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DNA barcoding and population genetic structure of Malaysian marine fishes

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DNA BARCODING AND POPULATION GENETIC STRUCTURE OF MALAYSIAN MARINE FISHES

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ABSTRACT

The development of a widely available global database of DNA barcodes has been proposed as a species-identification tool for large taxonomic assemblages of animals. The approach has particular value in revealing cryptic species, which typically have high incidence in marine environments. Despite the wealth of DNA barcode data for fish from many temperate regions, there are relatively few such data available for SE Asian waters. In Chapter 2, an initial reference DNA barcode library was produced for the marine fish Family Carangidae, one of the most commercially-important families from the Indo-Malay Archipelago (IMA). Thirty-species of Family Carangidae were collected from the IMA to examine the accuracy of DNA barcoding concepts and protocols, such as ease of amplification of the barcode gene cytochrome *c* oxidase I (*COI*), and implementation of the 'barcoding gap' concept for species delimitation. All described species formed monophyletic clusters in Neighbour-joining phylogenetic tree, although three species representing complexes of six potential cryptic species were detected.

Within 723 individuals, three described species (*Atule mate, Selar crumenophthalmus* and *Seriolina nigrofasciata*) exhibited conspecific divergences up to ten times greater (4.32-4.82%) than mean estimates (0.24-0.39%) indicating a discrepancy with assigned morphological taxonomic identification, and the existence of cryptic species within the IMA. Additional conspecifics sequences available from other geographical regions revealed the existence of several more complexes of potentially cryptic species outside the IMA. However, to explain the hypothesis of species richness in the IMA, it is necessary to sample the whole family across their broad geographic range. Such information will contribute to the development of an integrated taxonomic framework, thus informing management strategies for subsequent conservation and management of Carangidae. Additionally, the results will provide greater understanding of recruitment, and processes driving species diversification in the IMA.

The effectiveness of molecular methods in detecting population structure of marine fish was examined in Chapter 3. Identification of population structure of fisheries stocks is important in the IMA because a large proportion of the IMA fisheries occur as mixed-stocks. *Selar crumenophthalmus* (pelagic), *Atule mate* (moderately pelagic) and *Selaroides leptolepis* (demersal) are commercially-important Carangidae with contrasting habitat use. It is unknown whether these three species in Malaysian waters form single respective stocks, or are genetically subdivided into distinct populations. Population structure inferred from nuclear as well as mtDNA markers was lower in the pelagic (*Selar crumenophthalmus*) and moderately pelagic *(Atule mate*) species than in the demersal species, *Selaroides leptolepis*, which is consistent with the hypothesis that pelagic and/or moderately pelagic species will display less genetic divergence compared to demersal species due to their potential to undertake long-distance migrations in oceanic waters. This chapter also examined population genetic structure of Indo-Malay *Atule mate* with samples from Kuwait. All analyses showed significant genetic differences between samples from these two localities. Within IMA itself, there were two mitochondrial lineages detected in *Atule mate* suggesting the existence of potential cryptic species. However, there were lack of genetic differentiation in *Selar crumenophthalmus* and *Selaroides leptolepis*. Such core information is required for their effective conservation and management since rates of harvesting have been reported as continuing to decline.

In Chapter 4, we focussed on a highly mobile pelagic species, *Selar crumenophthalmus,* to investigate its phylogeographic patterns and population genetic structuring within the IMA. It is important to know whether a population consists of one homogeneous population, or many discrete populations associated with different geographic areas. Samples were collected from six geographic regions; the South China Sea, Straits of Malacca, Sulu Sea, Celebes Sea, Andaman Sea and the Bismarck Sea. Both mtDNA and nuclear DNA data showed low genetic differentiation among localities with low F_{ST}

values indicating extensive gene flow within regions. Such data can provide a better understanding of potential isolation mechanisms of pelagic marine species, as well as to assist fisheries managers in designing suitable management plans in the IMA.

This thesis includes 1 published paper:

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CHAPTER 1

GENERAL INTRODUCTION

1.1 MARINE BIODIVERSITY

Biodiversity is the science that attempts to document and understand spatial patterns of biological diversity. It was defined by the Biodiversity Convention (2007) as the variability among living organisms from all sources including *inter alia*, terrestrial, marine and other aquatic ecosystem and the ecological complexes of which they are part (Angel, 1993). It is generally accepted that biodiversity is a hierarchical concept, where diversity is considered on several levels, most commonly the molecular, species and ecosystem levels (Magurran, 2004).

Genetic diversity is the most basic level of biodiversity, which is found within a species. This type of diversity refers to any variation among individuals within a population in their nucleotides, genes, chromosomes, or whole genomes. It is reflected by the level of similarity or differences in the genetic makeup of individuals, populations and ultimately, species. These similarities and differences are represented by differences in the sequences of nucleotides that form the DNA within the cells of the organisms. Nucleotide variation is measured for discrete sections of the chromosomes, called genes. Thus, each gene comprises a hereditary section of DNA that occupies a specific place of the chromosome, and controls a particular characteristic of an organism (Gray, 1997). A population is usually defined as a group of individuals that can interbreed and, if sexually reproducing, can exchange genetic material. Different populations tend to diverge genetically due to limited genetic flow or through mutations, with divergence, resulting from one or a combination of the effects of natural selection, genetic drift, demographic effects and the accumulation of selectively neutral mutations through time. This leads to the presence of unique genetic characteristics, which distinguishes members of a given population from those of any other population. The extent to which such population differentiation is associated with genetically-based variance in ecological traits can determine response to natural and man-made environmental change (Carvalho, 1993).

Species diversity refers to the variety of species in a certain region, and varies greatly among taxonomic groups and among geographic areas. It is influenced by species richness and evenness. Communities with more species are considered to be more diverse while evenness measures the variance in the abundance of individuals per species within the area. Grassle and Maciolek (1992) suggested that there might be 10 million undescribed organisms in the deep sea alone. In both, marine and terrestrial realms, diversity of the smaller organisms is much less well understood than the larger, often more charismatic organisms. It is the origins and extinctions of species that are the main components determining biological diversity. However, the contribution of species to overall diversity is not equal. Organisms that differ widely from each other (e.g. phylogenetic diversity) will contribute more to overall diversity compared to species that are more closely related. This illustrates that species richness may not be the best estimate for species diversity, and it may be essential to use different indices depending upon the questions being addressed (Magurran, 2004).

