene seem to coincide with the time period of major cladogenetic events, there seems to be no causal link between climatic change and speciation, as the evidence is anecdotal. Finally, I identified multiple major ecoregions with high levels of species richness and genetic diversity throughout the known range of the Corydoradinae that are currently under threat from anthropogenic disturbance.

5.6.1 A Synthesis of Corydoradinae Diversification

Throughout the process of Corydoradinae diversification, neutral processes acted at all temporal stages as a constant background, generating minor variation. Major morphological divergence via divergent selection between niches in allopatry or sympatry (leading to different snouted species with varying body size), occurred at the onset of diversification leading to the separation of different lineages. This allowed resource partitioning and niche differentiation in sympatry, leading to ecological stability within Corydoradinae communities. Ecologically important morphological traits were subsequently conserved within most lineages. Genome duplication occurred, causing a shift in diversification rates, as duplicate genes led to new function in adaptive traits (such as colour patterns, toxins and armour). Genome duplications, changes in morphospace and major lineage separations occurred during time periods of major climatic fluctuation. Furthermore, climate change may have triggered these duplications, leading to extinction during warm climatic periods and speciation during cool climatic periods. Subsequent changes in genome sizes and chromosome complements between

species throughout Corydoradinae diversification contribute further to reproductive isolation. Different species become isolated due to large-scale paleogeographic and hydrological changes, and small-scale isolation within basins, while maintaining a stable presence within core Amazonian region. Dispersal is limited by the availability of niches to which the Corydoradinae are already well adapted, allowing some species to colonize new habitats while others go extinct. Species with sufficient morphological and genomic differences coexist successfully, while competitive exclusion acts on unsuccessful competitors leading to local extinction. Mimetic interactions within communities arise leading to mutualism. Multiple colour patterns develop amongst allopatric mimicry rings leading to further reproductive isolation, while genome duplication continues within some lineages fueling further diversification (Figure 20).

Figure 20- Speciation & Coexistence



Sources Cited:

Abell, R., J. D. Allan, and B. Lehner. 2007. Unlocking the potential of protected areas for freshwaters. Biological Conservation 134:48-63.

Abell, R., M. L. Thieme, C. Revenga, M. Bryer, M. Kottelat, et al. 2008. Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. Bioscience 58:403-414.

Agostinho, A. A., S. M. Thomaz, and L. C. Gomes. 2005. Conservation of the biodiversity of Brazil's inland waters. Conservation Biology 19:646-652.

Albert, J. S., and R. E. Reis. 2011. Historical biogeography of neotropical freshwater fishes. University of California Press, San Francisco.

Alevizon, W. S. 1976. Mixed schooling and its possible significance in a tropical Western Atlantic parrot-fish and surgeon-fish. Copeia:796-798.

Alexandrou, M. A., C. Oliveira, M. Maillard, R. A. McGill, J. Newton, S. Creer, and M. I. Taylor. 2011. Competition and phylogeny determine community structure in Mullerian co-mimics. Nature 469:84-8.

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proceedings of the National Academy of Sciences of the United States of America 106:13410-13414.

Allen, J. A. 1988. Frequency-Dependent Selection by Predators. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 319:485-503.

Almany, G. R., L. F. Peacock, C. Syms, M. I. McCormick, and G. P. Jones. 2007. Predators target rare prey in coral reef fish assemblages. Oecologia 152:751-761.

Amores, A., A. Force, Y. L. Yan, L. Joly, C. Amemiya, A. Fritz, R. K. Ho, J. Langeland, V. Prince, Y. L. Wang, M. Westerfield, M. Ekker, and J. H. Postlethwait. 1998. Zebrafish hox clusters and vertebrate genome evolution. Science 282:1711-1714.

Antonelli, A., A. Quijada-Mascarenas, A. J. Crawford, J. M. Bates, P. M. Velazco, and W. Wuster. 2010. Molecular studies and phylogeography of Amazonian tetrapods and their relation to geological and climatic models. *In* Amazonia: lanscape and species evolution (C. a. W. F. Hoorn, ed.) Wiley-Blackwell.

Armbruster, J. W., and L. M. Page. 1996. Convergence of a cryptic saddle pattern in benthic freshwater fishes. Environmental Biology of Fishes 45:249-257.

Axenrot, T. E., and S. O. Kullander. 2003. *Corydoras diphyes* (Siluriformes: Callichthyidae) and *Otocinclus mimulus* (Siluriformes: Loricariidae), two new species of catfishes from Paraguay, a case of mimetic association. Ichthyological Exploration of Freshwaters 14:249-272.

Ayres, J. M., and T. H. Cluttonbrock. 1992. River Boundaries and Species Range Size in Amazonian Primates. American Naturalist 140:531-537.

Barbosa, A., L. Litman, and R. T. Hanlon. 2008. Changeable cuttlefish camouflage is influenced by horizontal and vertical aspects of the visual background. Journal of Comparative Physiology a-Neuroethology Sensory Neural and Behavioral Physiology 194:405-413.

Barnes, C., S. Jennings, N. V. C. Polunin, and J. E. Lancaster. 2008. The importance of quantifying inherent variability when interpreting stable isotope field data. Oecologia 155:227-235.

Barthem, R. B., M. C. L. Ribeiro, and M. Petrere. 1991. Life Strategies of Some Long-Distance Migratory Catfish in Relation to Hydroelectric Dams in the Amazon Basin. Biological Conservation 55:339-345.

Bates, H. W. 1862. Contributions to an insect fauna of the Amazon Valley. Lepidoptera: Heliconidae. Transactions of the Entomological Society 23:495-566.

Baxter, S. W., R. Papa, N. Chamberlain, S. J. Humphray, M. Joron, et al. 2008. Convergent evolution in the genetic basis of Mullerian mimicry in heliconius butterflies. Genetics 180:1567-77.

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, and H. Macleod. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. Journal of Animal Ecology 73:1007-1012.

Bearhop, S., S. Waldron, S. C. Votier, and R. W. Furness. 2002. Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. Physiological and Biochemical Zoology 75:451-458.

Beccaloni, G. W. 1997. Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host-plant height. Biological Journal of the Linnean Society 62:313-341.

Bennett, M. D. 1987. Variation in genomic form in plants and its ecological implications. New Phytologist 106:177-200.

Bermingham, E., and A. P. Martin. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. Molecular Ecology 7:499-517.

Blanc, G., and K. H. Wolfe. 2004. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell 16:1667-1678.

Bojsen, B. H., and R. Barriga. 2002. Effects of deforestation on fish community structure in Ecuadorian Amazon streams. Freshwater Biology 47:2246-2260.

Bonaparte, C. L. 1838. A new systematic arrangement of vertebrate animals. Transactions of the LInnean Society of London 18:247-304.

Bond, A. B., and A. C. Kamil. 2002. Visual predators select for crypticity and polymorphism in virtual prey. Nature 415:609-613.

Bonnet, E., and Y. Van de Peer. 2002. zt: a software tool for simple and partial Mantel tests. Journal of Statistical Software 7:1-12.

Braasch, I., F. Brunet, J. N. Volff, and M. Schartl. 2009. Pigmentation Pathway Evolution after Whole-Genome Duplication in Fish. Genome Biology and Evolution 1:479-493.

Bridle, J. R., and C. D. Jiggins. 2000. Adaptive dynamics: is speciation too easy? Trends in Ecology & Evolution 15:225-226.

Britto, M. R. 2003. Phylogeny of the subfamily Corydoradinae Hoedeman, 1952 (Siluriformes : Callichthyidae), with a definition of its genera. Proceedings of the Academy of Natural Sciences of Philadelphia 153:119-154.

Brooker, R. W., F. T. Maestre, R. M. Callaway, C. L. Lortie, L. A. Cavieres, et al. 2008. Facilitation in plant communities: the past, the present, and the future. Journal of Ecology 96:18-34.

Brower, A. V. Z. 1996. Parallel race formation and the evolution of mimicry in *Heliconius* butterflies: A phylogenetic hypothesis from mitochondrial DNA sequences. Evolution 50:195-221.

Brown, W. L., and E. O. Wilson. 1956. Character Displacement. Systematic Zoology 5:49-64.

Caley, M. J., and D. Schluter. 2003. Predators favour mimicry in a tropical reef fish. Proceedings of the Royal Society of London Series B-Biological Sciences 270:667-672.

Callaway, R. M. 1995. Positive interactions among plants. Botanical Review 61:306-349.

Cardoso, Y. P., and J. I. Montoya-Burgos. 2009. Unexpected diversity in the catfish *Pseudancistrus brevispinis* reveals dispersal routes in a Neotropical center of endemism: the Guyanas Region. Molecular Ecology 18:947-964.

Cavender-Bares, J., K. H. Kozak, P. V. A. Fine, and S. W. Kembel. 2009. The merging of community ecology and phylogenetic biology. Ecology Letters 12:693-715.

Chan, K. M. A., and B. R. Moore. 2005. SymmeTREE: whole-tree analysis of differential diversification rates. Bioinformatics 21:1709-1710.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31:343-+.

Chiachio, M. C., C. Oliveira, and J. I. Montoya-Burgos. 2008. Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). Molecular Phylogenetics and Evolution 49:606-617.

Chouteau, M., K. Summers, V. Morales, and B. Angers. 2011. Advergence in Müllerian mimicry: the case of the poison dart frogs of Northern Peru revisited. Biol Lett.

Clabaut, C., P. M. E. Bunje, W. Salzburger, and A. Meyer. 2007. Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. Evolution 61:560-578.

Coburn, M. M., and P. G. Grubach. 1998. Ontogeny of the Weberian apparatus in the armored catfish *Corydoras paleatus* (Siluriformes: Callichthyidae). Copeia 1998:301-311.

Cockerell, T. D. 1925. A Fossil Fish of the Family Callichthyidae. Science 62:397-8.

Colinvaux, P. A. 1993. Pleistocene biogeography and diversity in tropical forest of South America. *In* Biological relationships between Africa and South America (P. Goldblatt, ed.) Yale University Press, New Haven.

Cope, E. D. 1872. On the fishes of the Ampiyacu River. Proceedings of the Academy of Natural Sciences of Philadelphia 23:250-294.

Cott, H. B. 1940. Adaptive coloration in animals. Methuen & Co, London.

Counterman, B. A., F. Araujo-Perez, H. M. Hines, S. W. Baxter, C. M. Morrison, et al. 2010. Genomic hotspots for adaptation: the population genetics of Müllerian mimicry in *Heliconius erato*. Plos Genetics 6:e1000796.

Crisp, M. D., S. A. Trewick, and L. G. Cook. 2011. Hypothesis testing in biogeography. Trends in Ecology & Evolution 26:66-72.

Crow, K. D., and G. P. Wagner. 2006. What is the role of genome duplication in the evolution of complexity and diversity? Molecular Biology and Evolution 23:887-892.

Cuthill, I. C., M. Stevens, J. Sheppard, T. Maddocks, C. A. Parraga, and T. S. Troscianko. 2005. Disruptive coloration and background pattern matching. Nature 434:72-74.

Cuthill, I. C., M. Stevens, A. M. M. Windsor, and H. J. Walker. 2006. The effects of pattern symmetry on detection of disruptive and background-matching coloration. Behavioral Ecology 17:828-832.

Dalerum, F., and A. Angerbjorn. 2005. Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144:647-658.

Darst, C. R., and M. E. Cummings. 2006. Predator learning favours mimicry of a less-toxic model in poison frogs. Nature 440:208-211.

Darst, C. R., P. A. Menendez-Guerrero, L. A. Coloma, and D. C. Cannatella. 2005. Evolution of dietary specialization and chemical defense in poison frogs (Dendrobatidae): A comparative analysis. American Naturalist 165:56-69.

Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. J. Murray, London.

David, L., S. Blum, M. W. Feldman, U. Lavi, and J. Hillel. 2003. Recent duplication of the, common carp (*Cyprinus carpio* L.) genome as revealed by analyses of microsatellite loci. Molecular Biology and Evolution 20:1425-1434.

Dinerstein, E., M. D. Olson, D. J. Graham, A. L. Webster, S. A. Primm, M. P. Bookbinder, and G. Ledec. 1995. Una evaluacion del estado de conservacion de las ecoregions terrestres de America Latina y el Caribe. World Wildlife Foundation and World Bank, Washington, D.C.

Dobzhansky, T. 1964. Biology, Molecular and Organismic. American Zoologist 4:443-452.

Drummond AJ, A. B., Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A. 2009. Geneious v4.7

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7.

Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. I. Kawabata, D. J. Knowler, C. Leveque, R. J. Naiman, A. H. Prieur-Richard, D. Soto, M. L. J. Stiassny, and C. A. Sullivan. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182.

Dumbacher, J. P., and R. C. Fleischer. 2001. Phylogenetic evidence for colour pattern convergence in toxic pitohuis: Mullerian mimicry in birds? Proceedings of the Royal Society of London Series B-Biological Sciences 268:1971-1976.

Dunham, R. A., D. P. Philipp, and G. S. Whitt. 1980. Levels of Duplicate Gene-Expression in Armored Catfishes. Journal of Heredity 71:248-252.

Edgar, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5:1-19.

Elias, M., Z. Gompert, C. Jiggins, and K. Willmott. 2008. Mutualistic interactions drive ecological niche convergence in a diverse butterfly community. PLoS Biology 6.

Elias, M., Z. Gompert, K. Willmott, and C. Jiggins. 2009. Phylogenetic community ecology needs to take positive interactions into account: Insights from colourful butterflies. Communicative & Integrative Biology 2:113-6.

Emerson, B. C., and R. G. Gillespie. 2008. Phylogenetic analysis of community assembly and structure over space and time. Trends in Ecology & Evolution 23:619-630.

Endler, J. A. 1980. Natural Selection on Color Patterns in *Poecilia reticulata*. Evolution 34:76-91.

Endler, J. A. 1981. An Overview of the Relationships between Mimicry and Crypsis. Biological Journal of the Linnean Society 16:25-31.

Endler, J. A. 1982a. Alternative hypotheses in Biogeography- A synopsis of the symposium. American Zoologist 22:349-354.

Endler, J. A. 1982b. Convergent and Divergent Effects of Natural Selection on Color Patterns in two Fish Faunas. Evolution 36:178-188.

Endler, J. A. 2006. Commentary - Disruptive and cryptic coloration. Proceedings of the Royal Society B-Biological Sciences 273:2425-2426.

Endler, J. A., and J. Mappes. 2004. Predator mixes and the conspicuousness of aposematic signals. American Naturalist 163:532-547.

Endler, J. A., and M. Thery. 1996. Interacting effects of lek placement, display behavior, ambient light, and color patterns in three neotropical forest-dwelling birds. American Naturalist 148:421-452.

Endler, J. A., D. A. Westcott, J. R. Madden, and T. Robson. 2005. Animal visual systems and the evolution of color patterns: Sensory processing illuminates signal evolution. Evolution 59:1795-1818.

Eric, G., E. Moodie, P. F. Moodie, and T. E. Reimchen. 2007. Stable isotope niche differentiation in sticklebacks with symmetric and asymmetric pectoral fins. Biological Journal of the Linnean Society 92:617-623.

Erwin, T. L. 1979. Thoughts on the evolutionary history of ground beetles: hypotheses generated from comparative faunal analyses of lowland forest sites in temperate and tropical regions. Pages 539-592 *in* Carabid beetles: their evolution, natural history, and classification (T. L. Erwin, G. E. Ball, and D. R. Whitehead, eds.). Dr. W. Junk, The Hague.

Esguicero, A. L. H., and M. Arcifa. 2010. Fragmentation of a Neotropical migratory fish population by a century-old dam. Hydrobiologia 638:41-53.

Fawcett, J. A., S. Maere, and Y. Van de Peer. 2009. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event.

Proceedings of the National Academy of Sciences of the United States of America 106:5737-5742.

Fearnside, P. F. 2006a. Containing destruction from Brazil's Amazon highways: now is the time to give weight to the environment in decision-making. Environmental Conservation 33:181-183.

Fearnside, P. M. 2006b. Dams in the Amazon: Belo Monte and Brazil's hydroelectric development of the Xingu River basin. Environmental Management 38:16-27.

Fenerich, P. C., F. Foresti, and C. Oliveira. 2004. Nuclear DNA content in 20 species of Siluriformes (Teleostei : Ostariophysi) from the Neotropical region. Genetics and Molecular Biology 27:350-354.

Ferraris, C. J. 2007. Checklist of catfishes, recent and fossil (Osteichthyes : Siluriformes), and catalogue of siluriform primary types. Zootaxa:1-548.

Ferris, S. D., and G. S. Whitt. 1979. Evolution of the differential regulation of duplicate genes after polyploidization. Journal of Molecular Evolution 12:267-317.

FitzJohn, R. G. 2010. Quantitative Traits and Diversification. Systematic Biology 59:619-633.

FitzJohn, R. G. 2011. Diversitree: Comparative phylogenetic analysis of diversification in R, version 0.6-3.

Force, A., M. Lynch, F. B. Pickett, A. Amores, Y. L. Yan, and J. Postlethwait. 1999. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 151:1531-1545.

Foresti, F., L. F. Almeida Toledo, and S. A. Toledo Filho. 1989. Supernumerary chromosome system, C-banding pattern characterization and multiple nucleolus organizer regions in Moenkhausia sanctaefilomenae (Pisces, Characidae). Genetica 79:107-114.

Fox, C. W., Roff D. A., and Fairbairn D. J. 2001. Evolutionary Ecology: Concepts and case studies. Oxford University Press, Oxford, UK.

Franks, D. W., and J. Noble. 2004. Warning signals and predator-prey coevolution. Proceedings of the Royal Society of London Series B-Biological Sciences 271:1859-1865.

Fuller, I. A., and H.-G. Evers. 2005. Identifying Corydoradinae Catfish. Ian Fuller Enterprises, Kidderminster.

Furlong, R. F., and P. W. H. Holland. 2004. Polyploidy in vertebrate ancestry: Ohno and beyond. Biological Journal of the Linnean Society 82:425-430.

Galbreath, P. F., and G. H. Thorgaard. 1995. Saltwater performance of all-female triploid Atlantic salmon. Aquaculture 138:77-85.

Genner, M. J., S. J. Hawkins, and G. F. Turner. 2003. Isotopic change throughout the life history of a Lake Malawi cichlid fish. Journal of Fish Biology 62:907-917.

Genner, M. J., G. F. Turner, S. Barker, and S. J. Hawkins. 1999. Niche segregation among Lake Malawi cichlid fishes? Evidence from stable isotope signatures. Ecology Letters 2:185-190.

Godin, J.-G. 1986. Antipredator function of shoaling in teleost fishes: a selective review. Nat Can 113:241-250.

Godinho, A. L., and B. Kynard. 2009. Migratory Fishes of Brazil: Life History and Fish Passage Needs. River Research and Applications 25:702-712.

Gosline, W. A. 1940. A revision of the Neotropical catfishes of the family Callichthyidae. Stanford Ichthyological Bulletin 2:1-36.

Grant, P. R., and B. R. Grant. 2008. How and Why Species Multiply: The Radiation of Darwin's Finches. Princeton University Press, Princeton.

Gregory, T. R., and J. D. S. Witt. 2008. Population size and genome size in fishes: a closer look. Genome 51:309-313.

Greven, H., T. Flasbeck, and D. Passia. 2006. Axillary glands in the armoured catfish *Corydoras aeneus* (Callichthyidae, Siluriformes). Verhandlungen der Gesellschaft für Ichthyologie Band 6:65-69.

Gross, K. 2008. Positive interactions among competitors can produce species-rich communities. Ecology Letters 11:929-936.

Groves, C. R., D. B. Jensen, L. L. Valutis, K. H. Redford, M. L. Shaffer, J. M. Scott, J. V. Baumgartner, J. V. Higgins, M. W. Beck, and M. G. Anderson. 2002. Planning for biodiversity conservation: Putting conservation science into practice. Bioscience 52:499-512.

Guase, G. F. 1934. The struggle for existence. Williams and Wilkins, Baltimore.

Haffer, J. 1969. Speciation in Amazonian Forest Birds. Science 165:131-&.

Haffer, J. 1997. Alternative models of vertebrate speciation in Amazonia: An overview. Biodiversity and Conservation 6:451-476.

Halas, D., D. Zamparo, and D. R. Brooks. 2005. A historical biogeographical protocol for studying biotic diversification by taxon pulses. Journal of Biogeography 32:249-260.

Hall, J. P. W., and D. J. Harvey. 2002. The phylogeography of Amazonia revisited: New evidence from riodinid butterflies. Evolution 56:1489-1497.

Hanlon, R. T., L. A. Conroy, and J. W. Forsythe. 2008. Mimicry and foraging behaviour of two tropical sand-flat octopus species off North Sulawesi, Indonesia. Biological Journal of the Linnean Society 93:23-38.

Hanlon, R. T., and J. B. Messenger. 1988. Adaptive coloration in young cuttlefish (Sepia officinalis L) - the morphology and development of body patterns and their relation to behavior. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 320:437-&.

Hardie, D. C., T. R. Gregory, and P. D. N. Hebert. 2002. From pixels to picograms: A beginners' guide to genome quantification by Feulgen image analysis densitometry. Journal of Histochemistry & Cytochemistry 50:735-749.

Hardman, M., and L. M. Hardman. 2008. The relative importance of body size and paleoclimatic change as explanatory variables influencing lineage diversification rate: An evolutionary analysis of bullhead catfishes (Siluriformes : Ictaluridae). Systematic Biology 57:116-130.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129-131.

Harrod, C., J. Grey, T. K. McCarthy, and M. Morrissey. 2005. Stable isotope analyses provide new insights into ecological plasticity in a mixohaline population of European eel. Oecologia 144:673-683.

Harzhauser, M., A. Kroh, O. Mandic, W. E. Piller, U. Gohlich, M. Reuter, and B. Berning. 2007. Biogeographic responses to geodynamics: A key study all around the Oligo-Miocene Tethyan Seaway. Zoologischer Anzeiger 246:241-256.

Heimberg, A. M., L. F. Sempere, V. N. Moy, P. C. J. Donoghue, and K. J. Peterson. 2008. MicroRNAs and the advent of vertebrate morphological complexity. Proceedings of the National Academy of Sciences of the United States of America 105:2946-2950.

Hinegardner, R. 1968. Evolution of Cellular DNA Content in Teleost Fishes. American Naturalist 102:517-&.

Hinegardner, R., and D. E. Rosen. 1972. Cellular DNA content and evolution of Teleostean fishes. American Naturalist 106:621-644.

Hoedeman, J. J. 1952. Notes on the ichthyology of Surinam (Dutch Guiana). The catfish genera *Hoplosternum* and *Callichthys*, with key to the genera and groups of the family Callichthyidae. Beaufortia 12:1-12.

Hoegg, S., H. Brinkmann, J. S. Taylor, and A. Meyer. 2004. Phylogenetic timing of the fish-specific genome duplication correlates with the diversification of teleost fish. Journal of Molecular Evolution 59:190-203.

Hoorn, C. 1993. Marine Incursions and the Influence of Andean Tectonics on the Miocene Depositional History of Northwestern Amazonia - Results of a Palynostratigraphic Study. Palaeogeography Palaeoclimatology Palaeoecology 105:267-309.

Hoorn, C., F. P. Wesselingh, H. ter Steege, M. A. Bermudez, A. Mora, J. Sevink, et al. 2010. Amazonia Through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. Science 330:927-931.

Hubert, N., F. Duponchelle, J. Nunez, C. Garcia-Davila, D. Paugy, and J. F. Renno. 2007. Phylogeography of the piranha genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the Neotropical ichthyofauna. Molecular Ecology 16:2115-2136.

Hubert, N., and J. F. Renno. 2006. Historical biogeography of South American freshwater fishes. Journal of Biogeography 33:1414-1436.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754-755.

Hufton, A. L., and G. Panopoulou. 2009. Polyploidy and genome restructuring: a variety of outcomes. Curr Opin Genet Dev 19:600-6.

Hutchinson, G. E. 1947. A Note on the Theory of Competition between 2 Social Species. Ecology 28:319-321.

Hutchinson, J. J., and C. N. Trueman. 2006. Stable isotope analyses of collagen in fish scales: limitations set by scale architecture. Journal of Fish Biology 69:1874-1880.

Huysentruyt, F., and D. Adriaens. 2005. Adhesive structures in the eggs of Corydoras aeneus (Gill, 1858; Callichthyidae). Journal of Fish Biology 66:871-876.

Ihering, R. 1907. Diversas espécies novas de peixes nematognathas do Brasil. Notas prelim Museum Paulista 1:13-39.

Isbrucker, I. J. H. 2001. Catalogue of genera and species of Corydoradinae Hoedeman, 1952 (Teleostei, Ostariophysi, Callichthyidae), including type localities, type specimens, and etymology. *In* Breeding Corydoradinae Catfishes (I. A. Fuller, ed.) Ian Fuller Enterprises, Worcestershire.

Jardine, T. D., D. F. Cartwright, J. P. Dietrich, and R. A. Cunjak. 2005. Resource use by salmonids in riverine, lacustrine and marine environments: Evidence from stable isotope analysis. Environmental Biology of Fishes 73:309-319.

Jiggins, C. D., C. Estrada, and A. Rodrigues. 2004. Mimicry and the evolution of premating isolation in *Heliconius melpomene* Linnaeus. Journal of Evolutionary Biology 17:680-691.

Jiggins, C. D., R. Mallarino, K. R. Willmott, and E. Bermingham. 2006. The phylogenetic pattern of speciation and wing pattern change in neotropical *Ithomia* butterflies (Lepidoptera : Nymphalidae). Evolution 60:1454-1466.

Jiggins, C. D., R. E. Naisbit, R. L. Coe, and J. Mallet. 2001. Reproductive isolation caused by colour pattern mimicry. Nature 411:302-305.

Johnson, K. R., J. E. Wright, and B. May. 1987. Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics 116:579-591.

Johnson, M. T. J., and J. R. Stinchcombe. 2007. An emerging synthesis between community ecology and evolutionary biology. Trends in Ecology & Evolution 22:250-257.

Jones, D. A., M. Rothschild, and J. Parsons. 1962. Release of hydrocyanic acid from crushed tissues of all stages in life-cycle of species of Zygaeninae (Lepidoptera). Nature 193:52-&.

Jones, R. N., and H. Rees. 1982. B Chromosomes. Academic Press, New York.

Joron, M., and J. L. Mallet. 1998. Diversity in mimicry: paradox or paradigm? Trends Ecol Evol 13:461-6.

Joron, M., R. Papa, M. Beltran, N. Chamberlain, J. Mavarez, et al. 2006. A conserved supergene locus controls colour pattern diversity in *Heliconius* butterflies. PLoS Biology 4:1831-1840.

Kaatz, I. M., and P. S. Lobel. 1999. Acoustic behavior and reproduction in five species of *Corydoras* catfishes (Callichthyidae). Biological Bulletin 197:241-242.

Kaatz, I. M., and P. S. Lobel. 2001. Intra- and interspecific differences in sound production in the neotropical catfish genus *Corydoras* (Callichthyidae). American Zoologist 41:1489-1489.

Kiehl, E., C. Rieger, and H. Greven. 2006. Axillary gland secretions contribute to the stress-induced discharge of a bactericidal substance in *Corydoras sterbai* (Callichthyidae, Siluriformes). Verhandlungen der Gesellschaft für Ichthyologie Band 6:111-115.

Kodandaramaiah, U. 2010. Use of dispersal-vicariance analysis in biogeography - a critique. Journal of Biogeography 37:3-11.

Kohda, M., M. Tanimura, M. Kikuenakamura, and S. Yamagishi. 1995. Sperm drinking by female catfishes - a novel mode of insemination. Environmental Biology of Fishes 42:1-6.

Kohda, M., K. Yonebayashi, M. Nakamura, N. Ohnishi, S. Seki, D. Takahashi, and T. Takeyama. 2002. Male reproductive success in a promiscuous armoured catfish *Corydoras aeneus* (Callichthyidae). Environmental Biology of Fishes 63:281-287.

Kovach, W. L. 1999. MVSP—A MultiVariate Statistical Package for Windows, version 3.1. Kovach Computing Services.

Kozak, K. H., C. H. Graham, and J. J. Wiens. 2008. Integrating GIS-based environmental data into evolutionary biology. Trends in Ecology & Evolution 23:141-148.

Kozak, K. H., and J. J. Wiens. 2010. Niche Conservatism Drives Elevational Diversity Patterns in Appalachian Salamanders. American Naturalist 176:40-54.

Kraaijeveld, K. 2010. Genome Size and Species Diversification. Evolutionary Biology 37:227-233.

Krakauer, D. C. 1995. Groups confuse predators by exploiting perceptual bottlenecks - a connectionist model of the confusion effect. Behavioral Ecology and Sociobiology 36:421-429.

Kramer, D. L., and M. McClure. 1981. The transit cost of aerial respiration in the catfish *Corydoras aeneus* (Callichthyidae). Physiological Zoology 54:189-194.

Krause, J., and G. D. Ruxton. 2002. Living in groups. Living in groups:i-xiv, 1-210.

Lacépède, B. G. E. 1803. Histoire naturelle des poissons. P. Plassan, Paris.

Ladich, F. 2000. Acoustic communication and the evolution of hearing in fishes. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 355:1285-1288.

Landeau, L., and J. Terborgh. 1986. Oddity and the confusion effect in predation. Animal Behaviour 34:1372-1380.

Le Comber, S. C., and C. Smith. 2004. Polyploidy in fishes: patterns and processes. Biological Journal of the Linnean Society 82:431-442.

Leggatt, R. A., and G. K. Iwama. 2003. Occurrence of polyploidy in the fishes. Reviews in Fish Biology and Fisheries 13:237-246.

Lemey, P., A. Rambaut, A. J. Drummond, and M. A. Suchard. 2009. Bayesian Phylogeography Finds Its Roots. Plos Computational Biology 5:-.

Lindstrom, L., R. V. Alatalo, A. Lyytinen, and J. Mappes. 2001. Strong antiapostatic selection against novel rare aposematic prey. Proceedings of the National Academy of Sciences of the United States of America 98:9181-9184.

Linnaeus, C. 1758. Systema Naturae. Holmia.

Losos, J. B., and D. Schluter. 2000. Analysis of an evolutionary species-area relationship. Nature 408:847-850.

Lovejoy, N. R., J. S. Albert, and W. G. R. Crampton. 2006. Miocene marine incursions and marine/freshwater transitions: Evidence from Neotropical fishes. Journal of South American Earth Sciences 21:5-13.

Lovejoy, N. R., and M. L. G. de Araujo. 2000. Molecular systematics, biogeography and population structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. Molecular Ecology 9:259-268.

Lovejoy, N. R., S. C. Willis, and J. S. Albert. 2010. Molecular signatures of Neogene biogeographic events in the Amazon fish fauna. *In* Amazonia, Landscape and Species Evolution: A Look into the Past (C. M. a. W. Hoorn, F. P., ed.) Blackwell Publishing, London, UK.

Loytynoja, A., and M. C. Milinkovitch. 2001. SOAP, cleaning multiple alignments from unstable blocks. Bioinformatics 17:573- 574.

Ludwig, A., N. M. Belfiore, C. Pitra, V. Svirsky, and I. Jenneckens. 2001. Genome duplication events and functional reduction of ploidy levels in sturgeon (Acipenser, Huso and Scaphirhynchus). Genetics 158:1203-1215.

Lundberg, J. G. 1997. Freshwater fishes and their paleobiotic implications. Pages 67-91. *In* Vertebrate paleontology in the Neotropics: The Miocene fauna of La Venta, Colombia (R. F. Kay, R. H. Madden, R. L. Cifelli, and J. J. Flynn, eds.). Smithsonian Institute Press, London.

Lundberg, J. G. 1998. The temporal context for the diversification of neotropical fishes. *In* Phylogeny and classification of neotropical fishes (R. R. E. Malabarba L.R., Vari R.P., Lucena Z.M.S., and Lucena C.A.S., ed.) Editoria Universitaria, Porto Allegre, Brasil.

Lundberg, J. G., L. G. Marshall, J. Guerrero, M. C. Malabarba, and F. Wesselingh. 1998. The stage for neotropical diversification: a history of tropical South American rivers. *In* Phylogeny and classification of neotropical fishes (R. R. E. Malabarba L.R., Vari R.P., Lucena Z.M.S., and Lucena C.A.S., ed.) Editoria Universitaria, Porto Allegre, Brasil.

Lundberg, J. G., J. P. Sullivan, R. Rodiles-Hernandez, and D. A. Hendrickson. 2007. Discovery of African roots for the Mesoamerican Chiapas catfish, *Lacantunia enigmatica*, requires an ancient intercontinental passage. Proceedings of the Academy of Natural Sciences of Philadelphia 156:39-53.

Luo, J., P. F. Stadler, S. P. He, and A. Meyer. 2007. PCR survey of Hox genes in the goldfish *Carassius auratus auratus*. Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 308B:250-258.

Luz-Agostinho, K. D. G., A. A. Agostinho, L. C. Gomes, and H. F. Julio. 2008. Influence of flood pulses on diet composition and trophic relationships among piscivorous fish in the upper Parana River floodplain. Hydrobiologia 607:187-198.

Lynch, M. 2007. Origins of Genome Architecture. Sinauer Associates, Massachussetts.

Lynch, M., and J. S. Conery. 2000. The evolutionary fate and consequences of duplicate genes. Science 290:1151-1155.

Lynch, M., and J. S. Conery. 2003. The evolutionary demography of duplicate genes. Journal of Structural and Functional Genomics 3:35-44.

Lynch, M., and A. G. Force. 2000. The origin of interspecific genomic incompatibility via gene duplication. American Naturalist 156:590-605.

Mable, B. K. 2004. 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. Biological Journal of the Linnean Society 82:453-466.

MacAvoy, S. E., S. A. Macko, and G. C. Garman. 2001. Isotopic turnover in aquatic predators: quantifying the exploitation of migratory prey. Canadian Journal of Fisheries and Aquatic Sciences 58:923-932.

Maddison, W. P., and D. R. Maddison. 2010. Mesquite: a modular system for evolutionary analysis, version 2.74.

Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. Systematic Biology 56:701-710.

Magallon, S., and M. J. Sanderson. 2001. Absolute diversification rates in angiosperm clades. Evolution 55:1762-1780.

Malabarba, M. C., L. R. Malabarba, and C. Del Papa. 2010. *Gymnogeophagus eocenicus*, n. sp (Perciformes: Cichlidae), an Eocene Cichlid from the Lumbrera Formation in Argentina. Journal of Vertebrate Paleontology 30:341-350.

Mallet, J., M. Beltran, W. Neukirchen, and M. Linares. 2007. Natural hybridization in heliconiine butterflies: the species boundary as a continuum. BMC Evol Biol 7:28.

Mallet, J., and K. Dasmahapatra. 2011. Evolutionary Biology: Catfish Mimics. Nature 469:41-42.

Mallet, J., C. D. Jiggins, and W. McMillan. 1996. Evolution: Mimicry meets the mitochondrion. Current Biology 6:937-940.

Mallet, J., and M. Joron. 1999. Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation. Annual Review of Ecology and Systematics 30:201-233.

Mallet, J., W. O. McMillan, and C. D. Jiggins. 1998. Mimicry and warning color at the boundary between races and species. Endless Forms:390-403.

Mank, J. E., and J. C. Avise. 2006. Cladogenetic correlates of genomic expansions in the recent evolution of actinopterygiian fishes. Proceedings of the Royal Society B-Biological Sciences 273:33-38.

Mappes, J., N. Marples, and J. A. Endler. 2005. The complex business of survival by aposematism. Trends in Ecology & Evolution 20:598-603.

Marek, P. E., and J. E. Bond. 2009. A Mullerian mimicry ring in Appalachian millipedes. Proceedings of the National Academy of Sciences of the United States of America 106:9755-9760.

Marples, N. M., D. J. Kelly, and R. J. Thomas. 2005. Perspective: The evolution of warning coloration is not paradoxical. Evolution 59:933-940.

Mathis, A., and D. P. Chivers. 2003. Overriding the oddity effect in mixed species aggregations: group choice by armored and non-armored prey. Behav Ecol 14:334-339.

Mavarez, J., C. A. Salazar, E. Bermingham, C. Salcedo, C. D. Jiggins, and M. Linares. 2006. Speciation by hybridization in *Heliconius* butterflies. Nature 441:868-871.

May, R. M. 1981. The role of theory in ecology. American Zoologist 21:903-910.

Mayhew, P. 2006. Discovering Evolutionary Ecology: bringing together ecology and evolution. Oxford University Press, Oxford, UK.

Mayr, E. 1942. Systematics and the origin of species, from the viewpoint of a zoologist. Harvard University Press.

McShea, D. W., and R. N. Brandon. 2010. Biology's First Law. The University of Chicago Press, Chicago.

Merilaita, S., and J. Lind. 2006. Great tits (*Parus major*) searching for artificial prey: implications for cryptic coloration and symmetry. Behavioral Ecology 17:84-87.

Merilaita, S., and G. D. Ruxton. 2007. Aposematic signals and the relationship between conspicuousness and distinctiveness. Journal of Theoretical Biology 245:268-277.

Messenger, J. B. 2001. Discovering what cephalopods see. Advances in Ethology:44.

Milinkovitch, M. C., R. Helaers, and A. C. Tzika. 2010. Historical Constraints on Vertebrate Genome Evolution. Genome Biology and Evolution 2:13-18.

Miller, K. G., M. A. Kominz, J. V. Browning, J. D. Wright, G. S. Mountain, M. E. Katz, P. J. Sugarman, B. S. Cramer, N. Christie-Blick, and S. F. Pekar. 2005. The phanerozoic record of global sea-level change. Science 310:1293-1298.

Mittelbach, G. G., D. W. Schemske, H. V. Cornell, A. P. Allen, J. M. Brown, et al. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters 10:315-331.

Mittermeier, R. A., G. A. B. Da Fonseca, A. B. Rylands, and K. Brandon. 2005. A brief history of biodiversity conservation in Brazil. Conservation Biology 19:601-607.

Montoya-Burgos, J. I. 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes : Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Molecular Ecology 12:1855-1867.

Morrone, J. J. 2009. Evolutionary biogeography: An integrative approach with case studies. Columbia University Press, New York.

Müller, F. 1878. Über die vortheile der mimicry bei schmetterlingen. Zoologischer Anzeiger 1:54-55.

Nelson, J. 2006. Fishes of the World. John Wiley & Sons Inc, New Jersey.

Nijssen, H. 1970. Revision of the Surinam catfishes of the genus *Corydoras* (Pisces; Siluriformes; Callichthyidae). Beaufortia 18:1-75.

Nijssen, H., and I. J. H. Isbrucker. 1980a. The identity of Corydoras nattereri with the description of *Corydoras prionotos* new species (Pisces, Siluriformes, Callichthyidae). Beaufortia 30:1-19.

Nijssen, H., and I. J. H. Isbrucker. 1980b. A review of the genus *Corydoras* Lacepede, 1803 (Pisces, Siluriformes, Callichthyidae). Bijdragen Tot De Dierkunde 50:190-220.

Nogueira, C., P. A. Buckup, N. A. Menezes, O. T. Oyakawa, T. P. Kasecker, M. B. Ramos Neto, and J. M. da Silva. 2010. Restricted-range fishes and the conservation of Brazilian freshwaters. Plos One 5:e11390.

Ohno, S. 1970. Enormous diversity in genome sizes of fish as a reflection of natures extensive experiments with gene duplication. Transactions of the American Fisheries Society 99:120-&.

Oliveira, C., L. F. Almeida-Toledo, L. Mori, and S. A. Toledo-Filho. 1992. Extensive chromosomal rearrangements and nuclear-DNA content changes in the evolution of the armored catfishes genus *Corydoras* (Pisces, Siluriformes, Callichthyidae). Journal of Fish Biology 40:419-431.

Oliveira, C., L. F. Almeida-Toledo, L. Mori, and S. A. Toledo. 1993a. Cytogenetic and DNA content in 6 genera of the family Callichthyidae (Pisces, Siluriformes). Caryologia 46:171-188.

Oliveira, C., L. F. Almeida-Toledo, L. Mori, and S. A. Toledo-Filho. 1993b. Cytogenetic and DNA content studies of armored catfishes of the genus *Corydoras* (Pisces, Siluriformes, Callichthyidae) from the southeast coast of Brazil. Revista Brasileira De Genetica 16:617-629.

Oliveira, C., L. F. A. Toledo, F. Foresti, and S. A. Toledo. 1988. Supernumerary chromosomes, robertsonian rearrangement and multiple NORs in *Corydoras aeneus* (Pisces, Siluriformes, Callichthyidae). Caryologia 41:227-236.

Oliveira, C., L. F. D. A. Toledo, and S. D. A. Toledo Filho. 1990. Comparative cytogenetic analysis of three cytotypes of *Corydoras nattereri* (Pisces, Siluriformes, Callichthyidae). Cytologia (Tokyo) 55:21-26.

Otto, S. P. 2007. The evolutionary consequences of polyploidy. Cell 131:452-462.

Otto, S. P., and J. Whitton. 2000. Polyploid incidence and evolution. Annual Review of Genetics 34:401-437.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. Bioinformatics 20:289-290.

Parenti, L. R., and M. C. Ebach. 2009. Comparative biogeography: discovering and classifying biogeographical patterns of a dynamic earth. University of California Press, Berkeley.

Parrish, J. D. 1989. Fish Communities of Interacting Shallow-Water Habitats in Tropical Oceanic Regions. Marine Ecology-Progress Series 58:143-160.

Pasteur, G. 1982. A Classificatory Review of Mimicry Systems. Annual Review of Ecology and Systematics 13:169-199.

Paxton, C. G. M. 1997. Shoaling and activity levels in *Corydoras*. Journal of Fish Biology 51:496-502.

Peng, Z. G., J. Wang, and S. P. He. 2006. The complete mitochondrial genome of the helmet catfish *Cranoglanis bouderius* (Silurifonnes : Cranoglanididae) and the phylogeny of otophysan fishes. Gene 376:290-297.

Pereira, L. H. G., F. Foresti, and C. Oliveira. 2009. Genetic structure of the migratory catfish *Pseudoplatystoma corruscans* (Siluriformes: Pimelodidae) suggests homing behaviour. Ecology of Freshwater Fish 18:215-225.

Perga, M. E., and D. Gerdeaux. 2005. 'Are fish what they eat' all year round? Oecologia 144:598-606.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293-320.

Pianka, E. R. 2000. Evolutionary Ecology, 6th edition. Benjamin-Cummings, Addison-Wesley-Longman, San Francisco.

Pie, M. R., R. A. Torres, and D. M. A. Brito. 2007. Evolution of genome size in fishes: a phylogenetic test of the Hinegardner and Rosen hypothesis. Genetica 131:51-58.

Pinnegar, J. K., and N. V. C. Polunin. 1999. Differential fractionation of delta C-13 and delta N-15 among fish tissues: implications for the study of trophic interactions. Functional Ecology 13:225-231.

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25:1253-1256.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179-189.

Pough, F. H. 1988. Mimicry of vertebrates: Are the rules different? The American Naturalist 131:S67-S102.

Poulton, E. B. 1890. The colours of animals: their meaning and use especially considered in the case of insects. Trench, Trubner and Co Ltd, London.

Power, M. E. 1984. Depth Distributions of Armored Catfish - Predator-Induced Resource Avoidance. Ecology 65:523-528.

Prudic, K. L., J. C. Oliver, and F. A. H. Sperling. 2007. The signal environment is more important than diet or chemical specialization in the evolution of warning coloration. Proceedings of the National Academy of Sciences of the United States of America 104:19381-19386.

Pruzsinszky, I., and F. Ladich. 1998. Sound production and reproductive behaviour of the armoured catfish *Corydoras paleatus* (Callichthyidae). Environmental Biology of Fishes 53:183-191.

Rabosky, D. L. 2006. LASER: A Maximum Likelihood Toolkit for Detecting Temporal Shifts in Diversification Rates From Molecular Phylogenies. Evolutionary Bioinformatics 2:247-250.

Rabosky, D. L. 2009a. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. Ecology Letters 12:735-743.

Rabosky, D. L. 2009b. Ecological Limits on Clade Diversification in Higher Taxa. American Naturalist 173:662-674.

Rabosky, D. L., and M. E. Alfaro. 2010. Evolutionary Bangs and Whimpers: Methodological Advances and Conceptual Frameworks for Studying Exceptional Diversification. Systematic Biology 59:615-618.

Rambaut, A., and A. J. Drummond. 2004. Tracer v1.4.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics 33:589-639.

Randall, J. E. 2005. A review of mimicry in marine fishes. Zoological Studies 44:299-328.

Raufaste, N., and F. Rousset. 2001. Are partial mantel tests adequate? Evolution 55:1703-1705.

Ree, R. H., B. R. Moore, C. O. Webb, and M. J. Donoghue. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. Evolution 59:2299-2311.

Ree, R. H., and S. A. Smith. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Systematic Biology 57:4-14.

Reis, R. E. 1998a. Anatomy and phylogenetic analysis of the neotropical callichthyid catfishes (Ostariophysi, Siluriformes). Zoological Journal of the Linnean Society 124:105-168.

Reis, R. E. 1998b. Systematics, Biogeography, and the Fossil Record of the Callichthyidae: A Review of the Available Data. Pages 603. *In* Phylogeny and Classification of Neotropical Fishes (R. R. E. Malabarba L.R., Vari R.P., Lucena Z.M.S., and Lucena C.A.S., ed.) EDIPUCRS, Porto Allegre, Brasil.

Reis, R. E., M. L. Zelditch, and W. L. Fink. 1998. Ontogenetic allometry of body shape in the neotropical catfish *Callichthys* (Teleostei: Siluriformes). Copeia 1998:177-182.

Revenga, C., I. Campbell, R. Abell, P. de Villiers, and M. Bryer. 2005. Prospects for monitoring freshwater ecosystems towards the 2010 targets. Philosophical Transactions of the Royal Society B-Biological Sciences 360:397-413.

Revenga, C., S. Murray, J. Abramovitz, and A. Hammond. 1998. Watersheds of the world: ecological value and vulnerabilityWorld Resources Institute, Washington.

Ricklefs, R. E. 2010. Evolutionary diversification, coevolution between populations and their antagonists, and the filling of niche space. Proceedings of the National Academy of Sciences of the United States of America 107:1265-1272.

Ritland, D. B. 1991. Revising a classic butterfly mimicry scenario - demonstration of Mullerian mimicry between florida viceroys (*Limenitis archippus floridensis*) and queens (*Danaus gilippus berenice*). Evolution 45:918-934.

Roberge, J. M., and P. Angelstam. 2004. Usefulness of the umbrella species concept as a conservation tool. Conservation Biology 18:76-85.

Robinson-Rechavi, M., B. Boussau, and V. Laudet. 2004. Phylogenetic dating and characterization of gene duplications in vertebrates: The cartilaginous fish reference. Molecular Biology and Evolution 21:580-586.

Robinson-Rechavi, M., O. Marchand, H. Escriva, P. L. Bardet, D. Zelus, S. Hughes, and V. Laudet. 2001. Euteleost fish genomes are characterized by expansion of gene families. Genome Research 11:781-788.

Ronquist, F. 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Systematic Biology 46:195-203.

Rowland, H. M., E. Ihalainen, L. Lindstrom, J. Mappes, and M. P. Speed. 2007. Co-mimics have a mutualistic relationship despite unequal defences. Nature 448:64-67.

Rubenstein, D. R., and K. A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology and Evolution 19:256-263.

Ruber, L., E. Verheyen, and A. Meyer. 1999. Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. Proceedings of the National Academy of Sciences of the United States of America 96:10230-10235.

Rundle, H. D., and P. Nosil. 2005. Ecological speciation. Ecology Letters 8:336-352.

Ruxton, G. D., T. N. Sherratt, and M. P. Speed. 2004. Avoiding Attack. Oxford University Press, Oxford.

Rybczynski, S. M., D. M. Walters, K. M. Fritz, and B. R. Johnson. 2008. Comparing trophic position of stream fishes using stable isotope and gut contents analyses. Ecology of Freshwater Fish 17:199-206.

Sanders, K. L., A. Malhotra, and R. S. Thorpe. 2006. Evidence for a Mullerian mimetic radiation in Asian pitvipers. Proceedings of the Royal Society B-Biological Sciences 273:1135-1141.

Sanderson, M. J. 2003. r8s: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19:301-302.