Species richness, or the number of species within a certain area, is one of the most straightforward ways to measure biological diversity. However, counting the exact number of species occurring in an area is a difficult task because a majority of the species are likely to be very small, and difficult to identify and count in the field. Another highly important metric of species diversity is endemism. The uniqueness of an area may be assessed by the number of endemic species found there. A species is endemic to a particular area if it occurs only in that area and nowhere else. The degree of endemism is an indication of an area's evolutionary importance in a wider context. Sites rich in endemic species can be viewed as areas of importance for speciation, or refuges for relict species (Gray, 1997). For example, in the Indian Ocean, of the 482 coral species recorded, 27% occur only at a single site (Sheppard, 1994) and of the 1200 species of echinoderms found at 16 sites, 47% occurred at only at a single site (Clark and Rowe, 1971).

Spectacular biodiversity exists in tropical marine ecosystems. Within the tropics, the Indo-Pacific region has much higher species diversity than the Caribbean because the Indo-Pacific, as an older region, has had more time for speciation events to occur. Most marine species diversity is benthic rather than pelagic (Angel, 1993). The pelagic realm has an enormous volume compared with the benthic realm. Angel (1993) also estimated that there are probably only 1,200 oceanic fish species compared with 13,000 coastal species.

One 'mega-diverse' tropical region, where the ranges of many tropical marine species overlap in a centre of maximum marine biodiversity, is located in the Indo-Malay Archipelago (IMA). This region is also referred to as the East Indies Triangle, and includes Malaysia, the Philippines, Indonesia, and Papua New Guinea (Figure 1.1). Due to its fauna depending to a large extent on the presence of coral reefs, it has been referred to as the Coral Triangle (Reaka *et al*., 2008). Coral reefs in this triangle harbour a high diversity of marine habitats that can accommodate many species of invertebrates, but studies that present accurate data on levels of diversity of these species are scarce (Porter and Tougas, 2001). In spite of this incomplete information, it is clear that Indo-West Pacific coral reefs are the world's most speciose marine ecosystems (Hughes *et al*., 2002). Over 50% of the Indo-West Pacific reef area occurs in the central Indo-Pacific, the border area of the eastern Indian Ocean and the western Pacific, including the seas of East and Southeast Asia, New Guinea, and Australia. Ekman (1953) considered the Malay Archipelago as the faunistic centre of the Indo-West Pacific where species dispersed to peripheral areas (Hoeksema, 2007). The archipelago consists of over 25,000 islands of different sizes, shapes and geological origins, spread over a distance of around 5,000 km from east to west. Coral reefs fringing the islands are separated by open water, frequently subjected to strong and complex currents, so that the region does not provide a continuous, but instead a rather patchy coral reef habitat (Timm and Kochzius, 2008).

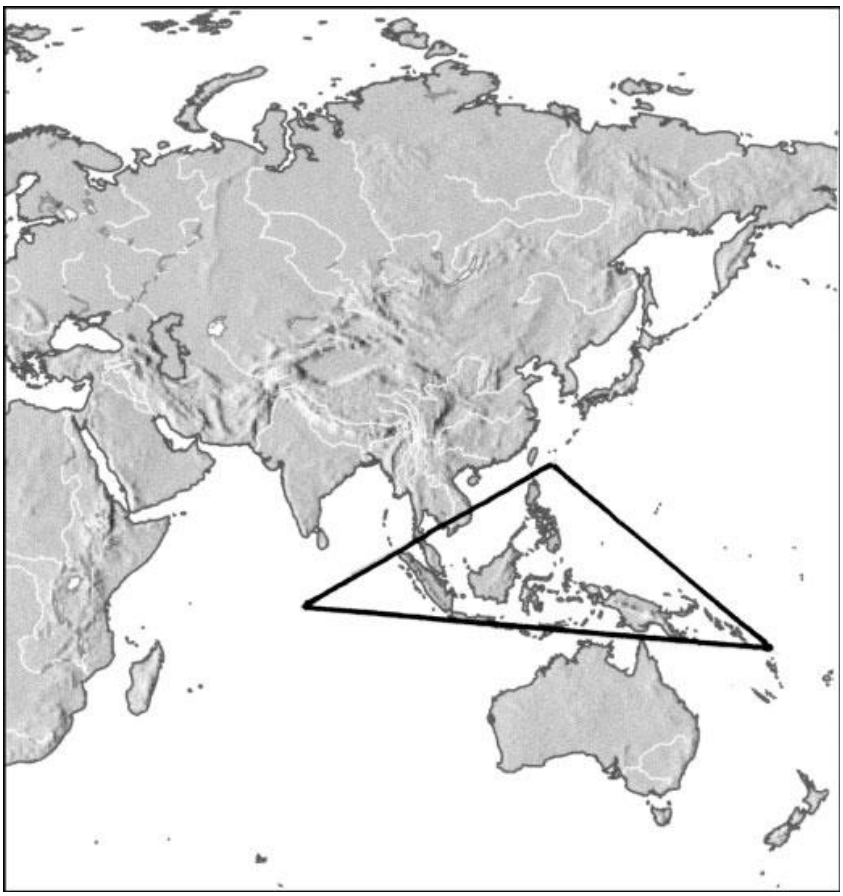


Figure 1.1 The East Indies Triangle in the Indo-West Pacific Ocean, a centre of origin for tropical marine species (Briggs, 2005).

Various hypotheses giving rise to this extraordinary species richness have been proposed (Reaka *et al*., 2008). Species richness might either be the result of diversification within the region and subsequent species dispersion to marginal locations ('Centre of Origin'; Briggs, 2005); by speciation in several areas peripheral to the central region, and these species subsequently extending their ranges into the region by way of prevailing currents, for example ('Centre of Accumulation'; Jokiel and Martinelli, 1992); or finally, the result of an overlap of the faunas from the Indian and Pacific Ocean ('Centre of Overlap'; Woodland, 1983). Recently, two of these theories in particular have been widely addressed (Hubert *et al*., 2012); the Centre of Overlap and the Centre of Origin hypotheses, both of which postulate contrasting patterns of species ranges and distribution of species richness. The former proposes geographic

isolation and allopatric speciation with midpoint ranges of species distributions falling on each side of the Indo-Malay Archipelago, with overlap across the IMA. This hypothesis is based on the premise that the isolating mechanisms is the shallow Sunda and Sahul shelves of Indonesia, Malaysia and Northern Australia, known as Indo-Pacific Barrier (IPB). Under this hypothesis, the faunas of the Pacific and Indian Oceans gained distinction during historical low sea-level stands when dispersal was restricted between ocean basins. Following sea-level rise, the geographical ranges of sister taxa formerly separated by the IPB expanded and eventually came to overlap in the IMA hotspot. Large-scale genetic structure is expected to result from geographic isolation and cryptic species may be expected to exhibit allopatric distributional ranges, potentially overlapping in the IMA. The Centre of Origin hypothesis proposes high rate of speciation centred in the IMA, with new species radiating out from this centre. Ekman (1986) suggested that the decline in species richness with distance from the IMA is an artifact of prevailing currents that impede outward dispersal. The elevation of speciation rate is explained by a few mechanisms, including the fracturing of populations as a result of the geological complexity of the region and eustatic sea-level changes (McManus, 1985); intense competiton (Briggs, 2005) and differing selection pressures in a highly heterogeneous environment (Rocha and Bowen, 2008). Large-scale genetic structure is expected to be shallow as a consequence of high connectivity and larval dispersal across the IMA. In Chapter 2, we test whether there is any evidence of highly divergent cryptic lineages in sympatry, as predicted by the Centre-of-Origin hypothesis.

1.2 BIOGEOGRAPHY AND GEOLOGICAL HISTORY OF SOUTHEAST ASIA

1.2.1 Biogeography

Southeast (SE) Asia is a sub-region of Asia, comprising the countries that are geographically south of China, east of India and north of Australia and New Guinea. It consists of two geographic regions: the mainland, and island arcs or archipelagos to the east and southeast. The mainland comprises Myanmar, Cambodia, Laos, Thailand, Vietnam and Peninsular Malaysia, while the maritime section consists of Brunei, East Timor, Indonesia, Malaysia (Sabah and Sarawak), the Philippines and Singapore (Figure 1.2). The SE Asian Archipelago is the largest aggregation of islands in the world. It includes most of the world's largest islands namely Borneo, Sumatra and Java, each surrounded by a constellation of lesser islands. New Guinea lies on the Sahul continental shelf with Australia and shares much of its fauna with that island-continent. Likewise, Borneo, Sumatra and Java lie on the continental shelf of Asia that is called the Sunda Shelf. Between these two continental shelves are the oceanic islands of Wallacea, which originated primarily as island arcs at pressure points between sliding oceanic plates; these tectonic forces have caused geological uplift and volcanism (Heaney, 1986). The Archipelago consists of over 25,000 islands of different sizes and topologies, and geological evidence indicates that several historical groups exist; some islands are strictly oceanic (e.g parts of the Philippines), some are fragments of once-larger islands or landmasses (e.g Sulawesi), and some, such as Borneo and Sumatra, had land-bridge connections to the Asian mainland (Heaney, 1986). These islands are distributed over a distance of around 5,000 km from east to west (Hall and Blundell, 1996; Tomascik *et al.*, 1997).

The geological and climatological complexity of the SE Asian region has most likely contributed to the tremendous biological diversity of the region. The diversity of the terrestrial biota probably results from the meeting and mixing of the flora and faunas

from two major zoogeographic regions, and the opportunities for isolation and speciation on the islands of SE Asia (Hall, 2002). In the terrestrial realm, Wallace's Line separates terrestrial flora and fauna into Asian (west) and Australasian (east) elements (Figure 1.3). Marine organisms, by contrast, are expected to show a north-east to south-west division perpendicular to Wallace's Line (Benzie, 1998). The two potential causes for Wallace's Line are: firstly, the ongoing collision of Asian and Australasian tectonic elements beginning ca. 15 million years ago (Mya), which brought terrestrial organisms from different realms into close contact (Hall, 2002); and secondly, periods of lowered sea-levels (up to at least 120 m below present levels) associated with Pleistocene glaciations (2.4 Ma to 10 000 years ago) which resulted in the formation of land-bridges between mainland Asia, Borneo and other western Indonesia islands (e.g. Java and Sumatra), and among some Philippine islands (Voris, 2000).

Figure 1.2 Topological map of Southeast Asia including parts of continental Asia and Australia.