Sandin, S. A., and S. W. Pacala. 2005. Fish aggregation results in inversely density-dependent predation on continuous coral reefs. Ecology 86:1520-1530.

Sands, D. D. 1994. The behaviour and evolutionary ecology of *Corydoras adolfoi* and *Corydoras imitator*: studies on two species of sympatric catfish from the upper Rio Negro, Brazil. PhD Thesis Liverpool University.

Santini, F., L. J. Harmon, G. Carnevale, and M. E. Alfaro. 2009. Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. BMC Evolutionary Biology 9.

Santos, J. C., L. A. Coloma, and D. C. Cannatella. 2003. Multiple, recurring origins of aposematism and diet specialization in poison frogs. Proceedings of the National Academy of Sciences of the United States of America 100:12792-12797.

Sazima, I., L. N. Carvalho, F. P. Mendonca, and J. Zuanon. 2006. Fallen leaves on the water-bed: diurnal camouflage of three night active fish species in an Amazonian streamlet. Neotropical Ichthyology 4:119-122.

Schaefer, H. M., and N. Stobbe. 2006. Disruptive coloration provides camouflage independent of background matching. Proceedings of the Royal Society B-Biological Sciences 273:2427-2432.

Schaefer, S. A. 1998. Conflict and Resolution: Impact of new taxa on phylogenetic studies of neotropical cascudinhos (Siluroidei: Loricariidae). *In* Phylogeny and classification of neotropical fishes (R. R. E. Malabarba L.R., Vari R.P., Lucena Z.M.S., and Lucena C.A.S., ed.) Editoria Universitaria, Porto Alegre, Brasil.

Scheel, J. J., V. Simonsen, and A. O. Gyldenholm. 1972. The karyotypes and some electrophoretic patterns of fourteen species of the genus *Corydoras*. Journal of Zoological Systematics and Evolutionary Research 10:144-152.

Schluter, D. 2000. The ecology of adaptive radiation. The ecology of adaptive radiation.:i-viii, 1-288.

Schluter, D. 2009. Evidence for Ecological Speciation and Its Alternative. Science 323:737-741.

Shannon, C. E., and W. Weaver. 1949. The mathematical theory of communication. The University of Illinois, Urbana.

Sherratt, T. N., and C. D. Beatty. 2003. The evolution of warning signals as reliable indicators of prey defense. American Naturalist 162:377-389.

Shimabukuro-Dias, C. K., C. Oliveira, and F. Foresti. 2004a. Cytogenetic analysis of five species of the subfamily Corydoradinae (Teleostei : Siluriformes : Callichthyidae). Genetics and Molecular Biology 27:549-554.

Shimabukuro-Dias, C. K., C. Oliveira, R. E. Reis, and F. Foresti. 2004b. Molecular phylogeny of the armored catfish family Callichthyidae (Ostariophysi, Siluriformes). Molecular Phylogenetics and Evolution 32:152-163.

Simpson, E. H. 1949. Measurement of Diversity. Nature 163:688-688.

Soltis, D. E., R. J. A. Buggs, J. J. Doyle, and P. S. Soltis. 2010. What we still don't know about polyploidy. Taxon 59:1387-1403.

Stachowicz, J. J. 2001. Mutualism, facilitation, and the structure of ecological communities. Bioscience 51:235-246.

Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688-2690.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. Systematic Biology 57:758-771.

Stanley, S. M. 1979. Macroevolution: Pattern and Process. W.H. Freeman, San Francisco.

Stebbins, G. L., and F. J. Ayala. 1981. Is a new evolutionary synthesis necessary. Science 213:967-971.

Stevens, M., I. C. Cuthill, C. A. Parraga, and T. Troscianko. 2006. The effectiveness of disruptive coloration as a concealment strategy. Visual Perception, Pt 2: Fundamentals of Awareness: Multi-Sensory Integration and High-Order Perception 155:49-64.

Storchova, Z., A. Breneman, J. Cande, J. Dunn, K. Burbank, E. O'Toole, and D. Pellman. 2006. Genome-wide genetic analysis of polyploidy in yeast. Nature 443:541-547.

Strauss, R. E. 1985. Evolutionary allometry and variation in body form in the South-American catfish genus *Corydoras* (Callichthyidae). Systematic Zoology 34:381-396.

Svanback, R., P. Eklov, R. Fransson, and K. Holmgren. 2008. Intraspecific competition drives multiple species resource polymorphism in fish communities. Oikos 117:114-124.

Symula, R., R. Schulte, and K. Summers. 2001. Molecular phylogenetic evidence for a mimetic radiation in Peruvian poison frogs supports a Müllerian mimicry hypothesis. Proceedings of the Royal Society B-Biological Sciences 268:2415-2421.

Taylor, J. S., Y. Van de Peer, and A. Meyer. 2001. Genome duplication, divergent resolution and speciation. Trends in Genetics 17:299-301.

Templeton, A. R. 2009. Statistical hypothesis testing in intraspecific phylogeography: nested clade phylogeographical analysis vs. approximate Bayesian computation. Molecular Ecology 18:319-331.

Theodorakis, C. W. 1989. Size segregation and the effects of oddity on predation risk in minnow schools. Anim. Behav. 38:496-502.

Thieme, M., B. Lehner, R. Abell, S. K. Hamilton, J. Kellndorfer, G. Powell, and J. C. Riveros. 2007. Freshwater conservation planning in data-poor areas: An example from a remote Amazonian basin (Madre de Dios River, Peru and Bolivia). Biological Conservation 135:484-501.

Turner, J. R. G. 1976. Adaptive radiation and convergence in subdivisions of butterfly genus *Heliconius* (Lepidoptera-Nymphalidae). Zoological Journal of the Linnean Society 58:297-308.

Turner, J. R. G. 1984. Mimicry: the palatibility spectrum and its consequences. Pages 141-161. *In* The biology of butterflies (R. I. Vane-Wright, and P. R. Ackerly, eds.). Academic Press, London.

Van de Peer, Y., S. Maere, and A. Meyer. 2009. OPINION The evolutionary significance of ancient genome duplications. Nature Reviews Genetics 10:725-732.

Vari, R. P. 1988. The Curimatidae, a lowland Neotropical fish family (Pisces: Characiformes); distribution, endemism, and phylogenetic biogeography. *In* Proceedings of a workshop on Neotropical distribution patterns (P. E. Vanzolini, and R. Heyer, eds.). Academia Brasiliera de Ciencias, Rio de Janeiro.

Volff, J. N. 2005. Genome evolution and biodiversity in teleost fish. Heredity 94:280-294.

Waddington, C. H. 1969. Towards a Theoretical Biology. Edinburgh University Press, Edinburgh.

Wallace, A. R. 1852. On the monkeys of the Amazon. Proceedings of the Zoological Society of London 20:107-110.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475-505.

Weitzman, S. H. a. W. M. 1982. Biogeography and evolutionary diversification in Neotropical freshwater fishes, with comments on the Refuge Theory. *In* Biological diversification in the tropics (G. T. Prance, ed.) Columbia University Press, New York.

West, J. B., G. J. Bowen, T. E. Cerling, and J. R. Ehleringer. 2006. Stable isotopes as one of nature's ecological recorders. Trends in Ecology and Evolution 21:408-414.

White, M. J. D. 1973. The Chromosomes, 6th edition. Chapman & Hall, London.

Wiens, J. J. 2004. Speciation and ecology revisited: Phylogenetic niche conservatism and the origin of species. Evolution 58:193-197.

Wiens, J. J., D. D. Ackerly, A. P. Allen, B. L. Anacker, L. B. Buckley, et al. 2010. Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters 13:1310-1324.

Wiens, J. J., and M. J. Donoghue. 2004. Historical biogeography, ecology and species richness. Trends Ecol Evol 19:639-44.

Wiens, J. J., and C. H. Graham. 2005. Niche conservatism: Integrating evolution, ecology, and conservation biology. Annual Review of Ecology Evolution and Systematics 36:519-539.

Williams, P. 2007. The distribution of bumblebee colour patterns worldwide: possible significance for thermoregulation, crypsis, and warning mimicry. Biological Journal of the Linnean Society 92:97-118.

Willmott, K. R., and J. Mallet. 2004. Correlations between adult mimicry and larval host plants in ithomiine butterflies. Proc Biol Sci 271 Suppl 5:S266-9.

Wilson, S. E., J. A. Allen, and K. P. Anderson. 1990. Fast movement of densely aggregated prey increases the strength of anti-apostatic selection by wild birds. Biol. J. Linn. Soc 41:375-380.

Winemiller, K. O., H. Lopez-Fernandez, D. C. Taphorn, L. G. Nico, and A. B. Duque. 2008. Fish assemblages of the Casiquiare River, a corridor and zoogeographical

filter for dispersal between the Orinoco and Amazon basins. Journal of Biogeography 35:1551-1563.

Wolfe, K. H. 2001. Yesterday's polyploids and the mystery of diploidization. Nature Reviews Genetics 2:333-341.

Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences of the United States of America 106:13875-13879.

Woodward, G., and A. G. Hildrew. 2002. Food web structure in riverine landscapes. Freshwater Biology 47:777-798.

Wright, J. J. 2009. Diversity, phylogenetic distribution, and origins of venomous catfishes. BMC Evolutionary Biology 9.

Wright, J. J. 2011. Conservative Coevolution of Mullerian Mimicry in a Group of Rift Lake Catfish. Evolution 65:395-407.

Wüster, W., C. S. E. Allum, I. B. Bjargardottir, K. L. Bailey, K. J. Dawson, et al. 2004. Do aposematism and Batesian mimicry require bright colours? A test, using European viper markings. Proceedings of the Royal Society of London Series B-Biological Sciences 271:2495-2499.

Yi, S., and J. T. Streelman. 2005. Genome size is negatively correlated with effective population size in ray-finned fish. Trends in Genetics 21:643-646.

Zachos, J., M. Pagani, L. Sloan, E. Thomas, and K. Billups. 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science 292:686-693.

Zachos, J. C., G. R. Dickens, and R. E. Zeebe. 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. Nature 451:279-283.

Zelditch, M. L., D. L. Swiderski, D. H. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier Academic Press, Oxford, UK.

Appendices:

Appendix 1: Systematic relationships and a new classification of the Corydoradinae (Hoedeman, 1952)

It is clear from the molecular phylogeny that the Corydoradinae are in need of taxonomic revision. The need for this revision has been recognized for some time (Isbrucker, 2001), although it is only now that the genetic relationships among species are clear, allowing species to be grouped into phylogenetically meaningful and monophyletic groups. Here I present a revised systematic classification of Corydoradinae species (both described and undescribed taxa), based on molecular phylogenetics and geometric morphometrics.

Lineage 1

The basal 'saddle nosed' species remain as *Corydoras,* as first described by Lacépède in 1803 (Lacépède, 1803). *C. geoffroy* would remain the type species for the genus. Long snouted 'Saddle nosed' species such as *Corydoras fowleri* occur at the base of the Corydoradinae. All 'saddle nosed' species occur within this lineage, and genetic differences among species are in general very large i.e. many species diverged a long time ago. Species included in this lineage include:

Described species:

C. coriatae, C. fowleri, C. semiaquilus, C. treitlii, C. narcissus, C. serratus, C. simulatus, C. amapaensis, C. solox, C. cortesi, C. septentrionalis, C. stenocephalus, C. aurofrenatus, C. ellisae, C. blochi, C. pastazensis, C. acutus, C. areio, C. cervinus, C. geoffroy, C. heteromorphus, C. maculifer, C. negro, C. sarareensis, C. vittatus, C. ourastigma, C. oxyrhynchus, C. orcesi, C. saramaccensis

Undescribed:

c8, c16, c24, c28, c29, c38, c42, c47, c51, c53, c61, c63, c77, c78, c86, c92, c94, c95, c99, c109, c115, c116, c124, c127, c145, c146, c149, c153, cw5, cw11, cw12, cw17, cw53, cw55, cw59

Lineage 2

Next most recently evolved are the *Aspidoras.* This group would remain as *Aspidoras* (Ihering, 1907) with the designated type species: *A. rochai.* All known *Aspidoras* belong to lineage 2 with the exception of *A. pauciradiatus*. Furthermore, recently described *C. gladysae* and *C. petrarcini* seem closely related to *Aspidoras*, yet they lack certain synapomorphies that define *Aspidoras*, thereby potentially requiring a new generic name (Calvino and Alonso, 2009).

Described species:

A. albater, A. belenos, A. brunneus, A. carvalhoi, A. depinnai, A. eurycephalus, A. fuscoguttatus, A. lakoi, A. maculosus, A. menezesi, A. microgaleus, A. poecilius, A. psammatides, A. raimundi, A. rochai, A. spilotus, A. taurus, A. velites, A. virgulatus

Undescribed species:

c35, c36, c37, c118, c119, c125, c158, cw52

Lineage 3

All known *Scleromystax* belong to lineage 3 and thereby this groups would remain as *Scleromystax* (Günther, 1864)with the designated type species: *S. barbatus*.

Described species:

S. barbatus, S. macropterus, S. prionotus, S. kronei, S. salmacis, S. lacerdai

Undescribed species:

c112, c113, cw38, cw42

Lineage 4

This lineage includes the dwarf species, and therefore I would resurrect the genus name of *Microcorydoras* (Myers, 1953), with the designated type species: *C. hastatus*. Not all species within this group are dwarfs per se, but they are closely related and share similar colour pattern throughout larval development.

Described species:

C. hastatus, C. pygmeaus, C. mamore, C. guapore, C. paucerna.

Lineage 5

Lineage 5 contains species that have been known as the '*elegans*' group *sensu* Nijssen (Nijssen, 1970) with some additions and corrections. A revision would involve the resurrection of the genus name *Gastrodermus* (Cope, 1878), with the designated type species: *C. elegans*. *C. gracilis* is the basal species in this lineage and *A. pauciradiatus* also belongs to this lineage rather than *Aspidoras* (Lineage 2).

Described species:

C. gracilis, C. sp. A. paucirdiatus, C. nijsseni, C. bilineatus, C. elegans, C. nanus, C. napoensis, C. undulatus

Undescribed species:

c41, c88, c89, c123, c126, c132, cw8, cw18, cw19, cw22, cw29, cw33, cw44, cw48, cw56

Lineage 6

Species within this group have always been classified under the genus *Corydoras*, with no synonymous disused generic names available. Thereby, it would be necessary to describe a new genus with a new type species.

Described species:

C. carlae, C. cochui, C. nattereri, C. potaroensis, C. diphyes, C. ehrhardti, C. micracanthus, C. paleatus, C. flaveolus, C. reynoldsi, C. tukano, C. albolineatus, C. longipinnis, C. ortegai, C. steindachneri

Undescribed species:

c7, c40, c73, c114, c144, cw3, cw24

Lineage 7

This lineage comprises all species from the '*aeneus*' group. A revision would involve the resurrection of the genus name *Osteogaster* (Cope, 1871), with the designated type species: *C. eques*. The most basal species in this group are *C. melanotaenia* and *C. aeneus* from Trinidad, which are found in the Orinoco drainage. *C. zygatus* and *C. rabauti* are found within this group and appear to be more closely related to each other than they are to other species in the lineage. Most closely related to these species are *C. aeneus* from the Parana drainage that were originally known as *C. macrosteus*. The Amazonian species form a group within the lineage, with *C. aeneus*

from Suriname and Guyana separate from species from Peru where the 'laser' species are found. These are quite closely related.

Described species:

C. rabauti, C. aeneus (spp), *C. eques, C. melanotaenia, C. zygatus, C. schultzei, C. venezuelanus*

Undescribed species:

cw7, cw9, cw10, cw14, cw16, cw23, cw26, cw41, cw43

Lineage 8

This lineage comprises mainly the 'intermediate long-snouts' - the long snouted but deep bodied species, but also includes *Brochis,* that was recently synonimized with *Corydoras* (Britto, 2003). A revision would involve the resurrection of the name *Brochis* (Cope, 1871), with the designated type species: *B. splendens*. Furthermore, another three genera would have to named for sub-clades within this species rich lineage.

Described species:

Sub-clade 1:

Brochis: *B. britskii, B. multiradiatus, B. splendens*

Sub-clade 2:

C. garbei, C. difluviatilis, C. filamentosus

Sub-clade 3:

C. latus, C. sodalis, C. reticulatus, C. geryi, C. pantanalensis

Sub-clade 4:

C. crypticus, C. imitator, C. virginiae, C. amandajanea, C. condisciplus, C. ornatus, C. orphnopterus, C. pulcher, C. agassizii, C. ambiacus, C. crimmeni, C. delphax, C. ephippifer, C. incolicana, C. robustus, C. leopardus, C. gomezi, C. haraldschultzi, C. isbrueckeri, C. noelkempffi, C. pinheiroi, C. robinae, C. seussi, C. spectabilis, C. approuaguensis, C. filamentosus, C. sychri, C. melanistius, C. lamberti, C. spilurus, C. bifasciatus

Undescribed species:

c9, c10, c13, c18, c34, c39, c49, c52, c57, c66, c67, c68, c71, c74, c75, c81, c87, c97, c98, c101, c102, c103, c110, c117, c122, c128, c130, c131, c135, c138, c141, c143, c152, c155, c156, c157, c159, cw2, cw6, cw13, cw20, cw25, cw34, cw35, cw40, cw45, cw57, cw58

Lineage 9

Species in lineage 9 are the classic 'short snouted' species such as C. adolfoi. A revision would involve the resurrection of name *Hoplosoma* (Agassiz, 1846), with the designated type species: *C. punctatus*. This is a very species rich lineage and man of the species are relatively recently evolved. There is a large diversity of colour patterns within this lineage and some colour patterns have evolved multiple times such as the '*arcuatus*' pattern, that appears to have evolved at least 4 times within this lineage.

Described species:

C. boesemani, C. arcuatus, C. adolfoi, C. davidsandsi, C. duplicareus, C. melini, C. metae, C. panda, C. gossei, C. burgessi, C. griseus, C. oiapoquensis, C. baderi, C. concolor, C. axelrodi, C. armatus, C. atropersonatus, C. kanei, C. loretoensis, C. loxozonus, C. bicolor, C. brevirostris, C. evelynae, C. leucomelas, C. parallelus, C. schwartzi, C. habrosus, C. sterbai, C. trilineatus, C.

araguaiaensis, C. bondi, C. breei, C. copei, C. coppenamensis, C. cruziensis, C. julii, C. multimaculatus, C. osteocarus, C. paragua, C. polystictus, C. punctatus, C. sipalwini, C. caudimaculatus, C. similis, C. weitzmani, C. urucu, C. xinguensis, C. sanchesi, C. surinamensis, C. boehlkei

Undescribed species:

c3, C6, c14, c19, c21, c30, c33, c43, c44, c45, c48, c54, c62, c65, c76, c84, c85, c90, c91, c96, c100, c104, c120, c121, c129, c133, c134, c136, c137, c139, c140, c142, c147, c148, c150, c151, c154, cw1, cw4, cw15, cw21, cw27, cw28, cw30, cw31, cw32, cw36, cw37, cw39, cw46, cw47, cw49, cw50, cw51, cw54, cw60

Sources Cited for Appendix 1:

Agassiz, L. 1846. Nomenclatoris Zoologici. Index univer- salis, continens nomina systematica classium, ordinum, familiarum et generum animalius omnium, tam viven- tium quam fossilius, secundum ordinem alphabeticum unicum disposita. Soloduri.

Britto, M. R. 2003. Phylogeny of the subfamily Corydoradinae Hoedeman, 1952 (Siluriformes : Callichthyidae), with a definition of its genera. Proceedings of the Academy of Natural Sciences of Philadelphia 153:119-154.

Calvino, P. A., and F. Alonso. 2009. Two new species of the genus Corydoras (Ostariophysi: Siluriformes: Callichthyidae) from northwestern Argentina, and redescription of C. micracanthus Regan, 1912. Revista del Museo Argentino de Ciencias Naturales 11:199-214.

Cope, E. D. 1871. Some anatomical points of importance in the classification of the siluroids of the Amazon. Proceedings of the Academy of Natural Sciences of Philadelphia 23:112-113.

Cope, E. D. 1878. Synopsis of the fishes of the Peruvian Amazon, obtained by Professor Orton during his expedition on 1873 and 1877. Proceedings of the American Philosophical Society 17:673-701.

Günther, A. C. L. G. 1864. Catalogue of the fishes in the British Museum. Catalogue of the Physostomi, conatining the families Siluridae, Characinidae, Haplochitonidae, Sternoptychidae, Scopelidae, Stomiatidae in the collection of the British Museum.

Hoedeman, J. J. 1952. Notes on the ichthyology of Surinam (Dutch Guiana). The catfish genera Hoplosternum and Callichthys, with key to the genera and groups of the family Callichthyidae. Beaufortia 12:1-12.

Ihering, R. 1907. Diversas espécies novas de peixes nematognathas do Brasil. Notas prelim Museum Paulista 1:13-39.

Isbrucker, I. J. H. 2001. Catalogue of genera and species of Corydoradinae Hoedeman, 1952 (Teleostei, Ostariophysi, Callichthyidae), including type localities, type specimens, and etymology*in* Breeding Corydoradinae Catfishes (I. A. Fuller, ed.) Ian Fuller Enterprises, Worcestershire.

Lacépède, B. G. E. 1803. Histoire naturelle des poissons. P. Plassan, Paris.

Myers, G. S. 1953. A note on the habits and classification of Corydoras hastatus. Aquarium Journal 24:268-270.

Nijssen, H. 1970. Revision of the Surinam catfishes of the genus *Corydoras* (Pisces; Siluriformes; Callichthyidae). Beaufortia 18:1-75.

Appendix 2: Genome duplication in amphibians and fish: An extended synthesis

A.2.1 Abstract

Whole genome duplication (leading to polyploidy) is widely accepted as an important evolutionary force in plants, but it is less recognised as a driver of animal diversification. Nevertheless, it occurs across a wide range of animals; this review investigates why it is particularly common in fish and amphibians, while rare among other vertebrates. We review the current geographic, ecological, and phylogenetic distributions of sexually reproducing polyploid taxa before focusing more specifically on what factors drive polyploid formation and establishment. In summary: 1) Polyploidy is phylogenetically restricted in both amphibians and fishes, although entire fish, but not amphibian, lineages are derived from polyploid ancestors. (2) Although mechanisms such as polyspermy are feasible, polyploid formation appears to occur principally through unreduced gamete formation, which can be experimentally induced by temperature or pressure shock in both groups. (3) External reproduction and fertilization in primarily temperate freshwater environments potentially exposes zygotes to temperature stress, which can promote increased production of unreduced gametes. (4) Large numbers of gametes and group breeding in relatively confined areas could increase the probability of compatible gamete combinations in both groups. (5) Both fish and amphibians have a propensity to form reproductively successful hybrids; although the relative frequency of autopolyploidy versus allopolyploidy is difficult to ascertain, multiple origins involving hybridization has been confirmed for a number of species in both groups. (6) Problems with establishment of polyploid lineages associated with minority cytotype exclusion could be overcome in amphibians via assortative mating by acoustic recognition of the same ploidy level, but less attention has been given to chemical or acoustic mechanisms that might operate in fish. (7) There is no strong evidence that polyploid fish or amphibians currently exist in more extreme environments than their diploid progenitors or have broader ecological ranges. (8) Although pathogens could play a role in the relative fitness of polyploid species, particularly given duplication of genes involved in immunity, this remains an understudied field in both fish and amphibians. (9) As in plants, many duplicate copies of genes are retained for long periods of time, indicative of selective maintenance of the duplicate copies, but we find no physiological or other reasons that could explain an advantage for allelic or genetic complexity. (10) Extant polyploid species do not appear

to be more or less prone to extinction than related diploids in either group. We conclude that, while polyploid fish and amphibians share a number of attributes facilitating polyploidy, clear drivers of genome duplication do not emerge from the comparison. The lack of a clear association of sexually reproducing polyploids with range expansion, harsh environments, or risk of extinction could suggest that stronger correlations in plants may be driven by shifts in mating system more than ploidy. However, insufficient data currently exist to provide rigorous tests of these hypotheses and we make a plea for zoologists to also consider polyploidy as a possibility in continuing taxonomic surveys.

A.2.2 Introduction

Genome sequencing has revealed that across both plant and animal kingdoms, the vast majority of genes are organized in multiple sets rather than single copies (Blanc et al., 2000; Donoghue and Purnell, 2005; Edgell et al., 2000; Wessler and Carrington, 2005; Wolfe and Shields, 1997). This extensive gene duplication is hypothesized to have arisen through multiple rounds of whole genome duplication (WGD, leading to polyploidy), and is thought to be fundamental for speciation, diversification of gene functions, and shaping genomic architecture across major eukaryotic groups (Blanc et al., 2003; Chen, 2007; Leitch et al., 2004; Lynch, 2002; Ramsey and Schemske, 2002; Wessler and Carrington, 2005). It is now accepted that two rounds of WGD occurred during the early diversification of chordates and vertebrates, with strong evidence supporting a subsequent teleost fish-specific genome duplication (FSGD Dehal and Boore, 2005; Hoegg and Meyer, 2005; Larhammar et al., 2009; Putnam et al., 2008; Van de Peer et al., 2009). More recent WGD events have also occurred, resulting in species that are currently functionally polyploid. Since some degree of diploidization and loss of duplicate gene expression inevitably follows WGD (Adams, 2007; Ferris and Whitt, 1977d; Flagel and Wendel, 2010), it is often difficult to distinguish "ancient" (i.e., paleopolyploid) from "recent" events. Nevertheless, in this review we define "extant" polyploid species as those having twice the chromosome number of close relatives, sometimes retaining at least some pairing of multiple chromosome copies during meiosis, and retaining evidence of duplicate gene expression distributed throughout the genome, with most having some recognizably distinctive features from their closely associated diploids. It is the distributions and characteristics of these extant polyploids that are the focus of this review.

There are no comprehensive surveys of the prevalence of polyploidy in animals but it has been documented across a wide range of taxa, including insects, crustaceans, molluscs, fish, amphibians, reptiles and (to a lesser extent) mammals (reviewed in Bogart, 1980; Gregory and Mable, 2005; Le Comber and Smith, 2004; Lewis, 1980; Otto and Whitton, 2000). Polyploidy is most common in organisms that do not regulate their internal temperature (i.e., plants and ectothermic animals), and are therefore directly exposed to

changes in their environments. However, since polyploidy is not widespread amongst ectothermic vertebrates in general, it begs the question of why some groups are polyploid and others not. Although it is possible that intrinsic mechanisms regulating genome integrity constrain polyploid speciation, it is also possible that ecological factors (i.e. living in habitats or conditions that favour polyploidy), in combination with the inherently stochastic nature of establishment of polyploid lineages (i.e., formation in the midst of diploid progenitors: (Husband, 2000; Levin, 1975); producing balanced chromosome sets) are responsible.

In this review we question why, among vertebrates, polyploidy is most frequent among fish and amphibians. Our purpose is to evaluate the phylogenetic and geographic distributions, hypothesized origins, reproductive biology and ecology of sexually reproducing polyploid fish and amphibians, to better understand the potential drivers of polyploidy. Kejnovsky et al. (2009) recently contrasted differences between mammals and plants that might make the former less prone to polyploidy, but our objective is to emphasize what features shared by fish and amphibians might promote polyploid formation and establishment. We focus solely on bisexually reproducing polyploids in order to avoid confounding environmental and geographic effects related to breeding system variation rather than polyploidy, which has been a problem for interpreting patterns in plants, ostracods and insects (reviewed in Mable, 2003). We first set the historical context for polyploid discoveries in fish and amphibians, with an overview of the types of data that have been used to identify them and summarize the current phylogenetic distribution of extant polyploid fish and amphibians, before focusing on factors that favour polyploid formation and establishment.

We thus surveyed the distribution of polyploidy in anurans (frogs and toads) compared to fishes and exploited comprehensive databases summarizing their taxonomy and distribution (Amphibian Species of the World, (Frost, 2010) http://research.amnh.org/vz/herpetology), ecology and life history (Amphibiaweb http://amphibiaweb.org/), and conservation status (International Union for Conservation of Nature Redlist of Endangered species, http://www.iucnredlist.org). For fishes, genome size and karyotype data were obtained from the animal genome size database

(http://www.genomesize.com/), while taxonomic, ecological and biogeographic information was mined from Fishbase (Froese and Pauly, 2008) (www.fishbase.org). Since ploidy state is not an attribute that is available for most species in these databases, we first compiled a list of known extant polyploid species from the primary literature (please see SI Tables A1 and A2, where the relevant references are cited), before using the databases to update species designations and phylogenetic distributions, examine geographic distributions and ecological preferences and assess endangered species status.

We conclude that the most striking feature shared by polyploid fish and amphibians is external reproduction in freshwater environments, predominantly in regions where temperature fluctuations during the breeding season are common. It would thus be tantalizing to speculate that this could support previous hypotheses that rates of polyploid formation and establishment are associated with periods of climatic change and/or currently unstable or extreme environments (e.g., Hagerup, 1932) because polyploids are genetically more resilient than their diploid progenitors or able to exploit more extreme habitats. We don't find evidence that bisexual polyploids have broader ecological ranges or distributions or lower risk of extinction than their diploids relatives but insufficient data currently exist to provide robust tests and to fully understand the potential impacts of climate change on rates of polyploid speciation.

A.2.3 Historical Context

While polyploid animals are now well documented, the existence of the first polyploid vertebrate (the *Amybstoma jeffersonianum* complex of salamanders: (Uzzell, 1964) was not accepted by the scientific community until 1964. The first polyploid frogs (*Odontophyrnus americanus* and *Ceratophrys ornata*) were described in 1966 (Saez and Brum-Zorrilla, 1966), but despite providing clear figures showing multiple sets of chromosomes and multivalent formation during meiosis, the authors rather remarkably concluded that "it does not mean that we believe in the existence of polyploidy". Bogart (1967) later confirmed that these were both octoploid species that reproduced bisexually. Earlier research on fish also suggested that polyploidy played a major role in the evolution of the Salmonidae (Svärdson, 1945) and the genus *Coregonus* (Kupka, 1948) but these were discounted by some researchers, and polyploidy in Salmonidae as of 1967 was "not considered to be a biological fact" (reviewed by Bogart, 1967). Despite the initial excitement of these early discoveries, polyploidy has never really emerged to the forefront of attention by animal evolutionary biologists.

In a thorough and insightful review based on experimental induction of polyploidy in amphibians compared to plants and insects, Gerhard Fankhauser (an embryologist from Princeton University) predicted that polyploidy was likely to be evolutionarily important in vertebrates (Fankhauser, 1945). He suggested that "the following data should be gathered: 1) occurrence and frequency of polyploidy in different groups of animals; 2) origin of these exceptional individuals, in other words, the mechanisms that are responsible for their production; 3) the effectiveness of methods for the experimental induction of polyploidy; 4) the general effects of polyploidy on cell size, body size, and viability, and on the general physiology and biochemistry of the organism; 5) the occurrence of qualitative effects, which are added to the more obvious quantitative consequences." Half a century later the answers to Fankhauser's queries remain largely unanswered. This is partly a result of the view that polyploidy could not be important in animals because: 1) they have too much developmental complexity compared to plants; 2) sex determining mechanisms in vertebrates and *Drosophila* are expected to be disrupted by changes in dosage (Mable, 2004; Muller, 1925; Orr, 1990); and 3) regulation

of body size in the developing ovum could be altered by cell size increases and dosage (Manevto, 1945).

The possibility that polyploidy has played an important role in animal evolution has recently received more attention (Donoghue and Purnell, 2005; Gregory and Mable, 2005; Volff, 2005) but discoveries of unrecognized polyploids remain rare, possibly because few researchers look for them, but also due to the difficulty of identifying cryptic polyploids (see Identification of Polyploids). Thus, there is likely to be a considerable underestimate of the distribution and frequency of polyploidy in animals. In contrast, polyploid plants are the focus of modern genomic research not only due to their economic importance, but also due to the much larger than expected genomic signatures of ancient WGD events that opens opportunities for studying changes in gene expression following polyploidization. A special issue on polyploidy in New Phytologist (Ainouche and Jenczewski, 2010) emphasizes the contributions of rapid advances in genome sequencing technologies to understanding such genomic consequences of polyploidy. However, even in plants, we still do not have a complete understanding of the factors that promote the formation and establishment of polyploidy in the wild, the role that ecology plays in polyploid speciation, and whether polyploidy accelerates diversification rates or is an evolutionary dead end (reviewed in Levin, 2002; Soltis et al., 2010).

A.2.4 Identification of Polyploids

Extant polyploids have been identified using a variety of techniques, including chromosome counts, detection of multivalent formation during meiosis, banding patterns in markers such as allozymes, cell size comparisons, and genome size estimations. However, none of these techniques are incontrovertible and controversies over polyploid status often remain unresolved (e.g., Viscacha rat Gallardo et al., 2004; Svartman et al., 2005). This is reflected in the wide range of estimates of polyploid frequencies in plants (Hilu, 1993; Jenkins and Rees, 1991; Masterson, 1994; Otto and Whitton, 2000; Soltis and Soltis, 1999). Most recently, based on detailed phylogenetic comparisons, it has been estimated that 15 % of flowering plant and 31 % of fern speciation events have been accompanied by a ploidy increase (Wood et al., 2009). However, despite increased rigour of analytical methods, this study relied on inferring ploidy level primarily from chromosome data, which can be problematic (Otto and Whitton, 2000). No single method works for all groups, with differences among plants, fish and amphibians in the predominant methods used to infer ploidy status. Most documented cases of polyploidy in animals have used a pluralistic approach, rather than relying on a single method. Below we survey the major types of methods that historically have been used to identify polyploids.

A.2.4.1 Cytogenetics

The oldest methods for identifying polyploids are through cytogenetic assessment of chromosome numbers, banding and meiotic configuration patterns. Theory suggests that allopolyploids segregate disomically, as they present two sets of homeologous chromosomes that are unlikely to pair at meiosis, while autopolyploids segregate polysomically because their chromosomes pair at random and form multivalents during meiosis (Chenuil et al., 1999). In practice, the reestablishment of disomic inheritance in ancient polyploids (deWet, 1980) or polysomic inheritance in allopolyploids arising from close relatives sharing partly homologous chromosomes (Stebbins, 1950), means that

there is likely to be a continuum between strictly disomic and strictly polysomic inheritance (see review by Soltis *et al.*, 2010). In general, the demonstration of tetravalents during meiosis and tetrasomic inheritance provide good evidence of polyploid status but lack of tetravalent formation does not necessarily mean that a species is diploid, because even young polyploids experience some degree of diploidization (e.g., Le Comber et al., 2010). Additional difficulties arise when chromosomal rearrangements (through Robertsonian fissions and fusions) lead to changes in chromosome number that are not related to genome duplication. Since increases in genomic content can be increased through other means than duplication (particularly in noncoding regions), genome size also is not always a reliable estimate of WGD. Genome sequencing studies have confirmed that transposable elements (which can result in large differences in genome sizes without changes in chromosome numbers) may confound relationships between DNA content and chromosome numbers (e.g., Parisod et al., 2010a).

Nevertheless, at least in anurans, karyotyping remains the most reliable method of detecting polyploidy. This is facilitated by the conservation of basal chromosome number (range: 9-13) and the presence of large chromosomes that allow banding patterns and rDNA distributions to be used to indicate changes in chromosome morphology. In fact, Bogart (1991) noted that species groups where Robertsonian changes in chromosomes (i.e. fusions or fissions) are common do not tend to include polyploids and polyploid species often share a high degree of apparent synteny (based on chromosome banding patterns) as their diploid progenitors. All known polyploid anurans have an even replication of chromosome number compared to closely related diploids (SI Table A1), often with highly similar chromosome morphologies, based on banding patterns and rDNA locations (Bogart, 1980; Stöck et al., 2005).

In fishes, the situation is more complicated (SI Table A2). Recent analyses of whole genome sequences and ancestral reconstruction suggest that the ancestor of all teleost fishes had a haploid chromosome complement of 12-13 chromosomes that increased to 23-24 chromosomes after the teleost specific duplication (Jaillon et al., 2004; Kasahara et al., 2007; Nakatani et al., 2007). The modal chromosome number for acanthopterygian fishes is 48 (Mank and Avise, 2006), with counts ranging from 22 to 250 and counts of

'diploid' species ranging between 22 and 78. Genome size is correlated with chromosome number when all species are investigated, but this relationship is weaker (although remains significant) when polyploids are removed from the analysis. Mank & Avise (2006), suggest that 7–20 polyploidization events have occurred in extant ray-finned fish lineages based on analysis of chromosome numbers, although this is almost certainly an underestimate, as many polyploid fishes have yet to be karyotyped and within polyploid lineages there is often evidence of multiple independent duplications (Alexandrou and Taylor, personal observation). Nevertheless, chromosome numbers have been used to identify polyploid fish species and even entire families. For example, the Catastomidae are all tetraploids, with a basal chromosome complement of 100, twice that of diploid cyprinids (Ferris, 1984).

A.2.4.2 Marker-based methods

Allozymes have been widely used to identify suspected polyploids (based on number of copies or dosage of bands), to assess modes of origin of polyploid taxa (allopolyploid or autopolyploid) based on patterns of duplicate gene expression and sharing of alleles with putative ancestors, to identify genomic composition of hybrids, and to make inferences about fates of duplicate genes. Since allozymes compare the protein products of expressed genes, they also have been used to assess the degree of duplicate gene expression across loci and tissue types (Allendorf, 1978; Bailey et al., 1978; Ferris and Whitt, 1977d). In fact, Susumo Ohno based his precocious predictions that vertebrates had experienced multiple rounds of whole genome duplication on patterns of duplicate gene expression in allozymes (Ohno et al., 1968). Despite the advent of more advanced technologies, allozymes remain one of the clearest methods for cheaply and rapidly characterizing polyploid genomes.

Since expertise in cytogenetics was also at its peak among evolutionary biologists when allozymes were popular, combining the two approaches was likely responsible for a peak in discovery of new polyploid amphibian species in the 1970s (SI Figure A1). Allozymes have been used to characterize the cryptic parental origins of previously identified

polyploid amphibians (e.g. *Tomopterna tandyi* complex: (Channing and Bogart, 1996); *Hyla versicolor* complex: (Ralin, 1978; Romano et al., 1987; Romano and Vaughn, 1985), discover that disomic, tetrasomic and intermediate patterns can be found within tetraploid families depending on the locus and tissue type examined (*Hyla versicolor*: (Danzmann and Bogart, 1983), and to identify which sexual species contribute to hybrid unisexual lineages (*Ambystoma laterale* complex: (Bi and Bogart, 2006; Bogart et al., 1985).

In fishes, allozymes have been used to identify polyploid lineages (e.g. Samonidae: (Allendorf and Thorgaard, 1984), to infer hybrid composition (e.g., tetraploid loaches: (Slechtova et al., 2003), to identify duplicated loci based on tissue-specific expression patterns (Ferris and Whitt, 1975; Ferris and Whitt, 1977b; Ferris and Whitt, 1977c; Ferris and Whitt, 1978; Ohno, 1970; Ohno, 1993; Ohno et al., 1968), to infer preferential expression of parental alleles in experimental crosses (e.g. crosses between carp and goldfish: (Danzmann and Down, 1982) and preferential pairing of homeologues (e.g., rainbow trout: (Allendorf and Danzmann, 1997), and to assess the proportion of duplicate genes that remain expressed in "old" polyploids (e.g., in the Salmonidae approximately 50% of duplicated allozyme loci remain detectable (Allendorf and Thorgaard, 1984); a similar proportion to other tetraploid fishes, which retain between 25-70% of duplicated loci (Li, 1980). An interesting point to note is that the study of the fate of duplicate genes based on allozymes in Cypriniformes (Ferris and Whitt, 1977a) provided evidence for the separation of function of duplicate copies by tissue type or developmental stage (now known as subfunctionalization), along with evolution of new functions or loss of functions.

DNA-based markers such as microsatellites can be used directly to provide evidence of duplicate genes rather than duplication of expressed products. However, fluorescent peak heights or stained-band intensities in microsatellites are not always directly proportional to dosage of starting products due to uneven amplification of alleles in PCR, so that copy numbers can be more difficult to infer than for allozymes. Nevertheless, the large numbers of alleles at microsatellite loci may make dosage less important, as polyploidy can be identified by the number of peaks rather than the differences in strength. Their

higher levels of variation also can be useful for inferring parental origin and segregation of alleles to assess inheritance patterns; in carp, around 60% of microsatellite loci still amplify duplicate copies despite the duplication event having occurred around 12 MYA (David et al., 2003). Microsatellites also have been used in conjunction with flow cytometry to establish ploidy levels and provide evidence of occasional sex in unisexual salamanders (Bi et al., 2007; Bi et al., 2009; Bogart et al., 2007; Ramsden et al., 2006).

A.2.4.3 Genome size

Variation in genome size has the potential to uncover polyploidy, but for many organisms, there is no relationship between DNA content and chromosome number (Gregory, 2005a; Gregory, 2005b), so it is important to confirm estimates with chromosome counts. There are also a variety of methods available to estimate genome sizes (e.g., flow cytometry, feulgen image analysis densitometry) and there can be difficulties calibrating estimates from different laboratories (Gregory, 2005a; Gregory, 2005b; Hardie et al., 2002; Leitch, 2007; Smith and Gregory, 2009). Methods that provide relative, rather than absolute, measures of DNA content can be less problematic for identifying suspected polyploids, particularly if they are conducted in the same laboratory.

In amphibians absolute genome size is not a good predictor of ploidy level, particularly in salamanders, which have a large range in genome sizes, even among diploids (Gregory, 2005a; Gregory, 2005b) Animal Genome Size Database. http://www.genomesize.com). In anurans, using genome size alone to infer ploidy is also equivocal. Diploid genome sizes vary widely, with diploids in the genus *Hyla* (c.a. 5.0 pg) having a genome size similar to tetraploid *Neobatrachus,* despite having the same basal chromosome number (2n = 24). At least in salamanders, differences in genome size of species with the same chromosome number have been found to be due to differences in repetitive DNA, rather than WGD (Baldari and Amaldi, 1976; Bozzoni and Beccari, 1978). Insufficient genomic data exist to assess whether this is also true in anurans or whether lineage specific paleopolyploidy has also occurred. Comparisons in genome size between previously identified diploid and tetraploid species pairs may be more illuminating with regards to the potential to use such data to infer ploidy levels. Data for the five diploid-

tetraploid pairs where data are available show an average tetraploid: diploid genome size ratio of 1.93 (SI Table A1). However, the same does not appear to be true for higher ploidy levels. In the genus *Ceratophrys*, for example, there are two octoploid species with the same chromosome number (8n = 104) but one (*C. aurita*) has a genome size of 6.34 pg, whereas the other (*C. ornata*) has 13.4 pg. Although a diploid or tetraploid progenitor has not been identified, a closely related diploid (*C. calcarata*) has a genome size of 2.3 pg; neither octoploid has 8x its DNA content. While this could reflect problems with genome size estimation by different authors, suggest that the genome size of the progenitor diploid was much higher, or that cryptic ancient polyploidy occurred in one "octoploid" lineage but not the other, it does highlight the need for caution. For *Xenopus*, genome size estimates for tetraploid species range between 3.0 and 4.1 pg, with an average ratio compared to *Silurana tropicalis* (the only extant diploid species) of 2.03 (range 1.8-2.3). However, this is not the likely progenitor, as it has a basal chromosome number of 2n = 20 compared to 4n = 36 in *Xenopus*. Again, higher ploidy levels show a non-linear increase in DNA content, with ratios of 3.5 in the octoploid *X. vestitus* and only 4.6 in the dodecaploid *X. ruwenzoriensis* compared to *S. tropicalis*.

Fish genome size varies considerably, with C-values ranging between 0.35 and 9.75 pg. C-values for teleosts range between 0.4 and 4.4 pg, with the average around 1.2 pg. However, the majority of species have C-values in the range 0.5 to 2.0 pg. The genome sizes of polyploid teleosts range from 1.36 to 3.75 pg, with a mean of 2.5 pg (Smith and Gregory, 2009). Smith & Gregory (2009) suggest that genome sizes are usually greater than 2.5 pg if polyploidy has occurred. Using genome size to infer polyploidy is more complicated in more basal groups of fishes, in which estimates may be confounded by transposable elements. For instance the cartilaginous fishes (Chondrychthyes) have C-values in the range 1.51-17 pg; although polyploidy has been suspected to have played a role in their early evolution (Kendall et al., 1994; Stingo and Rocco, 2001), repetitive elements are also likely to have led to increases (Kellogg et al., 1995; Olmo et al., 1982) or decreases (Leitch and Bennett, 1997) in genome size. Moreover, chondrichthyans and sarcopteryians have only four Hox clusters (Amemiya et al., 2008; Putnam et al., 2008; Venkatesh, 2007), whereas actinopterygians have seven or eight Hox clusters (Crow et al., 2006) which suggests that the large genome size of chondrichthyans may not be the

result of polyploidization. In summary, comparing genome size to chromosome counts can be informative for inferring ploidy status in fishes, but the more extensive rearrangements and lineage-specific polyploidy makes the classification of extant polyploidy less certain than for anurans (SI Table A2).

A.2.4.4 Cell size

Nucleus size is positively correlated with chromatin amount in polyploids (reviewed in Manevto, 1945). Cell size, however, is not always directly proportional to nuclear volume. In yeast, for example, whereas haploid cells tend to be smaller than diploid cells under reduced nutrient conditions, they can be the same volume when grown in rich medium (Weiss et al., 1975). The same can be true of higher ploidy levels – for animals with nucleated red blood cells, the erythrocyte volume of tetraploids is consistently higher than in diploids but is often less than the factor of two that might be expected with the doubling of DNA content (Mable, 2001). In frogs, tetraploid *Hyla versicolor* (Hylidae) can be reliably distinguished from their diploid progenitors, *Hyla chrysoscelis* (Matson, 1990) based on differences in erythrocyte size, but the volume of tetraploid cells is less than that expected from the DNA content (ratio 1.3-1.5). This means that triploids can be difficult to distinguish because the ranges in volume overlap (Mable, 1989). For octoploid *Odontophrynus americanus*, erythrocyte size varies between juveniles and adults, with those of juveniles comparable to those of adult erythrocytes of the diploid *O. cordobae* (Grenat et al., 2009).

In fishes, genome size and erythrocyte size are also correlated in both teleosts and cartilaginous fishes (Hardie and Hebert, 2003; Hardie and Hebert, 2004). Interestingly, cold water fish have larger cell sizes than warm water species when controlling for genome size (Hardie and Hebert, 2003). However, distinguishing whether this is primarily due to polyploidy or non-duplication based genome expansion is difficult. Nevertheless, cell size may be useful for differentiating between closely related diploid and polyploid species or forms (Felip et al., 2001).

A.2.4.5 Phenotypic Characteristics

Polyploidy may lead to an increase in the overall size of organisms. Such "gigantism" is prevalent among plants and insects but is not apparent in fish and amphibians. Polyploidy in fishes and amphibians appears to result in a reduction in the number of cells, so that even though cell size is increased, overall body size remains the same as in diploids (reviewed in Bogart, 1980). Most bisexually reproducing amphibians are found in close association with related and morphologically similar diploids, which have often been implicated in their formation (reviewed by Bogart, 1980). Polyploid anurans tend to have similar body size as the diploids (Bachman and Bogart, 1975; Fankhauser, 1945) and do not seem to experience radical changes in physiology; for example, *Hyla versicolor* have similar metabolic rates as their diploid progenitors (Kamel et al., 1985). However, species-specific mating calls used by females to choose mates have been used to identify cryptic polyploids, based on polymorphism in mating calls among populations that were otherwise indistinguishable, with ploidy status subsequently confirmed by allozymes and/or chromosome counts (Barrio, 1980; Bogart and Wasserman, 1972; Haddad et al., 1994; Roberts, 1997b; Stöck and Grosse, 2003; Vigny, 1979; Wasserman, 1970). In gray treefrogs (*Hyla versicolor* complex), females prefer calls of their own ploidy level (Gerhardt, 1974; Gerhardt, 1982; Gerhardt, 2005a; Gerhardt, 2005b; Klump and Gerhardt, 1987), and some characters of the mating call (e.g., pulse rate) change as a direct consequence of the increase in cell size arising from polyploidy (Bogart, 1980; Bogart and Wasserman, 1972; Holloway et al., 2006; Keller and Gerhardt, 2001), although this has not been found in all polyploid groups (e.g., Neobatrachus Roberts, 1997b). In some frog species, differences in colour patterns or morphometric measurements can also be used to distinguish some species that differ in ploidy (e.g., Castellano et al., 1998; Mahony et al., 1986; Stöck et al., 2005) but such differences are likely to have arisen from adaptation post speciation, rather than the genome duplication event itself.