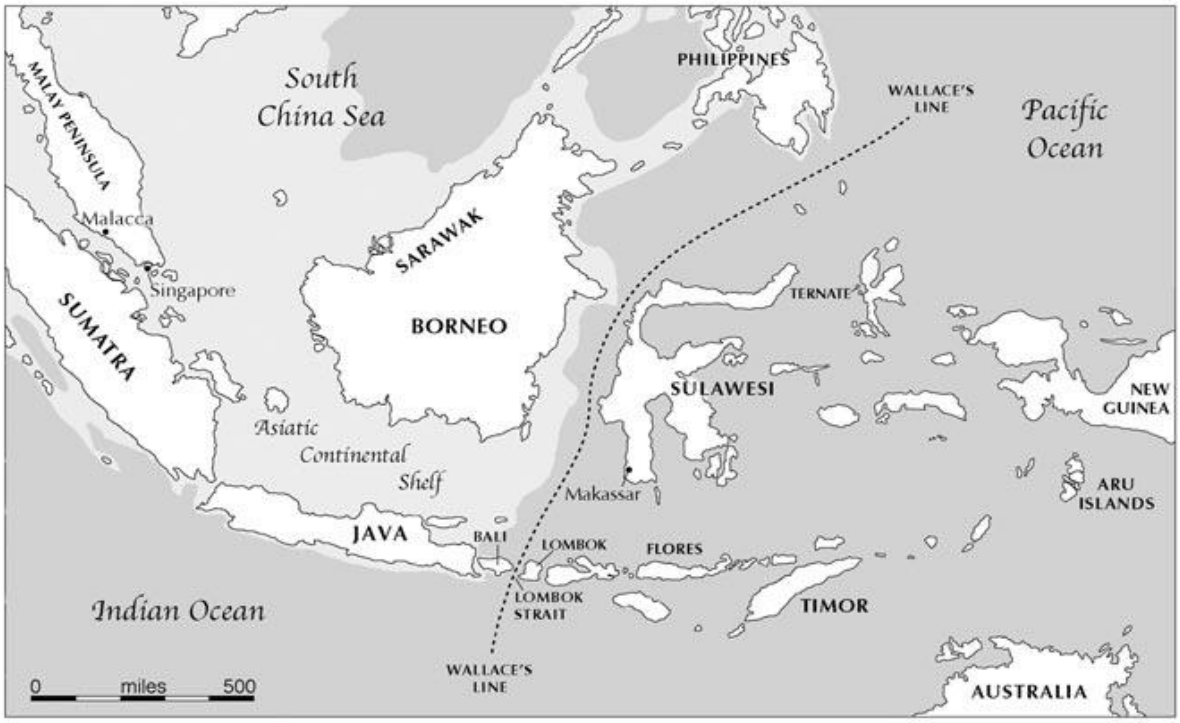


Figure 1.3 Wallace's Line separates terrestrial flora and fauna into Asian (west) and Australasian (east) elements. Pleistocene emergence of the Sunda Shelf (Sundaland) and parts of the Philippines illustrated in light grey shading.

1.2.2 A brief history of the geological history of Southeast Asia

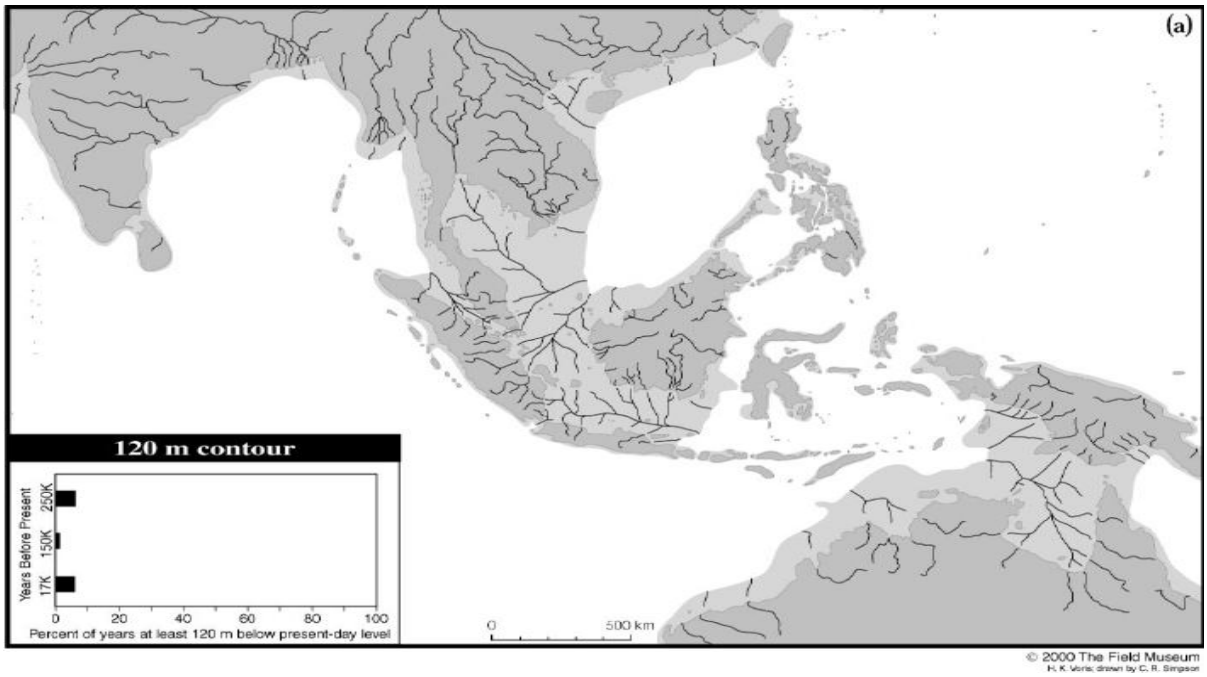
The geological and maritime events that led to the formation of SE Asia are some of the most complex in the earth's history. This involved plate tectonics of the Indian, Pacific and Philippine ocean plates and the Eurasian, Indian and Australian land plates, and also the emergence and disappearance of various seas (Hall, 1998). There have been conflicting theories among geologists regarding the individual events and their chronological sequence mostly due to insufficient data (Rangin *et al*., 1990; Lee and Lawver, 1994; Hall, 1996). However, it is now widely accepted that three major events in the Cenozoic era resulted in the current geography of the region: the collision of India with Eurasia during the Eocene about 45 Mya, the collision of the Australian plate with the Philippine sea arc plate during the Miocene about 25 Mya, causing further

rotation of SE Asian microcontinental fragments, and finally the collision of the Philippine arc with Eurasia in Taiwan about 5 Mya (Hall, 1996, 1998, 2001).