There has been less focus in fish on using morphological or behavioural cues to identify polyploids but there is the possibility that differences in quantity of chemical products that are produced in direct proportion to cell size or genome copy could be useful. As far

as we are aware, no comparisons have been made between olfactory signal components in relation to ploidy in fish or amphibians that primarily use odour cues.

A.2.4.6 Identifying Allo vs Autopolyploids

Determining the origin of polyploid organisms, and whether they have arisen via autopolyploidy or allopolyploidy is crucial to our understanding of polyploid evolution. However, distinguishing between these mechanisms is difficult, due to the continuum between disomic and polysomic inheritance that exists in most polyploid species, regardless of whether they arose through hybridization or from a single progenitor species. As Soltis et al. (2010) eloquently discuss, there is also a different perspective between systematists (who are interested in whether polyploids arose from different species) and geneticists (who are interested in segregation patterns during meiosis). Nevertheless, considerable effort has been devoted to testing polyploid origins based on inheritance patterns and more rigorous statistical methodologies are in development (reviewed by (Parisod et al., 2010b; Soltis et al., 2007). For example, by incorporating Bayesian statistics, Olson (1997) presented a method that allows simultaneous assessment of disomic vs tetrasomic inheritance, rather than performing goodness of fit tests separately for each model. Based on only two allozyme loci, they demonstrated disomic inheritance (allopolyploidy) in *Astilbe biternata* (Saxifragacea) using a very small sample size. For DNA-based approaches such as microsatellites, Bayesian methods have been proposed that use large numbers of microsatellite loci and large numbers of individuals, to evaluate models of inheritance without use of progeny arrays (e.g., Catalan et al., 2006).

More flexible statistical approaches that consider a range of inheritance patterns have also been proposed. For example, based on the number of homoeologous copies present in each species for a set of neutral markers, Chenuil et al. (1999) developed a method that does not require the assumption that allopolyploidy leads to multisomic inheritance. Based on this, using five microsatellite loci in eight cyprinid species (with 1-3 representatives of each) of the genus *Barbus* (Cypriniformes), the hypothesis that

European tetraploid barbs originated through autopolyploidy was rejected. These tests have proven particularly useful in cases where hybridization between particular taxa is known and well documented. Investigations of patterns of inheritance within the polyploid cyprinid *Cyprinus carpio* using 59 microsatellite markers suggested a hybrid origin (David *et al.*, 2003). Stift et al. (2008) describe a likelihood-based method incorporating intermediate inheritance patterns, as well as more complicated patterns due to double reduction, which provides a more realistic assessment of segregation patterns in polyploids.

A.2.5 Current taxonomy and phylogenetic distribution of polyploids

One feature shared by fish, amphibians and plants is their complex and dynamic taxonomic history. Ichthyologists, herpetologists and botanists historically have tended to share a passion for systematics and it has been common for species to be reclassified multiple times not only among species and genera but also among families. This has been particularly true since molecular characters have been widely used to resolve phylogenies; whole genome studies will result in further revisions. It can, therefore, be difficult to sort through original reports of polyploidy in the face of changing taxonomy. Here we review the distributions of extant known polyploid anurans and fish, considering changing species designations and relationships among families or higher levels of classification. As a result of this review, it is interesting to note the absence of polyploid vertebrates from certain regions. Notably, there are no recorded polyploid fishes in Australia or Antarctica (possibly an artefact, as these areas are understudied), while, in contrast to plants, very few extend their ranges into polar and arctic areas. There are polyploid frogs across all temperate and tropical continents, including Australia, but amphibians in general do not occur in the Antarctic or Arctic so their absence there is less surprising.

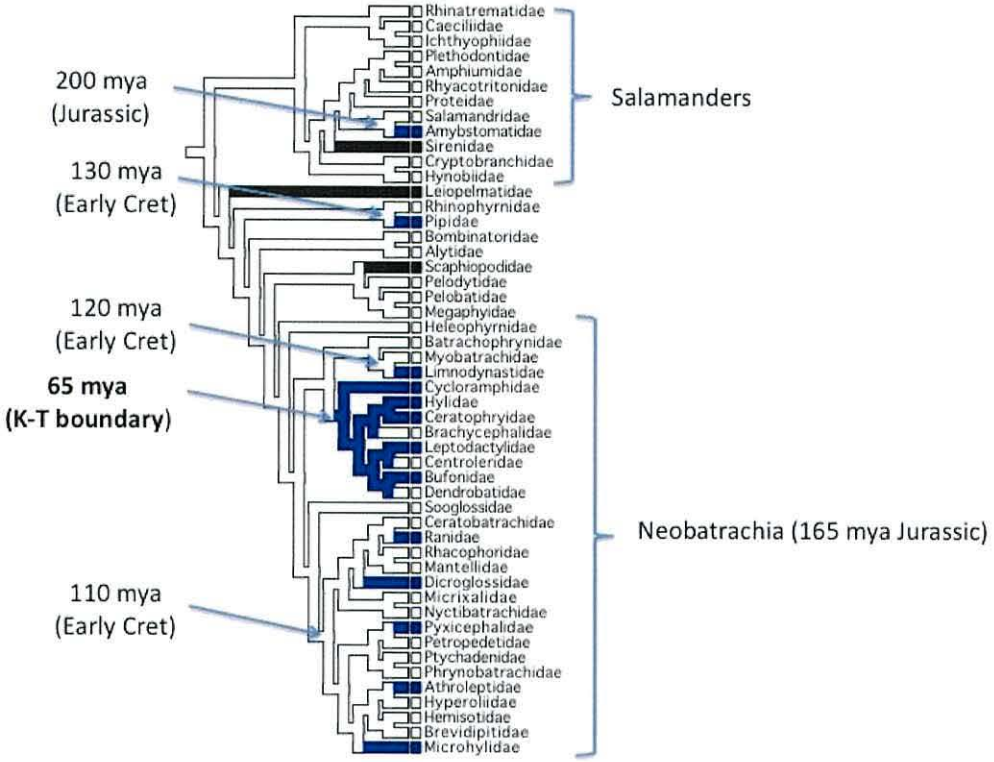
A.2.5.1 Anurans

Since polyploid frogs live in close proximity to their diploid relatives (Bogart, 1980), we provide a list of bisexual polyploids and their closely related diploids (SI Table A1). Wherever possible, credit has been given to the authors who first described a species as polyploid. Since the taxonomy of many anurans remains controversial (e.g., Roelants et al., 2007; Wiens, 2007), we provide the current classifications provided in the Frost Amphibian species of the world database (largely based on the revised taxonomy provided in Frost et al. (2006), as well as the species definitions at the time that polyploids were originally identified.

Polyploidy has arisen independently in multiple amphibian families. The majority of species in the basal family Pipidae are tetraploid or higher (Figure A1). The model Pipid

species *Xenopus laevis* was originally thought to be diploid but it is now recognized as an ancient tetraploid, with extensive, but incomplete diploidization across much of its genome (Kobel and Du Pasquier, 1986); octoploids and dodecaploids also occur in the family (see Evans et al., 2008; Evans et al., 2005; Evans et al., 2004). Polyploidy has also been suggested in other basal groups (Leiopelmatidae Green et al., 1984) and the entire Sirenidae family may be ancient polyploids (Morescalchi and Olmo, 1974), but these reports remained unconfirmed. In the more derived groups (e.g., Hylidae, Ranidae, Microhylidae), polyploid species are more scattered. Bisexually reproducing polyploid anurans have been confirmed across eight traditional families (Gregory and Mable, 2005) but taxonomic revisions suggested in the Frost database (Frost, 2010) mean that the 43 polyploids are now distributed across 12 families, with 19 in the family Pipidae (plus some unnamed species); eight in Leptodacylidae (now divided into four families); four each in Myobatrachidae (now Lymnodynastidae) and Bufonidae; three in Microhylidae; two in Hylidae; and one each in Dicroglossidae, Arthroleptidae, and Ranidae. Several new polyploid anuran species have been reported (*Chiasmocleis leucosticta*, *Cophixalus pansus, Scaphiophryne gottlebei*, *Ceratophyrs joazeirensis, Pleurodema cordobae*) since the summary in Otto & Whitton (2000), along with a number of new *Xenopus* species (Evans *et al.*, 2004) and the surprising finding of sexually reproducing triploid toads in the *Bufo viridis* complex (Stöck et al., 2002; Stöck et al., 2006). Species status has also been given to diploids in what were previously mixed complexes of diploids and polyploids (e.g., *Odontophrynus americanus*, *Phyllomedusa burmeisteri* complex, *Bufo viridis* complex) and a cryptic octoploid (*Pleurodema cordobae*) has recently been discovered in populations of *Pleurodema kriegi* (see SI Table A1 for references).

Figure A1- Family-Level Amphibian Phylogeny



Family-level amphibian tree, redrawn from Roelants et al. (2007), indicating families where polyploid species are known (blue branches) and estimated dates of divergence of clades that include polyploid lineages. Suspected but unconfirmed cases of polyploid species (black branches) have been reported in the Leiopelmatidae and Scaphiopidae. The entire family Sirenidae has been reported as anciently polyploid but this has yet to be confirmed.

Possibly as a result of the practical difficulties of dealing with duplicated gene sequences, but also the fact that polyploid taxa are not strictly appropriate for phylogenetic analyses because they don't arise via cladogenesis, polyploid species (and their progenitors) tend to be under-represented in molecular phylogenies (e.g., Faivovich et al., 2005; Frost et al., 2006). This, combined with lack of knowledge on the nature of origins for most species (auto or allopolyploid; single vs multiple), makes it difficult to evaluate hypotheses about dates of origins of particular species pairs. There are also discrepancies between recent phylogenetic hypotheses for amphibians (e.g., Frost *et al.*, 2006; Roelants *et al.*, 2007). However, Roelants et al. (2007) present an analysis of diversification rates and predicted divergence dates within amphibians that allows some insights. Based on this family-level phylogeny, we plotted the phylogenetic distribution of families that include polyploids and highlight the approximate dates of divergence of families that include polyploids from their closest relatives (Figure A1). It is important to note that this is not an indication of when polyploid species arose, just when the families in which they are found diverged from families where polyploidy has not been identified. The clustering of divergence times in different lineages that include polyploids could suggest that climatic conditions in the early Cretaceous and beginning of the Paleogene favoured speciation by polyploidy. It is intriguing to note that the Pipidae (African polyploids in the genus *Xenopus*) and Limnodynastidae (Australian polyploids in the genus *Neobatrachus*) both diverged in the early Cretaceous and both of these families include multiple polyploid species, some of which appear to have speciated as polyploids or have no extant diploid progenitors (Evans et al., 2004; Mable and Roberts, 1997; Mahony et al., 1986). Among the Ranoids (early Cretaceous diversification), polyploids are found in families within each of the major clades (ranids, dicroglossids, pyxicephalids, arthroleptids and microhylids), which could suggest that conditions favourable for polyploidy existed before their divergence. Particularly intriguing is the 65 mya divergence (corresponding to the Cretaceous-Tertiary boundary) of the clade including Hylids, Ceratophrids, Cycloramphids, Leptodacytlids and Bufonids, all of which contain multiple independent diploid-polyploid species pairs. It would be tempting to speculate that the environmental instability during the K-T boundary promoted polyploid speciation, as has been suggested for plants (Fawcett et al., 2009). Dates of divergence

predicted for *Hyla versicolor* (last post-glacial period, ca. 12,000-35,000 ya Otto et al., 2007), and species in the Pipidae (maximum 65 mya and most species thought to have arisen before the Pleistocene (Evans et al., 2005; Evans et al., 2004), correspond to times when climatic conditions were likely highly variable. However, similarly to plants (Soltis et al., 2004), many of the polyploid anurans are thought to have had multiple origins (Espinoza and Noor, 2002; Gerhardt, 2005b; Holloway et al., 2006; Mable and Roberts, 1997; Ptacek et al., 1994; Stöck et al., 2005) and so polyploidy may be an ongoing process. Even if complete phylogenies or genomic evidence for duplicate genes were available, dating origins precisely thus could remain difficult. As Doyle and Egan (2010) point out, the divergence of homoeologous copies (duplicate copies from each progenitor parent) in an allopolyploid tracks the divergence of diploid species, not the origin of the polyploid and autopolyploid origins could be even more difficult to infer, so that skepticism about estimated dates is warranted.

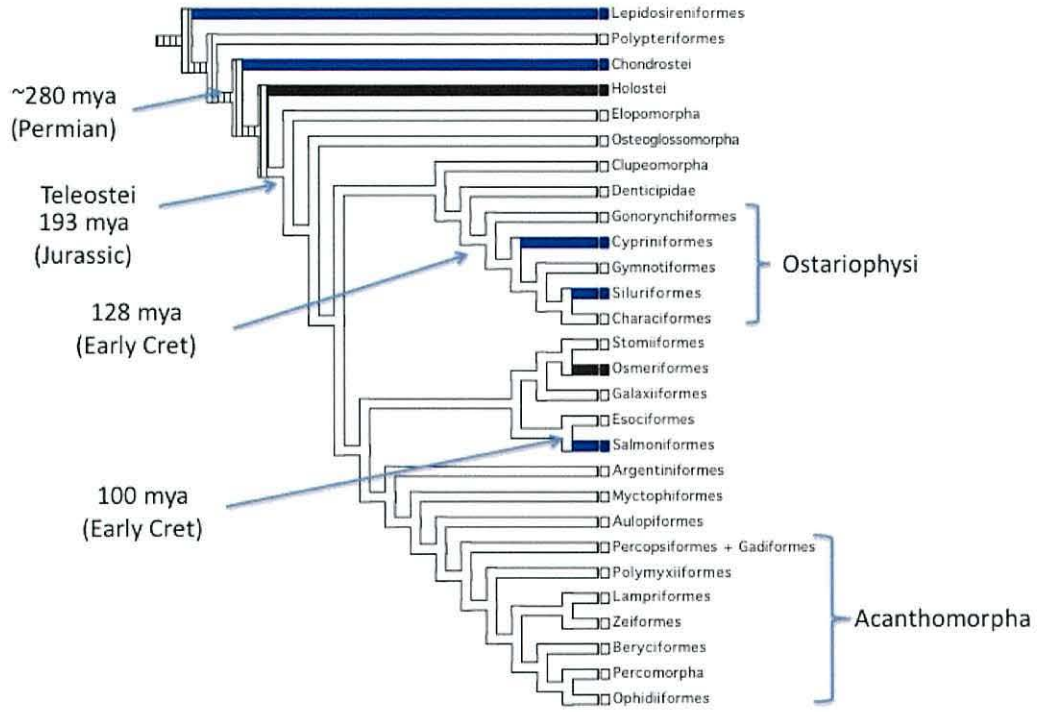
A.2.5.2 Fishes

For fishes, diploid ancestors of extant polploids are often not identifiable but, unlike amphibians, there are entire polyploid families. Based on karyotyping and genome size analyses, extreme cytogenetic variation has been found in certain lineages of fish (Hinegard, 1968; Hinegard and Rosen, 1972) (Venkatesh, 2003) and bisexually reproducing extant polyploids occur in a wide range of actinopteriygiian families, including the Acipenseridae (Birstein et al., 1997; Ludwig et al., 2001), Cyprinidae (David *et al.*, 2003), Catostomidae (Ferris, 1984), Callichthyidae (Oliveira et al., 1992), and Salmonidae (Johnson et al., 1987). Using the Cyprinidae and Salmonidae to illustrate attributes of polyploidy in fishes, Le Comber & Smith (2004) conclude that polyploidy may have been of considerable importance in the evolution of fishes. Polyploidy has also evidently played a role in the evolution of some lungfish species (Lepidoseriniformes), particularly within the genus *Protopterus*, with c-values surpassing 80 pg.

Although fish phylogenies also remain uncertain, we used the well-calibrated phylogeny presented in Santini et al. (2009) to illustrate relationships among the major fish orders

that include polyploids and plot rough divergence times (Figure A2). One large assemblage of fishes (primarily freshwater) referred to as the Ostariophysi contain multiple examples of extant polyploid families (Cyprinidae, Catostomidae, Cobitidae, Callichthyidae), constituting a significant proportion of the known polyploid actinopterygiian families within one massive clade. In addition, spontaneous polyploids have been reported in Gymnotiformes and Characiformes, which are also within the Ostariophysi (Figure A2). Of the remaining polyploid fishes, all are brackish, anadromous, or strictly freshwater species. With few exceptions, polyploidy is more common amongst early diverging teleosts and relict bony fishes than later diverging teleost lineages such as the Perciformes (Leggatt and Iwama, 2003). Thus, after the original three rounds of ancient genome duplications, subsequent polyploidization events seem to have occurred within particular families, while the majority of fish genomes remain functionally diploid.

Figure A2- Order-Level Fish Phylogeny



Phylogeny of fish orders, redrawn from Santini et al. (2009), indicating orders where polyploid species are known and estimated dates of divergence. A single family in the Percomorphs contains polyploid species (Channidae) but divergence of this lineage from relatives that do not include polyploids have not been clearly established. There is a notable absence of polyploids within the Acanthomorpha Within the Ostariophysi, spontaneous polyploids have been reported within the Gymnotiformes and Characiformes.

The order Acipenseriformes (sturgeons) is distributed across North America, Europe and Asia, and includes species with multiple levels of ploidy, with diploid species containing 120 chromosomes, tetraploids with 250 chromosomes, and even functional octaploids with 500 chromosomes (Birstein *et al.*, 1997). They are considered ancient relicts, with strikingly similar species preserved in the fossil record as far back as 200 million years (Bemis and Kynard, 1997). A study of genome duplication events and functional reduction of ploidy levels in sturgeon has revealed that gene silencing, chromosomal rearrangements, and transposition events are likely to be the dominant mechanisms that have shaped Acipenseriforme genomes (Ludwig *et al.*, 2001). Within this study, microsatellite analyses show that the maximum ploidy level for the Acipenseriformes is tetraploid and not octaploid, conflicting with original estimates (Birstein *et al.*, 1997). These differences may be due to the extinction of the original diploid Acipenseriforme ancestor, as the oldest extant genus within the order (Polyodon) contains species whose chromosomes can be easily arranged into quartets (suggesting that Polyodon may actually be tetraploid). Furthermore, *Acipenser sturio* (presumed to be diploid with 116 chromosomes) has a C-value approximately double the size of its closest relatives. These basal species are thought to be ancient tetraploids functioning in a diploidized state (Vasil'ev, 2009). Thus, there are a number of unresolved issues within this ancient order of fishes that require further in-depth analyses, including complete taxonomic coverage, further karyotypes and genome size estimates.

The order Cypriniformes contains the largest diversity of fish polyploids known to date, with over 250 recognized polyploid species spread across North America, Europe, Africa and Asia. Most cypriniform polyploids are tetraploids or hexaploids, with chromosome complements ranging between 100-150, while one species (*Ptychobarbus dipogon*) has an amazing 446 chromosomes. The largest family of freshwater fishes, the Cyprinidae (Teleostei: Cypriniformes), contains many species and genera with great cytogenetic variation. Two other families seem to contain a large number of tetraploid species, notably the Catostomidae and the Cobitidae; however, due to the complexity of Cypriniform systematics, this is likely to change in the future (Ferris, 1984; Saitoh et al., 2010; Suzuki and Taki, 1996). A molecular phylogenetic study based on cytochrome b and rRNA sequence data has revealed that a single polyploidization event occurred in the

lineage leading to the Botiinae (Cobitidae), suggesting a single origin for this monophyletic tetraploid assemblage (Slechtova et al., 2006). Many cyprinid genera are composed of stable polyploid series, including: *Barbus*, *Labeobarbus, Luciobarbus, Pseudobarbus, Spinibarbus, Diptychus, Carrasius, Capoeta, Tor, Cyprinus, Schizothorax, Sinocyclocheilus* and more. The genus *Barbus* consists is of particular interest as it contains at least 350 species and is well known for its morphological variation, wide distribution and the existence of diploid, tetraploid and hexaploid species (Berrebi, 1995; Klose et al., 1969; Machordom and Doadrio, 2001). In a study focusing on the evolutionary history and modes of speciation within the genus *Barbus*, Machordom & Doadrio (2001) reconstructed phylogenetic relationships based on three mitochondrial genes in an effort to infer patterns and processes of polyploidy. Although the authors focus on systematic relationships within *Barbus*, they propose that genome duplication within this genus may be considered as a homoplasic character, since it must have occurred over at least three independent periods and/or in three independent African regions. However, the lack of nuclear markers within this study make it difficult to cross-validate relationships and potentially infer a history of hybridization through topological incongruence. Another phylogenetic study relying solely on cytochrome b comes to similar conclusions concerning the multiple origins of African barbs (Tsigenopoulos et al., 2002); yet, once again, the authors focus more on the systematic relationships of the genus rather than hypotheses dealing with polyploidization events. Within Asia, the Yunnan province of China seems to contain an amazing diversity of cyprinid polyploids, but the biogeography of these fishes remains understudied. Given the diversity of cypriniform polyploids, combining comprehensive molecular phylogenies with more karytoyping and genome size estimates and other techniques should help to resolve the complex history of genome duplications within the group.

Within the Siluriformes (Catfishes), the species rich genus *Corydoras* (Callichthyidae: Corydoradinae) from the neotropical region is the most well studied and diverse group of polyploids with an impressive range of genome sizes and karyotypic variability (Fenerich et al., 2004; Hinegard and Rosen, 1972; Oliveira et al., 1993a; Oliveira et al., 1993b; Oliveira et al., 1992; Oliveira et al., 1988; Shimabukuro-Dias et al., 2004). The latter research supports the existence of multiple polyploid groups within the genus with

chromosomes ranging between 2n=44-132 and c-value between 1.3-8.75pg. The primary mechanisms presumed to have shaped the complex genomic variability within these lineage include: Robertsonian translocations, fissions, fusions, inversions and polyploidy followed by DNA loss (Oliveira et al., 1992; Oliveira et al., 1993c). Despite the recent publication of a comprehensive molecular phylogenetic framework (Alexandrou et al., 2011), many species remain without cytogenetic information, making it difficult to infer ploidy levels within the genus. Given karyotypic variability, it is also possible that several species of *Hypostomus, Plecostomus, Trichomycterus* and *Wallago* are polyploid, but this remains to be confirmed (Fenerich et al., 2004; Rab, 1981). Other polyploid catfish species that have been revealed at an intraspecific level include: *Heteropneustes fossilis* (Pandian and Koteeswaran, 1999) with a haploid c-value of 9.75pg (largest of all actinopterygyians) and *Clarias batrachus* (Mustafa and Shams, 1982), yet these are isolated examples in comparison to the genus *Corydoras*.

Tetraploidy is an ancestral condition of the Salmonidae, originally shown via linkage analyses (Johnson *et al.*, 1987) and microsatellite data (Gharbi et al., 2006; Sakamoto et al., 2000), but also confirmed by PCR amplification of non-orthologous sequences in different taxa (Angers et al., 2002). The Salmoniformes are all polyploid, having undergone a genome duplication after their separation from the Esociformes, between 45 and 100 million years ago (Allendorf and Thorgaard, 1984; de Boer et al., 2007; Santini et al., 2009). Most salmonids have a haploid c-value of approximately 3pg, while karyotypes range between 2n= 56-104. Similar to the Acipenseriformes, salmonids primarily occupy temperate regions; however, some species from the genus *Coregonus* can also be found within polar climates. The extreme migratory behaviour of salmonids might be related to the original ancestral shift in ploidy level. Recent evidence from molecular phylogenetic analyses supports the Esociformes (Esocidae and Umbridae composed strictly of freshwater species) as the sister group of the Salmonidae (Broughton et al., 2010).

Finally, several species from the genus *Channa* (Channidae) are the sole polyploid representatives of the order Perciformes (Banerjee et al., 1988); an extremely diverse lineage estimated to contain in excess of 10,000 species. Despite karyotypes of 78 and

104 chromosomes, genome size estimates for these species are lacking, making it difficult to accurately assess ploidy status (Rishi and Haobam, 1984). It is very likely that a bias in taxonomic sampling and a lack of more recent cytogenetic investigations have partially led to the pattern of imbalance in polyploidy among different taxonomic groups of fishes; and, given the diversity of Perciformes, species with duplicated genomes may yet be discovered.

A.2.6 What factors favour polyploid formation?

While polyploidy is not quite as rare as often suggested in animals, it is obvious that extant polyploidy is restricted to particular groups. In this section we focus on the drivers that might promote formation of polyploid lineages, emphasizing traits shared by fish and amphibians. We specifically question whether there are intrinsic features or ecological preferences shared by fish and amphibians (or at least those that give rise to polyploid lineages) that make them more prone to the initial formation of polyploids. The majority of polyploidization events in both plants and animals are thought to have been the result of unreduced gamete formation (Hagerup, 1932; Husband and Schemske, 2000; Rabe and Haufler, 1992; Ramsey, 2007; Ramsey and Schemske, 1998; Winge, 1917) but other mechanisms, such as polyspermy are also possible. Unlike plants, somatic polyploidization has not been described in animals. Under conditions where the frequency of these types of events is highest, it seems probable that opportunities for formation of polyploids would be maximized. We also consider other factors that might increase opportunities for the formation of successful polyploid individuals, such as gamete production, reproductive environment, and propensity for hybridization.

A.2.6.1 Frequency of Unreduced Gametes

Although unreduced gametes occur spontaneously in most vertebrates, they appear to produce viable progeny mainly in ectotherms. Artificial experimentation suggests that the ease of unreduced gamete formation is particularly high in fish and amphibians (Fankhauser, 1945). The absence of the pachytene checkpoint, which is a meiotic surveillance system present in many animals that would normally prevent the formation of unreduced gametes, has been suggested as a possible reason for the high prevalence of polyploidy in plants (Li et al., 2009). The authors suggest that perhaps this system is absent or defective in the animals that do give rise to polyploids but this has not yet been evaluated. There also might be other features of gametogenesis or the fertilization process that increase the potential for producing unreduced gametes.

Oogenesis is basically the same in all amphibians: primary oocytes undergo meiotic

division to yield secondary oocytes and the first polar bodies; activation of the egg by the sperm stimulates the second reduction division of the secondary oocyte (most amphibian eggs are arrested in metaphase II), resulting in an ovum and a secondary polar body (Duellman and Trueb, 1986). Polyploidy in amphibians can be experimentally induced by cold or pressure shock of females prior to fertilization. This is thought to disrupt spindle formation during meiosis, and result in retention of the 2nd polar body, which would lead to a diploid ovum, producing triploid gametes if fertilized by haploid sperm. For example, in *Xenopus,* unreduced gametes are known to be produced at a rate of about 10% in artificial hybridizations between species (Tymowska, 1991) and this can be increased by cold or pressure shock (Kobel and Du Pasquier, 1986). It is apparently not as easy to induce the formation of unreduced (diploid) sperm experimentally and surveys of ploidy have not found as high a frequency of spontaneously formed diploid sperm as diploid eggs in natural populations of anurans (Bogart, 1980). In temperate anurans, sperm cells mature uniformly throughout the testes but in tropical species that breed throughout the year, testes contain sperm cells in various stages of maturation (Duellman and Trueb, 1986). Although the origins are unknown for most tetraploid taxa, one possibility is through an intermediate triploid stage (e.g., Cunha et al., 2011; Fankhauser, 1945), since triploids only require unreduced gametes to be formed by one parent. Tetraploid gametes could be formed by disruption of the first mitotic division after fertilization. However, experimental attempts to synthesize tetraploid, rather than triploid, gametes in anurans have not been highly successful, so it has been inferred that an intermediate triploid stage is critical (Bogart, 1980).

Particularly in temperate or dry regions, anurans often breed during times when temperatures are unstable, which could increase the frequency of production of unreduced gametes. For example, the threshold for breeding in *H. chrysoscelis* (which is the diploid thought to have given rise to multiple independent lineages of *H. versicolor*) is at a water temperature of 15°C, which normally occurs in the early spring, when low temperatures and frosts are still likely to occur. Nevertheless, other sympatric species do not produce tetraploid lineages, even though experimental induction of unreduced gametes has been demonstrated (e.g., Richards and Nace, 1977), suggesting that temperature variation alone cannot explain tetraploidy in the grey treefrogs.

Experimental hybridization studies demonstrate that it is possible to produce viable polyploid offspring in a number of frog species where natural polyploids do not exist (e.g., Fankhauser, 1945; Nishioka and Ueda, 1983), suggesting that there are not intrinsic blocks to production of polyploid lineages in other anuran species.

In fishes, although 37 different ways of inducing polyploidy have been described (Pandian and Koteeswaran, 1998), polyploids have most commonly been induced by either temperature or pressure shock, with cold shock typically favoured for warm water species and warm shock favoured for cold water species (Donaldson et al., 2008). Temperature shock induces polyploidy by one of two mechanisms (1) causing the retention of the second meiotic polar body or (2) blocking the first mitotic division (Tiwary et al., 2004). High pressure of between 400-600 atmospheres may also induce polyploidy. Polyploidization (triploids and tetraploids) has been induced in fishes through temperature shock in plaice (Pleuronectes platessa Purdom, 1972), common carp (Cyprinus carpio Gervai et al., 1980), grass carp (Ctenopharyngodon idella Cassani and Caton, 1985), rainbow trout (Thorgaard et al., 1981), Channel catfish (Ictalurus punctatus Wolters et al., 1981), turbot (Scophthalmus maximus Piferrera et al., 2003), tilapia (Oreochromis aureus Don and Avtalion, 1988), and a cyprinid loach (Misgurnus anguillicaudatus Chao et al., 1986). While pressure shock is not relevant to polyploidy formation in wild systems, cold or heat shocks may occur naturally through changes in thermoclines, water movements such as flooding or snow melting, heavy precipitation or rapid changes in seasonal temperatures (Donaldson *et al.*, 2008).

A.2.6.2 Polyspermy

One route to polyploidy that does not involve unreduced gametes is through polyspermy - the fertilization of a single egg with more than one sperm. In many fishes and amphibians there is ample opportunity for multiple sperm to come into contact with an egg. However, in fishes, with the exception of the elasmobranchs only a single sperm actually penetrates. A range of physical and chemical mechanisms prevent polyspermy in animals, but the underlying mechanisms appear to be relatively conserved across the

animal kingdom (Wong and Wessel, 2006). Physiological polyspermy is the condition where multiple sperm fuse with an egg, but only one male pronucleus is merged with the haploid egg nucleus. Polyspermy is common in many urodeles, where 90–100% of all eggs may be polyspermic (Elinson, 1986; Iwao, 1989). However, despite multiple sperm penetrating the egg, only a single sperm fuses with the pro-nucleus and additional cytoplasmic sperm nuclei are subsequently suppressed (Elinson, 1986; Fankhauser, 1932; Iwao, 1989; Iwao and Elinson, 1990). Anurans use a diverse variety of mechanical methods to block polyspermy including reorganization of the egg extracellular matrix and hydroscopic swelling of the outer jelly layers to create barrier against sperm (Elinson, 1986; Hedrick and Nishihara, 1991).

The micropile is the point of entry through the corian for sperm attempting to fertilize a teleost fish egg. In teleosts there is a single micropile per egg (Hart, 1990); however, in the more ancient sturgeon and paddlefish (Acipenseriformes) there are several micropiles (Ciereszko et al., 1996; Hart, 1990) that represent an evolutionary mid-point between sperm storage in the elasmobranchs and a single micropile in the teleosts. In fishes, the chorion is an important physical barrier to polyspermy, and unfertilized eggs will be polyspermic if this is removed. The width of the micropile in most teleosts (with the exception of carp, where the micropile diameter is 2-3 times the width of the sperm Kudo, 1980) prevents multiple sperm entering the egg. Although sturgeons and paddlefish exhibit high levels of polyploidy and also have theoretically greater potential for polyspermy than teleosts due to multiple micropiles, polyspermy does not appear to be implicated in the genome duplications identified in other teleost lineages. There appear to be no difference in the mechanisms used by teleosts to prevent polyspermy in marine vs freshwater species suggesting that polyspermy is not a major driver in the differences in rates of polyploidy between marine and freshwater teleosts.

A.2.6.3 Gamete Production

Production of unreduced gametes does not necessarily mean that a reproductively viable polyploid individual will be formed, due to problems arising due to aneuploidy,

imbalances in chromosome numbers, altered dosage of parental proteins, and incompatibilities of parental genomes. Duplicating the chromosome complement increases the risk of problems during meiosis, particularly if more than two copies of each chromosome can pair. Aneuploidy tends to be even more common for unbalanced chromosome sets (triploid or pentaploid), so if most polyploids arise through an intermediate triploid stage, aneuploidy would be expected to be an inhibitory factor. Generation of sustained polyploid lineages is thus limited by the ability to produce offspring with an appropriate copy number of each chromosome in both the diploid progenitors and the newly arising polyploids. Due to the stochastic nature of chromosome pairing, it might be expected that, although a small proportion of gametes would end up with balanced sets, if they were the only forms that were viable, selection for full chromosome complements would be strong. This means that organisms (such as mammals) that produce few female gametes at one time would not be expected to form stable polyploid lineages (Mable, 2004). Fish and amphibians both tend to produce large numbers of both male and female gametes, which could facilitate generation of viable polyploid progeny.

Annual fecundity in amphibians ranges from one to potentially more than 80,000 offspring (Salthe and Mecham, 1974). Clutch sizes are not available for most of the polyploid anurans or, perhaps more importantly, their diploid counterparts, but Duellman & Trueb (Duellman and Trueb, 1986) report that while small clutches are produced by some species with terrestrial egg development and in ovoviviparous species, those with aquatic reproduction typically have clutch sizes in the range of 100's to many thousands (ranging to over 47,000 in *Rana catesbeiana*). The tetraploid *Tomopterna (Pyxicephalus) delalandii* has a clutch size of 2,500, and a survival rate of 19% (Wager, 1965), but this report was when it was still synonymized with its diploid progenitor so it isn't clear on which ploidy level the study was performed. Multiple mating opportunities per year increase the combinatorial aspects of fertilization and so could further increase the probability of producing viable, balanced gametes. However, since the vast majority of anurans produce large numbers of eggs, egg number alone cannot explain the success of polyploid formation in some groups. Nevertheless, high gamete numbers could enhance the probability of formation of balanced combinations of parental genomes.

The fecundities of fish can be equally impressive; egg numbers range from relatively few large eggs found in mouthbrooding cichlids (Kellogg et al., 1995; Taylor et al., 2003) to many millions of small eggs found in Atlantic sturgeon (Ryder, 1888) and cod (Thorsen et al., 2010). For example, egg numbers in *Esox lucius* can be as high as 300,000 to 400,000 per female (Billard, 1996) whereas salmonids have fewer eggs, with numbers ranging from 200-12,700 eggs per female (Scott and Crossman, 1973). Egg number and size is closely linked to breeding strategy and parental care (Kolm and Ahnesjo, 2005; Sargent et al., 1987; Wootton, 1984). For example, no parental care is provided by either sturgeons or paddlefishes, which produce hundreds of thousands to millions of eggs. The number of eggs produced by female fish is highly correlated with body size and additional trade-offs may be found with egg size vs egg number. If female gamete number is related to the propensity to form polyploids we might expect to find higher egg numbers in the ancestors of polyploid lineages or in the closest relatives of polyploid species than in lineages that do not include polyploids. However, while no exhaustive phylogenetic treatment has been conducted, this does not appear to be the case. While many freshwater groups where polyploidy occurs have large numbers of eggs, so do marine species such as the gadidae where no polyploid species have been identified. Additionally, in general, clutch sizes are much larger in marine fishes than in freshwater species, with freshwater fish producing low numbers of large eggs and marine fish produce large numbers of small eggs (Elgar, 1990). As all known polyploid teleosts reproduce in freshwater, the relationship doesn't appear to hold. It could be that there is a threshold number of eggs that would be required to favour the production of stable polyploid lineages.

A.2.6.4 Reproductive Environment

The most obvious feature shared by polyploid fish and amphibians is that, nearly without exception, they reproduce in freshwater environments. Reproduction in aquatic environments in general exposes ectotherms to fluctuations in environmental conditions during the breeding season and freshwater habitats are known to be more variable than marine environments. During times of environmental instability such as postglacial

periods, variation in temperature during the breeding season thus could be substantial, and large numbers of individuals could be exposed to temperature fluctuations in a local area (reviewed in Mable, 2004). External fertilization in an aquatic environment also enhances mixing of male and female gametes, which would facilitate the probability of producing offspring with balanced chromosome set combinations. Broadcast sperm deposition in aquatic environments, when multiple individuals breed at the same time, also promotes multiple paternity, which could further allow selection of favourable gamete combinations or polyspermy.

In contrast to salamanders and caecilians (where no bisexually reproducing polyploids have been found), virtually all anurans reproduce using external fertilization; internal fertilization is only known in a few genera and none of the species are known polyploids (Duellman and Trueb, 1986). Although there is a trend towards terrestrialization of fertilization in anurans (Duellman and Trueb, 1986), and there is variation in reproductive modes within the families that include polyploids, as far as can be ascertained, all of the known polyploid species and their diploid progenitors use the generalist mode of reproduction, thought to be ancestral: eggs deposited and larvae developed in a lentic (still water) environment. It is interesting that in the Pipidae, while the genus *Xenopus* is exclusively polyploid and deposits eggs communally into an aquatic environment, polyploids are not known in the genus *Pipa*, which has indirect development via eggs deposited to the dorsum of the female. In a survey of 5,828 amphibian species, Vences & Kohler (2008) found that 4,117 species live in an aquatic environment during at least one life history stage, with 177 more being water dependent.

All of the known polyploid anurans are communal breeders, where multiple males and females congregate simultaneously to breed. This can be particularly dramatic in amphibians that live in dry environments, where breeding opportunities are limited by occasional periods of extensive precipitation. For example, in the Australian frog genus *Neobatrachus*, individuals remain buried in sandy soil even across the central deserts for most of the year and breed only during cyclones, which means that breeding does not occur every year but breeding choruses are large when conditions are appropriate (Roberts and Majors, 1993). This type of group breeding strategy would enhance the

potential for exposure of individuals to the same extreme conditions during breeding.

Many of the polyploid fish also breed communally and nearly all live in or return to freshwater to breed. The Salmonidae are anadromous and reproduce in freshwater, where females lay eggs in redds and these are fertilized by multiple males (Hutchings and Myers, 1988). Sturgeons migrate upstream in rivers to spawn if they are marine, or to shallow areas of lakes if they live in freshwater. Typically, several males spawn with a single female (Bruch and Binkowski, 2002). Some species of Cyprinifomes form breeding aggregations, as observed in the genus *Barbus*, where communal spawning in the lake Tana barbs occurs after a migration upstream from the lake (de Graaf, 2003). In the Corydoradinae, reproduction is also a communal process and can often be triggered under aquarium conditions by reducing the temperature of the water (Fuller, 2001), thereby simulating the effects of sudden rainfall after a long dry season. Again, the preponderance of communal breeding in freshwater environments across fish and anurans does not explain why some species are polyploid and others not, but it does enhance the probability of formation of viable gamete combinations.

A.2.6.5 Propensity for Hybridization

Although estimates of the relative frequency of autopolyploidization and allopolyploidization events remain rare, even in plants, polyploidy is often associated with hybridization. Both hybridization and polyploidy can involve dramatic and immediate changes in genome structure (Buggs et al., 2010; Chelaifa et al., 2010; Gaeta and Chris Pires, 2010; Gaeta et al., 2007; Landry et al., 2007; Marmagne et al., 2010; McClintock, 1978), which could alter adaptive responses to environmental change, so it is unclear whether it is hybridization or polyploidy that might allow invasion of "harsh" environments. A recent special issue on polyploidy in plants emphasized that it is predominantly hybridization and not genome doubling that explains the dramatic changes documented in recent studies (Ainouche and Jenczewski, 2010). Although many cases of hybrid animals have been linked to polyploidy, many have also been associated with asexual reproduction (reviewed by Dowling and Secor, 1997; White, 1973). It has been

suggested that autopolyploidy could be rarer in animals that reproduce strictly sexually because they would be too similar to their diploid counterparts to gain a competitive edge without the reproductive assurance and potential for increasing numbers of their own cytotype provided by self-fertilization and so hybridization would be required to produce competitively different lineages (White, 1973). As our tools for evaluating genome structure advance, footprints of hybridization are becoming more broadly apparent in animals as well as plants (Baack and Rieseberg, 2007; Bi and Bogart, 2006) and similar numbers of well-supported cases of homoploid hybrid speciation (i.e. without a change in genome copy number) have now been documented in both taxonomic groups (Gross et al., 2007; Mallet, 2007; Mavarez and Linares, 2008). Whitney et al. (2010) estimated that plant hybrids in the wild occur in 40% of families and 16% of genera (including polyploids), with a frequency of 0.09 hybrids per nonhybrid taxa. They found that hybridization propensity tended to be consistent across regions, suggesting that hybridization behaviour may be determined more by intrinsic properties of a group rather than environmental conditions. While this would reflect the rate of "successful" hybrid establishment, it does not necessarily mean that opportunities for hybridization would not be increased by climatic shifts; both habitat fragmentation and changes in temperature during times of environmental instability are likely to change species interactions by altering distributions and bringing new combinations of individuals together, which can increase rates of hybridization (Seehausen et al., 2008). In plants, polyploidization has been found to "rescue" otherwise incompatible combinations of hybrids in diploids, possibly due to the possibility of preferential pairing of homeologous chromosomes, which could more easily allow incompatible allelic combinations to be eliminated or down-regulated (Martinez-Perez et al., 2001; Mestiri et al., 2010) or induce epigenetic silencing due to the increase in chromosome number (Mittelsten Scheid et al., 1996). There is some evidence that the extent of genomic divergence between hybridizing species influences the likelihood of diploid versus polyploid hybrid speciation, with more divergent genomes more often giving rise to the latter (Chapman and Burke, 2007; Paun et al., 2009). However, recent analyses in plants suggest that the pattern might be driven more by restriction of parental divergence in the production of viable diploid hybrids rather than polyploidy "rescuing" more distant hybrids (Buggs et al., 2009; Buggs et al.,

2008).

In the frog genus *Hyla*, whereas hybridization between diploid taxa tends to be limited by genetic distance (Ralin, 1970), experimental crosses involving tetraploid females have been found to be more successful with distantly related than closely related diploids (Mable and Bogart, 1995). This might be due to inability to recognize "foreign" chromosomes and alter regulatory controls accordingly to reduce incompatibilities. Since the male genome is not expressed until post-gastrula in anurans, diploid hybrid combinations often fail until after this point, when incompatibilities between parental genomes would become apparent (Mecham, 1965). Some amphibians are quite prone to hybridization (e.g., Bufo Blair, 1972; Malone and Fontenot, 2008) but it is interesting that in the genus *Rana*, where hybridization is not as common (Green, 1985) no polyploids have been described, except for the *Rana esculenta* complex, which has a complex reproductive system (hybridogenesisVinogradov et al., 1990). Although autopolyploidy has been suggested for some species based on tetrasomic inheritance and limited genetic distinction from closely related diploids (e.g., Hyla versicolor Bogart, 1980), hybridization between closely related diploids cannot be ruled out in most cases (e.g., Dowling and Secor, 1997; Holloway et al., 2006; Ptacek et al., 1994) and hybridization among tetraploid lineages is ongoing (Espinoza and Noor, 2002). Most species of *Xenopus* are thought to have arisen through hybridization (Evans, 2008; Evans et al., 2005) and hybridization occurs naturally within ploidy levels of extant species (Fischer et al., 2000; Kobel et al., 1981), In addition, evidence for allopolyploid origins has been provided for species in the Bufonidae (Bogart, 1980; Stöck et al., 2006; Stöck et al., 2005), Microhylidae (Vences et al., 2002), Ranidae (Channing and Bogart, 1996) and Bogart 1996), and Myobatrachidae (Mable and Roberts, 1997; Roberts, 1997a; Roberts, 1997b; Roberts, 1996). Based on the production of polyploid individuals, there is some evidence that unreduced gamete formation is higher in artificially produced hybrids between distantly related parents (Bogart, 1980), but whether this is a cause (i.e. hybridization promotes unreduced gamete formation) or a consequence (i.e. unreduced gamete formation allows survival of otherwise incompatible hybrid combinations) of polyploidy remains unclear.

Of the known groups of polyploid fish, the Salmonidae are thought to be autopolyploid due to multivalents at some loci, particularly in males (Allendorf and Thorgaard, 1984; Hartley, 1987; Wright et al., 1983). However most other examples indicate allopolyploid origins. For example, the tetraploid form of the Japanese spined loach (*Cobitis* 'yamato complex') appears to have an allopolyploid origin (Kitagawa et al., 2003), resulting from the hybridization of *Cobitis biwae* and *Cobitis striata* (Saitoh *et al.*, 2010), while other species of polyploid *Cobitis* are gynogenetic (Juchno and Boron, 2006). Other groups likely to have had allopolyploid origins include the Catostomidae (Ferris, 1984; Ferris and Whitt, 1977b; Uyeno and Smith, 1972), which is comprised of more than 60 species. In the Cobitidae there are three independent groups of tetraploids; however, assigning allopolyploid origin to them is not certain (Vasil'ev et al., 1990). The European polyploid *Barbus* spp. (Cyprinidae) appear to have an allopolyploid origin (Chenuil *et al.*, 1999), as does the common carp (Cyprinus carpio David *et al.*, 2003). The Botiidae is comprised of diploid (Leptobotiinae) and tetraploid (Botiinae) taxa, with a single origin of tetraploidy (Slechtova *et al.*, 2006); however, the origins of the polyploidy event are not known. Polyploid origins of the Corydoradinae remain unknown and most certainly merit further investigation. Thus, although propensity for hybridization is not a limiting factor for fish or amphibians in the formation of allopolyploids, the proportion of polyploids with hybrid origins remains unclear.

A.2.7 What factors favour polyploid establishment?

Theoretical explanations for the existence of independently reproducing polyploid species have focused on the presumed difficulty in establishing reproductively independent lineages when initially outnumbered by their diploid progenitors (minority cytotype exclusion principle: (Husband, 2000; Levin, 1975). The ability of a polyploid organism to occupy a new niche is crucial because otherwise competition with the presumably well-adapted diploid progenitor will be particularly pronounced. Moreover, newly formed polyploid populations are likely to be small, potentially allowing drift to fix ecologically relevant traits rapidly (although selection will be weaker). Possible advantages have been thought to be due to increased genetic flexibility provided by extra genome copies and the potential for regulatory innovation provided by extensive gene duplication (e.g., Beçak and Kobashi, 2004; Levin, 1983; Soltis and Soltis, 2000), which could broaden ecological tolerances and result in polyploids being able to survive harsher conditions than their diploid progenitors. This competitive edge might be expected to increase during times of climatic instability or allow polyploids to invade harsher environments (e.g., Lumaret, 1988; Stebbins, 1950; Stebbins, 1971). Dynesius (2000), for example, suggested greater rates of polyploid formation during times of climatic oscillations due to their high adaptive potential to rapidly changing environmental conditions. An intriguing hypothesis based on comparative genomics in plants is that genome duplication events tended to be clustered around the Cretaceous-Tertiary boundary (K-T boundary), when many plant species went extinct (Fawcett *et al.*, 2009), suggesting that polyploidy might have increased survival during times of environmental upheaval or were the best adapted to expand into vacant niches exposed by extinction events. Although insufficient genomic data currently exists for animals to allow the same test to be conducted, fossil evidence suggests that neither freshwater fish nor amphibians experienced the scale of mass extinctions during this time that many endotherms did (e.g., Milner, 1998). We thus might expect to find larger numbers of polyploid taxa in extreme environments, at the edge of ranges, or generally in more variable environments. We might also expect polyploid taxa to be more resilient to both abiotic and biotic pressures (e.g., pathogens, predators), and to show lower rates of extinction than their diploid relatives. Since the extent of genomic novelty would be

expected to be higher in hybrid lineages, such patterns should be most pronounced for allopolyploids.

It is also possible that other factors that do not require intrinsic genetic advantages (such as assortative mating) have played a role. Moreover, there has been a contrasting view in the literature that polyploidy represents an evolutionary dead end and that the prevalence of diploidization reflects maladaptation of duplicated genomes (Stebbins, 1950). In this section we evaluate attributes of polyploid fish and anurans that might promote the establishment of polyploid lineages and question whether there is evidence that genome duplication events have been related to times of climatic change or that polyploids show advantages (or disadvantages) relative to their related diploids in terms of ecological ranges, pathogen tolerance, or extinction rates.

A.2.7.1 Assortative Mating

Assortative mating by cytotype (i.e. prezygotic isolation from diploid progenitors) could enhance the probability that newly arising polyploid lineages will become established. Based on reproductive barriers between diploid and autotetraploid individuals of the perennial plant *Chamerion angustifolium*, simulations indicated that prezygotic isolation will reduce the strength of minority disadvantage acting on tetraploids and increase the importance of differences in viability and fertility between ploidy levels in regulating polyploid establishment (Husband and Sabara, 2004). In anurans, premating and/or postmating isolating mechanisms may arise automatically with the change in cell size or gene copy number resulting from genome doubling (Bogart, 1980; Holloway et al., 2006; Keller and Gerhardt, 2001). Since most diploid-polyploid species pairs differ by mating call, assortative mating by call type could enhance the potential of newly arising polyploids to find mates, as the hearing mechanism in the female changes correspondingly and females choose males of their own ploidy level (Keller and Gerhardt, 2001).

In fish, mate choice experiments are not as extensive as in frogs and toads, but production of specific olfactory cues has the potential to provide signals that could vary in direct

proportion to ploidy level and so allow assortative mating. There is also evidence that sound production is more common in fish than previously suspected, including in *Corydoras* catfish (Kaatz and Lobel, 1999; Kaatz and Lobel, 2001; Pruzsinszky and Ladich, 1998), which include multiple independently derived polyploids. It would be interesting to determine whether mate choice by ploidy exists in fish and to identify the underlying mechanisms. In mammals, major urinary proteins (which are linked to the major histocompatibility complex, MHC) have been demonstrated to be involved in mating decisions (Knapp et al., 2006) and orthologues of MHC-linked odorant receptor genes have been identified in *Xenopus* and zebra fish (Santos et al., 2010). However, the MHC does not appear to be retained in duplicate in polyploid *Xenopus* (Du Pasquier et al., 2009; Kobel and Du Pasquier, 1986; Sammut et al., 2002; Shum et al., 1993), or salmon (Kruiswijk et al., 2004; Shiina et al., 2005) so it isn't clear whether there would be ploidy-related signals.

A.2.7.2 Increased Ecological Tolerance

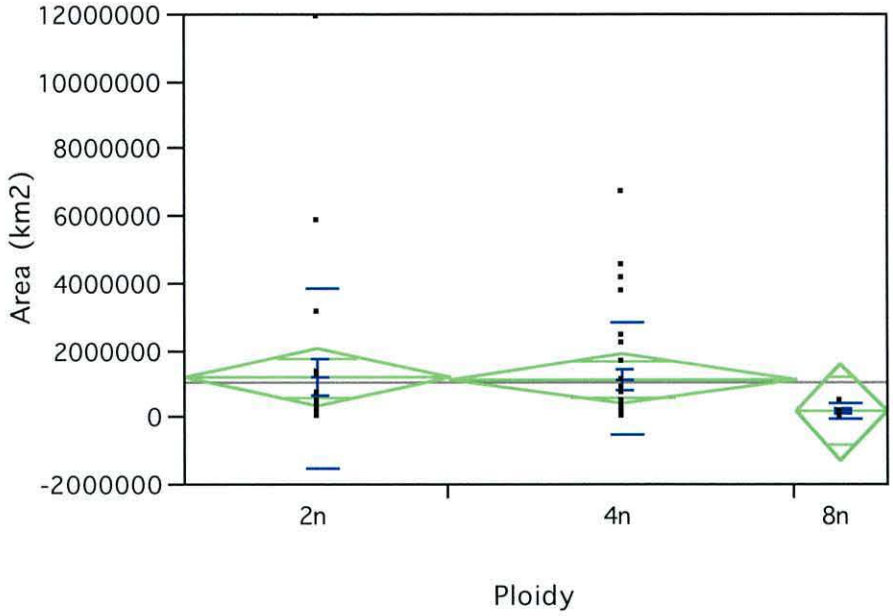
Even with assortative mating, establishment rates would be expected to be high only if polyploids had an initial fitness or competitive advantage over their diploid parents (Ramsey and Schemske, 2002). A long-standing observation is that polyploidy occurs more frequently at higher latitudes and higher altitudes, possibly because polyploids are genetically or physically more robust than their diploid counterparts (Ehrendorfer, 1980; Levin, 1983; Löve and Löve, 1943; Stebbins, 1971). However, in plants and some animals that show this distributional bias (e.g., Daphnia Dufresne and Hebert, 1997), polyploidy has also been associated with a shift in mating system towards autogamous reproduction (either through self-fertilization or parthenogenesis), which is thought to enhance dispersal abilities into novel environments because of relaxation of the need to find a suitable mating partner (reviewed in Mable, 2003). It also would provide newly arising polyploids with a higher chance of increasing to sufficient numbers that they could gain a competitive edge over their diploid progenitors. Whether polyploidy or the ability to reproduce without finding appropriate mating partners allows invasion of these potentially harsh environments is thus difficult to disentangle. This is particularly

difficult in plants, where transitions from outcrossing to selfing modes of reproduction have been described as the most common evolutionary transition among angiosperms (Bateman, 1952). There has also been suggestion that polyploids avoid competition following establishment by diversifying their ecological niches (Stebbins, 1950); again, invasiveness to new habitats has also been associated with shifts to selfing in plants. As early as 1940, Clausen et al. (1940; Clausen et al., 1945) suggested that it was by no means a general rule that polyploids occupy more extreme habitats than their diploid relatives but this view has held, at least partly because of the confounding effects of mating system. Soltis et al. (2010) also point out that it is difficult to define what "success" means in evolutionary terms and so whether or not polyploids are more successful than their progenitors is not a straight-forward question.

Nevertheless, vertebrates with strictly sexual reproduction may be better models to assess whether polyploidy, rather than mating system, allows range extensions to harsher environments. Based on digitizing areas in maps available through AmphibiaWeb, we found no significant difference in range sizes of polyploid anurans compared to their closest diploid relatives (Figure A3). While this is somewhat confounded by misclassification before ploidy was confirmed in cryptic diploid-polyploid species pairs, there is not a consistent pattern that would suggest an overall colonization advantage for polyploids. As for plants, some anuran species with higher ploidy levels are found in smaller ranges than their diploid counterparts, some are similar and some are larger. In addition, while some disjunction of ranges occurs, polyploids tend to exist in close geographical proximity to their diploid relatives. For example, for gray treefrogs, although tetraploids extend further north into colder environments and diploids extend further southeast into hotter environments, they overlap for most of their range. No differences in freeze tolerance have been demonstrated between the ploidy levels (Irwin and Lee, 2003), but the distribution of only the tetraploids north of the Great Lakes in eastern North America would be consistent with invasion of novel but more variable habitats after the last glacial period. It could, however, suggest that polyploidization initially occurred at the northern edge of the range after the last glacial maximum when environments were unstable and that the polyploids then expanded in both directions as the glacier receded, which would be consistent with estimates of speciation associated

with the Wisconsin glaciation (12,000-35,000 ybp). Otto et al. (2007) found that the tetraploid species occupies areas where climatic conditions are relatively severe (colder, drier, greater annual variation) whereas the diploid is more restricted in range, suggesting that large-scale climatic conditions have played a role in the establishment of the polyploid, in at least some portions of its range. For octoploid *Odontophrynus americanus*, a complex distribution pattern of populations with different ploidy levels exist, including areas of syntopy and sympatry, and cytogenetic variability, suggesting multiple origins of polyploids (Rosset et al., 2006). Overall, no obvious pattern emerges about relative distribution of diploid and polyploid species in amphibians that would suggest that polyploids expand into new environments or have broader ecological niches.

Figure A3- Anuran Polyploid Range Area



Range areas (km^2) for polyploid anurans compared to their closest diploid relatives, calculated by digitizing distribution maps provided through the AmphibiaWeb database http://amphibiaweb.org/index.html. There are no significant differences in range areas, although octoploid taxa tend to have smaller range areas than their diploid or tetraploid relatives, and several species have only been reported from single sites.

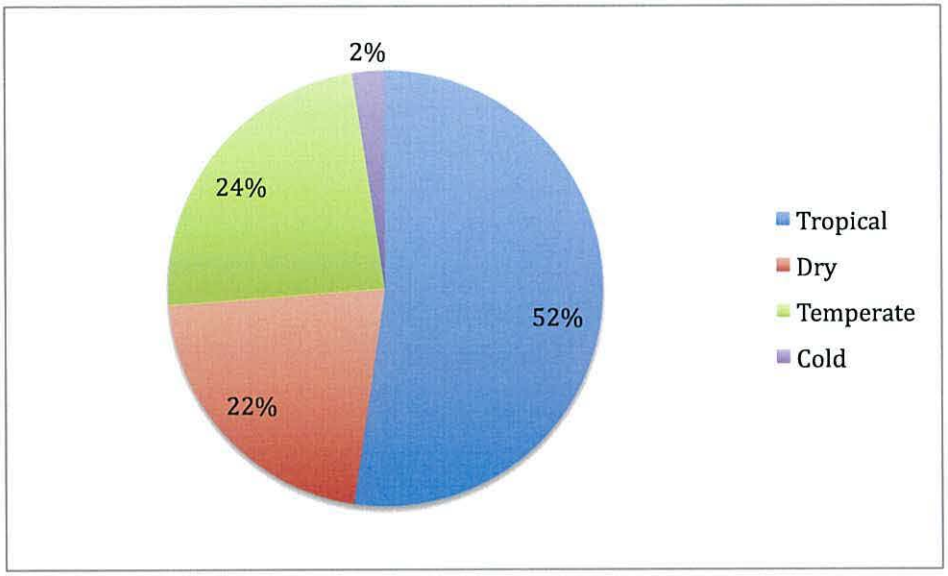
If polyploidy were associated with extreme environmental conditions, we might expect to find a higher proportion of polyploids in regions where climates are unstable or unpredictable. Since for amphibians, dry and cold environments could be considered harshest, we used the Köppen classification of climatic zones to evaluate whether polyploids tend to be found in extreme environments. Although 24% of polyploid anurans are found in temperate regions, 22% in dry regions and 2% in cold regions, the majority (52%) are found in the tropics (Figure A4a). However, in all cases, polyploids are found in the same climatic zones as their close diploid relatives. Of the species found in the tropics, two species are from Madagascar (*Cophixalus, Scaphiophryne*), where they are found at high elevations, where climatic conditions would likely be variable, even in the tropics. The remaining polyploids from tropical regions are in the family Pipidae, where polyploids occur at a range of elevations and habitat types. However, polyploidization within this group is relatively ancient (ranging from 2.7 mya for octoploid *X. wittei* and 42 mya for the ancestor of the current tetraploids in the genus *Xenopus*), so climatic conditions during times of speciation might have been very different than what they are currently (Evans et al., 2005; Evans et al., 2004). Few amphibians survive in cold environments so it is not surprising that only a single polyploid species (*H. versicolor*) is found under these conditions. Since in most cases polyploids do not have completely disjunct ranges from their diploid progenitors, there does not appear to be a dramatic advantage in terms of habitat exploitation.

For fish (Figure A4b), 46% of polyploidy species occur in temperate, 32% in subtropical and 21% in tropical regions. Mirroring the amphibians, only a small fraction of polyploid fishes occur in boreal (0.42%) or polar (0.83%) regions. It is also worthwhile to consider the habitat and life history strategies of polyploids. The vast majority of polyploid fish are dependent on freshwater: either living exclusively in freshwater (55%), migrating from marine to freshwater to breed (anadromous: 18%), or completing their entire lifecycle within rivers (potamodromous: 21%; SI Figure 2a). A small percentage are associated with brackish water (5.8%) and only a very few (0.4%) are catadromous (live in freshwater but migrate to a marine environment to breed). In addition, most polyploids either live near the bottom surface (benthopelagic: 61%) or in the bottom part of the water column (demersal: 36%) rather than on the surface (pelagic: 2.9%; SI Figure 2b).

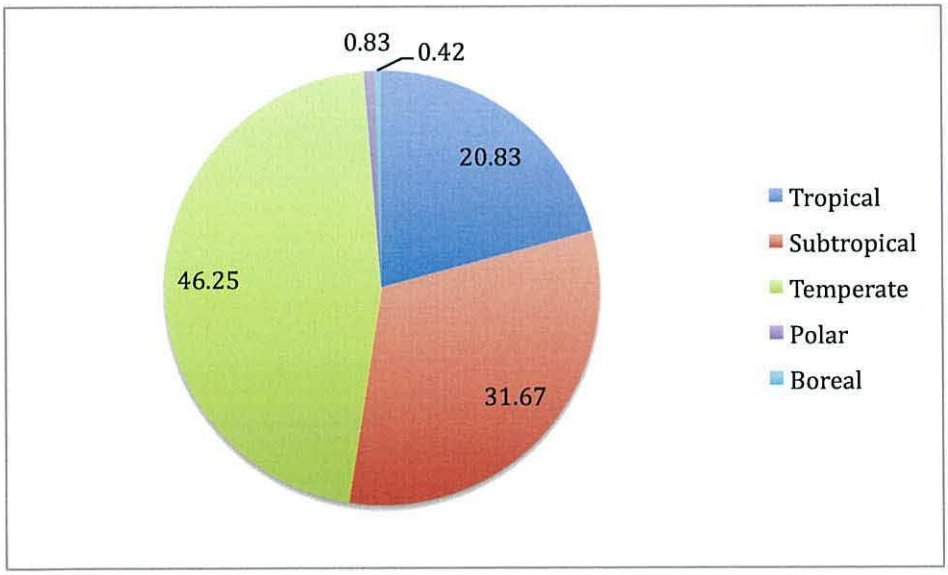
As niche shifts associated with ploidy change have been observed in some cases, it is interesting to note the possibility that the ancestors of sturgeons (Birstein et al., 2002) and salmonids (McDowall, 2001) were both strict freshwater inhabitants (Broughton *et al.*, 2010), with subsequent species occupying a broader niche (anadromous behaviour) and exhibiting greater environmental tolerance. While association with freshwater may indicate greater tolerance to fluctuating conditions, population sizes tend to be smaller in freshwater than marine fishes, thus potentially allowing for drift to maintain ploidy shifts at a greater rate than in marine species. Since most polyploid fish are not easily paired with diploid progenitors, it is more difficult to assess whether polyploids have larger ecological niches, but the complete lack of polyploids in more stable marine environments suggests association of polyploidy with environmental variability.

Figure A4- Climatic Distribution of Polyploids

(a)



(b)



(a) Distribution of polyploid anurans by major climatic zones, based on Köppen classification schemes. Note that there are no polyploid amphibians found in polar regions but there aren't any amphibians in general there and few species are found in cold regions. (b) Distribution of polyploid fish by major climatic zones. There are few polar species but a smaller proportion of tropical species than in amphibians.

A.2.7.3 Pathogen Pressures

It is possible that polyploids also might have increased adaptation to biotic pressures, such as those posed by pathogens. Since pathogen pressures are likely to change with environmental fluctuations and shifts to novel niches, newly arising polyploid lineages would likely be exposed to both novel and established pathogens. Particularly in the face of current concerns that global amphibian declines are related to changes in pathogen pressures (e.g., Green et al., 2002; St-Amour et al., 2008) there has been surprisingly little focus on resistance or response of polyploid taxa in relation to diploids. Frogs in the genus *Xenopus* have been implicated as reservoirs (i.e. they carry the pathogens but are not themselves greatly damaged by them) of the two most high profile disease agents currently thought to be threatening amphibians on a global basis: viruses in the family Iridoviridae (ranaviruses Robert et al., 2007) and the chytrid fungus species *Batrachochytrium dendrobatidis* (Weldon et al., 2004). For ranaviruses, this is due to the ability of adults to mount an effective immune response and clear the viruses (Robert *et al.*, 2007). The main vector is thought to be one of the tetraploid species, *X. laevis*, which has been used as an experimental developmental model for many years and has been commercially distributed worldwide. Although the only extant diploid species (*S. tropicalis* 2n = 20) has been found to be more susceptible than the tetraploid *X. laevis* (2n=36) to the type strain frog virus 3 (FV3; J. Robert, personal communication), it is not clear that this is due to polyploidy because the two species have different basal chromosome numbers. Although increased tolerance to pathogens has been suggested theoretically as a potential advantage of polyploids relative to their diploid progenitors (Guegan and Morand, 1996), this is also somewhat confounded by hybridization. The consequences of gene duplication for particular pathogen response genes have been investigated in economically important plants such as cotton (Wright et al., 1998), but since many cultivars arose through hybridization between species (allopolyploidy) rather than within a single species (autopolyploidy), both effects of combining genomes and cultivation history can obscure effects due to polyploidy itself. Hybridization has been considered in the transmission of pathogens between species (Cleaveland et al., 2007; Gonthier et al., 2007) but few studies have examined the consequences of genome interactions for pathogen response. A notable exception is the suite of studies focusing on

host-parasite co-evolution of monogeneans in relation to allopolyploid origins of their *Xenopus* hosts (Jackson and Tinsley, 2003; Jackson and Tinsley, 1998; Jackson and Tinsley, 2001). However, the long history of polyploidy in the host species makes it difficult to determine whether hybridization, polyploidy or other host factors are the most important in regulating this. Changes in pathogen distribution and virulence have also been linked to habitat and environmental changes (Bosch et al., 2007; Dionne et al., 2007; Laine, 2007) and so it is possible that at times when polyploid formation is most likely, there might also be exposure to new types of pathogen pressures. In these cases, it may be likely that newly formed polyploids benefit from increased pathogen resistance compared to diploid progenitors (Chevassus and Dorson, 1990; McDowall, 2001). Somewhat surprisingly, genes associated with immunity (at the major histocompatibility complex or MHC), which might be expected to benefit from higher diversity, are not always retained in duplicate in polyploid frogs (Du Pasquier et al., 2009; Kobel and Du Pasquier, 1986; Sammut et al., 2002; Shum et al., 1993), or fish (Kruiswijk *et al.*, 2004; Shiina *et al.*, 2005), so the relationship between pathogen response and ploidy remains unclear. In fish, despite focus on diseases of economically important salmonids, often in relation to MHC variation (e.g., Dionne, 2009; Dionne et al., 2007; Evans and Neff, 2009; Harris et al., 1998; Langefors et al., 2001; McClelland et al., 2003), there has been little emphasis on how polyploidy might influence pathogen responses.

A.2.7.4 Genomic Flexibility

Successful polyploid lineages might be those that can tolerate dramatic changes in genomic structure and regulatory divergence. Although duplicated genomes are thought to provide greater genetic flexibility and broader adaptive responses in general, reversion to effectively diploid segregation is apparent in most "old" polyploids and few polyploids retain duplicate gene expression across their genomes. Nevertheless, in animals rates of loss of duplicate gene expression have been far below expectations of neutral models (Allendorf, 1978; Ohno, 1970), so there is still potential that greater genomic flexibility exists in polyploids. For example, catostomid and salmonid fish retain approximately 50% duplicate gene expression, despite up to 100 million years of divergence as

polyploids (Bailey et al., 1978; Ferris and Whitt, 1977d) and many genes are retained in duplicate in polyploid series of *Xenopus* frogs (Hughes and Hughes, 1993). It has been suggested that genes involved in regulatory processes will be retained most frequently (Birchler and Veitia, 2007; Birchler and Veitia, 2010) and that selection for expression divergence is stronger than protein sequence divergence (Chain et al., 2008). There is also increasing evidence that epigenetic changes are abundant following polyploidization and hybridization (Bacquet et al., 2008; Beçak and Kobashi, 2004; Chen, 2007; Jackson and Chen, 2010; Matzke et al., 1999; Mittelsten Scheid et al., 1996; Paun et al., 2007; Rodin and Riggs, 2003; Xu et al., 2009), increasing the potential plasticity of duplicated genomes

Since hybrid ancestry itself might affect retention of duplicate genes and changes in expression (Evans, 2007), it is again difficult to separate the effects of genome duplication from the "genome shock" of hybridization. For example, Semon and Wolfe (2008) compared the expression profiles in 11 tissues of 1300 genes retained in duplicate in the tetraploid *X. laevis* relative to those in single copy in the diploid *S. tropicalis* and found a set of 68 genes that have undergone significant reduction in expression in at least two tissues. They found that slowly evolving genes tended to be more prone to subfunctionalization, which they concluded is due to allopolyploidization. They also found that the same orthologues found in zebrafish also tended to be retained in duplicate after the WGD at the base of the teleosts, suggesting that duplication of some types of genes could have selective advantages (or that some types of genes are not tolerated in duplicate). Polyploidy might be restricted to organisms with genomes flexible enough to tolerate the dramatic changes in genome structure and gene regulation that follow WGD and/or hybridization. Pandian & Koteeswaran (1998) remarked on the amazing ability of fish to tolerate genomes from haploid to heptaploid, genomic contributions from the male or female parent alone, and unequal contributions from parents belonging to the same or different species.

The first amphibian genome sequence has recently been published (Hellsten et al., 2010) (described as *Xenopus tropicalis* rather than *Silurana tropicalis*) but no tetraploids have yet been sequenced. Genome resources are more advanced in the fishes compared to

many other orders and so there is promise of assessing the consequences of WGD in detail. Draft genome sequences are available for *Fugu* (or Takifugu Aparicio et al., 2002) and Medaka (Kasahara *et al.*, 2007) and have been used to identify paleopolyploid events and the proto-karyotype of vertebrates (Dehal and Boore, 2005; Jaillon et al., 2004; Nakatani et al., 2007; Panopoulou et al., 2003; Panopoulou and Poustka, 2005; Putnam et al., 2008). The availability of an increasing number of fish genomes is allowing a better understanding than ever before about the role of gene and genome duplication in the evolution of fishes. There is now substantial evidence from remnant duplicate gene pairs suggesting that an ancient genome duplication event of tetraploidization (followed by diploidization) enabled the diversification of gene functions necessary to promote the explosive speciation in fish (Luo et al., 2007; Volff, 2005). The loss of certain genes, their subdivision, and acquisition of novel functions over evolutionary time seem to be linked with the evolution of fish variability (Lynch, 2007; Meyer and Schartl, 1999; Siegel et al., 2007; Vogel, 1998). The genomic complexity and plasticity of the teleosts might be the reason for their evolutionary success and astounding biological diversity (Luo et al., 2000; Meyer and Schartl, 1999), although this genomic plasticity might also come at a cost to diversity (Luo *et al.*, 2000). Yet, despite the indirect evidence, a link between a specific genome duplication event and an increase in overall complexity and diversity remains to be established (Donoghue and Purnell, 2005; Otto and Whitton, 2000). Crow & Wagner (2006) suggest that the probability of extinction was reduced by a factor of at least 5.5 in the lineages following the fish-specific genome duplication. However, correlations between specific duplications and increased diversity are problematic, as the genetic signature of single duplication events tends to be obscured by extensive genomic expansion, contraction, and subsequent gene loss (Blanc and Wolfe, 2004; Crow and Wagner, 2006). Nevertheless, rapid increase in genomic information should enable more precise evaluation of whether genomic constraints to polyploidy can explain why some species are polyploid and some not.

A.2.7.5 Risk of Extinction

If polyploids have some inherent competitive advantage compared to diploids, one might

expect that they would be less at risk of population declines and extinction than their diploid counterparts. Alternatively, if WGD comes at a cost, they might be more at risk. In plants, a positive correlation between risk of extinction and c-value has been found, but this was attributed to repetitive DNA elements rather than polyploidy (Vinogradov, 2003). Extinction risk with increased genome size has also been implicated in the species poor group of lungfishes, which have very large genomes littered with transposable elements (Kraaijeveld, 2010). We searched the IUCN redlist database for all known polyploid anurans and fish, in comparison to the diploids in the genera in which they occur. The list is proportionately more complete for anurans than for fish; nevertheless there were 41 polyploids and 283 diploids available for anurans and 101 polyploids and 311 diploids listed for fish. The number of species that were listed in each category was compared to the relative frequency of the ploidy types, using contingency chi-square (percentages are shown in Table A1, for more direct comparison). For anurans, there was no significant deviation from expectations ($p = 0.2$), and there were similar proportions of critically endangered diploids and polyploids (Table 1a). There were more polyploids of least concern, fewer vulnerable and fewer endangered, but more near threatened species than for diploids. None of the genera in which polyploids have been identified have extinct taxa listed on the IUCN database. Excluding data deficient taxa (of which there were more in diploids) did not change conclusions ($p = 0.48$). In contrast, for fish, there was a significant deviation from expectations ($p > 0.00001$), with more critically endangered, endangered and near threatened polyploid than diploid species but similar proportions of vulnerable, least concern and extinct species (Table 1b). Again, excluding data deficient taxa (of which there were again more in diploids) did not change conclusions ($p = 0.003$). In terms of population trends, there were no differences between polyploids and diploids for fish or amphibians ($p=0.11$ and 0.54 for amphibians and fish, respectively), but few were increasing in either group and there were many more diploids and polyploids with unknown status among fish and a smaller proportion of species classified as stable. For anurans, the only critically endangered polyploid was *X. longipes*, which has only been described from the type locality and it was listed as stable at the population level; the two endangered species (*X. gilli* and *Scaphiophryne gottlebei*) are both listed as decreasing; all near threatened species are decreasing (*Ceratophrys*

ornata, *Pleurodmea bibroni*, *Pleurodema kriegi*, *X. amieti*) as is the one vulnerable species (*Astylosternus diadematus*). These data suggest that there is not an overall advantage of being polyploid, in terms of risk of decline and extinction. In fact, in fish, there is some evidence that polyploids are at higher risk than diploids (Sturgeons); however, this pattern is complicated by the fact that some of the most "successful" polyploid lineages (e.g., salmonids and catastomids) do not include diploids for comparison. Overall, polyploidy does not seem to be a major factor explaining variation in risk of extinction in extant fish or amphibians.

Table A1- Population Trends of Frogs and Fish

	Frogs		**Fish**	
Population Trend	**Diploid**	**Polyploid**	**Diploid**	**Polyploid**
Decreasing	20.2	26.8	32.2	36.0
Stable	46.9	53.7	6.4	8.0
Increasing	1.1	4.9	1.0	2.0
Unknown	31.8	14.6	60.4	54.0

Population trends (i.e. stable, decreasing, increasing) in relation to ploidy. No significant differences were found for either frogs or fish ($p=0.11$ and 0.54, respectively; including unknowns made frogs marginally significant $p=0.048$ but not fish $p=0.63$).

A.2.8 Conclusions

Our updated survey of polyploidy in fish and anurans suggests that, even in these vertebrates where it is relatively common, it is restricted to certain groups. However, where it occurs, multiple origins are often apparent within certain lineages. In amphibians, except for the exclusively polyploid genus *Xenopus*, polyploidy seems to be restricted to individual species across a wide range of families (Figure A1; SI Table A1) and with no particular geographic pattern. In contrast, for fish, polyploidy seems to be more phylogenetically clustered (Figure A2), but where it occurs, it tends to be found in multiple species (SI Table A2). Closely associated diploid ancestors are not often apparent, perhaps due to the high dispersal rates of fishes. In addition, the identification of polyploidy in fish is complicated by multiple rounds of lineage-specific WGD and large variation in chromosome morphology, which means that a combination of genome size, chromosome counts, marker-based assessment of duplicate gene expression, and more fine-scale genomic information is often necessary to confirm polyploidy. In contrast, there is high conservation of chromosome morphology and numbers in anurans, and most polyploid species are still found in association with their diploid progenitors, making identification of polyploids more reliable based on karyotypes. Detection of polyploids in anurans has also been facilitated by differences in mating calls that can be used to identify otherwise cryptic species. Nevertheless, since cytogenetic surveys are no longer commonly performed, it is quite likely that we currently have an underestimate of the true frequency of polyploidy in both fish and amphibians.

The most striking feature shared by anurans and fish is that they breed in freshwater environments. They also tend to produce large number of gametes, have external fertilization and communal breeding and their type of gametogenesis enables the production of unreduced gametes. They also both have a propensity for hybridization, which is often involved in polyploid formation. These factors all should promote polyploid formation, particularly if environmental variability during the breeding season increases the production of unreduced gametes (Mable 2004). Nevertheless, diploids giving rise to polyploid lineages in anurans have similar breeding tactics as those that are exclusively diploid and there are many freshwater fish that are not polyploid. So, these factors might facilitate the formation of polyploids but cannot explain the establishment

of successful polyploid lineages in only certain taxa.

In anurans, establishment of polyploid lines could be enhanced by direct changes in the mating calls, which would enable assortative mating by cytotype that would decrease the potential barriers to polyploid speciation presented by being initially outnumbered by diploid progenitors. If this only occurred for particular species, this could explain why polyploidy occurs and often has multiple origins in some diploid-polyploid complexes, but not all polyploids seem to have this attribute. Polyploids don't seem to be restricted to certain geographic regions or climatic zones but there is some suggestion that they tend to be formed during times of climatic instability. However, the absence of dates for most polyploidization events and the exclusion of polyploid taxa from phylogenetic analyses (particularly in anurans) does not allow a rigorous test of this hypothesis. Comparing current distributions of polyploid anurans with their diploid progenitors does not suggest that polyploids have broader ranges or have always invaded harsher habitats than their diploid progenitors. Although this test is not possible for fish because their diploid progenitors often no longer exist, there also does not seem to be an overwhelming pattern based on distributional notes. It is possible that diploids that are able to invade harsh habitats are those with sufficient genomic flexibility to tolerate polyploidy, which would obscure differences between ploidy levels. It is also impossible to distinguish whether small range size reflects recent origins and exploitation of new habitats or contraction of previously larger ranges.

However, the occurrence of diploids that give rise to polyploids in the same habitats as closely related species where polyploidy does not become established (despite experimental demonstration that unreduced gamete formation can be induced), does not support this. In contrast, the phylogenetic clustering of polyploidy in fish suggests that genomic constraints could be important for successful polyploid establishment. We also find no evidence that polyploid species are more or less at risk of extinction than their diploid relatives, suggesting that the stronger trends found in plants could be driven largely by mating system, rather than polyploidy. Although there are not as many species to compare, it would be interesting to repeat these tests using unisexual vertebrates.

We conclude that, while there is a tantalizing suggestion that rates of polyploid formation

and establishment might be increased during times of environmental change, the data do not currently exist to fully evaluate this hypothesis. We also still don't have a very clear idea of what factors determine whether a given diploid can give rise to polyploid lineages or what determines the success of nascent polyploids. Particularly given concerns about climate change, experimental approaches to investigate whether tolerances are altered in polyploids compared to diploids (in terms of changes in both biotic and abiotic pressures) seem warranted. Re-initiating a focus on standardly measuring genome size and karyotyping in species surveys would help to determine the full extent of polyploidy, and including polyploid taxa in robustly dated family level phylogenies would enable evaluation of how often polyploidy is associated with drastic environmental change.

Sources Cited for Appendix 2:

Adams, K. L. 2007. Evolution of duplicate gene expression in polyploid and hybrid plants. Journal of Heredity 98:136-141.

Ainouche, M. L., and E. Jenczewski. 2010. Focus on polyploidy. New Phytologist 186:1-4.

Alexandrou, M., C. Oliveira, M. Maillard, R. McGill, J. Newton, S. Creer, and M. Taylor. 2011. Competition and phylogeny determine community structure in Mullerian co-mimics. Nature 469:84-88.

Allendorf, F. W. 1978. Protein polymorphism and the rate of loss of duplicate gene expression. Nature 272:76-78.

Allendorf, F. W., and R. G. Danzmann. 1997. Secondary tetrasomic segregation of MDH-B and preferential pairing of homeologues in rainbow trout. Genetics 145:1083-1092.

Allendorf, F. W., and G. H. Thorgaard. 1984. Tetraploidy and the Evolution of Salmonid fishes. Pages 1-53 *in* Evolutionary Genetics of Fishes (B. J. Turner, ed.) Plenum Press, New York.

Amemiya, C. T., S. J. Prohaska, A. Hill-Force, A. Cook, J. Wasserscheid, D. E. K. Ferrier, J. Pascual-Anaya, J. Garcia-Fernandez, K. Dewar, and P. F. Stadler. 2008. The amphioxus Hox cluster: Characterization, comparative genomics, and evolution. Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 310B:465-477.

Angers, B., K. Gharbi, and A. Estoup. 2002. Evidence of gene conversion events between paralogous sequences produced by tetraploidization in Salmoninae fish. Journal of Molecular Evolution 54:501-510.

Aparicio, S., J. Chapman, E. Stupka, N. Putnam, J. Chia, P. Dehal, A. Christoffels, S. Rash, S. Hoon, A. Smit, M. D. S. Gelpke, J. Roach, T. Oh, I. Y. Ho, M. Wong, C.

Detter, F. Verhoef, P. Predki, A. Tay, S. Lucas, P. Richardson, S. F. Smith, M. S. Clark, Y. J. K. Edwards, N. Doggett, A. Zharkikh, S. V. Tavtigian, D. Pruss, M. Barnstead, C. Evans, H. Baden, J. Powell, G. Glusman, L. Rowen, L. Hood, Y. H. Tan, G. Elgar, T. Hawkins, B. Venkatesh, D. Rokhsar, and S. Brenner. 2002. Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. Science 297:1301-1310.

Baack, E. J., and L. H. Rieseberg. 2007. A genomic view of introgression and hybrid speciation. Current Opinion in Genetics & Development 17:513-518.

Bachman, K., and J. P. Bogart. 1975. Comparative cytochemical measurements in the diploid-tetraploid species pair of hylid frogs *Hyla chrysoscelis* and *H. versicolor*. Cytogenetics and Cell Genetics 15:186-194.

Bacquet, C., T. Imamura, C. A. Gonzalez, I. Conejeros, G. Kausel, T. M. A. Neildez-Nguyen, A. Paldi, and M. H. Gallardo. 2008. Epigenetic processes in a tetraploid mammal. Mammalian Genome 19:439-447.

Bailey, G. S., R. T. M. Poulter, and P. A. Stockwell. 1978. Gene duplication in tetraploid fish: Model for gene silencing at unlinked duplicate loci. Proceedings of the National Academy of Sciences U.S.A. 11:5575-5579.

Baldari, C. T., and F. Amaldi. 1976. DNA reassociation kinetics in relation to genome size in four amphibian species. Chromosoma 59:13-22.

Banerjee, S. K., K. K. Misra, S. Banerjee, and S. P. Raychaudhuri. 1988. Chromosome numbers, genome sizes, cell volumes and evolution of snake-head fish (Family Channidae). Journal of Fish Biology 33:781-789.

Barrio, A. 1980. Una nueva especie de *Ceratophrys* (Anura, Ceratophryidae) del Dominio Chaqueno. Physis, Secc. C 39:21-30.

Bateman, A. J. 1952. Self-incompatibility systems in angiosperms. I. Theory. Heredity 6:285-310.

Beçak, M. L., and L. S. Kobashi. 2004. Evolution by polyploidy and gene regulation in *Anura*. Genetics and Molecular Research 3:195-212.

Bemis, W. E., and B. Kynard. 1997. Sturgeon rivers: An introduction to acipenseriform biogeography and life history. Environmental Biology of Fishes 48:167-184.

Berrebi, P. 1995. Speciation of the genus Barbus in the north Mediterranean basin: Recent advances from biochemical genetics. Biological Conservation 72:237-249.

Bi, K., and J. P. Bogart. 2006. Identification of intergenomic recombinations in unisexual salamanders of the genus *Ambystoma* by genomic in situ hybridization (GISH). Cytogenetic and Genome Research 112:307-312.

Bi, K., J. P. Bogart, and J. Fu. 2007. Intergenomic translocations in unisexual salamanders of the genus *Ambystoma* (Amphibia, Caudata). Cytogenetic and Genome Research 116:289-97.

Bi, K., J. P. Bogart, and J. Fu. 2009. An examination of intergenomic exchanges in *A. laterale*-dependent unisexual salamanders in the genus *Ambystoma*. Cytogenetic and Genome Research 124:44-50.

Billard, R. 1996. Reproduction of pike: gametogenesis, gamete biology and early development. Pages 69–101 *in* Pike Biology and Exploitation (J. F. Craig, ed.) Chapman & Hall, London.

Birchler, J. A., and R. A. Veitia. 2007. The gene balance hypothesis: from classical genetics to modern genomics. Plant Cell 19:395-402.

Birchler, J. A., and R. A. Veitia. 2010. The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution. New Phytol 186:54-62.

Birstein, V. J., P. Doukakis, and R. DeSalle. 2002. Molecular phylogeny of Acipenseridae: Nonmonophyly of Scaphirhynchinae. Copeia 2002:287-302.

Birstein, V. J., R. Hanner, and R. DeSalle. 1997. Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. Environmental Biology of Fishes 48:127-156.

Blair, W. F. 1972. 11. Evidence from hybridization. Pages 196-232 *in* Evolution in the genus *Bufo* University of Texas Press, Austin.

Blanc, G., A. Barakat, R. Guyot, R. Cooke, and M. Delseny. 2000. Extensive duplication and reshuffling in the *Arabidopsis* genome. The Plant Cell 12:1093-1101.

Blanc, G., K. Hokamp, and K. Wolfe. 2003. A recent polyploidy superimposed on older large-scale duplications in the *Arabidopsis* genome. Genome Research 13:137-144.

Blanc, G., and K. H. Wolfe. 2004. Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. Plant Cell 16:1667-1678.

Bogart, J. P. 1967. Chromosomes of the South American amphibian family Ceratophidae with a reconsideration of the taxonomic status of *Odontophrynus americanus*. Canadian Journal of Cytogenetics 9:531-542Canadian Journal of Genetics and Cytology.

Bogart, J. P. 1980. Evolutionary implications of polyploidy in amphibians and reptiles. Pages 341-378 *in* Polyploidy: Biological Relevance Plenum Press, New York and London.

Bogart, J. P. 1991. Chapter 11: The influence of life history on karyotypic evolution in frogs. Pages 233-258 *in* Amphibian Cytogenetics and Evolution (D. M. Green, and S. K. Sessions, eds.). Academic Press, New York.

Bogart, J. P., K. Bi, J. Fu, D. W. A. Noble, and J. Niedzwiecki. 2007. Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. Genome 50:119-137.

Bogart, J. P., L. E. Licht, M. J. Oldham, and S. J. Darbyshire. 1985. Electrophoretic identification of *Ambystoma laterale* and *Ambystoma texanum* as well as their

diploid and triploid interspecific hybrids (Amphibia: Caudata) on Pelee Island, Ontario. Can. J. Zool. 63:340-347.

Bogart, J. P., and A. O. Wasserman. 1972. Diploid-polyploid cryptic species pairs: a possible clue to evolution by polyploidization in anuran amphibians. Cytogenetics 11:7-24.

Bosch, J., L. M. Carrascal, L. Duran, S. Walker, and M. C. Fisher. 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? Proceedings of the Royal Society of London B Biological Sciences 274:253-260.

Bozzoni, I., and E. Beccari. 1978. Clustered and interspersed repetitive DNA sequences in four amphibian species with different genome size. Biochim Biophys Acta 520:245-52.

Broughton, R. E., J. S. Nelson, H.-P. Schultze, and M. V. H. Wilson. Year. Phylogeny of teleosts based on mitochondrial genome sequences *in* Origin and phylogenetic interrelationships of teleosts. Honoring Gloria Arratia. Proceedings of the international symposium at the ASIH Annual Meeting, St. Louis, Missouri:61-76.

Bruch, R. M., and F. P. Binkowski. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). Journal of Applied Ichthyology 18:570-579.

Buggs, R. J., S. Chamala, W. Wu, L. Gao, G. D. May, P. S. Schnable, D. E. Soltis, P. S. Soltis, and W. B. Barbazuk. 2010. Characterization of duplicate gene evolution in the recent natural allopolyploid *Tragopogon miscellus* by next-generation sequencing and Sequenom iPLEX MassARRAY genotyping. Molecular Ecology 19 Suppl 1:132-46.

Buggs, R. J., A. N. Doust, J. A. Tate, J. Koh, K. Soltis, F. A. Feltus, A. H. Paterson, P. S. Soltis, and D. E. Soltis. 2009. Gene loss and silencing in Tragopogon miscellus (Asteraceae): comparison of natural and synthetic allotetraploids. Heredity 103:73-81.

Buggs, R. J., P. S. Soltis, E. V. Mavrodiev, V. V. Symonds, and D. E. Soltis. 2008. Does phylogenetic distance between parental genomes govern the success of polyploids? Castanea 73:74-93.

Cassani, J. R., and W. E. Caton. 1985. Induced triploidy in grass carp, *Ctenopharvngodon idella*. Val. Aquaculture 46:37-44.

Castellano, S., C. Gicoma, T. Dujsebayeva, G. Oiderna, and E. Balletto. 1998. Morphometrical and acoustical comparisons between diploid and tetraploid green toads. Biological Journal of the Linnean Society 63:257-281.

Catalan, P., J. G. Segarra-Moragues, M. Palop-Esteban, C. Moreno, and F. Gonzalez-Candelas. 2006. A Bayesian approach for discriminating among alternative inheritance hypotheses in plant polyploids: The allotetraploid origin of genus *Borderea* (Dioscoreaceae). Genetics 172:1939-1953.

Chain, F. J., D. Ilieva, and B. J. Evans. 2008. Duplicate gene evolution and expression in the wake of vertebrate allopolyploidization. BMC Evolutionary Biology 8:43.

Channing, A., and J. P. Bogart. 1996. Description of a tetraploid *Tomopterna (A*nura: Ranidae) from South Africa. South African Journal of Zoology 31:80-85.

Chao, N.-H., S.-J. Chen, and I.-C. Liao. Year. Triploidy induced by cold shock in cyprinid loach, *Misgurnus anguillicaudatus*. *in* The First Asian Fisheries Forum, Manila, Philippines:101-104.

Chapman, M. A., and J. M. Burke. 2007. Genetic divergence and hybrid speciation. Evolution 61:1773-1780.

Chelaifa, H., A. Monnier, and M. Ainouche. 2010. Transcriptomic changes following recent natural hybridization and allopolyploidy in the salt marsh species *Spartina* x *townsendii* and *Spartina anglica* (Poaceae). New Phytologist 186:161-74.

Chen, Z. J. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annua Review of Plant Biology 58:377-406.

Chenuil, A., N. Galtier, and P. Berrebi. 1999. A test of the hypothesis of an autopolyploid vs. allopolyploid origin for a tetraploid lineage: application to the genus *Barbus* (Cyprinidae). Heredity 82:373-380.

Chevassus, B., and M. Dorson. 1990. Genetics of resistance to diseases in fishes. Aquaculture 85:83-107.

Ciereszko, A., K. Dabrowski, and S. I. Ochkur. 1996. Characterization of acrosin-like activity of lake sturgeon (*Acipenser fulvescens*) spermatozoa. Molecular Reproduction and Development 45:72–77.

Clausen, J., D. D. Keck, and W. Hiesey. 1940. Experimental studies on the nature of species. I. Effects of varied environments on western North American plants. Carnegie Institute, Washington.

Clausen, J., D. D. Keck, and W. M. Hiesey. 1945. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carnegie Institution of Washington, D.C. 564:1-174.

Cleaveland, S., T. Mlengeya, M. Kaare, D. Haydon, T. Lembo, M. K. Laurenson, and C. Packer. 2007. The conservation relevance of epidemiological research into carnivore viral diseases in the Serengeti. Conservation Biology 21:612-622.

Crow, K. D., P. F. Stadler, V. J. Lynch, C. Amemiya, and G. P. Wagner. 2006. The "fish-specific" Hox cluster duplication is coincident with the origin of teleosts. Molecular Biology and Evolution 23:121-136.

Crow, K. D., and G. P. Wagner. 2006. What is the role of genome duplication in the evolution of complexity and diversity? Molecular Biology and Evolution 23:887-892.

Cunha, C., I. Doadrio, J. Abrantes, and M. M. Coelho. 2011. The evolutionary history of the allopolyploid *Squalius alburnoides* (Cyprinidae) complex in the northern Iberian Peninsula. Heredity 106:100-112.

Danzmann, R. G., and J. P. Bogart. 1983. Further evidence for a polymorphism in gametic segregation in the tetraploid treefrog *Hyla versicolor* using a glutamate oxaloacetic transaminase locus. Genetics 103:753-769.

Danzmann, R. G., and N. E. Down. 1982. Isozyme expression in F1 hybrids between carp and goldfish. Biochemical Genetics 20:1-15.

David, L., S. Blum, M. W. Feldman, U. Lavi, and J. Hillel. 2003. Recent duplication of the, common carp (*Cyprinus carpio* L.) genome as revealed by analyses of microsatellite loci. Molecular Biology and Evolution 20:1425-1434.

de Boer, J. G., P. J. Ode, L. E. M. Vet, J. B. Whitfield, and G. E. Heimpel. 2007. Diploid males sire triploid daughters and sons in the parasitoid wasp Cotesia vestalis. Heredity 99:288-294.

de Graaf, M. 2003. Lake Tana's piscivorous barbs (Cyprinidae, Ethiopia): ecology, evolution, exploitation.University of Wageningen, Wageningen.

Dehal, P., and J. L. Boore. 2005. Two rounds of whole genome duplication in the ancestral vertebrate. PLOS Biology 3:e314.

deWet. 1980. Origins of polyploids. Pages 3-16 *in* Polyploidy: Biological relevance Plenum Press, New York and London.

Dionne, M. 2009. Pathogens as potential selective agents in the wild. Molecular Ecology 18:4523-4525.

Dionne, M., K. M. Miller, J. J. Dodson, F. Caron, and L. Bernatchez. 2007. Clinal variation in mhc diversity with temperature: Evidence for the role of host-pathogen interaction on local adaptation in Atlantic salmon. Evolution 61:2154-2164.

Don, J., and R. R. Avtalion. 1988. Production of viable tetraploid tilapias using the cold shock technique. Bamidgeh 40:17-21.

Donaldson, M. R., S. J. Cooke, D. A. Patterson, and J. S. Macdonald. 2008. Cold shock and fish. Journal of Fish Biology 73:1491-1530.

Donoghue, P. C. J., and M. A. Purnell. 2005. Genome duplication, extinction and vertebrate evolution. Trends in Ecology & Evolution 20:312-319.

Dowling, T. E., and C. L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics 28:593-619.

Doyle, J. J., and A. N. Egan. 2010. Dating the origins of polyploidy events. New Phytologist 186:73-85.

Du Pasquier, L., M. Wilson, and B. Sammut. 2009. The fate of duplicated immunity genes in the dodecaploid *Xenopus ruwenzoriensis*. Frontiers in Bioscience 14:177-91.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. John Hopkins Press, Baltimore.

Dufresne, F., and P. D. N. Hebert. 1997. Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran. Proceedings of the Royal Society of London, Series B 264:201-206.

Edgell, D. R., S.-B. Malik, and W. F. Doolittle. 2000. Evidence of independent gene duplications during the evolution of archaeal and eukaryotic family B DNA polymerases. Molecular Biology and Evolution 15:1207-1217.

Ehrendorfer, F. 1980. Polyploidy and distribution. Pages 45-60 *in* Polyploidy: Biological relevance Plenum Press, New York and London.

Elgar, M. A. 1990. Evolutionary compromise between a few large and many small eggs: comparative evidence in Teleost fish. Oikos 59:283-287.

Elinson, R. P. 1986. Fertilization in amphibians: The ancestry of the block to polyspermy. International Review of Cytology 101:59–100.

Espinoza, N., and M. Noor. 2002. Population genetics of a polyploid: is there hybridization between lineages of *Hyla versicolor*? Journal of Heredity 93:81-85.

Evans, B. J. 2007. Ancestry influences the fate of duplicated genes millions of years after polyploidization of clawed frogs (*Xenopus*). Genetics 176:1119-1130.

Evans, B. J. 2008. Genome evolution and speciation genetics of clawed frogs (*Xenopus* and *Silurana*). Frontiers in Bioscience 13:4687-4706.