1.2.2.1 Pleistocene conditions

The Pleistocene glaciations were arguably the most significant historical events to have occurred during the evolutionary lifespan of most extant species. Large areas of the world's landmasses were repeatedly buried under vast sheets of ice, causing drastic alterations on continental scales. It is estimated that up to 20 glaciations occurred during the Pleistocene (Martinson *et al*., 1987). Each glaciation spanned approximately 100,000 years, with interglacial periods lasting 10,000 to 12,000 years (Dawson, 1992). The growth and recession of continental glaciers during the Pleistocene were associated on a global basis with changes in sea level and temperature (Heaney, 1986). Sea levels in SE Asia were some 120 metres below the present level (BPL) during the Last Glacial Maximum (LGM) (some 17,000 year BP), but rapidly rose to approximately 5 metres above the present level some 5,000 years before present (YBP) before falling gradually to its present level. During the LGM, the bulk of the Sunda and Sahul shelves were largely exposed and formed large landmasses. Sunda land connected the islands of Borneo, Java and Sumatra with continental Asia. If one considers new contiguous shelf exposed south and east of the Isthmus of Kra, an additional 1.53 million sq km of land was annexed to SE Asia (Voris, 2000). This indicates that at the LGM, exposed land almost doubled in area, to 94% (3.2 million sq km) larger than compared to today. A consequence of this change in surface area is that several of the shallow seas in the Indonesian region did not exist at the LGM (De Decker *et al*., 2002). Sulawesi remained separated from Borneo by a narrow but very deep ocean trench, through which the Indonesian Throughflow still passes today. The Indonesian Throughflow carries a huge volume of fast-flowing water (~ 12 Sverdrup, Sv = 106 $m^3.s^{-1}$; Meyers *et al.* 1995) between the islands of Borneo and Bali to the west, and Sulawesi and Lombok to the east. The Makassar Strait, through which the Indonesian Throughflow travels, acts as

the western boundary for the Australian and Asian biotic transition zone (Wallacea) (Figure 1.3). To the east, the exposed Sahul Shelf broadly connected Australia and New Guinea and the Aru Islands (Sathiamurthy and Voris, 2006), and disconnected the marine passage through the Torres Strait. At 20 metres BPL, Voris (2000) demonstrated that there were no longer land bridges between the Malay Peninsula, Sumatra, Java and Borneo, although it is possible that the Malay Peninsula and Sumatra were still connected by a largely freshwater estuary (Figure 1.4)



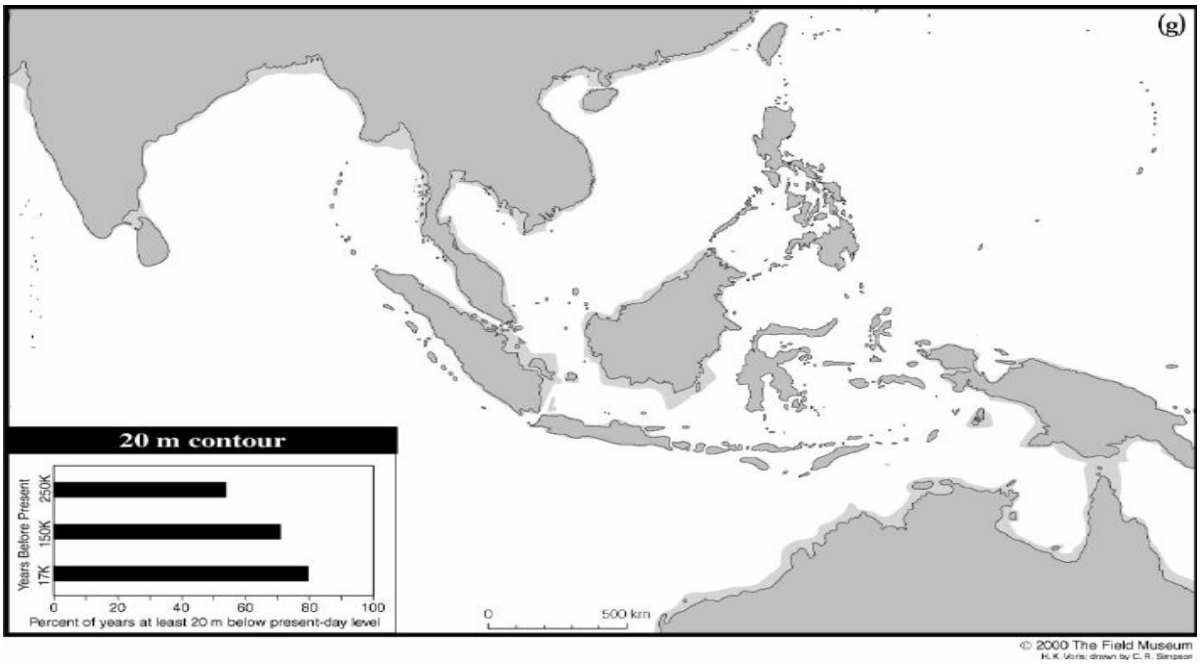


Figure 1.4 Maps of tropical Southeast Asia and Austral-Asia illustrating depth contours of 20 (bottom) and 120 m (top) below present level.

*The origin of the base maps is a Geographic projection in ArcView (Environmental Systems Research Institute, Inc.). Each map has a small horizontal bar graph in the lower left corner that provides estimates of the percentage of time that the sea level was at or below the level illustrated on the map during the past 17,000, 150,000 and 250,000 years. The percentage time estimates for 17,000, 150,000 and 250,000 year intervals are based on Fairbanks (1989), Bloom & Yonekura (1990) and Chappel & Shackelton (1986), respectively. Maps are from Voris *et al*., 2000.

1.2.2.2 Glacial impacts on marine taxa

Past geological and climatic events have undoubtedly played a major role in terrestrial and marine biogeography. Marine passages between the Pacific and Indian Oceans would have been drastically reduced when the Asian and Australasian elements collided in the Miocene, and almost completely interrupted when sea levels were lowered in the Pleistocene (Hall, 1998; Voris, 2000). Sea level fluctuations exposed the Sunda and Sahul continental shelves during the Pleistocene, resulting in the emergence of land barriers that isolated the South China Sea from the Indian Ocean at its southern limit and from the Sulu Sea eastbound (Rohfritsch and Borsa, 2005). This is thought to be the cause of a phylogeographic break between Indian and Pacific Ocean populations of several marine taxa at the species, subspecies or population levels, and also a factor contributing to the greater species diversity reported for the Indo-Malay region (Hewitt, 2000).

Numerous molecular phylogenetic and population genetic studies on various marine fishes and invertebrates have revealed a genetic discontinuity between the Indian and Pacific Oceans (Barber *et al*., 2002; Lourie and Vincent, 2004; Rohfritsch and Borsa, 2005; Crandall *et al*., 2008). This includes phylogeographic disjunction of barramundi (*Lates calcarifer*) on either side of the Torres Straits (Chenoweth *et al*., 1998), which formed a land barrier connecting Australia and New Guinea during the Pleistocene (Voris, 2000). The phylogeographic structure of false clown anemonefish (*Amphiprion ocellaris*) was also explained by sea level changes during the Pleistocene, rather than by contemporary geography. Significant differences in cytochrome *b* haplotype frequencies were found between *A.ocellaris* populations from the western edge of the Sunda Shelf and those from the rest of the Indo-Malay Archipelago, including South China Sea, Sunda Straits, Bali Strait, Sulu Sea and Sulawesi Sea (Nelson *et al*., 2000).

In contrast with the examples of inshore fishes and invertebrates, little genetic heterogeneity has been reported for pelagic fishes from the Indo-Pacific. There were no significant genetic differences found between Indian and Pacific Ocean populations of big-eye tuna (*Thunnus obesus*) (Chow *et al*., 2000) or Indo-Pacific sailfish (*Istiophorus platypterus*) (Graves and McDowell, 1995). Although a preliminary survey revealed a difference in genetic lineages of Indian scad mackerel (*Decapterus russelli*) (Perrin and Borsa, 2001), no genetic differences were evident at either mitochondrial or nuclear loci among round scad mackerel (*Decapterus macrosoma*) at the scale of Indo-Malay Archipelago populations (Borsa, 2003). Pelagic fishes spend their entire life in the open sea. Pleistocene changes in sea level may have caused temporary geographic isolation but it is expected that in the subsequent secondary contact between populations, the active dispersal in this continuous, pelagic habitat is more likely to have erased past genetic discontinuities than in the case of sedentary coastal species.

1.3 STATUS OF MARINE FISHERIES IN MALAYSIA

Marine fisheries are significant to the Malaysian economy for four main reasons: as a source of food and protein, as a major contributor to gross domestic product, as a source of employment, and for the generation of foreign exchange earnings. Because of its geomorphological features, Malaysia is endowed with various marine ecosystems suitable for the development of fishing industry. These ecosystems include mudflats, coral reefs, estuaries, mangrove swamps, coastal areas and continental shelves. The South China Sea, Strait of Malacca, Sulu Sea, Java Sea and Andaman Sea offer opportunities for exploitation of marine fish by Malaysia.

The Malaysian marine or capture fisheries are distributed primarily in the coastal waters within the 0 to 30 mile limit from the shoreline. Fishing methods employed are

varied, ranging from simple labour-intensive traditional gears such as lift net, bag net, barrier net, push net, traps and hook-and-line to modern, highly productive gears like trawling and purse-seining. While the traditional gears are operated using mainly small (below 40 Gross registered tonnes (GRT)) non-powered or outboard-powered fishing vessels, trawling and purse-seining, on the other hand, employ larger (above 40 GRT) more expensive fishing vessels (Mohsin and Ambak, 1996).

The fisheries sector can be divided into two distinct sub-sectors; small-scale or artisanal fishery and large scale or commercialised fishery. Small-scale fisheries are characterised by low capital investment, low productivity and little use of specialised skills. They are often labour intensive, with all members in the household being involved. Commercialised fishing is carried out in waters beyond inshore areas, characterised by the use of expensive technology and larger vessels capable of exploiting fish resources in waters beyond 12 miles (20km) from the coastline. Conventionally, the small-scale artisanal fishery is distinguished from the large-scale commercialised fishery on the basis of gear technology. Hence, it is customary to classify trawlers and purse-seiners as large-scale, while traditional gears such as lift net, bag net, barrier net, push net, traps and hook-and-lines are usually classified as small-scale (Ahmad *et al*., 2003).

1.3.1 Peninsular Malaysia

Although approximately 300 species of marine food fish are landed throughout Malaysia, only about 100 species are commonly displayed and sold in the local markets. Mohsin and Ambak (1996) managed to collect 712 species of fish, representing 28 orders and 138 families of which 300 species are commercially-important. The great variety of species that appear in the markets reflects not only the species diversity in the tropics, but also the customer's preference. The majority of these fishes can be grouped as reef fishes (snappers and basses), carangids (jacks,

pompanos and scads), mackerels (Spanish and Indian), tuna, clupeids (herring, sardines, anchovies), pomfrets, and shark and rays.

Based on the behavioural pattern of fish resources, marine fishing in Malaysia can be categorised into pelagic and demersal fisheries. Pelagic fisheries are mainly concentrated on the migratory species which dominate the fish landings in both the west and east coasts of Peninsular Malaysia. The top species in terms of landings are the Scombrids (*Rastrelligar kanagurta, R. bachysoma, Euthynnus affinis, Auxis thazard* and *Thunnus tonggol*) and the Carangids (*Decapterus spp., Atule mate, Selar* spp. and *Megalaspis cordyla*), while the demersal fisheries are mainly focussed on bottom-living crustaceans and fish. The major groups of demersal species landed in Peninsular Malaysia are Nemipteridae, Sciaenidae, Lutjanidae, Serranidae, Carangidae and Mullidae (Biusing, 2001).

The landing of fishes is not equally distributed, as their abundance varies depending on locality and seasonality. Pelagic fishes are more abundant in open seas such as the South China Sea and the Andaman Sea, and tend to show seasonality related to monsoonal changes. During the Northeast Monsoon (December to March), many of the offshore pelagic species tend to move closer to shore. Carangidae seem to dominate, and are widely distributed during the Northeast Monsoon, while certain pelagics such as *Decapterus* spp. and *Rastrelliger* spp. are also abundant during this season. On the east coast, gizzard shads (*Anodontostoma chachunda*) and mullets (*Liza* spp.) can be readily caught very close to shore. Tuna, Spanish mackerels and wolf herrings, however, are abundant during the Southwest Monsoon (April to November). Demersal fish, especially reef-related species, do not show a direct relationship with the monsoons. However, during the Northeast Monsoon, landings are low due to a decline in fishing intensity by handlines and bubu trap operations (Mohsin and Ambak, 1996).