Evans, B. J., T. F. Carter, M. L. Tobias, D. B. Kelley, R. Hanner, and R. C. Tinsley. 2008. A new species of clawed frog (genus *Xenopus*) from the Itombwe Massif, Democratic Republic of the Congo: implications for DNA barcodes and biodiversity conservation. Zootaxa:55-68.

Evans, B. J., D. B. Kelley, D. J. Melnick, and D. C. Cannatella. 2005. Evolution of RAG-1 in polyploid clawed frogs. Molecular Biology and Evolution 22:1193-1207.

Evans, B. J., D. B. Kelley, R. C. Tinsley, D. J. Melnick, and D. C. Cannatella. 2004. A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. Molecular Phylogenetics and Evolution 33:197-213.

Evans, M. L., and B. D. Neff. 2009. Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). Mol Ecol 18:4716-29.

Faivovich, J., C. F. B. Haddad, P. C. A. Garcia, D. R. Frost, J. A. Campbell, and W. C. Wheeler. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. Bulletin of the American Museum of Natural History:6-228.

Fankhauser, G. 1932. Cytological studies on egg fragments of the salamander *Triton* II. The history of the supernumerary sperm nuclei in normal fertilization and cleavage of fragments containing the egg nucleus. Journal of Experimental Zoology 62:185-235.

Fankhauser, G. 1945. The effects of changes in chromosome number on amphibian development. Quarterly Review of Biology 20:20-78.

Fawcett, J. A., S. Maere, and Y. Van de Peer. 2009. Plants with double genomes might have had a better chance to survive the Cretaceous-Tertiary extinction event. Proceedings of the National Academy of Sciences U.S.A. 106:5737-5742.

Felip, A., F. Piferrer, M. Carrillo, and S. Zanuy. 2001. Comparison of the gonadal development and plasma levels of sex steroid hormones in diploid and triploid sea bass, *Dicentrarchus labrax* L. Journal of Experimental Zoology 290:384-95.

Fenerich, P. C., F. Foresti, and C. Oliveira. 2004. Nuclear DNA content in 20 species of Siluriformes (Teleostei : Ostariophysi) from the Neotropical region. Genetics and Molecular Biology 27:350-354.

Ferris, S. D. 1984. Tetraploidy and the evolution of the catostomid fishes. Pages 55-93 *in* Evolutionary Genetics of Fishes (B. J. Turner, ed.) Plenum Press, New York.

Ferris, S. D., and G. S. Whitt. 1975. Gene duplication and functional diploidization in Catostomidae. Genetics 80:S30-S30.

Ferris, S. D., and G. S. Whitt. 1977a. Duplicate gene expression in diploid and tetraploid loaches. Biochemical Genetics 15:1097-1112.

Ferris, S. D., and G. S. Whitt. 1977b. Duplicate gene-expression in diploid and tetraploid Loaches (Cypriniformes, Cobitidae). Biochemical Genetics 15:1097-1112.

Ferris, S. D., and G. S. Whitt. 1977c. Evolution of duplicate gene-expression in Carp (*Cyprinus*-Carpio). Experientia 33:1299-1301.

Ferris, S. D., and G. S. Whitt. 1977d. Loss of duplicate gene expression after polyploidization. Nature 265:258-260.

Ferris, S. D., and G. S. Whitt. 1978. Phylogeny of the tetraploid catastomid fishes based on the loss of duplicate gene expression. Systematic Zoology 27:189-206.

Fischer, W. J., W. A. Koch, and A. Elepfandt. 2000. Sympatry and hybridization between the clawed frogs *Xenopus laevis laevis* and *Xenopus muelleri* (Pipidae). Journal of Zoology 252:99-107.

Flagel, L. E., and J. F. Wendel. 2010. Evolutionary rate variation, genomic dominance and duplicate gene expression evolution during allotetraploid cotton speciation. New Phytologist 186:184-93.

Froese, R., and D. Pauly. 2008. FishBase.

Frost, D. R. 2010. Amphibian Species of the World: an Online Reference. Version 5.4 (8 April, 2010). American Museum of Natural History, New York, USA.

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, A. Channing, M. Wilkinson, S. C. Donnellan, C. J. Raxworthy, J. A. Campbell, B. L. Blotto, P. Moler, R. C. Drewes, R. A. Nussbaum, J. D. Lynch, D. M. Green, and W. C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History:8-370.

Fuller, I. A. 2001. Breeding Corydoradine catfish. Ian Fuller Enterprises, Kidderminster.

Gaeta, R. T., and J. Chris Pires. 2010. Homoeologous recombination in allopolyploids: the polyploid ratchet. New Phytologist 186:18-28.

Gaeta, R. T., J. C. Pires, F. Iniguez-Luy, E. Leon, and T. C. Osborn. 2007. Genomic changes in resynthesized Brassica napus and their effect on gene expression and phenotype. Plant Cell 19:3403-3417.

Gallardo, M. H., G. Kausel, A. Jimenez, C. Bacquet, C. Gonzalez, J. Figueroa, N. Kohler, and R. Ojeda. 2004. Whole-genome duplications in South American desert rodents (Octodontidae). Biological Journal of the Linnean Society 82:443-451.

Gerhardt, H. C. 1974. Mating call differences between eastern and western populations of the treefrog *Hyla chrysoscelis*. Copeia 1974:534-536.

Gerhardt, H. C. 1982. Sound pattern recognition in some North American treefrogs (Anura: Hylidae): Implications for mate choice. Experimental Zoology 22:581-595.

Gerhardt, H. C. 2005a. Acoustic spectral properties in two cryptic species of grey treefrogs: implications for mate choice and sensory mechanisms. Animal Behaviour 70:39-48.

Gerhardt, H. C. 2005b. Advertisement-call preferences in diploid-tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): Implications for mate choice and the evolution of communication systems. Evolution 59:395-408.

Gervai, J., S. Páter, A. Nagy, L. Horváth, and V. Csényi. 1980. Induced triploidy in carp, *Cyprinus carpio* L. Journal of Fish Biology 17:667-671.

Gharbi, K., A. Gautier, R. G. Danzmann, S. Gharbi, T. Sakamoto, B. Hoyheim, J. B. Taggart, M. Cairney, R. Powell, F. Krieg, N. Okamoto, M. M. Ferguson, L. E. Holm, and R. Guyomard. 2006. A linkage map for brown trout (*Salmo trutta*): Chromosome homeologies and comparative genome organization with other salmonid fish. Genetics 172:2405-2419.

Gonthier, P., G. Nicolotti, R. Linzer, F. Guglielmo, and M. Garbelotto. 2007. Invasion of European pine stands by a North American forest pathogen and its hybridization with a native interfertile taxon. Molecular Ecology 16:1389-1400.

Green, D. E., K. A. Converse, and A. K. Schrader. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996-2001. Domestic Animal/Wildlife Interface: Issue for Disease Control, Conservation, Sustainable Food Production, and Emerging Diseases 969:323-339.

Green, D. M. 1985. Natural hybrids between the frogs *Rana cascadae* and *Rana pretiosa* (Anura: Ranidae). Herpetologica 41:262-267.

Green, D. M., J. Kezer, and R. A. Nussbaum. 1984. Triploidy in Hochstetters frog, *Leiopelma hochstteri*, from New Zealand. New Zealand Journal of Zoology 11:457-460.

Gregory, T. R. 2005a. Animal Genome Size Database.

Gregory, T. R. 2005b. The Evolution of the Genome. Elsevier Academic Press, Burlington, MA.

Gregory, T. R., and B. K. Mable. 2005. Polyploidy in Animals. Pages 428-517 *in* The Evolution of the Genome (T. R. Gregory, ed.) Elsevier Academic Press, London.

Grenat, P. R., C. Bionda, N. E. Salas, and A. L. Martino. 2009. Variation in erythrocyte size between juveniles and adults of *Odontophrynus americanus*. Amphibia-Reptilia 30:141-145.

Gross, B. L., K. G. Turner, and L. H. Rieseberg. 2007. Selective sweeps in the homoploid hybrid species *Helianthus deserticola*: evolution in concert across populations and across origins. Molecular Ecology 16:5246-5258.

Guegan, J.-F., and S. Morand. 1996. Polyploid hosts: strange attractors for parasites! Oikos 77:366-370.

Haddad, C. F. B., J. P. J. Pombal, and R. F. Batistic. 1994. Natural hybridization between diploid and tetraploid species of leaf-frogs, genus *Phyllomedusa (*Amphibia). Journal of Herpetology 28:425-430.

Hagerup, O. 1932. Uber polyploidie in *Beziehung* zu klima, okologie, und phylogenie. Hereditas 16:19-40.

Hardie, D. C., T. R. Gregory, and P. D. N. Hebert. 2002. From pixels to picograms: a beginner's guide to genome quantification by feulgen image analysis densitometry. Journal of Histochemistry and Cytochemistry 50:735-749.

Hardie, D. C., and P. D. N. Hebert. 2003. The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. Genome 46:683-706.

Hardie, D. C., and P. D. N. Hebert. 2004. Genome-size evolution in fishes. Canadian Journal of Fisheries and Aquatic Sciences 61:1636-1646.

Harris, P. D., A. Soleng, and T. A. Bakke. 1998. Killing of *Gyrodactylus salaris* (Platyhelminthes, Monogenea) mediated by host complement. Parasitology 117:137-143.

Hart, N. H. 1990. Fertilization in teleost fishes: Mechanisms of sperm–egg interactions. International Review of Cytology 121:1–66.

Hartley, S. E. 1987. The chromosomes of salmonid fishes. Biological Reviews 62:197-214.

Hedrick, J. L., and T. Nishihara. 1991. Structure and function of the extracellular matrix of anuran eggs. Journal of Electron Microscopy Techniques 17:319–335.

Hellsten, U., R. M. Harland, M. J. Gilchrist, D. Hendrix, J. Jurka, V. Kapitonov, I. Ovcharenko, N. H. Putnam, S. Shu, L. Taher, I. L. Blitz, B. Blumberg, D. S. Dichmann, I. Dubchak, E. Amaya, J. C. Detter, R. Fletcher, D. S. Gerhard, D. Goodstein, T. Graves, I. V. Grigoriev, J. Grimwood, T. Kawashima, E. Lindquist, S. M. Lucas, P. E. Mead, T. Mitros, H. Ogino, Y. Ohta, A. V. Poliakov, N. Pollet, J. Robert, A. Salamov, A. K. Sater, J. Schmutz, A. Terry, P. D. Vize, W. C. Warren, D. Wells, A. Wills, R. K. Wilson, L. B. Zimmerman, A. M. Zorn, R. Grainger, T. Grammer, M. K. Khokha, P. M. Richardson, and D. S. Rokhsar. 2010. The genome of the Western clawed frog *Xenopus tropicalis*. Science 328:633-6.

Hilu, K. W. 1993. Polyploidy and the evolution of domesticated plants. American Journal of Botany 80:1494-1499.

Hinegard, R. 1968. Evolution of cellular DNA content in Teleost fishes. American Naturalist 102:517-523.

Hinegard, R., and D. E. Rosen. 1972. Cellular DNA content and evolution of Teleostean fishes. American Naturalist 106:621-644.

Hoegg, S., and A. Meyer. 2005. Hox clusters as models for vertebrate genome evolution. Trends in Genetics 21:421-424.

Holloway, A. K., D. C. Cannatella, H. C. Gerhardt, and D. M. Hillis. 2006. Polyploids with different origins and ancestors form a single sexual polyploid species. American Naturalist 167:E88-E101.

Hughes, M. K., and A. L. Hughes. 1993. Evolution of duplicate genes in a tetraploid animal, *Xenopus laevis*. Molecular Biology and Evolution 10:1360-1369.

Husband, B. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. Proceedings of the Royal Society of London 267:217-223.

Husband, B. C., and H. A. Sabara. 2004. Reproductive isolation between autotetraploids and their diploid progenitors in fireweed, *Chamerion angustifolium* (Onagraceae). New Phytologist 161:703-713.

Husband, B. C., and D. W. Schemske. 2000. Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. Journal of Ecology 88:689-701.

Hutchings, J. A., and R. A. Myers. 1988. Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. Oecologia 75:169-174.

Irwin, J. T., and R. E. Lee, Jr. 2003. Geographic variation in energy storage and physiological responses to freezing in the gray treefrogs *Hyla versicolor* and *H. chrysocelis*. Journal of Experimental Biology 206:2859-2867.

Iwao, Y. 1989. An electrically mediated block to polyspermy in the primitive urodele *Hynobius nebulosus* and phylogenetic comparison with other amphibians. Developmental Biology 134:438–445.

Iwao, Y., and R. P. Elinson. 1990. Control of sperm nuclear behavior in physiologically polyspermic newt eggs: Possible involvement of MPF. Developmental Biology 142:301–312.

Jackson, J. A., and R. Tinsley. 2003. Parasite infectivity by hybridising host species: a linke between hybrid resistance and allopolyploid speciation? International Journal of Parasitology 33:137-144.

Jackson, J. A., and R. C. Tinsley. 1998. Paramphistome digeneans from *Xenopus* species (Pipidae) in Africa: taxonomy, host-specificity and biogeography. Systematic Parasitology 40:143-160.

Jackson, J. A., and R. C. Tinsley. 2001. Host-specificity and distribution of Cephalochlamydid Cestodes: correlation with allopolyploid evolution of Pipid Anuran hosts. Journal of Zoology 254:405-419.

Jackson, S., and Z. J. Chen. 2010. Genomic and expression plasticity of polyploidy. Current Opinion in Plant Biology 13:153-9.

Jaillon, O., J.-M. Aury, F. Brunet, J.-L. Petit, N. Stange-Thomann, E. Mauceli, L. Bouneau, C. Fischer, C. Ozouf-Costaz, A. Bernot, S. Nicaud, D. Jaffe, S. Fisher, G. Lutfalla, C. Dossat, B. Segurens, C. Dasilva, M. Salanoubat, M. Levy, N. Boudet, S. Castellano, V. Anthouard, C. Jubin, V. Castelli, M. Katinka, B. Vacherie, C. Biemont, Z. Skalli, L. Cattolico, J. Poulain, V. de Berardinis, C. Cruaud, S. Duprat, P. Brottier, J.-P. Coutanceau, J. Gouzy, G. Parra, G. Lardier, C. Chapple, K. J. McKernan, P. McEwan, S. Bosak, M. Kellis, J.-N. Volff, R. Guigo, M. C. Zody, J. Mesirov, K. Lindblad-Toh, B. Birren, C. Nusbaum, D. Kahn, M. Robinson-Rechavi, V. Laudet, V. Schachter, F. Quetier, W. Saurin, C. Scarpelli, P. Wincker, E. S. Lander, J. Weissenbach, and H. Roest Crollius. 2004. Genome duplication in the teleost fish *Tetraodon nigroviridis* reveals the early vertebrate proto-karyotype. Nature 431:946-957.

Jenkins, G., and H. Rees. 1991. Strategies of bivalent formation in allopolyploid plants. Proceedings of the Royal Society of London 243:209-214.

Johnson, K. R., J. E. Wright, Jr., and B. May. 1987. Linkage relationships reflecting ancestral tetraploidy in salmonid fish. Genetics 116:579-591.

Juchno, D., and A. Boron. 2006. Comparative histology of the testes of the spined loach *Cobitis taenia* L. and natural allotetraploids of *Cobitis* (Pisces, Cobitidae). Hydrobiologia 573:45-53.

Kaatz, I. M., and P. S. Lobel. 1999. Acoustic behavior and reproduction in five species of *Corydoras* catfishes (Callichthyidae). Biological Bulletin 197:241-242.

Kaatz, I. M., and P. S. Lobel. 2001. Intra- and interspecific differences in sound production in the neotropical catfish genus *Corydoras* (Callichthyidae). American Zoologist 41:1489-1489.

Kamel, S., J. E. Marsden, and J. H. Pough. 1985. Diploid and tetraploid gray treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*) have similar metabolic rates. Comparative Biochemistry and Physiology A 82:217-220.

Kasahara, M., K. Naruse, S. Sasaki, Y. Nakatani, W. Qu, B. Ahsan, T. Yamada, Y. Nagayasu, K. Doi, Y. Kasai, T. Jindo, D. Kobayashi, A. Shimada, A. Toyoda, Y. Kuroki, A. Fujiyama, T. Sasaki, A. Shimizu, S. Asakawa, N. Shimizu, S.-i. Hashimoto, J. Yang, Y. Lee, K. Matsushima, S. Sugano, M. Sakaizumi, T. Narita, K. Ohishi, S. Haga, F. Ohta, H. Nomoto, K. Nogata, T. Morishita, T. Endo, T. Shin-I, H. Takeda, S. Morishita, and Y. Kohara. 2007. The medaka draft genome and insights into vertebrate genome evolution. Nature 447:714-719.

Kejnovsky, E., I. J. Leitch, and A. R. Leitch. 2009. Contrasting evolutionary dynamics between angiosperm and mammalian genomes. Trends in Ecology & Evolution 24:572-581.

Keller, M. J., and H. C. Gerhardt. 2001. Polyploidy alters advertisement call structure in grey treefrogs. Proceedings of the Royal Society London, Series B 268:341-345.

Kellogg, K. A., J. A. Markert, J. R. Stauffer, and T. D. Kocher. 1995. Microsatellite variation demonstrates multiple paternity in lekking Cichlid fishes from Lake Malawi, Africa. Proceedings of the Royal Society of London, Series B 260:79-84.

Kendall, C., S. Valentino, A. B. Bodine, and C. A. Luer. 1994. Triploidy in a nurse Shark, *Ginglymostoma cirratum*. Copeia 1994:825–827.

Kitagawa, T., M. Watanabe, E. Kitagawa, M. Yoshioka, M. Kashiwagi, and T. Okazaki. 2003. Phylogeography and the maternal origin of the tetraploid form of the Japanese spined loach, *Cobitis biwae*, revealed by mitochondrial DNA analysis. Ichthyological Research 50:318-325.

Klose, J., U. Wolf, Hitzerot.H, H. Ritter, and S. Ohno. 1969. Polypolidization in fish family Cyprinidae Order Cypriniformes .2. Duplication of gene loci coding for Lactate Dehydrogenase (Ec - 1.1.1.27) and 6-Phosphogluconate Dehydrogenase (Ec - 1.1.1.44) in various species of Cyprinidae. Humangenetik 7:245-250.

Klump, G. M., and H. C. Gerhardt. 1987. Use of non-arbitrary acoustic criteria in mate choice by female gray treefrogs. Nature 326:286-288.

Knapp, L. A., J. Robson, and J. S. Waterhouse. 2006. Olfactory signals and the MHC: a review and a case study in *Lemur catta*. American Journal of Primatology 68:568-584.

Kobel, H. R., and L. Du Pasquier. 1986. Genetics of polyploid *Xenopus*. Trends in Genetics 2:310-315.

Kobel, H. R., L. Dupasquier, and R. C. Tinsley. 1981. Natural hybridization and gene introgression between *Xenopus gilli* and *Xenopus laevis laevis* (Anura, Pipidae). Journal of Zoology 194:317-322.

Kolm, N., and I. Ahnesjo. 2005. Do egg size and parental care coevolve in fishes? Journal of Fish Biology 66:1499-1515.

Kraaijeveld, K. 2010. Genome size and species diversification. Evolutionary Biology 37:227-233.

Kruiswijk, C. P., T. Hermsen, K. Fujiki, B. Dixon, H. F. J. Savelkoul, and R. J. M. Stet. 2004. Analysis of genomic and expressed major histocompatibility class Ia and

class II genes in a hexaploid Lake Tana African "large" barb individual. Immunogenetics 55:770-781.

Kudo, S. 1980. Sperm penetration and the formation of a fertilization cone in the common carp egg. Development Growth & Differentiation 22:403-414.

Kupka, E. 1948. Chromosomale versheidenheiten bei schweizerischen Coregonen. Revue Suisse Zoologische 55:285-293.

Laine, A. L. 2007. Detecting local adaptation in a natural plant-pathogen metapopulation: a laboratory vs. field transplant approach. Journal of Evolutionary Biology 20:1665-1673.

Landry, C. R., D. L. Hartl, and J. M. Ranz. 2007. Genome clashes in hybrids: insights from gene expression. Heredity 99:483-493.

Langefors, A., J. Lohm, M. Grahn, O. Andersen, and T. von Schantz. 2001. Association between major histocompatibility complex class IIB alleles and resistance to *Aeromonas salmonicida* in Atlantic salmon. Proceedings of the Royal Society of London, Series B 268:479-485.

Larhammar, D., G. Sundstrom, S. Dreborg, D. O. Daza, and T. A. Larsson. 2009. Major genomic events and their consequences for vertebrate evolution and endocrinology. Pages 201-208 *in* Trends in Comparative Endocrinology and Neurobiology (H. Vaudry, E. W. Roubos, G. M. Coast, and M. Vallarino, eds.). Blackwell Publishing, Oxford.

Le Comber, S. C., M. L. Ainouche, A. Kovarik, and A. R. Leitch. 2010. Making a functional diploid: from polysomic to disomic inheritance. New Phytologist 186:113-22.

Le Comber, S. C., and C. Smith. 2004. Polyploidy in fishes: patterns and processes. Biological Journal of the Linnean Society 82:431-442.

LeComber, S. 2004. Polyploidy in fishes: patterns and process. Biological Journal of the Linnean Society 82:431-442.

Leggatt, R. A., and G. K. Iwama. 2003. Occurrence of polyploidy in the fishes. Reviews in Fish Biology and Fisheries 13:237-246.

Leitch, A. R., D. E. Soltis, P. S. Soltis, I. J. Leitch, and J. C. Pires. 2004. Biological relevance of polyploidy: Ecology to genomics. Biological Journal of the Linnean Society 82.

Leitch, I. J. 2007. Genome sizes through the ages. Heredity 99:121-122.

Leitch, I. J., and M. D. Bennett. 1997. Polyploidy in angiosperms. Trends Plant Sci. 2:470-476.

Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. Taxon 24:35-43.

Levin, D. A. 1983. Polyploidy and novelty in flowering plants. American Naturalist 122:1-25.

Levin, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Oxford.

Lewis, W. H. 1980. Polyploidy: Biological Relevance. Plenum Press, New York and London.

Li, W.-H. 1980. Rate of gene silencing at duplicate loci: a theoretical study and interpretation of data from tetraploid fishes. Genetics 95:237-258.

Li, X. C., B. C. Barringer, and D. A. Barbash. 2009. The pachytene checkpoint and its relationship to evolutionary patterns of polyploidization and hybrid sterility. Heredity 102:24-30.

Löve, A., and D. Löve. 1943. The significance of differences in the distribution of diploids and polyploids. Hereditas 29:145-163.

Lovejoy, N. R., and M. L. G. de Araujo. 2000. Molecular systematics, biogeography and population structure of Neotropical freshwater needlefishes of the genus Potamorrhaphis. Molecular Ecology 9:259-268.

Ludwig, A., N. Belfiore, P. C, V. Svirsky, and I. Jenneckens. 2001. Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*). Genetics 158:1203-15.

Lumaret, R. 1988. Adaptive strategies and ploidy levels. Acta Oecologica-Oecologia Plantarum 9:83-93.

Luo, J., P. F. Stadler, S. P. He, and A. Meyer. 2007. PCR survey of Hox genes in the goldfish *Carassius auratus auratus*. Journal of Experimental Zoology Part B-Molecular and Developmental Evolution 308B:250-258.

Luo, J., J.-X. Yang, and Y.-P. Zhang. 2000. Genetic basis of fish diversity. Zoological Research 21:158-164.

Lynch, M. 2002. Genomics. Gene duplication and evolution. Science 297:945-947.

Lynch, M. 2007. The origins of genome architecture. Sinauer Associates, Inc., Sunderland, Mass.

Mable, B. K. 1989. Genetic investigation of interspecific hybrids between tetraploid and diploid species of treefrogs, genus *Hylain* Department of Zoology University of Guelph, Guelph.

Mable, B. K. 2001. Ploidy evolution in the yeast *Saccharomyces cerevisiae*: a test of the nutrient limitation hypothesis. Journal of Evolutionary Biology 14:151-170.

Mable, B. K. 2003. Breaking down taxonomic barriers in polyploidy research. Trends in Plant Science 8:582-589.

Mable, B. K. 2004. "Why polyploidy is rarer in animals than in plants" revisited: Myths and Mechanisms. Biological Journal of the Linnean Society 82:453-466.

Mable, B. K., and J. P. Bogart. 1995. Hybridization between tetraploid and diploid species of treefrogs (Genus *Hyla*). Journal of Heredity 86:432-440.

Mable, B. K., and J. D. Roberts. 1997. Mitochondrial DNA evolution of tetraploids in the genus *Neobatrachus* (Anura: Myobatrachidae). Copeia 1997:680-689.

Machordom, A., and I. Doadrio. 2001. Evolutionary history and speciation modes in the cyprinid genus *Barbus*. Proceedings of the Royal Society of London, Series B 268:1297-1306.

Mahony, M., S. C. Donnellan, and J. D. Roberts. 1986. An electrophoretic investigation of relationships of diploid and tetraploid species of Australian desert frogs *Neobatrachus* (Anura: Myobatrachidae). Australian Journal of Zoology 44:639-650.

Mallet, J. 2007. Hybrid speciation. Nature 446:279-83.

Malone, J. H., and B. E. Fontenot. 2008. Patterns of reproductive isolation in toads. PLOS One 3:Article No.: e3900.

Manevto, E. D. 1945. Polyploidy in animals Uspekhi Sovremennoi Biologii 20:307-324.

Mank, J. E., and J. C. Avise. 2006. Phylogenetic conservation of chromosome numbers in Actinopterygiian fishes. Genetica 127:321-327.

Marmagne, A., P. Brabant, H. Thiellement, and K. Alix. 2010. Analysis of gene expression in resynthesized *Brassica napus* allotetraploids: transcriptional changes do not explain differential protein regulation. New Phytologist 186:216-27.

Martinez-Perez, E., P. Shaw, and G. Moore. 2001. The Ph1 locus is needed to ensure specific somatic and meiotic centromere association. Nature 411:204-7.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science 264:421-423.

Matson, T. O. 1990. Erythrocyte size as a taxonomic character in the identification of Ohio *Hyla chrysoscelis* and *H. versicolor*. Herpetologica 46:457-462.

Matzke, M., O. Scheid, and A. Matzke. 1999. Rapid structural and epigenetic changes in polyploid and aneuploid genomes. BioEssays 21:761-7.

Mavarez, J., and M. Linares. 2008. Homoploid hybrid speciation in animals. Molecular Ecology 19:4181-4185.

McClelland, E. E., D. J. Penn, and W. K. Potts. 2003. Major histocompatibility complex heterozygote superiority during coinfection. Infection and Immunity 71:2079-86.

McClintock, B. 1978. Mechanisms that rapidly reorganize the genome. Stadler Genetics Symposium 10:25-48.

McDowall, R. M. 2001. Anadromy and homing: two life-history traits with adaptive synergies in salmonid fishes? Fish and Fisheries 2:78-85.

Mecham, J. S. 1965. Genetic relationships and reproductive isolation in southeastern frogs of the genera *Pseudacris* and *Hyla*. American Midland Naturalist 74:269-308.

Mestiri, I., V. Chague, A. M. Tanguy, C. Huneau, V. Huteau, H. Belcram, O. Coriton, B. Chalhoub, and J. Jahier. 2010. Newly synthesized wheat allohexaploids display progenitor-dependent meiotic stability and aneuploidy but structural genomic additivity. New Phytologist 186:86-101.

Meyer, A., and M. Schartl. 1999. Gene and genome duplication in vertebrates: the one-to-four (-to eight in fish) rule and the evolution of novel gene functions. Current Opinion in Cell Biology 11:699-704.

Milner, A. C. 1998. Timing and causes of vertebrate extinction across the Cretaceous-Tertiary boundary. Pages 247-257 *in* Meteorites: Flux with time and impact effects (M. M. Grady, R. Hutchison, G. J. H. McCall, and D. Rothery, eds.). Geological Society, London.

Mittelsten Scheid, O., L. Jakovleva, K. Afsar, J. Maluszynska, and J. Paszkowski. 1996. A change of ploidy can modify epigenetic silencing. Proceedings of the National Academy of Sciences U.S.A. 93:7114-9.

Morescalchi, A., and E. Olmo. 1974. Sirenids: a family of polyploid Urodeles? . Experientia 30:491-492.

Muller, H. J. 1925. Why polyploidy is rarer in animals than in plants. American Naturalist 59:346-353.

Mustafa, S., and N. Shams. 1982. Distribution of nucleic-acids in different tissues of catfish *Clarias batrachus*. Japanese Journal of Ichthyology 28:458-460.

Nakatani, Y., H. Takeda, Y. Kohara, and S. Morishita. 2007. Reconstruction of the vertebrate ancestral genome reveals dynamic genome reorganization in early vertebrates. Genome Research 17:1254–1265.

Nishioka, M., and H. Ueda. 1983. Studies on polyploidy in Japanese treefrogs. Sci. Rep. Lab. Amph. Biol. Hiroshima Univ. 6:207-252.

Ohno, S. 1970. Evolution by Gene Duplication. Springer-Verlag, New York.

Ohno, S. 1993. Patterns in genome evolution. Current Opinion in Genetics and Development 3:911-914.

Ohno, S., U. Wolf, and N. B. Atkin. 1968. Evolution from fish to mammals by gene duplication. Hereditas 59:169-187.

Oliveira, C., L. F. Almeida-Toledo, L. Mori, and S. A. Toledo-Filho. 1993a. Cytogenetic and DNA content studies of armoured catfishes of the genus *Corydoras* (Pisces, Siluriformes, Callichthyidae) from the southeast coast of Brazil. Brazilian Journal of Genetics 16:617-629.

Oliveira, C., L. F. Almeidatoledo, L. Mori, and S. A. Toledo. 1993b. Cytogenetic and DNA content in 6 genera of the family Callichthyidae (Pisces, Siluriformes). Caryologia 46:171-188.

Oliveira, C., L. F. Almeidatoledo, L. Mori, and S. A. Toledofilho. 1992. Extensive chromosomal rearrangements and nuclear-DNA content changes in the evolution of the armored catfishes genus Corydoras (Pisces, Siluriformes, Callichthyidae). Journal of Fish Biology 40:419-431.

Oliveira, C., F. Foresti, L. F. D. Almeida-Toledo, and I. Sociedade Brasileira De. 1993c. Karyotype, nucleolar organizing regions and C bands of *Corydoras* sp. of Araras (Pisces, Siluriformes, Callichthyidae). [Tenth Brazilian Ichthyology Meeting]|X Encontro Brasileiro de Ictiologia:173.

Oliveira, C., L. F. A. Toledo, F. Foresti, and S. A. Toledo. 1988. Supernumerary chromosomes, Robertsonian rearrangement and multiple NORs in Corydoras aeneus (Pisces, Siluriformes, Callichthyidae). Caryologia 41:227-236.

Olmo, E., V. Stingo, O. Cobror, T. Capriglione, and G. Odierna. 1982. Repetitive DNA and polyploidy in Selachians. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 73:739-745.

Olson, M. 1997. Bayesian procedures for discriminating among hypotheses with discrete distributions: inheritance in the tetraploid *Astilbe biternata*. Genetics 147:1933-42.

Orr, H. A. 1990. "Why polyploidy is rarer in animals than in plants" revisited. American Naturalist 6:759-770.

Otto, C. R. V., J. W. Snodgrass, D. C. Forester, J. C. Mitchell, and R. W. Miller. 2007. Climatic variation and the distribution of an amphibian polyploid complex. Journal of Animal Ecology 76:1053-1061.

Otto, S. P., and J. Whitton. 2000. Polyploidy: incidence and evolution. Annual Review of Genetics 34:401-437.

Pandian, T. J., and R. Koteeswaran. 1998. Ploidy induction and sex control in fish. Hydrobiologia 384:167-243.

Pandian, T. J., and R. Koteeswaran. 1999. Natural occurrence of monoploids and polyploids in the Indian catfish, *Heteropneustes fossilis*. Current Science 76:1134-1137.

Panopoulou, G., S. Hennig, D. Groth, A. Krause, Poustka AJ, R. Herwig, M. Vingron, and H. Lehrach. 2003. New evidence for genome-wide duplications at the origin

of vertebrates using an amphioxus gene set and completed animal genomes. Genome Research 13:1056–1066.

Panopoulou, G., and A. J. Poustka. 2005. Timing and mechanism of ancient vertebrate genome duplications—the adventure of a hypothesis. Trends in Genetics 21:559–567.

Parisod, C., K. Alix, J. Just, M. Petit, V. Sarilar, C. Mhiri, M. Ainouche, B. Chalhoub, and M. A. Grandbastien. 2010a. Impact of transposable elements on the organization and function of allopolyploid genomes. New Phytologist 186:37-45.

Parisod, C., R. Holderegger, and C. Brochmann. 2010b. Evolutionary consequences of autopolyploidy. New Phytologist 186:5-17.

Paun, O., M. F. Fay, D. E. Soltis, and M. W. Chase. 2007. Genetic and epigenetic alterations after hybridization and genome doubling. Taxon 56:649-656.

Paun, O., F. Forest, M. F. Fay, and M. W. Chase. 2009. Hybrid speciation in angiosperms: parental divergence drives ploidy. New Phytologist 182:507-518.

Piferrera, F., R. M. Calb, C. Gómezb, B. C, and P. Martínez. 2003. Induction of triploidy in the turbot (*Scophthalmus maximus*): II. Effects of cold shock timing and induction of triploidy in a large volume of eggs. Aquaculture 220:821-831.

Pruzsinszky, I., and F. Ladich. 1998. Sound production and reproductive behaviour of the armoured catfish *Corydoras paleatus* (Callichthyidae). Environmental Biology of Fishes 53:183-191.

Ptacek, M., H. C. Gerhardt, and R. D. Sage. 1994. Speciation by polyploidy in treefrogs: multiple origins of the tetraploid, *Hyla versicolor*. Evolution 48:893-903.

Purdom, C. E. 1972. Induced polyploidy in plaice (*Pleuronectes platessa*) and Its hybrid with Flounder (*Platichthys flesus*). Heredity 29:11-24.

Putnam, N. H., T. Butts, D. E. K. Ferrier, R. F. Furlong, U. Hellsten, T. Kawashima, M. Robinson-Rechavi, E. Shoguchi, A. Terry, J.-K. Yu, E. g. l. Benito-Gutierrez, I.

Dubchak, J. Garcia-Fernandez, J. J. Gibson-Brown, I. V. Grigoriev, A. C. Horton, P. J. de Jong, J. Jurka, V. V. Kapitonov, Y. Kohara, Y. Kuroki, E. Lindquist, S. Lucas, K. Osoegawa, L. A. Pennacchio, A. A. Salamov, Y. Satou, T. Sauka-Spengler, J. Schmutz, T. Shin-I, A. Toyoda, M. Bronner-Fraser, A. Fujiyama, L. Z. Holland, P. W. H. Holland, N. Satoh, and D. S. Rokhsar. 2008. The amphioxus genome and the evolution of the chordate karyotype. Nature 453:1064-1071.

Rab, P. 1981. Karyotype of European catfish *Silurus glanis* (Siluridae, Pisces), with remarks on cytogenetics of Siluroid fishes. Folia Zoologica 30:271-279.

Rabe, E. J., and C. H. Haufler. 1992. Incipient polyploid speciation in the maidenhair fern (*Adiantum pedatum*; Adiantaceae)? American Journal of Botany 79:701-707.

Ralin, D. B. 1970. Genetic compatibility and a phylogeny of the temperate North American hylid faunaUniversity of Texas at Austin, Austin.

Ralin, D. B. 1978. "Resolution" of diploid-tetraploid treefrogs. Science 202:335-336.

Ramsden, C., K. Beriault, and J. P. Bogart. 2006. A nonlethal method of identification of *Ambystoma laterale*, *Ambystoma jeffersonianum* and sympatric unisexuals. Molecular Ecology Notes 6:261-264.

Ramsey, J. 2007. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). Heredity 98:143-150.

Ramsey, J., and D. W. Schemske. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annual Review of Ecology and Systematics 29:467-501.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. Annual Review of Ecology and Systematics 33:589-639.

Richards, C. M., and G. W. Nace. 1977. The occurrence of diploid ova in *Rana pipiens*. Journal of Herpetology 68:307-312.

Rishi, K. K., and M. S. Haobam. 1984. Karyotypes of three forms of fishes having high chromosome number. Int. J. Acad. Ichthyol Modinagar 5:139-144.

Robert, J., L. Abramowitz, J. Gantress, and H. D. Morales. 2007. *Xenopus laevis*: A possible vector of ranavirus infection? Journal of Wildlife Diseases 43:645-652.

Roberts, J. D. 1997a. Call evolution in Neobatrachus (Anura: Myobatrachidae): speculations on tetraploid origins. Copeia 1997:791-801.

Roberts, J. D. 1997b. Geographic variation in calls of males and determination of species boundaries in tetraploid frogs of the Australian genus *Neobatrachus* (Myobatrachidae). Australian Journal of Zoology 45:95-112.

Roberts, J. D., L.R. Maxson, and N. Plummer. 1996. Recent speciation in diploid-tetraploid burrowing frogs of the genus Neobatrachus (Anura: Myobatrachidae): immunological evidence. Pages 287-291 *in* Gondwanan Heritage: Past, Present and Future of the Western Australian Biota Surrey-Beatty, Chipping Norton, Australia.

Roberts, J. D., and C. M. Majors. 1993. Range extensions, range definitions and call structures for frogs from Western Australia. Records of the Western Australian Museum 16:315-322.

Rodin, S. N., and A. D. Riggs. 2003. Epigenetic silencing may aid evolution by gene duplication. Journal of Molecular Evolution 56:718-29.

Roelants, K., D. J. Gower, M. Wilkinson, S. P. Loader, S. D. Biju, K. Guillaume, L. Moriau, and F. Bossuyt. 2007. Global patterns of diversification in the history of modern amphibians. Proceedings of the National Academy of Sciences U.S.A. 104:887-892.

Romano, M. A., D. B. Ralin, S. I. Guttman, and J. H. Skillings. 1987. Parallel electromoph variation in the diploid-tetraploid gray treefrog complex. American Naturalist 130:864-878.

Romano, P. R., and J. C. Vaughn. 1985. Diploidization of rRNA gene function may proceed by ordered nucleotide sequence change at the genomic level in a tetraploid frog species. Journal of Cell Biology 101:452.

Rosset, S. D., D. Baldo, C. Lanzone, and N. G. Basso. 2006. Review of the geographic distribution of diploid and tetraploid populations of the *Odontophrynus americanus* species complex (Anura : Leptodactylidae). Journal of Herpetology 40:465-477.

Ryder, J. A. 1888. The sturgeon and sturgeon industries o f the eastern coast o f the United States, with an account of experiments bearing upon sturgeon culture. Bulletin of the United States Fish Commission 8:231-238.

Saez, F. A., and N. Brum-Zorrilla. 1966. Karyotype variation in some species of the genus *Odontophrynus* (Amphibia-Anura). Caryologia 19:7-24.

Saitoh, K., W. J. Chen, and R. L. Mayden. 2010. Extensive hybridization and tetrapolyploidy in spined loach fish. Molecular Phylogenetics and Evolution 56:1001-1010.

Sakamoto, T., R. Danzmann, K. Gharbi, P. Howard, i. A. Ozak, S. Khoo, R. Woram, N. Okamoto, M. Ferguson, L. Holm, R. Guyomard, and B. Hoyheim. 2000. A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. Genetics 155:1331-45.

Salthe, S. N., and J. S. Mecham. 1974. Reproductive and coutship pattersn. Pages 308-521 *in* Physiology of the Amphibia, Volume II (B. Lofts, ed.) Academic Press, New York.

Sammut, B., A. Marcuz, and L. Pasquier. 2002. The fate of duplicated major histocompatibility complex class Ia genes in a dodecaploid amphibian, *Xenopus ruwenzoriensis*. European Journal of Immunology 32:2698-709.

Santini, F., L. J. Harmon, G. Carnevale, and M. E. Alfaro. 2009. Did genome duplication drive the origin of teleosts? A comparative study of diversification in ray-finned fishes. BMC Evolutionary Biology 9:194.

Santos, P., T. Kellermann, B. Uchanska-Ziegler, and A. Ziegler. 2010. Genomic architecture of MHC-linked odorant receptor gene repertoires among 16 vertebrate species. Immunogenetics 62:569-584.

Sargent, R. C., P. D. Taylor, and M. R. Gross. 1987. Parental care and the evolution of egg size in fishes. American Naturalist 129:32-46.

Scott, W. B., and E. J. Crossman. 1973. The freshwater fishes of Canada. Fisheries Research Board of Canada Bulletin 173:381-381.

Seehausen, O. L. E., G. Takimoto, D. Roy, and J. Jokela. 2008. Speciation reversal and biodiversity dynamics with hybridization in changing environments. Molecular Ecology 17:30-44.

Semon, M., and K. H. Wolfe. 2008. Preferential subfunctionalization of slow-evolving genes after allopolyploidization in *Xenopus laevis*. Proceedings of the National Academy of Sciences U.S.A. 105:8333-8338.

Shiina, T., J. M. Dijkstra, S. Shimizu, A. Watanabe, K. Yanagiya, I. Kiryu, A. Fujiwara, C. Nishida-Umehara, Y. Kaba, I. Hirono, Y. Yoshiura, T. Aoki, H. Inoko, J. K. Kulski, and M. Ototake. 2005. Interchromosomal duplication of major histocompatibility complex class I regions in rainbow trout (*Oncorhynchus mykiss*), a species with a presumably recent tetraploid ancestry. Immunogenetics 56:878-893.

Shimabukuro-Dias, C. K., C. Oliveira, and F. Foresti. 2004. Cytogenetic analysis of five species of the subfamily Corydoradinae (Teleostei : Siluriformes : Callichthyidae). Genetics and Molecular Biology 27:549-554.

Shum, B. P., D. Avila, L. Dupasquier, M. Kasahara, and M. F. Flajnik. 1993. Isolation of a classical MHC Class-I cDNA from an amphibian - Evidence for only one Class-I locus in the *Xenopus* MHC. Journal of Immunology 151:5376-5386.

Siegel, N., S. Hoegg, W. Salzburger, I. Braasch, and A. Meyer. 2007. Comparative genomics of ParaHox clusters of teleost fishes: gene cluster breakup and the retention of gene sets following whole genome duplications. BMC Genomics 8:312.

Slechtova, V., J. Bohlen, J. Freyhof, and P. Rab. 2006. Molecular phylogeny of the Southeast Asian freshwater fish family Botiidae (Teleostei : Cobitoldea) and the origin of polyploidy in their evolution. Molecular Phylogenetics and Evolution 39:529-541.

Slechtova, V., W. Luskova, V. Slechta, S. Lusk, and J. Pivonkova. 2003. Potential species identification by allozyme/protein markers in European spined loaches. Folia Biologica-Krakow 51:43-47.

Smith, E. M., and T. R. Gregory. 2009. Patterns of genome size diversity in the ray-finned fishes. Hydrobiologia 625:1-25.

Soltis, D. E., R. J. Buggs, J. J. Doyle, and P. S. Soltis. 2010. What we still don't know about polyploidy. Taxon 2:1-17.

Soltis, D. E., and P. S. Soltis. 1999. Polyploidy: recurrent formation and genome evolution. Trends in Ecology & Evolution 14:348-352.

Soltis, D. E., P. S. Soltis, D. W. Schemske, J. F. Hancock, J. N. Thompson, B. C. Husband, and W. S. Judd. 2007. Autopolyploidy in angiosperms: have we grossly underestimated the number of species? Taxon 56:13-30.

Soltis, D. E., P. S. Soltis, and J. A. Tate. 2004. Advances in the study of polyploidy since Plant speciation. New Phytologist 161:173-191.

Soltis, P. S., and D. E. Soltis. 2000. The role of genetic and genomic attributes in the success of polyploids. Proceedings of the National Academy of Sciences USA 97:7051-7057.

St-Amour, V., W. M. Wong, T. W. J. Garner, and D. Lesbarreres. 2008. Anthropogenic influence on prevalence of 2 amphibian pathogens. Emerging Infectious Diseases 14:1175-1176.

Stebbins, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York and London.

Stebbins, G. L. 1971. Chromosomal evolution in higher plants. Edward Arnold, London.

Stift, M., C. Berenos, P. Kuperus, and P. H. van Tienderen. 2008. Segregation models for disomic, tetrasomic and intermediate inheritance in tetraploids: A general procedure applied to *Rorippa* (yellow cress) microsatellite data. Genetics 179:2113-2123.

Stingo, V., and L. Rocco. 2001. Selachian cytogenetics: a review. Genetica 111:329-347.

Stöck, M., and W.-R. Grosse. 2003. Die Bufo viridis-Untergruppe in Mittel- und Zentralasien: Eine übersich zu Verbreitung, Polyploidie, Paarungsfuren und Taxonomie. Mertensiella 14:179-217.

Stöck, M., D. K. Lamatsch, C. Steinlein, J. T. Epplen, W.-R. Grosse, R. Hock, T. Klapperstück, K. P. Lampert, U. Scheer, M. Schmid, and M. Schartl. 2002. A bisexually reproducing all-triploid vertebrate. Nature Genetics 30:325-328.

Stöck, M., C. Moritz, M. Hickerson, D. Frynta, T. Dujsebayeva, V. Eremchenko, J. R. Macey, T. J. Papenfuss, and D. B. Wake. 2006. Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Molecular Phylogenetics and Evolution 41:663-689.

Stöck, M., C. Steinlein, D. K. Lamatsch, M. Schartl, and M. Schmid. 2005. Multiple origins of tetraploid taxa in the Eurasian *Bufo viridis* subgroup. Genetica 124:255-272.

Suzuki, A., and Y. Taki. 1996. Tetraploidization in the cobitid subfamily Botinae (Pisces, Cypriniformes). Cytobios 85:229-245.

Svärdson, G. 1945. Chromosome studies on Salmonidae. Report on the Swedish States Institute for Freshwater Fishery Research Drottingholm 23:1-151.

Svartman, M., G. Stone, and R. Stanyon. 2005. Molecular cytogenetics discards polyploidy in mammals. Genomics 85:425-430.

Taylor, M. I., J. I. Morley, C. Rico, and S. Balshine. 2003. Evidence for genetic monogamy and female-biased dispersal in the biparental mouthbrooding cichlid *Eretmodus cyanostictus* from Lake Tanganyika. Molecular Ecology 12:3173-3177.

Thorgaard, G. H., M. E. Jazwin, and A. R. Stier. 1981. Polyploidy Induced by Heat-Shock in Rainbow-Trout. Transactions of the American Fisheries Society 110:546-550.

Thorsen, A., P. R. Witthames, G. Marteinsdottir, R. D. M. Nash, and O. S. Kjesbu. 2010. Fecundity and growth of Atlantic cod (Gadus morhua L.) along a latitudinal gradient. Fisheries Research 104:45-55.

Tiwary, B. K., R. Kirubagaran, and A. K. Ray. 2004. The biology of triploid fish. Reviews in Fish Biology and Fisheries 14:391-402.

Tsigenopoulos, C., P. Rab, D. Naran, and P. Berrebi. 2002. Multiple origins of polyploidy in the phylogeny of southern African barbs (Cyprinidae) as inferred from mtDNA markers. Heredity 88:466-73.

Tymowska, J. 1991. Polyploidy and cytogenetic variation in frogs of the genus *Xenopus*. Pages 259-297 *in* Amphibian Cytogenetics and Evolution (D. M. Green, and L. K. Sessions, eds.). Academic Press, New York.

Uyeno, T., and G. R. Smith. 1972. Tetraploid origin of karyotype of Catostomid fishes. Science 175:644-646.

Uzzell, T. M., Jr. 1964. Relations of the diploid and triploid species of the *Ambystoma jeffersonianum* complex (Amphibia, Caudata). Copeia 1964:257-300.

Van de Peer, Y., S. Maere, and A. Meyer. 2009. OPINION The evolutionary significance of ancient genome duplications. Nature Reviews Genetics 10:725-732.

Vasil'ev, V. P. 2009. Mechanisms of polyploid evolution in fish: Polyploidy in Sturgeons. Biology, Conservation and Sustainable Development of Sturgeons 29:97-117.

Vasil'ev, V. P., Y. D. Vasil'eva, and A. G. Osinov. 1990. Reticulate evolution in vertebrates: the diploid-triploid-tetraploid complexes in the genus *Cobitis* (Cobitidae). III. Origin of the triploid form. Journal of Ichthyology 30:22-31.