1.3.2 Sabah and Sarawak

As in the case of Peninsular Malaysia, various fishing gears are deployed in Sabah and Sarawak. However, the major proportion of fish landings is from trawls, gill nets and purse seines. Gill nets are second in importance to trawls, contributing about 31% of the total fish catches (Department of Fisheries Statistics, 2007). In Sarawak, the important pelagics are *Scomberomorus* spp., *Rastrelligar* spp., *Megalaspis cordyla*, *Ilisha elongate*, *Coilia macrognathus* and *Hilsa* spp. *Hilsa* spp., locally known as Terubuk is conspicuously prominent compared to Peninsular Malaysia and Sabah. They are caught in fairly large quantities from May to July with a peak in July. *Ilisha elongate*, *Coilia macrognathus* and *Scomberomorus* spp. are abundant from March to September, peaking in June to July. Other pelagics include *Euthynnus affinis*, *Chirocentrus dorab*, *Sardinella* spp. and *Atule mate*. *Anodontostoma chachunda* and *Liza* spp. are less abundant, but are prominent during the Northeast Monsoon. Sharks are also more prominent in Sarawak than in the Peninsular, and are mainly landed from March to September using gill nets. On the other hand, the important pelagic species in Sabah are *Thunnus obesus*, *Scomberomorus* spp*., Decapterus macrosoma, Rastrelliger kanagurta, Sardinella* spp., *Megalaspis cordyla, Liza* spp., *Atule mate* and *Sphyraena* spp. The main fishing season for these species is from March to September. As in the case of Sarawak, *Liza* spp. are mainly caught during the Northeast Monsoon (Biusing, 2001).

As for demersal fish, the most abundant species in Sarawak are *Johnius* spp., *Harpadon nehereus* and *Muraenesox* spp. They are landed all year round by trawls. In Sarawak, the demersal fishery is less important than the pelagic fishery. However, in Sabah, the demersal fishery is more important. The landings of the reef-associated species are quite significant and are mainly caught by handlines. The most important genus in Sabah is *Lutjanus* followed by *Epinephaelus, Nemipterus, Paracaesio, Arius, Carcharhinus* and *Dasyatis* (Mohsin and Ambak, 1996).

Having considered the fishery status in Malaysia, I will now focus the target group of the current study, the commercially-important fish family, Family Carangidae.

1.4 FAMILY CARANGIDAE

1.4.1 General information and distribution

The Carangidae are an important group of largely piscivorous predators found in the coastal waters of all subtropical and tropical seas. Randall *et al*. (1990) estimated a total of 25 genera and approximately 140 species in this family. The family is very important as food fishes, and play a significant role in the commercial fisheries of Malaysia. Carangidae encompasses a diverse group of fishes known variously by such common names as jacks, trevallies, amberjacks, pompanos, scads, kingfish, pilotfish and rainbow runners (Ambak and Mohsin, 1996).

1.4.2 Taxonomic description and morphology

Members of the family Carangidae are characterized by an anal fin with two anterior spines (one spine in *Elagatis* and *Seriolina*) separated from the rest of the fin, but which often become embedded with age. The caudal peduncle is very slender, and the caudal fin is deeply forked. The dorsal fin is generally divided into an anterior portion with four to eight spines and a posterior portion with one spine and 17 to 44 soft rays. In many carangids the last rays of the dorsal and anal fins are detached and form one to nine small posterior finlets. Pectoral fins are often long and falcate. The eye is usually protected by a transparent adipose eyelid, a thickened transparent skin covering much of the eyeball with only a small opening in the centre (Honebrink, 2000).

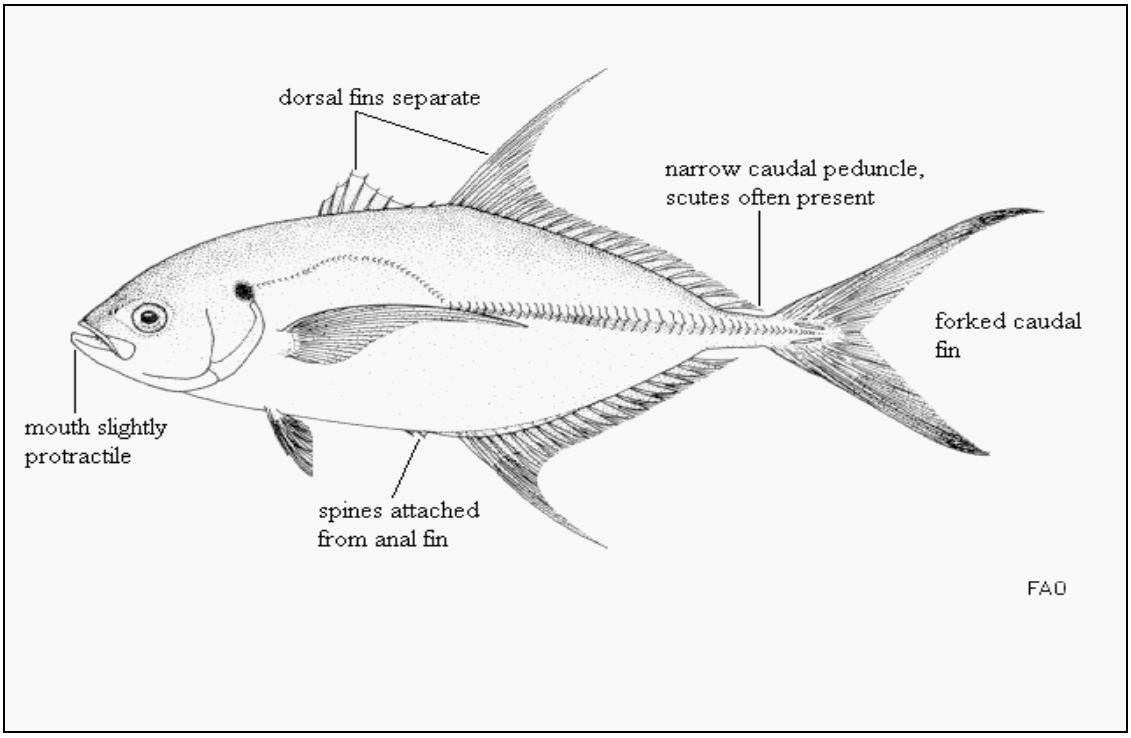


Figure 1.5 Diagnostic characteristics typical of the family Carangidae (Chan *et al*., 1984).