Vences, M., G. Aprea, T. Capriglione, F. Andreone, and G. Odierna. 2002. Ancient tetraploidy and slow molecular evolution in *Scaphiophryne*: ecological correlates of speciation mode in Malagasy relict amphibians. Chromosome Research 10:127-136.

Vences, M., and J. Kohler. 2008. Global diversity of amphibians (Amphibia) in freshwater. Hydrobiologia 595:569-580.

Venkatesh, B. 2003. Evolution and diversity of fish genomes. Current Opinion in Genetics & Development 13:588-592.

Venkatesh, B. 2007. Elephant shark (*Callorhinchus milii*) genome analysis. Genes & Genetic Systems 82:503-503.

Vigny, C. 1979. The mating calls of 12 species and sub-species of the genus *Xenopus* (Amphibia: Anura). Journal of Zoology 188:103-122.

Vinogradov, A. E. 2003. Selfish DNA is maladaptive: evidence from the plant Red List. Trends in Genetics 19:609-614.

Vinogradov, A. E., L. J. Borkin, R. Gunther, and J. M. Rosanov. 1990. Genome elimination in diploid and triploid *Rana esculenta* males: cytological evidence from DNA flow cytometry. Genome 33:619-627.

Vogel, G. 1998. Evolutionary biology - Doubled genes may explain fish diversity. Science 281:1119-1121.

Volff, J. N. 2005. Genome evolution and biodiversity in teleost fish. Heredity 94:280-294.

Wager, V. A. 1965. The frogs of South Africa. Purnell & Sons, Capetown.

Wasserman, A. O. 1970. Polyploidy in the common tree toad *Hyla versicolor* LeConte. Science 167:385-386.

Weiss, R. L., J. R. Kukora, and J. Adams. 1975. The relationship between enzyme activity, cell geometry, and fitness in *Saccharomyces cerevisiae*. Proceedings of the National Academy of Sciences U.S.A. 72:794-798.

Weldon, C., L. H. du Preez, A. D. Hyatt, R. Muller, and R. Speare. 2004. Origin of the amphibian chytrid fungus. Emerging Infectious Diseases 10:2100-2105.

Wessler, S. R., and J. C. Carrington. 2005. The consequences of gene and genome duplication in plants. Current Opinion in Plant Biology 8:119-121.

White, M. J. D. 1973. Animal Cytology and Evolution, 3 edition. University Press, Cambridge.

Whitney, K. D., J. R. Ahern, L. G. Campbell, L. P. Albert, and M. S. King. 2010. Patterns of hybridization in plants. Perspectives in Plant Ecology, Evolution and Systematics 12:175-182.

Wiens, J. J. 2007. Review of "The amphibian tree of life" by Frost et al. . Quarterly Review of Biology 82:55-56.

Winge, Ö. 1917. The chromosomes. Their numbers and general importance. Compt. Rend. Trav. Lab. Carlsberg 13:131-175.

Wolfe, K. H., and D. C. Shields. 1997. Molecular evidence for an ancient duplication of the entire yeast genome. Nature 387:708-713.

Wolters, W. R., G. S. Libey, and C. L. Chrisman. 1981. Induction of triploidy in channel catfish. Transactions of the American Fisheries Society 110:310-312.

Wong, J. L., and G. M. Wessel. 2006. Defending the zygote: search for the ancestral animal block to polyspermy. Current Topics in Developmental Biology 72:1-151.

Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg. 2009. The frequency of polyploid speciation in vascular plants. Proceedings of the National Academy of Sciences U.S.A. 106:13875-9.

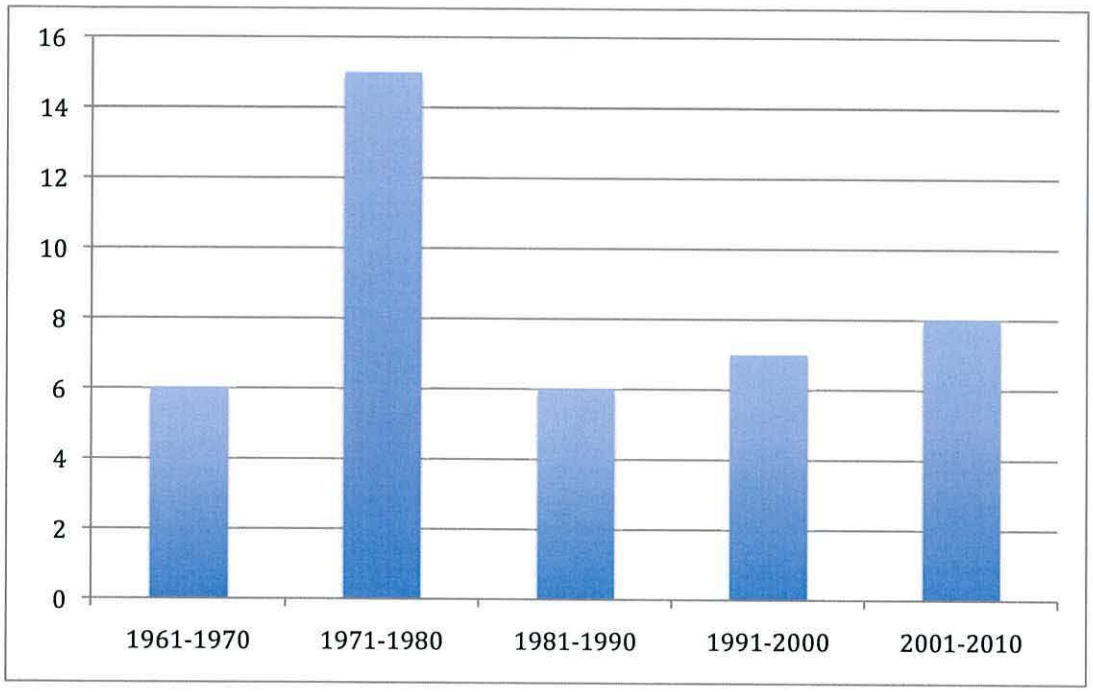
Wootton, R. J. 1984. Introduction: tactics and strategies in fish reproduction. Pages 1-12. *in* Fish Reproduction: strategies and tactics. (G. W. Potts, and R. J. Wootton, eds.). Academic Press,, London.

Wright, J. E., K. Johnson, A. Hollister, and B. May. 1983. Meiotic models to explain classical linkage, pseudolinkage, and chromosome-pairing in tetraploid derivative Salmonid genomes. Isozymes-Current Topics in Biological and Medical Research 10:239-260.

Wright, R. J., P. M. Thaxton, K. M. El-Zik, and A. H. Paterson. 1998. D-subgenome bias of Xcm resistance genes in tetraploid *Gossypium* (cotton) suggests that polyploid formation has created novel avenues for evolution. Genetics 149:1987-1996.

Xu, Y., L. Zhong, X. Wu, X. Fang, and J. Wang. 2009. Rapid alterations of gene expression and cytosine methylation in newly synthesized *Brassica napus* allopolyploids. Planta 229:471-483.

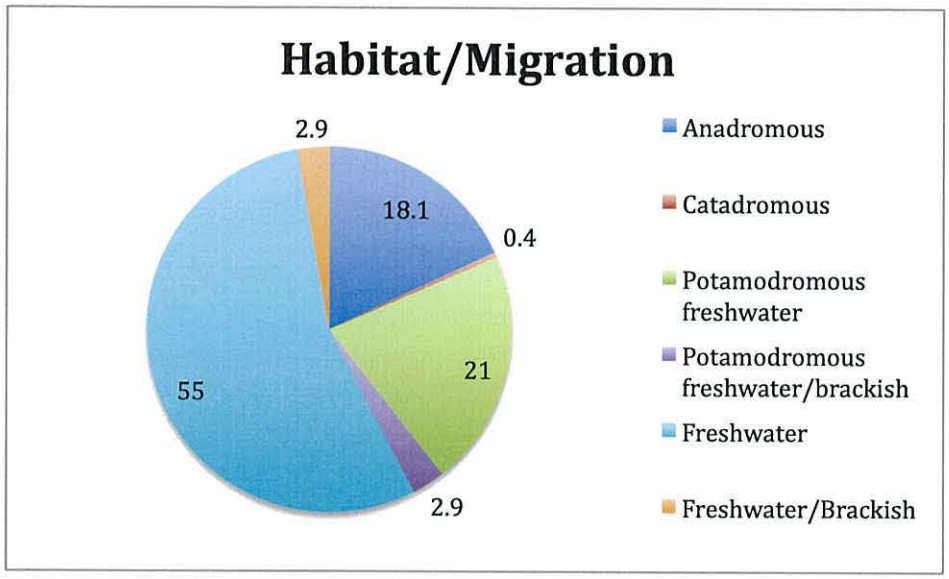
SI Figure A1- Distribution of Polyploid Descriptions



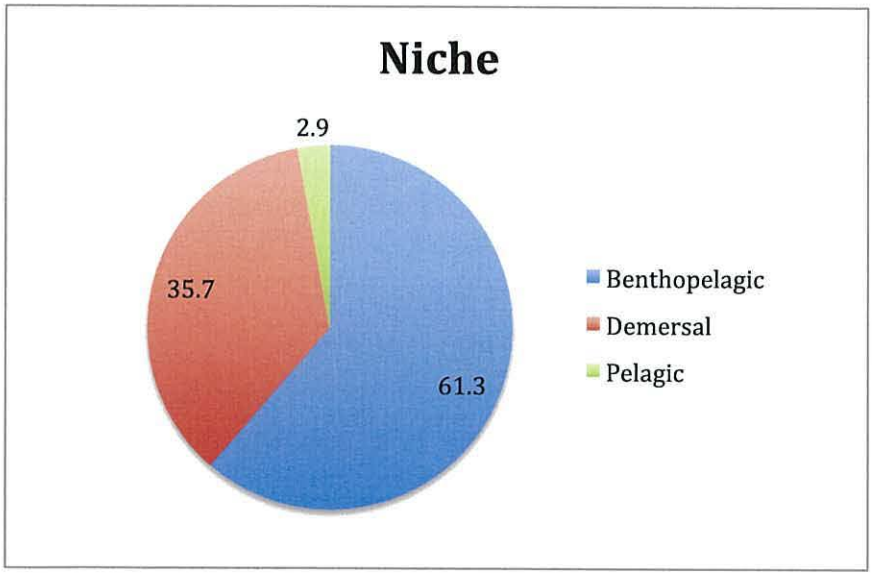
Distribution of descriptions of new polyploid anuran species by decade. The first naturally occurring polyploid amphibians were described in 1964 (*Ambystoma Jeffersonian* complex of salamanders) and the first polyploid anuran species (*Odontophrynus americanus*) was reported in 1966. The peak of new descriptions was in the 1970's, when allozymes and cytogenetics were at their peak.

SI Figure A2- Polyploid Habitats, Migration and Niche

(a)



(b)



(a) Distribution of polyploid fish by habitats and breeding site for migratory species. The vast majority of polyploid fish are dependent on freshwater: either living exclusively in freshwater, migrating from marine to freshwater to breed (anadromous) or completing their entire lifecycle within rivers (potamodromous). A small percentage are associated with brackish water and only a very few are catadromous (live in freshwater but migrate to a marine environment to breed. (b) Distribution of polyploid fish by niche type.). Most polyploids either live near the bottom surface (benthopelagic) or in the bottom part of the water column (demersal) rather than on the surface (pelagic).

SI Table A1- Table of Amphibian Polyploids

Family (Revised)	Species (revised)	Citation for Polyploidy	Citation for Species Name	Citation for Revised Names	Ploidy	Genome size	Notes on Origins	Centre of Distribution	Climate	Red list status	Population Trend	Distribution
Arthropleptidae	***Astylosternus diadematus***	Bogart & Tandy 1981	Werner 1898		4n=54		no known diploid; Duellman& Trueb (1986) list as 2n with high chromosome numbers	(4.033 10.567)	tropical	vulnerable		Africa: Western and southwestern Cameroon, and possibly in extreme eastern Nigeria, at elevations of 250-1100 m
Arthropleptidae	*Astylosternus batesi*		Boulenger 1900		2n=26 or 28?					least concern	decreasing	Africa: Cameroon south of Sanaga River, Equatorial Guinea, Gabon, Southwestern Central African Republic, and Mayombe hills of extreme western Dem. Rep. Congo, sea level to 1000 m elevation
Arthropleptidae	*Astylosternus montanus*		Amiet 1978		2n=26 or 28?					near threatened	decreasing	Africa: Cameroon range and along the northern and southern edges of the Adamauoa Plateau, in the submontane zone; Obudu Plateau of eastern Nigeria
Bufonidae	*Bufo (Amietophrynus) kerinyagae*		Keith 1968	Frost et al. 2006	2n=20		*Bufo latifrons complex; formerly confused with B. asmarae (Bogart 1980)*		tropical	least concern	unknown	Africa: Discontinuous in montane grassland and forest-edge of central Ethiopia on both sides of rift valley, Kenya highlands, Mont Elgon in SE Uganda and rim of Ngorogor crater in Tanzania
Bufonidae	*Bufo (Amietophrynus) regularis*		Reuss 1833	Frost et al. 2006	2n=20					least concern	stable	Africa: Subsaharan west Africa to the oases of Djanet in Algeria and Gat in Libya to northern Nilotic Egypt, western Ethiopia southward to northwestern angola, northeastern Congo, Uganda and southern Kenya in savanna and farmbush; introduced cape verde
Bufonidae	***Bufo (Amietophrynus) asmarae***	Bogart & Tandy 1976	Tandy et al. 1982	Frost et al. 2006	4n=40		most similar to B. kerinyagae and B. regularis;likely Bufo D in Bogart & Tandy (1976); named by Tandy et al. (1982)	(9.7091 39.6826)		least concern	unknown	Africa: Highlands of Ethiopia, both sides of rift valley; western pop extending north into Eritrea, elevations 2000-3000 m in Ethiopia and down to 200 m in Eritrea

Bufonidae	*Bufo (Pseudepidalia) viridis*		Laurenti 1768	Frost et al. 2006	2n=22	3.82-6.84	diploid in B. viridis group		cold, temperate, dry	least concern	decreasing	Eurasia: Northeastern borderlands of France, western Germany, and northern Italy easts and south to Greece (including Crete) and through Europe through Russia to Kazakhstan
Bufonidae	***Bufo danatensis (Pseudepidalea oblongus)***	Stöck et al. 2001a	Pisanets 1978	Frost et al. 2006	4n=44	12.03	allopolyploid--separate origin from P. pewzowi (Bogart 1980)	(35.7465 59.0625)		least concern	stable	Eurasia: East of central Iranian deserts (Khorasan) to the north along the Kopet-Dagh range to Kyuren Dagh, probably east into western Afghanistan
Bufonidae	*(B. oblongus danatensis)*											
Bufonidae	***Pseudepidalea pewzowi***	Stöck et al.2001b	Bedriaga 1898		4n=44		*B. viridis group; allopolyploid (Stöck et al. 2005; 2006); removed from synonymy with B. viridis by Stöck et al. (2001b)*	not in database		not listed		Eurasia: isolated oasis pops along margins of Tarim Basin, northern and eastern slope (and likely southern slope) of the Tien Shan and the northern slope of Kuen-Lun and eastern Pamirs, Xinjiang, China; east to western Mongolia; west through Kygystan to eastern Uzbekistan, Tajikistan and possibly extreme northern Afghanistan
Bufonidae	***Bufo pseudoraddei baturae (Pseudepidalea pseudoraddei)***		Mertens 1971		3n=33		*B. viridis group; triploid species now not subspecies*	(34.4522 71.2793)		least concern	stable	Himalayan moist-temperate forest in northern Pakistan and likely into adjacent Afghanistan and India
Dicroglossidae	***Dicroglossus (Hoplobatrachus) occipitalis***	Bogart & Tandy 1976	Deckert 1938	Dubois 1992	2n=26; 4n=52		two sibling species, one of which, in Liberia is tetraploid (Bogart & Tandy 1976)	(8.4072 14.5898)	tropical	least concern	decreasing	Africa: Gambia and extreme southwestern Sahara to Ethiopia (including isolated pops in SW Libya, Air mountains of Niger, nothern Mali), south through subsaharan Africa to northern Zambi, western Angola, and western Rep. Congo
Hylidae	***Hyla versicolor***	Wasserman 1970	Le Conte 1825		4n=48	9.20,10.30	calls, mtDNA; segmental polyploids?; multiple origins from two lineages at least three times (Holloway et al. 2006)	(41.5744 - 84.8145)	cold, temperate	least concern	stable	North America: eastern North Dakota, Minnesota, Wisconsin, Michigan, south and east southeast to Maryland thence south to south-central TX and northern Florida--provisional due to confusion with H. versicolor
Hylidae	*Hyla chrysoscelis*		Cope 1880	Johnson 1966	2n=24	4.67				least concern	stable	North America: eastern TX north and northeast to Minnesota and Virginia and thence to Maine and in Canada in southern quebec, southern Ontario and southeastern Manitoba--provisional due to confusion

												with H. chrys
Hylidae	*Phyllomedusa distincta*		Bokermann 1966		2n=26					least concern	decreasing	South America: Southeastern Brazil (Sao Paulo and Santa Catarina);in the P. burmeisteri group
Hylidae	***Phyllomedusa tetraploidea***	Beçak et al. 1970	Pombal & Haddad 1992		4n=52		in P. burmeisteri group and derived by tetraploidy from P. distincta	(-24.0465 -51.1523)	temperate	least concern	stable	South America: Interior regions of the states of Sao Paulo and Parana, Brazil; Misiones, Argentina; extreme southeastern Paraguay (Itapua); see reference list for locality references
Hylidae	*Phyllomedusa burmeisteri*		Boulenger 1882		2n=26; 4n=52	6.58, 13.02	diploid and tetraploid genome sizes; tetraploid value is likely tetraploidea (Haddad et al. 1994)			least concern	stable	South America: Eastern Brazil
Leptodactylidae (Craugastoridae)	***Eleutherodactylus (Haddadus) binotatus***	Beçak & Beçak 1974	Stejneger 1904	Hedges et al. 2008	4n=44			(-20.9614 -42.7148)	temperate and tropical	least concern	decreasing	South America: Southeastern Brazil
Leptodactylidae (Leiuperidae)	***Pleurodema bibroni***	Barrio & de Chieri 1970	Tschudi 1838	Grant et al. 2006	4n=44		some taxonomic confusion; see also Barrio (1977)	(-30.1451 -53.6133)	temperate	near threatened	decreasing	South America: Southern Uruguay (isolated recored in northern Uruguay) and northeaster Rio Grande do Sul, Brazil and presumably at least in western part of Santa Catarina, Brazial
Leptodactylidae (Leiuperidae)	***Pleurodema kriegi***	Barrio & de Chieri 1970	di Tada et al. 1976		4n=44			(-31.8962 -64.8303)	temperate	near threatened	decreasing	South America: Montane landscapes in central-western part of province of Cordoba, Argentina, 1800-2600 m elevation
Leuiperidae	***Pleurodema cordobae***	Valetti et al. 2009	Valetti et al. 2009		8n=88		cryptic species with P. kriegi	(-32.3995 -64.9264)	temperate	not in database	not in database	South America: Known only from the type locality (Los Tabaquillos, Sierra de Comechingones, Córdoba province, Argentina, ca. 2105 m elevation).
Leptodactylidae (Ceratophryidae)	*Ceratophrys cranwelli*		Barrio 1980	Frost et al. 2006	2n=26					least concern	decreasing	South America: Northern Argentina, SW Uruguay, Paraguay, Bolivia, SE Brazil; Chacoan region of Argentina, Bolivia, Brazil, Paraguay

Leptodactylidae (Ceratophryidae)	***Ceratophrys ornata***	Bogart 1967	Günther 1849	Frost et al. 2006	8n=104	13.4	no known diploid; calls, karyotype; morphology--differences magnified in zone of contact; inviability of hybrids; chromosome numbers originally described in Saez and Brum-Zorrilla (1966) but tetraploidy was rejected as an explanation	(-35.3532 -61.084)	temperate	near threatened	decreasing	South America: Pampean region of Argentina (Buenos Aires, Cordoba, Entre Rios, La Pampa, Mendoza, and Santa Fe provinces), Uruguay, and Rio Grande de Sul, Brazil, 0-500 m elevation
Leptodactylidae (Ceratophryidae)	***Ceratophrys dorsata (aurita)***	Beçak et al. 1967	Wied-Neuwied 1824; Bokermann 1966	Barrio 1980	8n=104	6.34	no known diploid (Barrio 1980)	(-21.4531 -43.7695)	temperate	least concern	stable	South America: Minas Gerais and Bahia to Rio Grande do Sul, Brazil
Leptodactylidae (Ceratophryidae)	*Ceratophrys calcarata*		Boulenger 1890		2n=26	1.89, 2.75						
Leptodactylidae (Ceratophryidae)	***Ceratophyrs joazeirensis***	Vieira et al. 2006	Mercadal de Barrio 1986		8n=104		most closely related to C. aurita; chromosomes similar size and morphology to C. aurita, C. ornata and C. cranwelli	(-8.4072 - 38.3203)	temperate, semi-arid savanna and hypoxerophylic Caatinga	data deficient	unknown	South America: Known from the vicinity of type locality (Joazeiro, Bahia, Brazil), north to Municipality of Triunfo, Pernambuco, to the municipality of Araruna, northern Paraíba; contiguous with C. aurita in Brazil (Caatinga and NE Atlantic Forest)
Leptodactylidae (Cycloramphibidae)	***Odontophrynus americanus***	Beçak et al. 1966	Miranda-Ribeiro 1920	Frost et al. 2006	4n=44	3.09, 3.44		(-24.287 - 54.4043)	temperate	least concern	stable	South America: Central and northern Argentina, southern Paraguay, southern Brazil, and Uruguay
	Odontophrynus cordobae		Martino & Sinsch 2002		2n=22					least concern	unknown	South America: Departments of Cordoba and Santiago el Estero in northern Argentina
	Odontophrynus cultripes		Reinhardt & Lütken 1862		2n=22	2.07, 2.44						

Microhylidae	*Chiasmocleis schubarti*		Bokermann 1952		2n=24				temperate	least concern	decreasing	South America: Atlantic Rainforest fragments in the states of Espírito Santo and Minas Gerais in southeastern Brazil; also reported from Guaratinga Municipality, Bahia.
Microhylidae	***Chiasmocleis leucosticta***	Kasahara & Haddad 1997	Parker 1934		4n=48		origins unknown	(-24.6071 -48.4277)	temperate	least concern	decreasing	South America: States of Santa Catarina and São Paulo, Brazil.
Microhylidae	***Cophixalus pansus (Aphantophryne pansa)***	Kuramoto & Allison 1989	Zweifel 1956	Zweifel & Parker 1989	4n=52		not clear which diploid related to but C. riparius has most similar karyotype (Kuramoto and Alison 1989)	(-8.5647 147.3981)	tropical	least concern	stable	Oceania: High elevation in Owen Stanley Range and disjunct to northwest in mountains south of Wau, Morobe Province, Papua New Guinea
Microhylidae	*Cophixalus riparius*		Zweifel 1962		2n=26			(-6.3808 145.7886)	tropical	leat concern	stable	Oceania: Central mountain ranges of Papua New Guinea from Southern Highlands and Western Highlands provinces southeastward to vicinity of Wau, Morobe Province, 1800-2800 m elevation
Microhylidae	***Scaphiophryne gottlebei***	Vences et al. 2002	Busse & Böhome 1992		4n=52		likely allotetraploid but no diploid partner known (Vences et al. 2002)	(-10.7628 23.8105)	tropical?	endangered	decreasing	Africa: Only polyploid known from Madagascar; Isalo Massif region often in deep canyons, Fianarantso Province, southern Madagascar, 700-1000 m elevation
Microhylidae	*Scaphiophryne madagascariensis*		Boulenger 1882		2n=26					near threatened	decreasing	Africa: High elevation in both forest and above the tree line on the eastern slopes of mountains in southern Madagascar. Ankarata mountains of central Madagascar 1300-2000 m elevation
Microhylidae	*Scaphiophryne spinosa*		Steindachner 1876		2n=26					least concern	unknown	Africa: Mid-altitude rainfores localities along eastern coast of Madagascar
Ranidae (Pyxicephalidae)	*Pyxicephalus (Tomopterna) delalandii*		Tschudi 1838	Dubois 1987	2n=26; 4n=52		polyploids used to be in same taxon			least concern	stable	Africa: Namaqualand, southward in sandy areas through Western Cape, and eastwards along the coast to Cape St. Francis, Rep. South Africa; possibly into adjacent Namibia

Ranidae (Pyxicephalidae)	***Tomopterna tandyi***	Channing & Bogart 1996	Channing & Bogart 1996		4n=52		allopolyploid: T. delalandii x T. cryptotis; morphologically indistinguishable from parental species (Channing and Bogart 1996)	(-25.2447 22.5879)	dry and temperate	least concern	stable	Africa: Broad band from Eastern Cape coast and between Port Elizabeth and the Kei River mouht; northwards to highlands around Vaal River and Pietersburg, Rep. South Africa; north and west to Grootfontein and Hardap in Namibia to southwestern Angola; northward in a very poorly understood range through Tanzania and Kenya; possibly in Lesotho
Myobatrachidae (Lymnodynastidae)	*Neobatrachus albipes*		Roberts et al. 1991	Frost et al. 2006	2n=24					least concern	stable	Oceania: Southern Western Australia from Wyalkatchem, Bruce Rock, Narembeen, Quairading, Dongalocking, and the Stirling ranges in the west, east to Coolgardie and Cape Aird and south of the great eastern highway; also reported at Junanan Rock
Myobatrachidae (Lymnodynastidae)	***Neobatrachus aquilonius***	Mahony & Roberts 1986	Tyler et al. 1981		4n=48			(-19.8081 127.0898)	dry	least concern	stable	Oceania:Isolated localities in south-central to northern WA, to the Barkly Tablelands, Northern Territory, Australia
Myobatrachidae (Lymnodynastidae)	***Neobatrachus centralis****	Mahony & Robinson 1980	Parker 1940		4n=48	5.23	mistakenly described as 2n in many other reports but correctly described in Roberts and Majors (1993); Mable and Roberts (1997)	(-27.4888 139.834)	dry and temperate	least concern	stable	Oceania:Poorly documented region in south-central WA, southern NT, central and northern South Australia to extreme southwestern Queenland
Myobatrachidae (Lymnodynastidae)	*Neobatrachus fulvus*		Mahony & Roberts 1986		2n=24					least concern	stable	Oceania:Central coastal WA from Shark Bay north to the North West cape
Myobatrachidae (Lymnodynastidae)	***Neobatrachus kunapalari***	Roberts et al. 1991	Mahony & Robinson 1980		4n=48			(-31.3536 118.916)	dry	least concern	stable	Oceania:Southwestern half of WA south of Menzies and Wubin to edge of the Nullarbor plain
Myobatrachidae (Lymnodynastidae)	*Neobatrachus pelobatoides*		Littlejohn & Main 1960		2n=24					least concern	stable	Oceania:Southwestern WA from Nerren Nerren, Perenjori, Dalwallinu, Mount Elvire, and Ponier Rocks in north and east, south to Buckingham, Stirling ranges and Israelite bay
Myobatrachidae (Lymnodynastidae)	*Neobatrachus pictus*		Peters 1863		2n=24	2.77				least concern	stable	Oceania:Southeastern SA and adjacent southwestern Victoria, with one record in extreme southwestern New South Wales, Australia

Myobatrachidae (Lymnodynastidae)	***Neobatrachus sudelli***	Mahony & Robinson 1980	Lamb 1911		4n=48	5.23		(-32.9902 146.8652)	dry	least concern	stable	Oceania:Southeastern Australia from southern Queensland south through central and western NSW into western Victoria and lower southeast of SA
Myobatrachidae (Lymnodynastidae)	*Neobatrachus sutor**		Main 1957		2n=24		mistakenly described as 4n in many other reports but correctly described in Roberts and Majors (1993); Mable and Roberts (1997)			least concern	stable	Oceania:Southern half of WA (except for Perth region and southern coast) to extreme northwest of SA and southwest of NT
Myobatrachidae (Lymnodynastidae)	*Neobatrachus wilsmorei*		Parker 1940		2n=24					least concern	stable	Oceania:Western WA: on the west coast from the Lyndon River south to the Irwin River and east to lake Nabberu, Banjawarn, and Yundamindra in the north, south to Carnarvon, Gnoolowa Hill, Morowa, Paynes Find and Kalgoorlie
Pipidae	*Xenopus (Silurana) tropicalis*			Gray 1864	2n=20	1.5,1.74,1.93			tropical	least concern	decreasing	Africa: Forested West Africa from Senegal to Cameroon
Pipidae	*Silurana cf tropicalis*		Evans et al. 2004		2n=20				tropical	least concern	stable	
Pipidae	***Xenopus (Silurana) epitropicalis***	Kobel & Du Pasquier 1986	Fischberg et al. 1982	Cannatella & Trueb 1988	4n=40			(-0.6152 19.1162)	tropical			Africa:Cameroon south and east through the Congo Basin (Gabon and western Dem. Rep. Congo and eastward to Garamba) to extreme northeastern Angols
Pipidae	***Xenopus (Silurana) new tetraploid1***	Evans et al. 2004			4n=40				tropical			
Pipidae	***Xenopus (Silurana) new tetraploid2***	Evans et al. 2004			4n=40				tropical			
Pipidae	***Xenopus amieti***	Kobel & Du Pasquier 1980	Kobel et al. 1980		8n=72			(5.3891 10.2365)	tropical	near threatened	decreasing	Africa:Volcanic highlands of western Cameroon including the Manengouba Highlands and the Bamileke and Bamenda Plateaus, 1100-1900 m

Pipidae	***X. cf fraseri 1***	Loumont 1983	Boulenger 1905		4n=36	3.11	*Xenopus fraseri forms part of a diploid-polyploid cryptic species group with chromosome numbers of 2n=36 (Xenopus fraseri), 2n=72 (Xenopus amieti, Xenopus andrei, Xenopus boumbaensis), and 2n=108 (Xenopus ruwenzoriensis) (Kobel et al. 1980; Loumont 1983)*		tropical	least concern	stable	Africa:Forested West Africa from Cameroon and Bioka (Equatorial Guinea) eastward throughout the congo river basin to the Zaire-Uganda border, and southward to Angola
Pipidae	***X. cf fraseri 2***	Evans et al. 2004			4n=36				tropical	least concern	stable	
Pipidae	***Xenopus new tetraploid***	Evans et al. 2004			4n=36				tropical			
Pipidae	***X. andrei***	Tymowska 1991	Loumont 1983		8n=72		mating call. See Kobel et al. (1996)	(2.2406 13.2715)	tropical	least concern	stable	Africa:Coastal Cameroon, northern Gabon and western central African republic; possibly into Equatorial guinea and Rep. Congo
Pipidae	***x. borealis***	Tymowska & Fischberg 1973	Parker 1936		4n=36	3.48,3.56		(-1.0107 35.8813)	tropical	least concern	unknown	Africa:Savanna in northern Tanzania and central and northern Kenya, above 1500 m elevation; one record for southern Tanzania
Pipidae	***X. boumaensis***	Loumont 1983	Loumont 1983		8n=72		A mating call of a single note (found elsewhere within the genus only in Xenopus borealis; see Loumont (1983)		tropical	data deficient	unknown	Africa:Known only from type locality in equatorial forest, Cameroon
Pipidae	***X. clivii***	Tymowska & Fischberg 1973	Peracca 1898		4n=36	3.9,4.14			tropical	least concern	decreasing	Africa:Centred on the Ethiopian plateau, but also found in Eritrea, and expected to occur in the Lake Turkana region of northwestern Kenya and immediately adjacetn Sudan, from about 820-2745 m elevation
Pipidae	***X. gilli***	Kobel et al. 1981	Rose & Hewitt 1927		4n=36	3.11	Kobel, du Pasquier, and Tinsley (1981) demonstrated natural hybridization and gene introgression between this species and Xenopus laevis		tropical	endangered	decreasing	Africa:Restricted to the Cape Flats and Cape Peninsula together with isolated inland localities on the southwestern Western Cape, Rep. South Africa, 10-140 m

							(both with 2n = 36 chromosomes)					
Pipidae	***X. laevis***	Tymowsk & Kobel 1972	Steindach ner 1882		4n=36	3.0-3.84	Bisbee et al (1977) suggested that the chromosome number 2n=36, basic to several species, may reflect ancient tetraploidy and that total genome duplication occurred in an ancestor of the Xenopus laevis group		dry, tropical, temperat e	least concern	increasing	Africa:Extreme southern Angola south to Cape Region of Rep. South Africa thence east and north in savanna habitats in north-east-central Central African Republic and Southern Sudan nd then west to Nigeria; introduced in southern California, Arizona, Mexico, Chile, France, Mexico, Italy and Java, Indonesia, as well as Ascension Island; range extends over 40° latitude, occupying cooler upland regions between the rainforests of the west and the hotter, drier savannas of the east and north
Pipidae	***X. largeni***	Tinsley 1995	Tinsley 1995		4n=36				tropical	data deficient	unknown	Africa:Known only from region of type locality (between Dodola and Aselle) and at 2500 in the Arussi Mountains, Ethiopia
Pipidae	***X. longipes***	Loumon & Kobel 1991	Loumont & Kobel 1991		12n=10 8				tropical	critically endangere d	stable	Africa:Lake Oku in the volcanic highlands of western Cameroon, 2200 m elevation
Pipidae	***X. muelleri***	Tymowsk & Kobel 1972	Boulenge r 1882		4n=36	3.52-4.08	described as X. m. petersii in the original publication		dry	least concern	stable	Africa:Relatively arid savanna from Burkina Faso eastward across Sudan-Guinea zone to northeastern Dem. Rep. Congo, then south along East African coastal belt from extreme southern Kenya through Tanzania into northwestern border areas of Rep. South Africa, Botswana, and Caprivi region of Namibia; isolated record in the Ennedi (northeastern Chad)
Pipidae	***X. pygmaeus***	Loumont 1986	Loumont 1986		4n=36		A diploid species in the Xenopus fraseri group according to original publication		tropical	least concern	stable	Africa:Congo river basin in southern Cen. African Rep. and northern Dem. Rep. congo, presumably into adjacent northeast Rep. Congo
Pipidae	***X. ruwenzoriensis***	Tymowsk a & Fischberg 1973	Tymowsk a & Fischberg 1973		12n=10 8	7.84,7.95	Distinguished by chromosome number (2n=108), hexaploid with respect to Xenopus laevis		tropical	data deficient	unknown	Africa:Foothills of Ruwenzori Mountains, on border of Uganda and Dem. Rep. Congo
Pipidae	***X. vestitus***	Tymowsk a et al. 1977	Laurent 1972		8n=72	5.91,6.28	Chromosome number (2n=72) tetraploid with respect to Xenopus laevis		tropical	least concern	stable	Africa:Highland swamps and lakes bordering Western Rift in southwestern Uganda, Rwanda and eastern Dem. Rep. Congo and rivers draining Virunga volcanoes

Pipidae	***X. wittei***	Tinsley et al. 1979	Tinsley et al. 1979		8n=72		Closely related to Xenopus vestitus with similar karyotypes (2n=72); suggested to share an allopolyploid origin with one ancestor in common		tropical	least concern	stable	Africa:Highland swamps and lakes bordering Western Rift in southwestern Uganda, Rwanda nad Kabasha Escarpment, eastern Dem. Rep. Congo
Pipidae	***X. itonbwensis***	Evans et al. 2008	Evans et al. 2008		4n=36?				tropical	not listed		Africa: Known only from the type locality (Miki Town, South Kivu Province, Dem. Rep. Congo, elevation 2000 m
Pipidae	***X. (l.) petersii***	Channing 2001	Channing 2001		4n=36		Removed from the synonymy of Xenopus laevis by Channing (2001)		tropical	least concern	stable	Africa:Northern Namibia and northern Botswana through Angola and Zambia north to southern Dem. Rep. Congo, Rep. Congo and southern Gabon; possibly to Malawi and western Zimbabwe and Tanzania
Pipidae	***X. (l.) victorianus***	Channing & Howell 2005	Ahl 1924		4n=36		See comments under Xenopus laevis (Channing & Howell 2006)		tropical	least concern	increasing	Africa:Aquatic habitats in arid savanna to forest in northern Tanzania, Burundi, Rwanda, eastern Dem. Rep. Congo, Uganda and adjacent Sudan to Kenya
Other species listed in Otto & Whitton 2001												
Bufonidae	*Bufo boreas-punctatus hybrds*	Feder 1979							temperate and cold			North America: Very confused taxonomy--Western North America from Southeastern Alaska through Western Canada and western USA to northern New Mexico, outhern Colorado, southern Utah, southern Nevade, and norhtenr Baja California,; populations in south-central Wyoming and northenr New Mexico now extinct, as are those for most of central Utah
Leptodactylidae	*Eupsophus vertebralis*	Formas 1993							temperate	near threatened	decreasing	South America: Forests in the region of Santiago de Chile to Chiloe, Chile; reported from Puert Blest, Rio Negro Province, Argentina, 50-1000 m elevation

Leiopelmatidae	*Leiopelma hochstetteri*	Green et al. 1984				8.63			temperate	vulnerable	decreasing	Oceania: North and South Auckland, including Rangitoto Range, Coromandel Peninsula and Great Barrier Island; Gisborne (Huaiarau and Raukamara Range), New Zealand

* these species were reversed in the original publication, in Otto & Whitton 2001, and in genome size estimates but are correctly described in later publications by J.D. Roberts

Summary of known polyploid anurans (bold face type), along with their closest known diploid relatives, indicating original taxonomy (genus and family) at the time that the polyploids were described, as well as revised taxonomy. References are provided for the first report of polyploidy for each species, as well as those recommending changes to the original taxonomy. Chromosome numbers were taken from the original ploidy descriptions; genome sizes were obtained from the Animal Genome Size database (http://www.genomesize.com/) but are not available for most of the species listed. Notes on origins of the polyploids were taken from the primary literature. Coordinates for the centre of distributions of polyploid taxa were taken from the maps available through Amphibiaweb (http://amphibiaweb.org/); Krüppen classifications were used to characterize the climates in the relevant regions. Endangered species status and population trends were obtained from the IUCN database (International Union for Conservation of Nature Redlist of Endangered species, http://www.iucnredlist.org). Descriptions of species distributions were obtained from the Amphibian Species of the World database (Frost et al. 2010; http://research.amnh.org/vz/herpetology/amphibia/).

References for SI Table 1A:

Ahl, E. (1924). Über eine Froschsammlung aus Nordost-Afrika und Arabien. Mitteilungen aus dem Zoologischen Museum in Berlin 11, 1-12.

Amiet, J.-L. (1978). Les Astylosternus du Cameroun (Amphibia, Anura, Astylosterninae). Annales de la Faculte des Sciences du Cameroun. Yaoundé 23-24, 99-227.

Barrio, A. (1977). Contributions to elucidate the taxonomic status of Pleurodema bibroni and Pleurodema kriegi Amphibia Anura Leptodactylidae. Physis Seccion C los Continentes y los Organismos Terrestres 37, 311-331.

Barrio, A. (1980). Una nueva especie de Ceratophrys (Anura, Ceratophryidae) del Dominio Chaqueno. Physis, Secc. C 39, 21-30.

Barrio, A. & de Chieri, P.R. (1970). Estudios citogeneticos sobre elgenero Pleurodema y sus consecuencias evolutivas (Amphibia, Anura, Leptodactylidae). Physis, Buenos Aires 30, 309-319.

Becak, M.L. & Becak, W. (1974). Diploidization in Eleutherodactylus (Leptodactylidae-Amphibia). Experientia 30, 624-625.

Becak, M.L., Becak, W. & Rabello, M.N. (1967). Further Studies on Polyploid Amphibians (Ceratophrydidae) .I. Mitotic and Meiotic Aspects. Chromosoma 22, 192-&.

Beçak, M.L., Beçak, W. & Rabello, M.N. (1966). Cytological evidence for constant tetraploidy in the bisexual South American frog Odontophrynus americanus. Chromosoma 19, 188-193.

Beçak, M.L., Danaro, L. & Beçak, W. (1970). Polyploidy and mechanisms of karyotypic diversification in Amphibia. Cytogenetics 9, 225-238.

Bedriaga, J.V. (1898). Amphibien und Reptilien, Wissenschaftliche Resultate der von N. M. Przewalski nach Central-Asien unternommenen Reisen, & c./Nauchnuie Rezul'tatui puteshestvii N. M. Przheval'skagho po tzentral'noi Azii, & c. Volume 3, Zoologischer Theil, Part 1. Akadamie der Wissenschaften, St. Petersburg.

Bisbee, C.A., Baker, M.A., Wilson, A.C., Hadjiazimi, I. & Fischberg, M. (1977). Albumin phylogeny for clawed frogs (Xenopus). Science 195, 785-787.

Bogart, J.P. (1967). Chromosomes of the South American amphibian family Ceratophidae with a reconsideration of the taxonomic status of Odontophrynus americanus. Canadian Journal of Cytogenetics 9, 531-542Canadian Journal of Genetics and Cytology.

Bogart, J.P. (1980). Evolutionary implications of polyploidy in amphibians and reptiles. In Polyploidy: Biological Relevance: 341-378. (Ed). New York and London.: Plenum Press.

Bogart, J.P. (1981). Chromosome studies in Sminthillus from Cuba and Eleutherodactylu from Cuba and Puerto Rico (Anura: Leptodactylidae). Royal Ontario Museum Publications in Life Science 130, 1-22.

Bogart, J.P. & Tandy, M. (1976). Polyploid amphibians: three more diploid-tetraploid cryptic species of frogs. Science 193, 334-335.

Bokerman, W.C.A. (1952). Microhylidae de coleçao do Departamento de Zoologia (Amphibia-Anura). Papeis Avulsos de Zoologia. São Paulo 10, 271-292.

Bokerman, W.C.A. (1966). Lista anotada das localidades tipo de anfíbios Brasileiros. .

Boulenger, G.A. (1882). Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum. Second Edition. London: Taylor and Francis.

Boulenger, G.A. (1890). Second report on additions to the batrachian collection in the Natural-History Museum. Proceedings of the Zoological Society of London 1890, 323-328.

Boulenger, G.A. (1900). A list of the batrachians and reptiles of the Gaboon (French Congo), with descriptions of new genera and species. Proceedings of the Zoological Society of London 1900, 33-456.

Boulenger, G.A. (1905). On a collection of batrachians and reptiles made in South Africa by Mr. C. H. B. Grant, and presented to the British Museum by Mr. C. D. Rudd. Proceedings of the Zoological Society of London 1905, 248-255.

Busse, K. & Böhome, W. (1992). Two remarkable frog discoveries of the genera Mantella (Ranidae: Mantellinae) and Scaphiophryne (Microhylidae: Scaphiophryninae) from the west coast of Madagascar. Revue Française d'Aquariologie, Herpétologie 19, 57-64.

Cannatella, D.C. & Trueb, L. (1988). Evolution of pipoid frogs - intergeneric relationships of the aquatic frog family Pipidae (Anura). Zoological Journal of the Linnean Society 94, 1-38.

Channing, A. (2001). Amphibians of central and southern Africa. Ithaca: Cornell University Press.

Channing, A. & Bogart, J.P. (1996). Description of a tetraploid Tomopterna (Anura: Ranidae) from South Africa. South African Journal of Zoology 31, 80-85.

Channing, A. & Howell, K.M. (2005). Amphibians of East Africa. Frankfurt: Edition Chimaira.

Cope, E.D. (1880). On the zoological position of Texas. Bulletin of the United States National Museum 17, 1-51.

Deckert, K. (1938). Beiträge zur Osteologie und Systematik ranider Froschlurche. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 1938, 127-184.

di Tada, I.E., M. M. Salusso, and R. A. Martori (1976). Lista de los batracios de Córdoba. Boletin de la Academia Nacional de Ciencia. Córdoba 51, 325-362.

Dubois, A. (1987). Miscellanea taxinomica batrachologica (I). Alytes (Paris) 5, 7-95.

Dubois, A. (1992). Notes on the classificaiton of Ranidae (Amphibia, Anura). Bulletin Mensuel de la Societe Linneenne de Lyon 61, 305-352.

Duellman, W.E. & Trueb, L. (1986). Biology of Amphibians. Baltimore: John Hopkins Press.

Evans, B.J., Carter, T.F., Tobias, M.L., Kelley, D.B., Hanner, R. & Tinsley, R.C. (2008). A new species of clawed frog (genus Xenopus) from the Itombwe Massif, Democratic Republic of the Congo: implications for DNA barcodes and biodiversity conservation. Zootaxa 55-68.

Evans, B.J., Kelley, D.B., Tinsley, R.C., Melnick, D.J. & Cannatella, D.C. (2004). A mitochondrial DNA phylogeny of African clawed frogs: phylogeography and implications for polyploid evolution. Molecular Phylogenetics and Evolution 33, 197-213.

Feder, J.H. (1979). Natural hybridization and genetic divergence between the toads Bufo boreas and Bufo punctatus. Evolution 33, 1089-1097.

Fischberg, M., Colombelli, B. & Picard, J.-J. (1982). Diagnose préliminaire d'une espèce nouvelle de Xenopus du Zaïre. Alytes 1, 53-55.

Fischberg, M. & Kobel, H.R. (1978). 2 New polyploid Xenopus species from Western Uganda. Experientia 34, 1012-1014.

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., De Sa, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. & Wheeler, W.C. (2006). The amphibian tree of life. Bulletin of the American Museum of Natural History 8-370.

Grant, T., Frost, D.R., Caldwell, J.P., Gagliardo, R., Haddad, C.F.B., Kok, P.J.R., Means, D.B., Noonan, B.P., Schargel, W.E. & Wheeler, W.C. (2006). Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). Bulletin of the American Museum of Natural History 299, 6-262.

Gray, J.E. (1864). Notice of a new genus (Silurana) of frogs from West Africa. Annals and Magazine of Natural History, Series 3 14, 314-316.

Green, D.M., Kezer, J. & Nussbaum, R.A. (1984). Triploidy in Hochstetters frog, Leiopelma hochstteri, from New Zealand. New Zealand Journal of Zoology 11, 457-460.

Günther, A.C.L.G. (1849). Catalogue of the Batrachia Salientia in the collection of the British Museum. London: Taylor and Francis.

Haddad, C.F.B., Pombal, J.P.J. & Batistic, R.F. (1994). Natural hybridization between diploid and tetraploid species of leaf-frogs, genus Phyllomedusa (Amphibia). Journal of Herpetology 28, 425-430.

Hedges, S.B., Duellman, W.E. & Heinicke, M.P. (2008). New World direct-developing frogs (Anura: Terrarana): molecular phylogeny, classification, biogeography, and conservation. Zootaxa 1737, 1-182.

Holloway, A.K., Cannatella, D.C., Gerhardt, H.C. & Hillis, D.M. (2006). Polyploids with different origins and ancestors form a single sexual polyploid species. American Naturalist 167, E88-E101.

Johnson, C. (1966). Species recognition in the Hyla versicolor complex. Texas Journal of Science 18, 361-364.

Kasahara, S. & Haddad, C.F.B. (1997). Karyotypes of two Brazilian microhylid frogs of the genus Chiasmocleis, including a new case of polyploidy. Journal of Herpetology 31, 139-142.

Keith, R. (1968). A new species of Bufo from Africa, with comments on the toads of the Bufo regularis complex. American Museum Novitates 2345, 1-22.

Kobel, H.R. & Du Pasquier, L. (1986). Genetics of polyploid Xenopus. Trends in Genetics 2, 310-315.

Kobel, H.R., du Pasquier, L., Fischberg, M. & Gloor, H. (1980). Xenopus amieti sp. nov. (Anura: Pipidae) from the Cameroons, another case of tetraploidy. Revue Suisse de Zoologie 87, 919-926.

Kobel, H.R., Dupasquier, L. & Tinsley, R.C. (1981). Natural hybridization and gene introgression between Xenopus gilli and Xenopus laevis laevis (Anura, Pipidae). Journal of Zoology 194, 317-322.

Kuramoto, M. & Allison, A. (1989). Karyotypes of microhylid frogs of Papua New Guinea and their systematic implications. Herpetologica 45, 250-259.

Lamb, J. (1911.). Description of three new batrachians from southern Queensland. Annals of the Queensland Museum 10, 26-28.

Laurent, R.F. (1972). Amphibiens. Exploration du Parc National des Virunga. Deuxième Série. Bruxelles 22, 1-125.

Laurenti, J.N. (1768). Specimen Medicum, Exhibens Synopsin Reptilium Emendatum cum Experimentis Circa Venena et Antidota Reptilium Austriacorum. Wien, Austria: Joan. Thom. nob. de Trattnern.

Le Conte, J.E. (1825). Remarks on the American species of the genera Hyla and Rana. Annals of the Lyceum of Natural History of New-York 1, 278-282.

Littlejohn, M.J. & Main, A.R. (1960). Male brooding call as an aid to identifying southwestern Australian frogs. Western Australian Naturalist 7, 128-131.

Loumont, C. (1983). 2 New species of Xenopus from Cameroon (Amphibia, Pipidae). Revue Suisse de Zoologie 90, 169-177.

Loumont, C. (1986). Xenopus pygmaeus, a new diploid Pipid frog from rain-forest of Equatorial Africa. Revue Suisse de Zoologie 93, 755-764.

Loumont, C. & Kobel, H.R. (1991). Xenopus longipes sp-Nov, a new polyploid pipid from Western Cameroon. Revue Suisse de Zoologie 98, 731-738.

Mable, B.K. & Roberts, J.D. (1997). Mitochondrial DNA evolution of tetraploids in the genus Neobatrachus (Anura: Myobatrachidae). Copeia 1997, 680-689.

Mahony, M. & Roberts, J.D. (1986). Two new species of desert burrowing frogs of the genus Neobatrachus (Anura: Myobatrachidae) from Western Australia. Records Western Australian Museum 13, 155-170.

Mahony, M.J. & Robinson, E.S. (1980). Polyploidy in the Australian Leptodactylid frog genus Neobatrachus. Chromosoma 81, 199-212.

Main, A.R. (1957). A new burrowing frog from Western Australia. Western Australian Naturalist 6, 23-24.

Martino, A.L. & Sinsch, U. (2002). Speciation by polyploidy in Odontophrynus americanus. Journal of Zoology London 257, 67-81.

Mercadal de Barrio, I.T. (1986). Ceratophrys joazeirensis sp. n. (Ceratophryidae, Anura) del noreste de Brasil. Amphibia-Reptilia 7, 313-334.

Mertens, R. (1971). Die Amphibien und Reptilien West-Pakistans. Senckenbergiana Biologica 52, 7-15.

Miranda-Ribeiro, A.D. (1920). Algumas consideracoes sobre o genero Ceratophrys e suas especies. Revista do Museu Paulista. São Paulo 12, 291-304.

Parker, H.W. (1934). A Monograph of the Frogs of the Family Microhylidae. London: Trustees of the British Museum.

Parker, H.W. (1936). Reptiles and amphibians collected by the Lake Rudoph Rift Valley Expedition, 1934. Annals and Magazine of Natural History, Series 10 18, 594-609.

Parker, H.W. (1940). Australasian frogs of the family Leptodactylidae. Novitates Zoologicae 42, 1-106.

Peracca, M.G. (1898). Descrizione di una nuova specie di amfibio del gen. Xenopus Wagl. dell'Eritrea. Bollettino dei Musei di Zoologia e Anatomia Comparata della R. Universita di Torino 13, 1-4.

Peters, W.C.H. (1863). Eine Übersicht der von Hrn. Richard Schomburgk an das zoologische Museum eingesandten Amphibien, aus Buchsfelde bei Adelaide in Südaustralien. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin 1863, 228-236.

Pisanets, E.M. (1978). [On a new polyploid species of Bufo danatensis Pisanets sp. n. from Turkmenistan] [In Ukranian with Russian and English summaries]. Dopovidi Akademiï nauk Ukraïnskoï RSR. Seriia B, Heolohichni, khimichni ta biolohichni nauky 3, 272-282.

Pombal, J.P., Jr. & Haddad, C.F.B. (1992). Species of the Phyllomedusa burmeisteri group from easter Brazil, with description of a new species (Amphibia, Hylidae). Revista Brasileira de Biologia 52, 217-229.

Reinhardt, J.T. & Lütken, C.F. (1862). Bidrag til Kundskab om Brasiliens Padder og Krybdyr. Förste Afdeling: Padderne og Öglerne. . Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn, Serie 2 3, 143-242.

Reuss, A. (1833). Zoologischen Miscellen. Reptilian. Saurier. Batrachier. Museum Senckenbergianum 1, 27-62.

Roberts, J.D., Mahony, M., Kendrick, P. & Majors, C.M. (1991). A new species of burrowing frog, Neobatrachus (Anura: Myobatrachidae), from the eastern wheatbelt of Western Australia. Records of the Western Australian Museum 15, 23-32.

Roberts, J.D. & Majors, C.M. (1993). Range extensions, range definitions and call structures for frogs from Western Australia. Records of the Western Australian Museum 16, 315-322.

Rose, W. & Hewitt, J. (1927). Description of a new species of Xenopus from the Cape Peninsula. Transactions of the Royal Society of South Africa 14, 343-346.

Saez, F.A. & Brum-Zorrilla, N. (1966). Karyotype variation in some species of the genus Odontophrynus (Amphibia-Anura). Caryologia 19, 7-24.

Steindachner, F. (1876). Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859 unter den Bafehlen des Commodore B. von Wüllerstorf-Urbair. Zologischer Theil. 1. Amphibien. Wien: K. K. Hof- und Staatsdruckerei.

Steindachner, F. (1882). Batrachologische Beiträge. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Mathematisch-Naturwissenschaftliche Classe 85, 188-194.

Stejneger, L. (1904). The herpetology of Porto Rico. 549-734.

Stöck, M., Frynta, D., Grosse, W.-R., Steinlein, C. & schmid, M. (2001a). A review of the distribution of diploid, triploid and tetraploid green toads (Bufo viridis complex) in Asia including new data from Iran and Pakistan. Asiatic Herpetological Research 9, 77-100.

Stöck, M., Günther, R. & Böhme, W. (2001b). Progress towards a taxonomic revision of the Asian Bufo viridis group: Current status of nominal taxa and unsolved problems (Amphibia: Anura: Bufonidae). Zoologische Abhandlungen. Staatliches Museum für Tierkunde in Dresden 51, 253-319.

Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebayeva, T., Eremchenko, V., Macey, J.R., Papenfuss, T.J. & Wake, D.B. (2006). Evolution of mitochondrial relationships and biogeography of Palearctic green toads (Bufo viridis subgroup) with insights in their genomic plasticity. Molecular Phylogenetics and Evolution 41, 663-689.

Stöck, M., Steinlein, C., Lamatsch, D.K., Schartl, M. & Schmid, M. (2005). Multiple origins of tetraploid taxa in the Eurasian Bufo viridis subgroup. Genetica 124, 255-272.

Tandy, M., Bogart, J.P., Largen, M.J. & Feener, D.J. (1982). A tetraploid species of Bufo (Anura-Bufonidae) from Ethiopia. Monitore Zoologico Italiano-Italian Journal of Zoology 17, 1-79.

Tinsley, R.C. (1995). A new species of Xenopus (Anura: Pipidae) from the highlands of Ethiopia. Amphibia-Reptilia 16, 375-388.

Tinsley, R.C. & Kobel, H.R. (1996). The Biology of Xenopus. 440.

Tinsley, R.C., Kobel, H.R. & Fischberg, M. (1979). The biology and systematics of a new species of Xenopus (Anura, Pipidae) from the highlands of central Africa. Journal of Zoology 188, 69-102.

Tschudi, J.J.V. (1838). Classification der Batrachier mit Berücksichtigung der fossilen Thiere dieser Abtheilung der Reptilien. Neuchâtel: Petitpierre.

Tyler, M.J., M. Davies and A.A. Martin. (1981). New and rediscovered species of frogs from the Derby-Broome area of Western Australia. Records of the Western Australian Museum 9, 147-172.

Tymowska, J. (1991). Polyploidy and cytogenetic variation in frogs of the genus Xenopus. In Amphibian Cytogenetics and Evolution: 259-297. Green, D.M. & L.K. Sessions (Ed). New York: Academic Press.

Tymowska, J. & Fischberg, M. (1973). Chromosome complements of genus Xenopus. Chromosoma 44, 335-342.

Tymowska, J., Fischberg, M. & Tinsley, R.C. (1977). Karyotype of tetraploid species Xenopus vestitus Laurent (Anura Pipidae). Cytogenetics and Cell Genetics 19, 344-354.

Tymowska, J. & Kobel, H.R. (1972). Karyotype analysis of Xenopus muelleri (Peters) and Xenopus laevis (Daudin), Pipidae. Cytogenetics 11, 270-278.

Valetti, J.A., Salas, N.E. & Martino, A.L. (2009). A new polyploid species of Pleurodema (Anura: Leiuperidae) from Sierra de Comechingones, Cordoba, Argentina and rediscription of Pleurodema kriegi (Muller, 1926). Zootaxa 2073, 1-21.

Vences, M., Aprea, G., Capriglione, T., Andreone, F. & Odierna, G. (2002). Ancient tetraploidy and slow molecular evolution in Scaphiophryne: ecological correlates of speciation mode in Malagasy relict amphibians. Chromosome Research 10, 127-136.

Vieira, K.D., Silva, A.P.Z. & Arzabe, C. (2006). Cranial morphology and karyotypic analysis of Ceratophrys joazeirensis (Anura : Ceratophryidae, Ceratophrynae): taxonomic considerations. Zootaxa 57-68.

Wasserman, A.O. (1970). Polyploidy in the common tree toad Hyla versicolor LeConte. Science 167, 385-386.

Werner, F. (1898). Ueber Reptilien und Batrachier aus Togoland, Kamerun und Tunis aus dem Kgl. Museum für Naturkunde in Berlin. Verhandlungen des Zoologisch-Botanischen Vereins in Wien 48, 191-213.

Wied-Neuwied, M.A.P. & zu, P. (1824). Verzeichnis der Amphibien welche in zweiten Bande der Naturgeschichte Brasiliens von Prinz Max von Neuwied werden beschreiben Werden. Isis von Oken 14, 661-673.

Zweifel, R.G. (1956). Results of the Archbold Expeditions. No. 72. Microhylid frogs from New Guinea, with descriptions of new species. American Museum Novitates 1766, 1-49.

Zweifel, R.G. (1962). Results of the Archbold Expeditions. No. 83. Frogs of the microhylid genus Cophixalus from the mountains of New Guinea. American Museum Novitates 2087, 1-26.

Zweifel, R.G. & Parker, F. (1989). New species of microhylid frogs from the Owen Stanley Mountains of Papua New Guinea and resurrection of the genus Aphantophryne. American Museum Novitates 2954, 1-20.

SI Table A2- Table of Fish Polyploids

IDENTIFICATION	Haploid C-value	Chrom num	Ploidy	Distribution	Environment	Climate	References
SARCOPTERYGII							
Lepidosireniformes, Protopteridae							
Protopterus annectens annectens	40.46	34.00	2xAncestor	Africa: Senegal, Niger, Gambia, Volta and Chad basins, also in temporary tributaries of Chari River in Western Sudan. Bandama and Comoé basins in Côte d'Ivoire and certain basins of Sierra Leone and Guinea	Demersal; potamodromous; freshwater	Tropical	Vervoort (1980)
Protopterus dolloi	81.60	68.00	4x	Africa: Ogowe, Kouilou-Niari, lower and Middle Congo River basins	Demersal; freshwater	Tropical	Vervoort (1980)
ATCTINOPTERYGII							
Amiiformes, Amiidae							
Amia calva	1.15	46.00	2xAncestor	North America: St. Lawrence River, Lake Champlain drainage of Quebec and Vermont west across southern Ontario to the Mississippi drainage in Minnesota	Demersal; freshwater	Temperate	Mirsky & Ris (1951)
Acipenseriformes, Polyodontidae							
Psephurus gladius		120	4x re-diploidized	Asia: China, endemic to the Yangtze River and its tributaries	Demersal; potamodromous; freshwater	Temperate	Zhang *et al.* (1999)
Polyodon spathula	1.95	120.00	4x re-diploidized	North America: Mississippi River system, including the Missouri River into Montana, the Ohio River, and their major tributaries	Demersal; potamodromous; freshwater	Temperate	Tiersch *et al.* (1989a)
Acipenseriformes, Acipenseridae							

Huso huso	1.80	118	4x re-diploidized	Basins of the Black and Caspian seas; rare in the Adriatic Sea;	Demersal; anadromous; freshwater; brackish; marine	Temperate	Fontana (1976)
Huso dauricus	1.89	120.00	4x re-diploidized	Asia: Amur basin, ascending far up to the Argun, Shilka, and Onon. In the Amur Liman to the Amur estuary in the Sea of Okhotsk. Adults inhabit some lakes, like Orel Lake above Nikolaevsk. Also in the Ussuri and Sungari, China	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1993)
Scaphirhynchus platorynchus	1.75	112.00	4x re-diploidized	North America: USA in Mississippi River basin from west Pennsylvania to Montana and south to Louisiana; Mobile Bay drainage, Alabama and Mississippi; upper Rio Grande, New Mexico.	Demersal; potamodromous; freshwater	Temperate	Ohno *et al.* (1969a)
Acipenser sturio	3.95	116	4x re-diploidized	Eastern Atlantic: Only in the Gironde-Garonne-Dordogne basin in France and in the Rioni basin in Georgia	Demersal; anadromous; freshwater; brackish; marine	Temperate	Vialli (1957a); Mirsky & Ris (1951)
Acipenser oxyrinchus oxyrinchus	2.19	112	4x re-diploidized	Western Atlantic: Hamilton River, Labrador, Newfoundland, Canada to northeastern Florida, USA. Occurs occasionally in Bermuda and French Guiana	Demersal; anadromous; freshwater; brackish; marine	Temperate	Hardie & Hebert (2003)
Acipenser nudiventris	1.97	118	4x re-diploidized	Europe and Former USSR: Caspian and Aral seas, very rarely in the Black Sea and Sea of Azov	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1993)
Acipenser ruthenus	1.87	118	4x re-diploidized	Eurasia: Basins of the Black and Caspian seas; Arctic drainages to White and Kara seas and the Sea of Azov	Demersal; potamodromous; freshwater; brackish	Temperate	Birstein *et al.* (1993)

Acipenser stellatus	2.35	118	4x re-diploidized	Europe, Former USSR and Asia: Basins of the Black, Azov, and Caspian seas	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1993)
Acipenser baerii baerii	4.15	248.00	4x-8x*	Former USSR and Asia: Siberia, rivers Ob, Irtysh, Yenisei, Lena, Kolyma. Some non-migratory populations exist in the Irtysh River system. Indigirka river	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1993)
Acipenser brevirostrum	6.54	360.00	8x-16x*	North America: St. John River in Canada to St. Johns River in Florida, USA	Demersal; anadromous; freshwater; brackish; marine	Temperate	Blacklidge & Bidwell (1993)
Acipenser brevirostrum (3x)	9.32	500*	8x-16x*	North America: St. John River in Canada to St. Johns River in Florida, USA	Demersal; anadromous; freshwater; brackish; marine	Temperate	Hardie & Hebert (2003)
Acipenser fulvescens	4.45	250*	4x-8x*	North America: St. Lawrence-Great Lakes, Hudson Bay, and Mississippi River basins.	Demersal; potamodromous; freshwater; brackish	Temperate	Blacklidge & Bidwell (1993)
Acipenser gueldenstaedtii	3.94	250.00	4x-8x*	Eurasia: Black Sea, Sea of Azov and Caspian Sea, entering all main rivers that empty into them (Don, Kuban, Danube, Dnieper (rare), Dniester).	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1993)
Acipenser medirostris	4.41	250*	4x-8x*	North America: Aleutian Islands and the Gulf of Alaska to Ensenada, Mexico	Demersal; anadromous; freshwater; brackish; marine	Temperate	Blacklidge & Bidwell (1993)

Acipenser mikadoi	7.1	500*	8x-16x*	Northwest Pacific: Bering Sea, Tumnin (Datta) river, to northern Japan and Korea.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1997)
Acipenser naccarii	2.85	239.00	4x-8x*	Europe: Adriatic Sea in Italy, Albania, Croatia, Bosnia-Herzegovina and Montenegro	Demersal; anadromous; freshwater; brackish; marine	Temperate	Fontana (1976)
Acipenser persicus		>200	4x-8x*	Caspian Sea and the rivers entering it. Also distributed along the eastern Black Sea	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1997)
Acipenser schrenckii		240	4x-8x*	Asia: Endemic to the Amur River system.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1997)
Acipenser sinensis		264	4x-8x*	Northwest Pacific: China and Japan (Sagami Sea), Korea, Pearl River and Chinese sea	Demersal; anadromous; freshwater; brackish; marine	Temperate	Birstein *et al.* (1997)
Acipenser transmontanus	4.775	230	4x-8x*	Eastern Pacific: Alaska Bay to Monterey, California, USA. Landlocked in Columbia River drainage, Montana, and perhaps Lake Shasta in California, USA. Translocated to lower Colorado River, Arizona in USA. Reported from northern Baja California, Mexico	Demersal; anadromous; freshwater; brackish; marine	Temperate	Blacklidge & Bidwell (1993)

Cypriniformes, Cyprinidae

Gobio gobio	1.4	50.00	2xAncestor	Europe: Atlantic Ocean, North and Baltic Sea basins, from Loire drainage eastward, eastern Great Britain, Rhône and Volga drainages, upper Danube and middle and upper Dniestr and Dniepr drainages; in Finland, north to about 61°N. Introduced to eastern and northern Italy, Ireland, Wales and Scotland. Eastern and southern limits unclear. Occurs as far east as Korea. Populations from the Iberian Peninsula and Adour basin in southern France refer to G. lozanoi. Populations from the Caspian basin may represent a distinct species	Benthopelagic; potamodromous; freshwater; brackish	Temperate	Hafez *et al.* (1978)
Ptychocheilus lucius	1.26	50.00	2xAncestor	North America: Colorado River drainage in Wyoming, Colorado, Utah, New Mexico, Arizona, Nevada and California, USA and Mexico. Now mostly restricted to Utah and Colorado and extirpated from the southern portion of the range by the construction of large dams on the Colorado and Gila Rivers.	Demersal; potamodromous; freshwater	Temperate	Gold (1994)
Aulopyge huegelii		100	4x	Europe: Croatia (Cetina, Krka and Zrmanja drainages) and Bosnia-Herzegovina (Karstic streams in Glamocko, Livanjsko and Duvanjsko poljes, Lakes Busko and Blidinje)	Benthopelagic; freshwater	Temperate	Hinegardner (1968)
Barbus andrewi		100.00	4x	Africa: Berg and Breë systems, south-western Cape Province, South Africa.	Benthopelagic; freshwater	Subtropical	Skelton & Naran (1995)
Barbus barbus	1.83	100-150	4x-6x	Europe: excluding the Italian, Greek and Iberian peninsulas	Benthopelagic; potamodromous; freshwater	Temperate	Mauro & Micheli (1979)
Barbus bynni bynni		150	6x	Africa: confined to the Nile system; also found in lakes that were once connected to the Nile.	Benthopelagic; freshwater	Subtropical	Golubtsov & Krysanov (1993)

Barbus bynni occidentalis	148	6x	Africa: Chad, Niger, Senegal, Volta, Ouémé and Ogun basins.	Benthopelagic; freshwater	Tropical	Guegan & Morand (1996)
Barbus bynni waldroni	150	6x	Africa: Côte d'Ivoire and Ghana.	Benthopelagic; potamodromous; freshwater	Tropical	Guegan & Morand (1996)
Barbus calidus	100.00	4x	Africa: tributaries of the Clanwilliam Olifants, western Cape, South Africa.	Benthopelagic; freshwater	Subtropical	REFERENCES WITHIN: Tsigenopoulos *et al.* (2002)
Barbus callensis	100.00	4x	Africa: Algeria, Morocco, Tunisia.	Benthopelagic; freshwater	Subtropical	Berrebi & Rab (1998)
Barbus canis	150.00	6x	Eurasia: common in the streams and lakes of the Jordan River basin	Benthopelagic; freshwater	Subtropical	Gorshkova *et al.* (2002)
Barbus cyclolepis	100.00	4x	Europe: Aegean Sea basin, from Vistonis drainage eastward (Greece, Bulgaria, Turkey). Black Sea basin, few rivers in Thracian Turkey, northwards to Istrance and Former USSR	Benthopelagic; freshwater	Temperate	Guegan & Morand (1996)
Barbus erubescens	100.00	4x	Africa: Twee River and its two source tributaries, the Middledeur and Suurvlei Rivers, South Africa. Also recorded from the Olifants River, southwestern Cape, South Africa	Benthopelagic; freshwater	Subtropical	REFERENCES WITHIN: Tsigenopoulos *et al.* (2002)
Barbus ethiopicus	150	6x	Africa: endemic to Ethiopia.	Benthopelagic; freshwater	Tropical	Golubtsov & Krysanov (1993)
Barbus fritschii	148-150	6x	Africa: Morocco, Trigadir-el-hor, Shishawa River and Wed Ksib.	Benthopelagic; freshwater	Temperate	Guegan & Morand (1996)
Barbus haasi	100.00	4x	Europe: Spain, from Llobregat to Riudecanyes drainages	Benthopelagic; freshwater	Temperate	Guegan & Morand (1996)
Barbus harterti	148-150	6x	Africa: Morocco, Oum Erbiah, Talmist River.	Benthopelagic; freshwater	Subtropical	Berrebi & Rab (1998)
Barbus meridionalis	100.00	4x	Europe: borders of France and Spain in the West to Romania, Ukraine and Poland in the East.	Benthopelagic; freshwater	Temperate	Cataudella *et al.* (1977b)

Barbus nasus		100.00	4x	Africa: Morocco.	Benthopelagic; freshwater	Subtropical	Guegan & Morand (1996)
Barbus parawaldroni		150	6x	Africa: rivers situated on the western part of Côte d'Ivoire and eastern part of Liberia: Cavally, Nipoué/Cess, Dodo, Tabou, Saint Paul, and Loffa.	Benthopelagic; freshwater	Tropical	Guegan & Morand (1996)
Barbus peloponnesius		100.00	4x	Europe: endemic to the western Greece from Kalamas to Pamissos drainages.	Benthopelagic; freshwater	Temperate	Fišter *et al.* (1999)
Barbus petitjeani		150	6x	Africa: Bafing River in the upper Senegal basin and from the upper Niger in Guinea.	Benthopelagic; freshwater	Tropical	Guegan *et al.* (1995)
Barbus plebejus		100.00	4x	Europe: Croatia, Italy, Slovenia and Switzerland. Asia: Turkey	Benthopelagic; potamodromous; freshwater	Temperate	Cataudella *et al.* (1977b)
Barbus reini		148-150	6x	Africa: Morocco.	Benthopelagic; freshwater	Subtropical	REFERENCES WITHIN: Tsigenopoulos *et al.* (2002)
Barbus sacratus		150	6x	Africa: Diogou, Diani and Simandou Rivers systems. Also known from the coastal Guinean basins, from the Tominé River to east of Liberia and from Côte d'Ivoire	Benthopelagic; freshwater	Tropical	Guegan & Morand (1996)
Barbus serra		100.00	4x	Africa: Olifants River system, western Cape Province, South Africa.	Benthopelagic; freshwater	Subtropical	REFERENCES WITHIN: Tsigenopoulos *et al.* (2002)
Barbus steindachneri	1.82	100.00	4x	Europe: Endemic to Portugal also reported to occur in Spain: Guadiana and Tago drainages	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Barbus trevelyani		100.00	4x	Africa: Keiskamma and Buffalo systems in the Ciskei and eastern Cape Province, South Africa	Benthopelagic; freshwater	Subtropical	REFERENCES WITHIN: Tsigenopoulos *et al.* (2002)
Barbus wurtzi		148	6x	Africa: numerous coastal basins from Konkouré to rest of Volta.	Benthopelagic; freshwater	Tropical	Guegan *et al.* (1995)

Capoeta capoeta sevangi		150	6x	Former USSR: Widespread in the Araks from the upper to the lower reaches, as well as in Chaldyr, Sevan and Aigergel lakes.	Benthopelagic; freshwater	Temperate	Kryzanov (1998)
Capoeta damascina		150	6x	Eurasia: Jordan River drainage basin, the entire Levant, Mesopotamia and parts of southern Turkey.	Benthopelagic; freshwater	Subtropical	Gorshkova *et al.* (2002)
Capoeta trutta		150	6x	Asia: Tigris-Euphrates basin.	Benthopelagic; freshwater	Subtropical	Kilic Demirok & Unlu (2001)
Capoeta umbla		150	6x	Asia: Tigris and Euphrates.	Benthopelagic; freshwater	Subtropical	Kilic Demirok & Unlu (2001)
Carassioides acuminatus		100	4x	Asia: Viet Nam and China	Benthopelagic; freshwater	Tropical	Gui *et al.* (1985)
Carassioides cantonensis		100	4x	Asia: Viet Nam and China.	Benthopelagic; freshwater	Tropical	Gui *et al.* (1985)
Carassius auratus auratus	2.00	100.00	4x	Asia: central Asia and China, and Japan	Benthopelagic; potamodromous; freshwater	Subtropical*	Wu & Yang (1980)
Carassius auratus auratus		206	8x	Asia: central Asia and China, and Japan	Benthopelagic; potamodromous; freshwater	Subtropical*	Zan *et al.* (1986)
Carassius auratus auratus	2.30	162.00	6x	Asia: central Asia and China, and Japan	Benthopelagic; potamodromous; freshwater	Subtropical*	Fan & Liu (1990)
Carassius auratus auratus	2.38	150.00	6x	Asia: central Asia and China, and Japan	Benthopelagic; potamodromous; freshwater	Subtropical*	De Smet (1981)
Carassius auratus auratus	2.56	150.00	6x	Asia: central Asia and China, and Japan	Benthopelagic; potamodromous; freshwater	Subtropical*	Suzuki *et al.* (1995)
Carassius auratus grandoculis	1.35-2.05	100.00	4x	Asia: Lake Biwa, Japan	Demersal; freshwater	Temperate	Suzuki *et al.* (1995)
Carassius auratus langsdorfii	1.70	100.00	4x	Asia: Japan	Demersal; freshwater	Temperate	Ojima & Yamamoto (1990)

Carassius auratus ssp.	1.94	100.00	4x	Asia	Demersal; freshwater	Temperate	Suzuki *et al.* (1995)
Carassius auratus ssp.1	1.50	100.00	4x	Asia	Demersal; freshwater	Temperate	Suzuki *et al.* (1995)
Carassius auratus ssp.2	1.85	100.00	4x	Asia	Demersal; freshwater	Temperate	Vinogradov (1988a)
Carassius carassius	2.14	100.00	4x	Eurasia: Spain across Europe and north-central Asia to northern China.	Benthopelagic; potamodromous; freshwater; brackish	Temperate	Suzuki *et al.* (1995)
Carassius cuvieri	2.40	100.00	4x	Asia: Japan and Taiwan	Demersal; freshwater	Temperate	Cui *et al.* (1991)
Carassius gibelio	3.06	150.00	6x	Asia: Siberia	Benthopelagic; potamodromous; freshwater; brackish	Temperate	Suzuki & Taki (1988)
Catlocarpio siamensis	1.76	98.00	4x	Asia: Maeklong, Mekong and Chao Phraya basins	Benthopelagic; potamodromous; freshwater	Tropical	Suzuki & Taki (1988)
Cyprinion semiplotum		100	4x	Asia: India, Nepal and upper Myanmar. Reported from Bhutan	Benthopelagic; freshwater	Tropical	Zan *et al.* (1986)
Cyprinus acutidorsalis		100	4x	Asia: China.	Benthopelagic; freshwater; brackish	Subtropical	Zan *et al.* (1986)
Cyprinus barbatus	1.75	100	4x	Asia: Erhai Lake (Mekong basin) in Yunnan Province, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus carpio carpio	1.70	100	4x	Europe to Asia: Europe, Russia, China, India and South-East Asia	Benthopelagic; potamodromous; freshwater; brackish	Subtropical*	Zan *et al.* (1986)
Cyprinus centralus		100	4x	Asia: Viet Nam	Benthopelagic; freshwater	Tropical	Zan & Song (1980a)
Cyprinus chilia	1.65	100	4x	Asia: known from most lakes on the Yunnan Plateau, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980a)

Cyprinus dai		100	4x	Asia: Viet Nam	Benthopelagic; freshwater	Tropical	Zan & Song (1980a)
Cyprinus daliensis	1.55	100	4x	Asia: Er Hai Lake (Mekong) in Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan *et al.* (1986)
Cyprinus fuxianensis		100	4x	Asia: China.	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus hyperdorsalis		100	4x	Asia: Viet Nam	Benthopelagic; freshwater	Tropical	Zan & Song (1980)
Cyprinus ilishaestomus		100	4x	Asia: Qiluhu Lake in Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus intha		100	4x	Asia: Salween basin and probably Mekong basin	Benthopelagic; freshwater	Tropical	Zan & Song (1980)
Cyprinus longipectoralis	1.85	100	4x	Asia: endemic to Erhai Lake in Yunnan Province, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus longzhouensis		100	4x	Asia: China.	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus megalophthalmus		100	4x	Asia: Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus micristius		100	4x	Asia: Dian chi Lake, Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus multitaeniata		100	4x	Asia: West River in China; Viet Nam	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus qionghaiensis		100	4x	Asia: China.	Benthopelagic; freshwater	Temperate	Zan & Song (1980)
Cyprinus quidatensis		100	4x	Asia: Viet Nam	Benthopelagic; freshwater	Tropical	Zan & Song (1980)
Cyprinus rubrofuscus	1.70	100	4x	Asia: Laos, Viet Nam and China. Amur to Red River drainages	Benthopelagic; freshwater; brackish	Tropical	Zan & Song (1980a)
Cyprinus yilongensis		100	4x	Asia: Yi-lung Lake, Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan & Song (1980)
Cyprinus yunnanensis		100	4x	Asia: Tunghai, Yunnan, China	Benthopelagic; freshwater	Subtropical	Hinegardner (1968)

Diptychus maculatus		100	4x	Asia: Pakistan, India, China (Tibet) and Nepal. Also former USSR	Benthopelagic; potamodromous; freshwater	Subtropical*	Mazik *et al.* (1989)
Diptychus sewerzowi		98-100	4x	Former USSR	Benthopelagic; freshwater	Temperate	Mazik (1982)
Diptychus sp.		98	4x		Benthopelagic; freshwater	Temperate	Zan *et al.* (1985)
Gymnocypris przewalskii		92	4x	Asia: China.	Benthopelagic; potamodromous; freshwater	Temperate	Yan *et al.* (2007)
Labeobarbus aeneus		148	6x	Africa: originally endemic to the Orange-Vaal River system, South Africa.	Benthopelagic; potamodromous; freshwater	Subtropical	Oellerman & Skelton (1990)
Labeobarbus capensis		150	6x	Africa: Clanwilliam Olifants River system, South Africa.	Benthopelagic; potamodromous; freshwater	Subtropical	Oellerman & Skelton (1990)
Labeobarbus intermedius		150	6x	Africa: widely distributed throughout Southern Ethiopia and into Northern Kenya, certainly as far as Lake Baringo.	Benthopelagic; freshwater	Tropical	Golubtsov & Krysanov (1993)
Labeobarbus kimberleyensis		148	6x	Africa: Orange-Vaal River system.	Benthopelagic; potamodromous; freshwater	Subtropical	Oellerman & Skelton (1990)
Labeobarbus marequensis		150	6x	Africa: Widely distributed from the middle and lower Zambezi south to the Pongolo system.	Benthopelagic; potamodromous; freshwater	Tropical	Naran *et al.* (2007)
Labeobarbus natelensis		150	6x	Africa: Natal, widespread from the Mkuze southwards to the Umtamvuna on Transkei border.	Benthopelagic; potamodromous; freshwater	Subtropical	Oellerman & Skelton (1990)
Labeobarbus polylepis		150	6x	Africa: the Limpopo, Incomati and Pongolo.	Benthopelagic; freshwater	Subtropical	Oellerman & Skelton (1990)
Luciobarbus bocagei	1.91	100.00	4x	Europe: Portugal and Spain	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)

Luciobarbus brachycephalus		100.00	4x	Europe and Asia: Kura-Aras river watersheds.	Benthopelagic; potamodromous; freshwater; brackish	Temperate	Vasil'ev (1985)
Luciobarbus comizo	1.36	100.00	4x	Europe: Portugal and Spain; Tajo, Guadiana and Guadalquivir	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Luciobarbus graellsii		100.00	4x	Europe: northern Spain, from Zaragoza to Bilbao.	Benthopelagic; non-migratory; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Luciobarbus guiraonis		100.00	4x	Europe: Spain: in streams draining to Mediterranean, between Vinalopo and (but not including) Ebro drainages, and in a few headwaters of Guadiana drainage	Benthopelagic; non-migratory; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Luciobarbus microcephalus	1.87	100.00	4x	Europe: Portugal and Spain; Tajo and Guadiana	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Luciobarbus mursa		100	4x	Asia	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Luciobarbus sclateri	1.83	100.00	4x	Europe: Southern Spain and Portugal from Segura to Mira drainages.	Benthopelagic; freshwater	Temperate	Collares-Pereira & Moreira da Costa (1999)
Neolissochilus sumatranus		98	4x	Asia: Sumatra	Benthopelagic; freshwater	Tropical	Arkhipchuk (1999)
Percocypris pingi		98	4x	Asia: Jinshajiang River, Shigu in Lijiang, Yunnan Province, China.	Benthopelagic; freshwater	Subtropical	Zan *et al.* (1984)
Percocypris regani		98	4x	Asia: China.	Benthopelagic; freshwater	Subtropical	Collares-Pereira (1994)
Procypris rabaudi		100	4x	Asia: middle and upper reaches of Yangtze River, China	Benthopelagic; freshwater	Subtropical	Yu *et al.* (1987)
Pseudobarbus afer		96-100	4x	Africa: Coastal rivers from Algoa Bay to Mossel Bay.	Demersal; freshwater	Subtropical	Naran *et al.* (2006)
Pseudobarbus asper		96-100	4x	Africa: Groot (Gamtoos system) and larger tributaries of the Gourits system, South Cape, South Africa.	Demersal; freshwater	Subtropical	Naran *et al.* (2006)

Pseudobarbus burchelli		96-100	4x	Africa: Breede River system, Cape Province, South Africa; also in the Nieuwejaar, Grashock and Kars Rivers to the west; Duivenhoks and Kaffirkuils Rivers to the east	Benthopelagic; freshwater	Subtropical	Naran *et al.* (2006)
Pseudobarbus burgi		96-100	4x	Africa: Occurs in the Berg River system and Verlorenvlei Rivers, southwest Cape.	Benthopelagic; freshwater	Subtropical	Naran *et al.* (2006)
Pseudobarbus phlegethon		96-100	4x	Africa: tributaries of the Clanwilliam Olifants River, Cape Province, South Africa.	Benthopelagic; freshwater	Subtropical	Naran *et al.* (2006)
Pseudobarbus quathlambae		96-100	4x	Africa: Headwater streams of the Orange River in Lesotho.	Benthopelagic; freshwater	Subtropical	Skelton & Naran (1995)
Pseudobarbus tenuis		96-100	4x	Africa: mountain tributaries of the Gourits and Keurbooms River systems, Cape Province, South Africa	Benthopelagic; freshwater	Subtropical	Naran et al. (2006)
Ptychobarbus dipogon		446	16x	Asia: Brahmaputra River in Tibet	Benthopelagic; freshwater	Subtropical	Cui et al. (1991)
Schizopyge niger		98	4x	Asia: Kashmir Valley in India and Azad Kashmir in Pakistan	Benthopelagic; potamodromous; freshwater	Tropical	Khuda-Bukhsh & Nayak (1982)
Schizothorax davidi		98	4x	Asia: Sichuan and Yunnan provinces, China.	Benthopelagic; freshwater	Subtropical	Yu et al. (1987)
Schizothorax grahami	3.25	148.00	6x	Asia: China	Benthopelagic; freshwater	Subtropical	Cui et al. (1991)
Schizothorax lissolabiatus		148	6x	Asia: China	Benthopelagic; freshwater	Subtropical	Zan et al. (1986)
Schizothorax oconnori	1.53	92.00	4x	Asia: Brahmaputra in Tibet	Benthopelagic; freshwater	Subtropical	Zan et al. (1986)
Schizothorax prenanti		148	6x	Asia: China	Benthopelagic; freshwater	Subtropical	Yu et al. (1987)
Schizothorax progastus		98	4x	Asia: India and Nepal and Bhutan	Benthopelagic; potamodromous; freshwater	Tropical	Gui et al. (1985)

Schizothorax sp.	3.50	148.00	6x	Asia	Benthopelagic; freshwater	Tropical/Subtropical	Zan et al. (1986)
Schizothorax yunnanensis yunnanensis		148.00	6x	Asia: Yunnan, Province, China	Benthopelagic; freshwater	Subtropical	Zan et al. (1986)
Schizothorax zarudnyi		96	4x	Asia: Sistan, Iran; Afghanistan	Benthopelagic; freshwater	Subtropical	Kalbassi *et al.* (2008)
Sinocyclocheilus grahami	2.35	96.00	4x	Asia: China.	Benthopelagic; freshwater; brackish	Temperate	Li & Zhou (1983b)
Sinocyclocheilus maculatus	2.30	96.00	4x	Asia: China.	Benthopelagic; freshwater	Tropical	Zan et al. (1984)
Sinocyclocheilus tingi		96.00	4x	Asia: Fuxian Lake in Yunnan, China	Benthopelagic; freshwater	Temperate	Zan et al. (1984)
Spinibarbus denticulatus		100.00	4x	Asia: Laos, northern Viet Nam and southeastern China, Hainan.	Benthopelagic; freshwater	Subtropical	Gui et al. (1985)
Spinibarbus caldwelli		100.00	4x	Asia	Benthopelagic; freshwater	Subtropical	Gui et al. (1985)
Spinibarbus hollandi		100.00	4x	Asia: Laos, Viet Nam, China and Taiwan.	Benthopelagic; freshwater	Subtropical	Gui et al. (1985)
Spinibarbus sinensis		100.00	4x	Asia: China.	Benthopelagic; freshwater	Subtropical	Yu et al. (1987)
Tor douronensis		100	4x	Asia: Thailand east to Viet Nam and south to Indonesia. Known from the Chao Phraya and Mekong	Benthopelagic; freshwater	Tropical	Zan et al. (1986)
Tor khudree		100	4x	Asia: India and Sri Lanka	Benthopelagic; freshwater	Subtropical	Khuda-Bukhsh & Nayak (1982)
Tor putitora		100	4x	Asia: Afghanistan, Pakistan, India, Nepal, Bangladesh, Bhutan and Mynmar	Benthopelagic; potamodromous; freshwater	Subtropical	Rishi & Haobam (1984)
Tor sinensis		100	4x	Asia: Mekong basin	Benthopelagic; potamodromous; freshwater	Subtropical	Zan et al. (1986)
Tor soro		100	4x	Asia: Indonesia, Malaya, Myanmar, Thailand and	Benthopelagic; freshwater	Tropical	Magtoon & Arai (1993)

				Indochina			
Tor tambra		100	4x	Asia: Java, Borneo, Malay Peninsula and the Mekong basin	Benthopelagic; freshwater	Tropical	Zan et al. (1986)
Tor tor		100	4x	Asia: Pakistan, India, Bangladesh, Myanmar, Nepal and Bhutan	Benthopelagic; potamodromous; freshwater	Subtropical	Khuda-Bukhsh & Nayak (1982)
Varicorhinus beso		150	6x	Africa: Lake Tsana, Blue Nile and Awash Rivers systems	Benthopelagic; freshwater	Subtropical	Golubtsov & Krysanov (1993)
Varicorhinus nelspruitensis		150	6x	Africa: rocky areas of the Incomati (Mozambique) and Pongolo (South Africa) Rivers systems	Benthopelagic; freshwater	Subtropical	Oellerman & Skelton (1990)
Zacco taliensis	3.45	148.00	6x	Asia: Erhai Lake in Yunnan, China	Benthopelagic; freshwater	Subtropical	Zan et al. (1985)
Cypriniformes, Gyrinocheilidae							
Gyrinocheilus aymonieri		48	2x	Asia: Mekong, Chao Phraya and Meklong basins; northern Malay Peninsula	Demersal; potamodromous; freshwater	Subtropical	Arai *et al.* (1988)
Cypriniformes, Catostomidae							
Carpiodes cyprinus	2.24	100.00	4x	North America: Great Lakes-St. Lawrence River, Hudson Bay and Mississippi River basins from Quebec to Alberta in Canada and south to Louisiana, USA; Atlantic Slope drainages from Delaware River to Altamaha River in USA; Gulf Slope drainages from Apalachicola River to Pearl River in USA	Demersal; freshwater	Temperate	Ferris (1984)

Carpoides carpio		100	4x	North America: Mississippi River basin from Pennsylvania to Montana, south to Louisiana, USA; Gulf Slope drainages from Calcasieu River in Louisiana to Rio Grande in Texas and New Mexico, USA; also in Mexico	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Catostomus catostomus catostomus	2.09	98.00	4x	North America: throughout most of Canada and Alaska; Atlantic Slope south to Delaware River drainage in New York, USA; Great Lakes basin; upper Monongahela River drainage in Maryland and West Virginia, USA; Missouri River drainage south to Nebraska and Colorado, USA. Also in Arctic basin of Siberia in Russia. Occurs in Columbia River System	Demersal; freshwater; brackish	Temperate	Hardie & Hebert (2003)
Catostomus clarki		98	4x	North America: lower Colorado River drainage including Pluvial White River and Meadow Valley Wash in Nevada, USA; Virgin River in Utah, Arizona and Nevada, USA; Bill Williams River in Arizona, USA; and Gila River in New Mexico and Arizona, USA and in northern Sonora, Mexico	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Catostomus commersonii	2.77	98.00	4x	North America: throughout most of Canada to the Atlantic Coast, south through North Carolina to New Mexico	Demersal; catadromous; freshwater, usually	Temperate	Ferris (1984)
Catostomus discobolus discobolus		100	4x	North America: Snake River basin in Wyoming, and Idaho, USA; Lake Bonneville basin in Idaho, Wyoming and Utah, USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Catostomus latipinnis		100	4x	North America: Colorado River drainage from southwestern Wyoming to southern Arizona in the USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)

Cycleptus elongatus		98	4x	North America: Mississippi River basin from Pennsylvania to central Montana and south to Louisiana, USA; Gulf Slope drainages in the USA from Mobile Bay, Alabama to Rio Grande in Texas and New Mexico, and in Mexico	Benthopelagic; freshwater	Temperate	Uyeno & Smith (1972b)
Erimyzon oblongus	1.86		4x	North America: Maine to Altamaha River in Georgia, USA; Lake Ontario drainage in New York, USA; from Chattahoochee River in Alabama and Escambia River in Florida to San Jacinto River in Texas, USA; lower Great Lakes and Mississippi River basins from Michigan and Wisconsin to Gulf of Mexico in USA	Demersal; freshwater	Temperate	Ferris (1984)
Erymizon succetta		100	4x	North America: Great Lakes and Mississippi River basin lowlands from southern Ontario in Canada to Wisconsin, USA and south to Gulf of Mexico in the USA; Atlantic Slope from southern Virginia to southern Florida in USA; Gulf Slope drainages from Charlotte Harbor, Florida to Guadalupe River in Texas, USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Hypentelium nigricans		100	4x	North America: Great Lakes, Hudson Bay and Mississippi River basins from New York in USA and Ontario in Canada to Minnesota and south to Alabama, Arkansas and Louisiana in USA; from Mohawk-Hudson River to Altamaha River in USA; from Pascagoula River to Comite River in USA	Demersal; potamodromous; freshwater	Temperate	Uyeno & Smith (1972b)

Moxostoma duquesnii		104	4x	North America: lower Great Lakes and Mississippi River basin from Ontario in Canada and from New York to southeastern Minnesota in USA and south to northern Alabama and eastern Oklahoma, USA; upper and middle Mobile Bay drainage in Georgia, Alabama and southeastern Tennessee in USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Moxostoma erythrurum	2.14	98.00	4x	North America: Great Lakes, Hudson Bay, and Mississippi River basins from New York in USA and Ontario in Canada to North Dakota and south to Alabama and Oklahoma in USA; isolated in southwestern Mississippi, USA; from Potomac River in Maryland to Roanoke River in North Carolina, USA; Mobile Bay drainage in USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Moxostoma macrolepidotum		98	4x	North America: Great Lakes-St. Lawrence River, Hudson Bay and Mississippi River basins from Quebec to Alberta in Canada and south to northern Alabama and Oklahoma in the USA; Atlantic Slope drainages from Hudson River in New York to Santee River in South Carolina, USA	Demersal; freshwater	Temperate	Uyeno & Smith (1972b)
Myxocyprinus asiaticus	2.02	100.00	4x	Asia: China (Yangtze River basin)	Demersal; potamodromous; freshwater	Subtropical	Suzuki (1992b)

Cypriniformes, Balitoridae

Acanthocobitis botia		50	2x	Asia: Indus basin in Pakistan to the Mae Khlong basin in Thailand through Ganges, Chindwin, Irrawaddy, Sitang and Salween basins. Recorded also from Yunnan, China	Demersal; freshwater	Tropical	Rishi *et al.* (1977)

Cypriniformes, Cobitidae

Botia birdi		98.00	4x	Asia: India and Pakistan. Widespread in the Indus drainage	Demersal; freshwater	Subtropical	Khuda-Bukhsh & Nayak (1982)
Botia dario		98.00	4x	Asia: India and Bangladesh. Reported from Bhutan	Demersal; freshwater	Subtropical	Barat (1985)
Chromobotia macracanthus	1.03*	98.00	4x	Asia: Indonesia (Sumatra and Borneo)	Demersal; freshwater	Tropical	Ojima & Yamamoto (1990)
Cobitis biwae (4x)	3.61	96.00	4x	Asia: Honshu, Shikoku, and Kyushu in Japan	Benthopelagic; freshwater	Temperate	Suzuki (1992b)
Cobitis elongatoides		98?	4x	Europe: Danube basin, Argesel River	Demersal; non-migratory; freshwater	Temperate	Kottelat & Freyhof (2007)
Cobitis matsubarai		94	4x	Asia: Japan	Demersal; freshwater	Temperate	Nakabo (2007)
Cobitis sinensis		98?	4x	Asia: China	Benthopelagic/Demersal; freshwater	Subtropical	Yu et al. (1987)
Cobitis sp. (4x)	3.82	98.00	4x	Asia/Europe	Benthopelagic; freshwater	Temperate/Subtropical	Vasil'ev *et al.* (1999)
Cobitis striata		98	4x	Asia: Japan and Korea	Benthopelagic; freshwater	Temperate	Ueno & Ojima (1976)
Cobitis taenia		94	4x	Europe and Asia: Spain to Siberia	Benthopelagic; potamodromous; freshwater	Temperate	Ueno & Ojima (1976)
Misgurnus anguillicaudatus (4x)	2.26	100.00	4x	Asia: Myanmar and Northeastern Asia and southward to Central China, Japan	Demersal; freshwater	Subtropical	Cui *et al.* (1991)
Misgurnus fossilis	2.60	100.00	4x	Europe and Asia: France to Russia	Demersal; potamodromous; freshwater	Temperate	Timofeeva & Kaviani (1964)
Sinibotia pulchra		100.00	4x	Asia: China and Viet Nam	Demersal; freshwater	Subtropical	Yu et al. (1987)
Sinibotia robusta		100.00	4x	Asia: China and Viet Nam	Demersal; freshwater; brackish	Subtropical	Yu et al. (1987)

Yasuhikotakia modesta	0.80	100.00	4x	Asia: Mekong and Chao Phraya basins, and Mae Khlong basin	Demersal; potamodromous; freshwater	Subtropical	Ojima & Yamamoto (1990)
Siluriformes, Heteropneustidae							
Heteropneustes fossilis	4.35	58	2x	Asia: Pakistan and Sri Lanka to Myanmar, Tamilnadu and Kerala	Demersal; freshwater; brackish	Tropical	Pandian & Koteeswaran (1999)
Heteropneustes fossilis	9.75	116	4x	Asia: Pakistan and Sri Lanka to Myanmar, Tamilnadu and Kerala	Demersal; freshwater; brackish	Tropical	Pandian & Koteeswaran (1999)
Siluriformes, Claridae							
Clarias batrachus	1.20	56.00	2x	Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java, Borneo	Demersal; potamodromous; freshwater; brackish	Tropical	Arkhipchuk (1999)
Clarias batrachus		100	4x	Asia: Mekong and Chao Phraya basins, Malay Peninsula, Sumatra, Java, Borneo	Demersal; potamodromous; freshwater; brackish	Tropical	Manna & Prasad (1971)
Siluriformes, Callichthyidae							
Hoplosternum littorale	0.68	60.00	2x	Most Cis-Andean South American river drainages north of Buenos Aires, Argentina	Demersal; potamodromous; freshwater	Subtropical	Porto & Feldberg (1992)
Corydoras simulatus	0.65	64.00	2x	Colombia, province of Meta, Rio Ocoa near Puerto Lopez, Rio Meta	Demersal; potamodromous; freshwater	Tropical	Oliveira *et al.* (1992)
Corydoras britskii	-	90	4x	Brazil, Est. Mato Grosso, upper Rio Paraguai system, near Pocone and Miranda, also common in parts of Pantanal	Demersal; potamodromous; freshwater	Tropical	Oliveira *et al.* (1993b)

Corydoras splendens	1.17	100.00	4x	Brazil, Rio Tocantins, Rio Paraguai near Caceres; Peru, Rio Ampiyacu, Rio Nanay, Rio Ucayali; Ecuador, Rio Napo	Demersal; potamodromous; freshwater	Tropical	Oliveira *et al.* (1993b)
Corydoras aeneus (4x)	3.30	134.00	6x	Peru	Demersal; potamodromous; freshwater	Tropical	Turner *et al.* (1992)
Corydoras aeneus (4x)	4.12	132.00	6x	Peru	Demersal; potamodromous; freshwater	Tropical	Ojima & Yamamoto (1990)
Corydoras aeneus (4x)	4.40	120.00	6x	Guyana	Demersal; potamodromous; freshwater	Tropical	Hinegardner (1968)
Corydoras arcuatus	2.27	46.00	4x re-diploidized	Peru, Loreto, Rio Yavari, Lago Matamata; Ecuador, Rio Napo; Brazil, border region close to Peru, up to Rio Negro, Sao Gabriel de Cachoeira; Colombia, Rio Purus drianage, around Humaita, Rio Caqueta, Rio Tacana	Demersal; potamodromous; freshwater	Tropical	Oliveira et al. (1992)
Corydoras elegans	3.00	50.00	?	Brazil, Est. Amazonas, Rio Amazonas, at Tefe. Widely distributed in Peruvian and Colombian Amazon and in Ecuador, Rio Aguarico and Rio Napo	Demersal; potamodromous; freshwater	Tropical	Hinegardner & Rosen (1972)
Corydoras flaveolus	2.46	58.00	4x re-diploidized	Brazil, state of Sao Paulo, Rio Piracicaba	Demersal; potamodromous; freshwater	Subtropical	Oliveira et al. (1992)
Corydoras julii	4.20	92.00	4x	Brazil, state of Maranhao, creek into Rio Parnaiba near Alto Parnaiba	Demersal; potamodromous; freshwater	Tropical	Hinegardner (1968)
Corydoras brevirostris	3.00	48.00	4x re-diploidized	Venezuela, Orinoco	Demersal; potamodromous; freshwater	Tropical	Hinegardner & Rosen (1972)
Corydoras metae	4.38	92.00	4x	Colombia, Rio Meta, Barrigon	Demersal; potamodromous; freshwater	Tropical	Oliveira et al. (1992)

Corydoras punctatus	2.90	46.00	4x re-diploidized	Suriname, Suriname river system, Marshall creek, east of Paranam-Afobaka road, 1.5 km north of Marchall village. Compagnie creek suriname river draiange	Demersal; potamodromous; freshwater	Tropical	Hinegardner (1968)
Corydoras reticulatus	0.98	74.00	?	Brazil, state of Para, Rio Amazonas at Monte Alegre; Peru, Loreto province, Rio Ampiyacu, Rio Nanay, Rio Blanco	Demersal; potamodromous; freshwater	Tropical	Oliveira et al. (1992)
Corydoras schwartzi	2.39	46.00	4x re-diploidized	Brazil, state of Amazonas, mouth of Rio Purus, into the Rio Solimoes	Demersal; potamodromous; freshwater	Tropical	Oliveira et al. (1992)
Corydoras trilineatus	2.45	46.00	4x re-diploidized	Peru, in the tributaries of the Rio Ampiyacu, Rio Yavari, Rio Nanay, Rio Huytoyacu, Rio Yasuni; Ecuador, Rio Pastaza; Brazil, state of Acre, Rio Acre drainage	Demersal; potamodromous; freshwater	Tropical	Oliveira et al. (1992)
Corydoras undulatus	3.00	50.00	?	Argentina, Buenos Aires, La Plata	Demersal; potamodromous; freshwater	Subtropical	Hinegardner (1968)
Corydoras nattereri	1.79	44	4x re-diploidized	Brazil, state of Rio de Janeiro, Rio Paraiba do Sul drainage, state of Sao Paulo, Rio Juquia	Demersal; potamodromous; freshwater	Tropical	Shimabukuro-Dias *et al.* (2004)
Corydoras difluviatilis	1.94	78	?	Brazil, state of Sao Paulo and Minas Gerais. Rio Parana and the upper Rio Sao Francisco-basin	Demersal; potamodromous; freshwater	Tropical	Shimabukuro-Dias *et al.* (2004)
Corydoras sodalis	-	74	?	Peru, Loreto province, Rio Yavari, Brazil, upper Rio Solimoes close to Peruvian border; Bolivia, Rio Beni drainage	Demersal; potamodromous; freshwater	Tropical	Shimabukuro-Dias *et al.* (2004)
Corydoras agassizii	-	98	4x	Brazil, Est. Amazonas, Rio Amazonas near Tabatinga; Peru, Rio Apiyacu	Demersal; potamodromous; freshwater	Tropical	Scheel *et al.* (1971)

Corydoras delphax	-	84	?	Colombia, Guainia, Rio Inirida system, Cano Bacon, Pueblo Bretania, also inhabiting parts of Colombian Orinodo drainage	Demersal; potamodromous; freshwater	Tropical	Kato & Ojima (1991)
Corydoras imitator	-	80	?	Brazil, Est. Amazonas, upper Rio Negro	Demersal; potamodromous; freshwater	Tropical	Sands (1994)
Corydoras pulcher	-	102	4x	Brazil, Amazonas state, Rio Purus, north of Labrea, Rio Amazonas system	Demersal; potamodromous; freshwater	Tropical	Shimabukuro-Dias *et al.* (2004)
Corydoras robinae	-	84	?	Brazil, state of Amazonas, Rio Aiuana, a southern tributary of the middle Rio Negro. Sao Gabriel de Cachoeira	Demersal; potamodromous; freshwater	Tropical	Shimabukuro-Dias *et al.* (2004)
Esociformes, Esocidae							
Esox lucius	1.15	50.00	2xAncestor	Circumpolar in fresh water. North America: Atlantic, Arctic, Pacific, Great Lakes and Mississippi River basins from Labrador to Alaska and south to Pennsylvania, Missouri and Nebraska, USA. Eurasia: France to eastern Siberia, south to northern Italy.	Demersal; potamodromous; freshwater; brackish;	Temperate	Vinogradov (1998a)
Esociformes, Umbridae							
Dallia pectoralis	1.26	78.00	2xAncestor	North America: Alaska from Colville River delta south to central Alaska Peninsula near Chignik; upstream in Yukon-Tanana drainage to near Fairbanks. Also Bering Sea islands and northeastern Siberia, Russia.	Demersal; freshwater	Temperate	Beamish *et al.* (1971)
Dallia spp.		74-78	2xAncestor		Demersal; freshwater	Temperate	Crossman & Ráb (1996)

Novumbra hubbsi	1.04	48	2xAncestor	North America: coastal lowlands of Olympic Peninsula in Washington, USA from Ozette Lake and Queets River drainage to upper Chehalis River drainage; occasionally in lower Deschutes River (Puget Sound drainage), as a result of floodwater exchange with Chehalis River.	Demersal; freshwater	Temperate	Crossman & Ráb (2001)
Umbra krameri		44	2xAncestor	Europe: Danube and Dniester river systems	Benthopelagic; non-migratory; freshwater	Temperate	Ráb (1981)
Umbra limi	2.70	22.00	2xAncestor	North America: St. Lawrence-Great Lakes, Hudson Bay (Red River) and Mississippi River basins from Quebec to Manitoba in Canada and south to Ohio, Tennessee and Arkansas, USA; Hudson River drainage (Atlantic Slope) in New York, USA. Isolated populations in Missouri River drainage of South Dakota and Iowa, USA.	Demersal; freshwater;	Temperate	Hinegardner (1968)
Umbra pygmaea		22	2xAncestor	North America: Atlantic and Gulf slopes from southeastern New York (including Long Island) to St.Johns River drainage in Florida and west to Aucilla River drainage in Florida and Georgia, USA.	Demersal; non-migratory; freshwater	Temperate	Ráb *et al.* (2002)
Salmoniformes, Salmonidae							
Brachymystax lenok		90	4x re-diploidized	Asia: Siberia, Korea and North China. A subspecies, Brachymystax lenok tsinlingensis is endemic to parts of the Taibaishan Mountain in the Qinling Mountains, particularly in the Heihe River at the eastern foot, the Shitouhe River at the northern foot and the Xushui and Taibaihe rivers at the southern foot of the mountain.	Benthopelagic; anadromous?: freshwater	Temperate	Viktorovsky *et al.* (1985)

Coregonus albula		80	4x re-diploidized	Europe and Asia: in lakes from England to northwest Russia. In the Baltic Sea: in the Bothnian Bay and eastern end of the Gulf of Finland.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Booke (1968)
Coregonus artedi	3.25	80	4x re-diploidized	North America: widely distributed in Canada and northern USA in St. Lawrence-Great Lakes, Arctic, and upper Mississippi River basins from Quebec to Northwest Territories and Alberta and south to northern Ohio, Illinois and Minnesota. Rare in Great Lakes	Pelagic-neritic; anadromous; freshwater; brackish; marine	Temperate	Lockwood & Bickham (1991)
Coregonus autumnalis autumnalis	3.22		4x re-diploidized	Europe: UK, Barents Sea and coasts and rivers of Siberia. Northern America: Murchison River in Northwest Territories, Canada to Point Barrow in Alaska; also in Mackenzie River in Northwest Territories and the lower Liard River, British Columbia	Pelagic-neritic; anadromous; freshwater; brackish; marine	Temperate	Booke (1968)
Coregonus clupeaformis	3.44	80.00	4x re-diploidized	North America: throughout Alaska and most of Canada south into New England, the Great Lakes basin, and central Minnesota. This species was stocked into high Andean lakes in two countries in southern Latin America	Demersal; anadromous; freshwater; brackish; marine	Temperate	Bargetzi (1958)
Coregonus fera	2.88		4x re-diploidized	Europe: Lake Geneva, Switzerland and France. Extinct!!	Benthopelagic; freshwater	Temperate	Booke (1968)
Coregonus hoyi	2.77	80.00	4x re-diploidized	North America: found only in Great Lakes (except Lake Erie) in Canada-USA and Lake Nipigon in Canada. This species is probably extirpated from Lakes Ontario and Nipigon, threatened in Lake Michigan, and declining in Lakes Superior and Huron.	Demersal; freshwater	Temperate	Booke (1968)

Coregonus kiyi	3.29		4x re-diploidized	North America: found only in Great Lakes (except Lake Erie), Canada-USA. This species is common in Lake Superior, extremely rare, possibly extirpated, in Lakes Huron and Ontario, and endangered in Lake Michigan.	Pelagic; freshwater	Temperate	Bargetzi (1958)
Coregonus lavaretus		96	4x re-diploidized	Europe: usage of name restricted to Lakes Genève (now extinct), Bourget, and Aiguebelette in the river Rhône basin	Demersal; anadromous; freshwater; brackish; marine	Temperate	Sola *et al.* (1989)
Coregonus macrophthalmus	3.02		4x re-diploidized	Europe: Lake Konstanz, Germany, Switzerland and Austria.	Pelagic; freshwater	Temperate	Lockwood *et al.* (1991b)
Coregonus nasus	3.54	96.00	4x re-diploidized	Arctic: east from Pechora River, Russia to northern America: Arctic drainages from Perry River in Northwest Territories, Canada to Kushokwim River (Bering Sea tributary), Alaska.	Demersal; anadromous; freshwater; brackish; marine	Polar	Booke (1968)
Coregonus oxyrhynchus		96	4x re-diploidized	Northeast Atlantic: Ireland to Netherlands, Germany and the countries along the coast of the Baltic Sea. Also found in several countries in central and eastern Europe. Maritime stocks may be extinct	Demersal; anadromous; freshwater; brackish; marine	Temperate	Karbe (1964)
Coregonus peled		80	4x re-diploidized	Europe and Asia: lakes and rivers from Mezen to Kolyma River, Russia.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Nygren *et al.* (1971b)
Coregonus pidschian		96	4x re-diploidized	Arctic: Sweden and Finland to the extreme northeast of Siberia, in the western portion of the Bering Sea basin, and partly in the basin of the Sea of Okhotsk. In Alaska in the tributaries of the Beaufort, Chukchi, and Bering seas. Additional populations in Poland and in the Alps	Demersal; anadromous; freshwater; brackish; marine	Polar	Karbe (1964)

Coregonus reighardi	3.01	80.00	4x re-diploidized	North America: Great Lakes, Canada and USA.	Demersal; freshwater	Temperate	Booke (1968)
Coregonus spp.		76-96	4x re-diploidized	North America/Europe	Pelagic/Demersal/Benthopelagic	Temperate/Polar	Rab & Jankun (1992)
Coregonus ussuriensis		80	4x re-diploidized	Former USSR: Lower and middle part of the Amur, Zeya, Ussuri, Lake Khanka, Amur Liman, southern part of the Sea of Okhotsk. Asia: China	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Viktorovsky & Maximova (1978)
Coregonus zenithicus	2.63	80.00	4x re-diploidized	North America: Great Slave Lake in Northwest Territories, Canada southeast through Hudson Bay and Great Lakes basin (except Lakes Ontario and Erie). Declining in Lakes Superior and Nipigon. Uncommon outside Great Lakes basin	Demersal; freshwater	Temperate	Johnson *et al.* (1987)
Hucho hucho		82-84	4x re-diploidized	Europe: Rivers of the Danube basin. Introduced into other European river basins when their numbers declined due to ecological changes in the Danube.	Benthopelagic; potamodromous freshwater	Temperate	Rab & Liehman (1982)
Hucho taimen		84	4x re-diploidized	Former USSR and Asia: Caspian Sea and Arctic drainages to Amur river.	Benthopelagic; potamodromous freshwater	Temperate	Frolov & Frolova (2000)
Oncorhynchus aguabonita	2.52		4x re-diploidized	North America: Upper Kern River basin, Tulare and Kern counties in California, USA.	Demersal; freshwater	Temperate	Johnson *et al.* (1987)
Oncorhynchus clarki clarki	2.62	64.00	4x re-diploidized	Eastern Pacific: northern parts of Prince William Sound, Alaska, south to the Eel River in northern California, USA and is found in most streams emptying into the Pacific.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Johnson *et al.* (1987)

Oncorhynchus gorbuscha	2.57	52.00	4x re-diploidized	Arctic, Northwest to Eastern Central Pacific: Alaska and the Aleutian Islands, drainages from Northwest Territories in Canada to southern California in USA. Western Pacific: Russian Federation, the Bering and Okhotsk seas ; eastern Korea and Hokkaido, Japan	Demersal; anadromous; freshwater; brackish; marine	Temperate*	Johnson *et al.* (1987)
Oncorhynchus keta	2.64	74.00	4x re-diploidized	North Pacific: Korea , Japan, Okhotsk and Bering Sea (Ref. 1998), Arctic Alaska south to San Diego, California, USA.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Hinegardner & Rosen (1972)
Oncorhynchus kisutch	3.00	60.00	4x re-diploidized	North Pacific: distributed from the Anadyr River in Russia south towards Hokkaido, Japan, and from Point Hope in Alaska southwards to Chamalu Bay in Baja California, Mexico.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Johnson *et al.* (1987)
Oncorhynchus masou macrostomus	2.96	66.00	4x re-diploidized	Northwest Pacific: endemic to western Japan, on the Pacific side of Honshu, Shikoku and on the Inland Sea of Japan side of Kyushu.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Ojima *et al.* (1963)
Oncorhynchus masou masou	2.53	66.00	4x re-diploidized	Northwest Pacific: Sea of Okhotsk and Sea of Japan; northern Japan and eastern Korea Peninsula.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Ojima & Yakamoto (1990)
Oncorhynchus masou rhodurus	2.16	66.00	4x re-diploidized	Asia: originally endemic to Lake Biwa, then transplanted to Lake Chûzenji and Lake Ashinoko of Japan	Demersal; anadromous; freshwater; brackish; marine	Temperate	Rasch (1985)
Oncorhynchus mykiss	2.60	60-90	4x re-diploidized	Southwest Atlantic: Argentina. Eastern Pacific: Kamchatkan Peninsula and have been recorded from the Commander Islands east of Kamchatka and sporadically in the Sea of Okhotsk as far south as the mouth of the Amur River along the mainland.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Ojima *et al.* (1963)

Oncorhynchus nerka	3.04	56.00	4x re-diploidized	North Pacific: northern Japan to Bering Sea and to Los Angeles, California, USA. Landlocked populations in Alaska, Yukon Territory and British Columbia in Canada, and Washington and Oregon in USA.	Pelagic-oceanic; anadromous; freshwater; brackish; marine	Temperate	Hinegardner & Rosen (1972)
Oncorhynchus tshawytscha	3.30	68.00	4x re-diploidized	Arctic, Northwest to Northeast Pacific: drainages from Point Hope, Alaska to Ventura River, California, USA; occasionally strays south to San Diego in California, USA. Also in Honshu, Japan, Sea of Japan, Bering Sea and Sea of Okhotsk. Found in Coppermine River in the Arctic.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate*	Booke (1968)
Parahucho perryi		62	4x re-diploidized	Northwest Pacific: Sea of Japan, from southern Kuril Islands and Primorskii Krai, Russia to Hokkaido.	Benthopelagic; anadromous freshwater; brackish; marine	Temperate	Anbinder *et al.* (1982)
Prosopium abyssicola		72	4x re-diploidized	North America	Benthopelagic/Pelagic	Temperate	Booke (1974)
Prosopium coulterii	2.53	82.00	4x re-diploidized	North America: three disjunct areas: Lake Superior in Ontario, Canada and Michigan, USA; Yukon River drainage in Yukon, Canada to Columbia River drainage in western Montana and Washington, USA; and Chignik, Naknek and Wood River drainages in southwest Alaska. Former USSR: Russia	Benthopelagic; freshwater	Temperate	Booke (1968)

Prosopium cylindraceum	2.48	78.00	4x re-diploidized	North America: Arctic and Pacific drainages from western Hudson Bay in Canada to Alaska and northern British Columbia, Canada; Arctic and Atlantic drainages from Labrador in Canada to Connecticut, USA and west through St. Lawrence-Great Lakes basin in Canada-USA (except Lake Erie). Northern Asia: widely distributed in Siberian rivers	Benthopelagic; potamodromous freshwater; brackish	Temperate	Booke (1974)
Prosopium gemmiferum		64	4x re-diploidized	North America: endemic to Bear Lake in southeastern Idaho and northern Utah, USA.	Pelagic; freshwater	Temperate	Booke (1974)
Prosopium spilonotus		74	4x re-diploidized	North America: endemic to Bear Lake, southeast Idaho and northern Utah in USA.	Benthopelagic; freshwater	Temperate	Booke (1974)
Prosopium williamsoni		78	4x re-diploidized	North America: Mackenzie River drainage in Northwest Territory, Canada south through western Canada and northwestern USA in the Pacific, Hudson Bay and upper Missouri River basins to Truckee River drainage in Nevada and Sevier River drainage in Utah, USA.	Benthopelagic; freshwater	Temperate	Booke (1974)
Salmo carpio		80	4x re-diploidized	Europe: Lake Garda, Italy.	Demersal; freshwater	Temperate	Vinogradov (1998a)
Salmo obtusirostris		82	4x re-diploidized	Europe: Croatia and Montenegro to eastern Albania (mostly in Adriatic Sea drainages). Now restricted to a 300 m long stream stretch in Krka (Croatia) (if not already extirpated). A single recent record (2004) from Zeta (Montenegro). Still frequent in Neretva drainage, especially in Buna and uppermost Jardo, Vrljika and Zrnovnica streams.	Benthopelagic; non-migratory; freshwater	Temperate	Berberovic & Sofradzija (1972)

Salmo salar	3.10	60.00	4x re-diploidized	Atlantic Ocean: temperate and arctic zones in northern hemisphere. In western Atlantic Ocean distributed in coast drainages from northern Quebec in Canada and Connecticut in USA to Argentina. In eastern Atlantic Ocean distributed in drainages from the Baltic states to Portugal. Landlocked stocks are present in Russia, Finland, Sweden and Norway and in North America	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Alfei *et al.* (1996)
Salmo spp.		78-84	4x re-diploidized	Europe/Atlantic	Benthopelagic/Demersal	Temperate	Hartley & Horne (1984b)
Salmo trutta fario	3.15	80.00	4x re-diploidized	Northeast Atlantic: southward to southern Norway; Iceland; southern Greenland. Non-migratory and land-locked relict populations south to the British Isles and central France. Reported from Greece, Estonia and Argentina. Elsewhere circumpolar.	Demersal; anadromous; freshwater; brackish; marine	Temperate	Alfei *et al.* (1996)
Salmo trutta macrostigma	3.19	80.00	4x re-diploidized	Europe: known only from the island of Corse in the Mediterranean.	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Vinogradov (1998a)
Salmo trutta trutta	3.07	80.00	4x re-diploidized	Europe and Asia: northwestern coast of Europe	Pelagic-neritic; anadromous; freshwater; brackish; marine	Temperate	Hartley & Horne (1984b)
Salvelinus alpinus alpinus	3.70	80.00	4x re-diploidized	Europe: northern Atlantic southward to southern Norway, also Iceland and southern Greenland. Isolated populations in Northern UK, Scandinavia, Finland and the Alps. Landlocked populations in Quebec, Canada and in Maine and New Hampshire in USA	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Hinegardner (1968)

Salvelinus fontinalis	3.50	84.00	4x re-diploidized	North America: most of eastern Canada from Newfoundland to western side of Hudson Bay; south in Atlantic, Great Lakes, and Mississippi River basins to Minnesota and northern Georgia in USA. South America: Argentina	Demersal; anadromous; freshwater; brackish; marine	Temperate	Ojima et al. (1963)
Salvelinus leucomaenis leucomaenis	3.75	84.00	4x re-diploidized	Northwest Pacific: Hokkaido, Japan and the northeastern Korean Peninsula through Sakhalin, the Kuril Islands to Kamchatka. Bering and Okhotsk seas	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate	Hardie & Hebert (2003)
Salvelinus malma malma		84	4x re-diploidized	Arctic, Northwest to Northeast Pacific: drainages from Alaska to Puget Sound, Washington, USA; formerly in McCloud River drainages in California, USA. Northwest Pacific: Korea to Bering Sea	Benthopelagic; anadromous; freshwater; brackish; marine	Temperate*	Hartley (1990)
Salvelinus namaycush	3.04		4x re-diploidized	North America: Widely distributed from northern Canada and Alaska south to New England in USA and Great Lakes basin in Canada-USA	Benthopelagic; non-migratory; freshwater	Temperate	Booke (1968)
Stenodus leucichthys	3.27	74.00	4x re-diploidized	North America and Asia: Arctic drainages from Anderson River in Northwest Territories, Canada to Kuskokwim River (Bering Sea tributary) in Alaska. Upstream in Mackenzie River and Yukon River drainages to British Columbia, Canada. Isolated population occurs in northern Caspian Sea and inflowing rivers. Populations from Kazakhstan, Russia and probably Azerbaijan are endangered	Demersal; anadromous; freshwater; brackish; marine	Temperate	Hardie & Hebert (2003)

Thymallus arcticus arcticus	1.99		4x re-diploidized	North America: widespread in Arctic drainages from Hudson Bay, Canada to Alaska and in Arctic and Pacific drainages to central Alberta and British Columbia in Canada; upper Missouri River drainage in Montana, USA. Formerly in Great Lakes basin in Michigan, USA. Asia: Siberia, Russia	Benthopelagic; freshwater	Boreal	Vinogradov (1998a)
Thymallus spp.		100-104	4x re-diploidized	North America/Europe	Benthopelagic; freshwater	Temperate/Boreal	Severin & Zinovyev (1982)
Thymallus thymallus	2.15	102.00	4x re-diploidized	Europe: England and France to the Ural Mountains in northwest Russia	Benthopelagic; non-migratory; freshwater; brackish	Temperate	Nygren et al. (1971a)

Perciformes, Channidae

Channa punctata	0.66	32.00	2x	Asia: Afghanistan, Pakistan, India, Sri Lanka, Nepal, Bangladesh, Myanmar and Yunnan in China	Demersal; potamodromous; freshwater; brackish	Tropical	Banerjee *et al.* (1988)
Channa striata	0.73	40.00	2x	Asia: Pakistan to Thailand and south China	Demersal; potamodromous; freshwater; brackish	Tropical	Banerjee *et al.* (1988)
Channa asiatica	0.81	44.00	2x	Asia: Yangtze River basin in central China, Taiwan, Hainan Island to the Red River basin of northern Viet Nam	Benthopelagic; freshwater	Tropical	Cui et al. (1991)
Channa argus argus	0.77	48.00	2x	Asia: China and western and southern Korea	Benthopelagic; freshwater	Temperate	Cui et al. (1991)
Channa orientalis		78	4x?	Asia: Afghanistan and Baluchistan southward to Sri Lanka and eastward to Indonesia	Benthopelagic; potamodromous; freshwater; brackish	Tropical	Manna & Prasad (1973)

Channa gachua	0.97	78	4x?	Asia: Sri Lanka to the Mekong (Xe Bangfai and Nam Theun basins) and Bali, Indonesia. Also Maharashtra, India	Benthopelagic; potamodromous; freshwater	Tropical	Banerjee et al. (1988)
Channa stewartii		104	6x?	Asia: Eastern Himalaya (India and Nepal)	Benthopelagic; freshwater	Tropical	Rishi & Haobam (1984)

Summary of known polyploid fish, along with a list of species where polyploidy has been suspected but not confirmed. A description of higher level classifications is provided for comparison with Figure 2. References are provided for the first report of polyploidy for each species. Chromosome numbers were taken from the original ploidy descriptions; genome sizes were obtained from the (http://www.genomesize.com/). Endangered species status and population trends were obtained from the IUCN Red list database. Notes on distributions, environment, and climate were obtained from Fishbase (Froese et al. 2008; www.fishbase.org).

References for SI Table A2:

Alfei, L., D. Cavallo, P. Eleuteri, M.G. Grollino, P.T. Colombari, A. Ferri, A. Onali, and R. De Vita (1996). Nuclear DNA content in *Salmo fibreni* in Lake Posta Fibreno, Italy. Journal of Fish Biology 48: 1051-1058.

Anbinder, E. M., Glubokovsky, M. K. & Pokozy, N. V. (1982). Karyotype of the Sakhalin trout *Hucho perryi*. Biologyia Morya 4, 59–60.

Arai, R., A. Suzuki and Y. Akai (1988) The karyotype and DNA value of a Cypriniform algae eater, *Gyrinocheilus aymonieri*. Japanese Journal of Ichthyology 34(4):515-517.

Arkhipchuk, V.V. (1999). Chromosome database. Database of Dr. Victor Arkhipchuk.

Banerjee, S.K., K.K. Misra, S. Banerjee, and S.P. Ray-Chaudhuri (1988). Chromosome numbers, genome sizes, cell volumes and evolution of snake-head fish (family Channidae). Journal of Fish Biology 33: 781-789.

Barat, A. (1985). A study of chromosomes in some Indian teleost (Pisces). Kalyani University, West Bengal. Ph.D. dissertation.

Bargetzi, J.-P. (1958). Application de méthodes d'analyse biochimique à une étude taxonomique: les corégones du lac de Neuchâtel. II. Cytologie et dosage de DNA. Experientia 14: 445-447.

Beamish, R.J., M.J. Merrilees, and E.J. Crossman (1971). Karyotypes and DNA values for members of the suborder Esocoidei (Osteichthyes: Salmoniformes). Chromosoma 34: 436-447.

Berberovic, L. and A. Sofradzija (1972). Pregled podataka o hromosomskim garniturama slatko-vodnih riba Jugodlavije Ichthyologia 4:1-21.

Berrebi, P. and Ráb, P. (1998). The 'Barbus' intermedius species flock in Lake Tana (Ethiopia): III – cytogenetic and molecular genetic data. Italian Journal of Zoology. 65, 15–20.

Birstein, V.J., A.I. Pletaev, and B.F. Goncharov (1993). DNA content in Eurasian sturgeon species determined by flow cytometry. Cytometry 14: 377-383.

Birstein, V.J., Hanner, R. and DeSalle, R. (1997). Phylogeny of the Acipenseriformes: Cytogenetic and molecular approaches. Environmental Biology of Fishes 48, 127–155.

Blacklidge, K.H. and C.A. Bidwell (1993). Three ploidy levels indicated by genome quantification in Acipenseriformes of North America. Journal of Heredity 84: 427-430.

Booke, H.E. (1968). Cytotaxonomic studies of the coregonine fishes of the Great Lakes, USA: DNA and karyotype analysis. Journal of the Fisheries Research Board of Canada 25: 1667-1687.

Booke, J. E. (1974). A cytotaxonomic study of roundfishes, genus Prosopium. Copeia 1974, 115–119.

Cataudella, S., L. Sola, R. Accame Muratori & E. Capanna (1977b). The chromosomes of 11 species of Ciprinidae and one Cobitidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. Genetica 47 (3): 161-171.

Collares-Pereira, M.J. (1994). Révison caryologique des barbinés et hypothèses concernant la plésiomorphie possible de l'état polyploïde chez les Cyprinidés. Bull. Fr. Pêche Piscic. 334 : 191-199.

Collares-Pereira, M.J. and L. Moreira da Costa (1999). Intraspecific and interspecific genome size variation in Iberian Cyprinidae and the problem of diploidy and polyploidy, with review of genome sizes within the family. Folia Zoologica 48: 61-76.

Crossman, E. J., Ráb, P. (1996). Chromosome-banding study of the Alaska blackfish, *Dallia pectoralis* (Euteleostei: Esocae) with implications for karyotype evolution and relationships of esocoid fishes. Canadian Journal of Zoology, 74 (1): 147 - 156.

Crossman, E. J., Ráb, P. (2001). Chromosomal NOR phenotype and C-banded karyotype of Olypmic mudminnow, *Novumbra hubbsi* (Euteleostei: Umbridae). Copeia, 2001, 3: 860 - 865.

Cui, J., X. Ren, and Q. Yu (1991). Nuclear DNA content variation in fishes. Cytologia 56: 425-429.

De Smet, W.H.O. (1981). The nuclear Feulgen-DNA content of the vertebrates (especially reptiles), as measured by fluorescence cytophotometry, with notes on the cell and chromosome size. Acta Zoologica et Pathologica Antverpiensia 76: 119-167.

Fan, Z. and G.Liu (1990). The ploidy and reproductive mechanism of crucian carp, *Carassius auratus gibelio*. Journal of Fish Biology 36: 415-419.

Ferris, S.D. (1984). Tetraploidy and the evolution of the catostomid fishes. In: Evolutionary Genetics of Fishes, edited by B.J. Turner. Plenum Press, New York. 55-93.

Fišter, S., Cakić, P., Kataranovski, D. (1999). Karyotype analysis of *Barbus barbus* L. and *Barbus peloponnesius* V. (Cyprinidae) and frequences of breaks and gap type structural chromosome changes in fishes from the river Vapa. Acta Veterinaria, 49, 385-392.

Fontana, F. (1976). Nuclear DNA content and cytometry of erythrocytes of Huso huso L., *Acipenser sturio* L. and *Acipenser naccarii* Bonaparte. Caryologia 29: 127-137.

Frolov, S. V. & Frolova, V. N. (2000). Polymorphism and karyotype divergence in taimens of the *Hucho* genus. Genetika 36, 237–240.

Gold, J.R. (1994). Cytosystematic evidence that the genus *Richardsonius* belongs in the western clade of phoxinin cyprinids. Copeia 1994: 815-818.

Golubtsov, A.S. and E.Y. Krysanov (1993). Karyological study of some cyprinid species from Ethiopia. The ploidy differences between large and small Barbus of Africa. Journal of Fish Biology. 42(3):445-455.

Gorshkova, G., Gorshkov, S. & Golani, D. (2002). Karyotypes of *Barbus canis* and *Capoeta damascina* (Pisces, Cyprinidae) from the Middle East. Italian Journal of Zoology 69: 191–194.

Guegan, J.F. & Morand, S. (1996). Polyploid hosts: strange attractors for parasites? Oikos 77: 366–370.

Guegan, J.F., Rab, P., Machordom, A. & Doadrio, I. (1995). New hexaploidy in 'large' African Barbus with some considerations on the origin of hexaploidy. Journal of Fish Biology 47: 192–198.

Gui, J.Y. Li, K. Li, Y. Hong and T. Zhou (1985). Studies on the karyotypes of Chinese cyprinid fishes. 6. Karyotypes of three tetraploid species in Barbinae and one tetraploid species in Cyprininae. Acta Genetica Sinica. 12(4):302-308.

Hafez, R., R. Labat, and R. Quillier (1978). Teneurs nucléaires en A.D.N. et relations évolutives dans la famille des cyprinides (Teleostei). Bulletin de la Societé d'Histoire Naturelle de Toulouse 114: 71-84.

Hardie, D.C. and P.D.N. Hebert (2003). The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. Genome 46: 683-706.

Hartley, S. E. & Horne, M. T. (1984b). Chromosome relationships in the genus *Salmo*. Chromosoma 90, 229–237.

Hartley, S.E. (1990). Variation in cellular DNA content in Arctic charr, *Salvelinus alpinus* (L.). Journal of Fish Biology 37: 189-190.

Hinegardner, R. (1968). Evolution of cellular DNA content in teleost fishes. American Naturalist 102: 517-523.

Hinegardner, R. and D.E. Rosen (1972). Cellular DNA content and the evolution of teleostean fishes. American Naturalist 106: 621-644.

Johnson, O.W., F.M. Utter, and P.S. Rabinovitch (1987). Interspecies differences in salmonid cellular DNA identified by flow cytometry. Copeia 1987: 1001-1009.

Kalbassi M.H., Hosseini S.V., and Tahergorabi R. (2008). Karyotype Analysis in *Schizothorax zarudnyi* from Hamoon Lake, Iran. Turkish Journal of Fisheries and Aquatic Sciences 8: 335-340.

Karbe, L. (1964). Die chromosomen verhaltnisse bei den Coregonen des bodensees und einiger weiterer voralpiner seen, ein beitrag zum problem der speziation in der gattung *Corregonus* Z. Zoology Systematics Evolution. (2):18-40.

Kato, F. and Y. Ojima (1991). Karyotypes of four species of *Corydoras* catfishes (Siluriformes, Callichthyidae). Chromosome Information Service, 52:26-29.

Khuda-Bukhsh, A.R. and K. Nayak (1982). Karyomorpological studies in two species of hill stream fishes from Kashmir, India: occurrence of a high number of chromosomes. Chromosome Information Service. Japan 33:12-14.

Kilic Demirok, N. & Ü nlu, E. (2001). Karyotypes of cyprinid fish *Capoeta trutta* and *Capoeta capoeta umbla* (Cyprinidae) from the Tigris River. Turkish Journal of Zoology 25: 389–393.

Kottelat, M. and J. Freyhof (2007). Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland. 646 p.

Kryzanov, E.Yu. (1999). Karyotypes of *Varicorhinus capoeta* and *Barbus goktschaicus* (Cypriniformes) from Lake Sevan. Journal of Ichthyology 39: 187–189.

Li, Y., Li, K. & Zhou, T. (1983b). Acta Genetica Sinica. (Beijing) 10(5): 384-389.

Lockwood, S.F. and J.W. Bickham (1991). Genetic stock assessment of spawning Arctic cisco (*Coregonus autumnalis*) populations by flow cytometric determination of DNA content. Cytometry 12: 260-267.

Lockwood, S.F., B.T. Seavey, R.E. Dillinger, and J.W. Bickham (1991b). Variation in DNA content among age classes of broad whitefish (*Coregonus nasus*) from the Sagavanirktok River delta. Canadian Journal of Zoology 69: 1335-1338.

Magtoon, W. and R. Arai (1993). Karyotypes and distribution of nucleolus organizer regions in cyprinid fishes from Thailand. Japanese Journal of Ichthyology. 40(1):77-85.

Manna, G. K., and R. Prasad. (1973). Chromosomes of three species of fish (*Channa*). Nucleus 16:150-157.

Manna, G.K. and R. Prasad (1971). A new perspective in the mechanism of evolution of chromosomes in fishes. p. 237-240. In J. Cytol. Genet. Congr./ Proc. 1st All Ind. Cong. Cytol. and Genet., Cytol. and Genet. Cong.

Mauro, M.L. and G. Micheli (1979). DNA reassociation kinetics in diploid and phylogenetically tetraploid cyprinidae. Journal of Experimental Zoology 208: 407-416.

Mazik, E.Y. (1982). Some data about chromosomal sets of the genus *Diptychus*. Theses of reports. Probl. bioecol. animals and plants and environment protection:33-34. Frunze.

Mazik, E.Y., A.T. Toktosunov and P. Ráb (1989). Karyotype study of four species of the genus *Diptychus* (Pisces, Cyprinidae), with remarks on polyploidy of schizothoracine fishes. Folia Zoologica. 38:325-332.

Mirsky, A. E. and H. Ris (1951). The desoxyribonucleic acid content of animal cells and its evolutionary significance. Journal of General Physiology 34: 451–462.

Nakabo, T. (2002). Fishes of Japan with pictorial keys to the species, English edition I. Tokai University Press, Japan, pp v-866.

Naran D., Skelton P.H., & Villet M.H. (2006). Karyology of the redfin minnows, genus *Pseudobarbus* Smith, 1841 (Teleostei: Cyprinidae): one of the evolutionary tetraploid lineages of South African barbines. African Zoology, 42(2): 254-260.

Naran D., Skelton P.H., & Villet M.H. (2007). Karyology of three evolutonarily heaxaploid southern African species of yellowfish, *Labeobarbus* Ruppel, 1836 (Cyprinidae). African Zoology, 42(2): 254-260.

Nygren, A., Leion, U., Nilsson, B. & Jahnke, M. (1971b). Cytological studies in *Coregonus* from Sweden. Annales Academiae Regiae Scientiarum Uppsaliensis 15, 5–20.

Nygren, A., Nilsson, B. & Jahnke, M. (1971a). Cytological studies in *Thymallus thymallus* and *Coregonus albula*. Hereditas 67, 269–274.

Oellermann, L.K. & Skelton, P.H. (1990). Hexaploidy in yellowfish species (Pisces:Cyprinidae) from southern Africa. Journal of Fish Biology 37: 105–115.

Ohno, S., J. Muramoto, C. Stenius, L. Christian, W.A. Kittrell, and N.B. Atkin (1969a). Microchromosomes in Holocephalian, Chondrostean and Holostean fishes. Chromosoma 26: 35-40.

Ojima, Y. and K. Yamamoto (1990). Cellular DNA contents of fishes determined by flow cytometry. La Kromosomo II 57: 1871-1888.

Ojima, Y., K. Maeki, S. Takayama, and S. Nogusa (1963). A cytotaxonomic study on the Salmonidae. The Nucleus 6: 91-98.

Oliveira, C., L.F. Almeida-Toledo, L. Mori, and S.A. Toledo-Filho (1992). Extensive chromosomal rearrangements and nuclear DNA content changes in the evolution of the armoured catfishes genus *Corydoras* (Pisces, Siluriformes, Callichthyidae). Journal of Fish Biology 40: 419-431.

Oliveira, C., L.F. Almeida-Toledo, L. Mori, and S.A. Toledo-Filho (1993b). Cytogenetic and DNA content in six genera of the family Callichthyidae (Pisces, Siluriformes). Caryologia 46: 171-188.

Pandian, T.J. and Koteeswaran, R. (1999). Natural occurrence of monoploids and polyploids in the Indian catfish *Heteropneustes fossilis*. Current Science 76.

Porto, J.I.R. and E. Feldberg (1992). Comparative cytogentic study of the armored catfishes of the genus *Hoplosternum* (Siluriformes, Callichthyidae). Revista Brasileira De Genetica. 15:359-367.

Ráb, P. (1981). Karyotype of European Mudminnow, *Umbra krameri*. Copeia, 4: 911-913.

Rab, P. & Jankun, M. (1992). Chromosome studies of coregonine fishes: a review. Pol Arch. Hydrobiol. 39, 523–465.

Rab, P. & Liehman, P. (1982). Chromosome study of Danube salmon *Hucho hucho* (Linnaeus, 1758) (Pisces: Salmonidae). Folia Zoologica 31, 181–190.

Ráb, P., Crossman, E.J., Reed, K.M., Rábová, M. (2002). Chromosomal characteristics of ribosomal DNA in two extant species of North American mudminows *Umbra pygmaea* and *U. limi* (Euteleostei: Umbridae). Cytogenetics and Genome Research, 98: 194 - 198.

Rasch, E.M. (1985). DNA "standards" and the range of accurate DNA estimates by Feulgen absorption microspectrophotometry. In: Advances in Microscopy, edited by R.R. Cowden and S.H. Harrison. Alan R. Liss, New York, pp.137-166.

Rishi, K.K. & Haobam, M.S. (1984). Karyotypes of three forms of fishes having high chromosome number. International Journal of Academic Ichthyology 5: 139–144.

Rishi, K.K., M.P. Sharma and R. Mankotia (1977). Somatic chromosomes of three Indian teleosts. Matsya. 3:6-9.

Sands, D.D. (1994). The Behaviour and Evolutionary Ecology of *Corydoras adolfoi* and *Corydoras imitator*: Studies on Two Species of Sympatric Catfish from the Upper Rio Negro, Brazil. PhD. Thesis, Liverpool, UK.

Scheel J.J., Simonsen V., and Gyldenholm A.O. (1971). The karyotypes and some electrophoretic patterns of fourteen species of the genus *Corydoras*. Z. zool. Syst. Evolut. - forsch. 10: 144-152.

Severin, S. O. & Zinovyev, E. A. (1982). Karyotypes of isolated populations of *Thymallus arcticus* (Pallas) in Ob River basin. Voprosy Ichtiologii, 22 (1), 27–35.

Shimabukuro-Dias C.K., Oliveira C., and Foresti F. (2004). Karyotype variability in eleven species of the catfish genus *Corydoras* (Siluriformes: Callichthyidae). Ichtyological Exploration of Freshwaters, Vol 15, No. 2, pp. 135-146.

Skelton, P.H. and D. Naran (1995). Polyploidy in southern African barbine cyprinids. p. 62. In: Abstr. VIII Congr. Soc. Europ. Ichthyol., Oviedo.

Sola, L., Natili, G. L. & Cataudella, S. (1989). Chromosome variability in introduced whitefishes (genus *Coregonus*) from 2 lakes in Central Italy. Copeia 1989, 189–192.

Suzuki, A. (1992b). Karyotype and DNA contents of the Chinese catostomid fish, *Myxocyprinius asiaticus*. Chromosome Information Service 53: 25-27.

Suzuki, A. and Y. Taki (1988). Karyotype and DNA content in the cyprinid *Catlocarpio siamensis*. Japanese Journal of Ichthyology 35: 389-391.

Suzuki, A., Y. Taki, M. Mochizuki, and J. Hirata (1995). Chromosomal speciation in Eurasian and Japanese Cyprinidae (Pisces, Cypriniformes). Cytobios 83: 171-186.

Tiersch, T. R., Chandler, R. W., Wachtel, S. S. & Elias, S. (1989a). Reference standards for flow cytometry and application in comparative studies of nuclear DNA content. Cytometry 10: 706–710.

Timofeeva, M.Y. and K.A. Kaviani (1964). Nucleic acids of unfertilized eggs and developing groundling embryos. Biokimiia 29: 96-100.

Tsigenopoulos, C.S., Rab P., Naran D. and Berrebi P. (2002). Multiple origins of polyploidy in the phylogeny of southern African barbs (Cyprinidae) as inferred from mtDNA markers. Heredity 88, 466-473.

Turner, B.J., N. Diffoot, and E.M. Rasch (1992). The callichtyid catfish *Corydoras aeneus* is an unresolved diploid-tetraploid sibling species complex. Ichtyological Exploration of Freshwaters 3: 17-23.

Ueno, K. & Y. Ojima (1976). Diploid-tetraploid complexes in the genus *Cobitis* (Cobitidae, Cyprinoidei). Proc. Japan Acad. 52 (8): 446-449.

Uyeno, T. & G.R. Smith (1972b). Tetraploid origin of the karyotype of catostomid fishes. Science 175: 644-646.

Vasil'ev, V.P. (1985). The evolutionary karyology of fishes. (Evoljuzionnaja kariologiya ryb). 300p. Moscow: Nauka.

Vasil'ev, V.P., A.E. Vinogradov, Y.M. Rozanov, and E.D. Vasil'eva (1999). Cellular DNA content in different forms of the bisexual-unisexual complex of spined loaches of the genus *Cobitis* and in Luther's spined loach *C. lutheri* (Cobitidae). Journal of Ichthyology.

Vervoort, A. (1980). Tetraploidy in Protopterus (Dipnoi). Experientia 36: 294–296.

Vialli, M. (1957a). Volume et contenu en ADN par noyau. Experimental Cell Research Supplement. 4: 284-293.

Viktorovsky, R. M. & Maximova, T. G. (1978). Chromosome set of Amur whitefish and some problems in the evolution of the whitefish karyotype. Tsitologiya 20, 967–970.

Viktorovsky, R. M., Makoedov, A. N. & Shevchishin, A. A. (1985). The chromosomal sets of *Brachymystax lenok* and *Hucho taimen* and the divergency of the salmonid genera. Tstiologiya 27, 703–709.

Vinogradov, A.E. (1998a). Genome size and GC-percent in vertebrates as determined by flow cytometry: the triangular relationship. Cytometry 31: 100-109.

Wu, Z. & Yang, H. (1980). Acta Genetica Sinica. (Beijing) 7(4): 370- 375.

Yan X., Shi J., Sun X., Liang L. (2007). Study on the karyotype of *Gymnocypris przewalskii*. Journal of Northeast Agricultural University, 05.

Yu X., Zhou T., Li K., Li Y. & Zhou M. (1987). On the karyosystematics of cyprinid fishes and a summary of fish chromosome studies in China. Genetica 72: 225-236.

Zan, R. and Song, Z. (1980a). Acta Genetica Sinica. (Beijing) 7(1): 72-77.

Zan, R. and Z. Song (1980). Studies of karyotypes of eight species of fishes in *Cyprinus* and *Anabarilius* Zool. Res. 1:141-150. Kunming.

Zan, R., Liu, W. & Song, Z. (1984). Zool. Res. (Kunming) 5(1) Supplement: 82-99.

Zan, R., Song, Z. & Liu, W. (1986). Studies on karyotypes and DNA contents of some cyprinoid fishes, with notes on fish polyploids in China. In: Indo-Pacific Fish Biology, (eds) T. Uyeno, R. Arai, T. Taniuchi & K. Matsuuram, pp. 877–885. Ichthyological Society of Japan, Tokyo.

Zan, R., W. Liu and Z. Song (1985). Tetraploid-hexaploid relationship in Schizothoracinae Acta genet. sin. 12:137-142.

Zhang, S. M., Yan, Y., Deng, H., Wang, D. Q., Wei, Q. W., Wu, Q. J. (1999). Genome size, ploidy characters of several species of sturgeons and paddlefishes with comment on cellular evolution of Acipenseriformes. Acta Zoologica Sinica. 45: 200–206.