Carangids also possess small cycloid scales, which in most species are modified into a row of enlarged scutes along the posterior straight portion of the lateral line. In some carangids, particularly the genera *Carangoides* and *Caranx*, the breast is only partially scaled, and the pattern of breast squamation is useful for species identification. Body shape is generally compressed but extremely variable, ranging from slender forms like *Decapterus* and *Elagatis* to deep-bodied forms like *Selene*. The premaxilla is usually protrusible. Teeth range from small and villiform to large and conical, and are located variously on the premaxillae, dentary, vomer, palatines, tongue, and pharyngeals (Gunn 1990).

1.4.3 Biology and life history

Most of the Carangids are widely distributed within the Indo-Pacific region, ranging from the Indian Ocean to waters of the Hawaiian and Marquesas Islands in the central Pacific Ocean (Berry *et al*., 1981). The species in this family usually occur individually and in schools of up to several hundred fish, and are generally found nearshore, at depths of less than 20 m, but occasionally to around 100 m (Mohsin and Ambak, 1996).

Some species spawn pelagically, whereas others spawn close to shore. Spawning seasons for most species are fairly long, generally peaking during summer months. Spawning in the wild has, however, been described for only a few species, but seems to occur both repeatedly and periodically (Thresher, 1984). von Westernhagen (1974) observed the spawning behaviour of some of the most common species (*Alectis indicus*, *Alectis crinitus*, *Caranx ignobilis*, *Caranx malabaricus*, *Caranx sexfasciatus* and *Gnathodon speciosus*). The fishes occured in groups of sometimes more than 100 individuals, gathering close to shore, then splitting into smaller groups of three to four, and descending to two or three meters from the seafloor. Males were easily recognized by their black and white head region, and black dorsal surface. Eventually one male would pair up with a female, the two would sink to just above the seafloor, and slowly swim around each other. While circling, they were observed to release eggs and sperm into the water, during which time they could be easily approached. The eggs of *Atule mate* were found in open areas of the bay where bottom depth was at least 10 m (Watson and Leis, 1974).

Very little information is available concerning larval behavior of Carangidae. Cha *et al*. (1994) sampled fish larvae off the Florida keys and found 92.4% of Carangid larvae occurred in the upper 25 m of the water column, and 100% occurred in the upper 50 m. Development from larval through juvenile toward adult stages proceeds rather

directly in Carangids, and adult characters are gradually acquired. There are no known sudden developmental rate changes between stages. Leis (1991) indicates that carangid fishes do not settle. Young juveniles often associate with floating or drifting objects, including jellyfish, clumps of algae and flotsam.

Carangids are noted for the changes they undergo with growth (Bohlke and Chaplin 1993), and these changes have likely been responsible for misidentification of specimens and likely contributed to some of the general taxonomic confusion that has occurred. An interesting example of change with growth occurs in juveniles of the African pompano (*Alectis ciliaris*) and Indian threadfish (*Alectis indicus*) (Figure 1.6), which are easily recognized by the presence of long filaments trailing from the first four or five rays of the dorsal and anal fins. These filaments may be twice the length of the body, and as the fish gets larger they gradually shorten and eventually disappear. It has not been determined what exactly happens to the filaments, or their possible function. Randall *et al*. (1990) and Myers (1991) speculate the filaments may serve to mimic the stinging tentacles of jellyfishes for protection from predators. Migration to inshore waters likely occurs when the fish are 21 to 50 mm standard length (SL) (Berry, 1959). Some forms, such as *Naucrates ductor* and golden trevally *Gnathanodon speciosus* do not lose their juvenile banded patterning. *Naucrates ductor* juveniles apparently do not move inshore as they grow, and continue to accompany larger objects, including sharks and other large carnivorous fishes (Bohlke and Chaplin, 1993).

Figure 1.6 The presence of long filaments in juvenile (left) *Alectis indicus*, and gradually shorten when the fish gets larger (right). Juvenile fish was collected from Tawau, Sabah while adults from Miri, Sarawak during our field work in June 2010.

A number of authors note that the young of many carangid species are known to enter estuaries. Blaber and Cyrus (1983) studied this phenomenon in estuaries of Natal, South Africa. They identified 40 species of Carangidae in the waters of Natal, of which 17 have been recorded in estuaries. All are euryhaline but *Caranx melampygus, Caranx papuensis* and *Scomberoides lysan* are only found in clear water. It was suggested that the more widespread estuarine distribution of juvenile *Caranx sexfasciatus* and *Caranx ignobilis* may be related to their turbidity tolerances. In Hawaii, some juvenile *Caranx ignobilis* and *Caranx melampygus* occupy estuaries opportunistically as nurseries before moving to nearshore ocean habitats (Smith and Parrish, 2001).

Pelagic marine fishes usually have high fecundity, very large population sizes, and high dispersal potential at egg, larval, and adult stages. Limited genetic differentiation between geographic populations would therefore be predicted, because of the high levels of gene flow facilitated by such traits (Rohfritsch and Borsa, 2005). Philopatric behaviour, gamete incompatibility, differences in spawning time or location and habitat selection could, however, be factors involved in inducing, maintaining or enforcing genetic differentiation. Therefore in Chapter 3 of the thesis, three species of

Carangidae (*Atule mate, Selar crumenophthalmus and Selaroides leptolepis*) with contrasting habitat use were selected for comparison of genetic differentiation and population structuring.

1.5 TAXONOMIC TOOLS

Early works of Avise (Avise *et al*., 1987; Avise, 2004) had showed that molecular markers have for several decades been used to identify and discriminate species. The mitochondrial and nuclear genes have several advantages over classical taxonomic characters because they are discrete, heritable and generally stable in relation to environmental change, and therefore they have the potential to provide resolution across a time scale (Hillis, 1987). They can be used to study many systematic problems, from studies of evolutionary processes to the phylogeny of life. Next sections will describe in more detail the nature of mitochondrial and nuclear genes and their diversity.

1.5.1 Mitochondrial (mt) DNA

Organellar DNA in eukaryotic cells includes the mitochondrial genome, a circular molecule with bacterial origins that ranges in size from 16 to 18kb in length. In vertebrates, the mitochondrial genome generally consists of 37 genes (13 protein-coding genes, 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and a non-coding region (control region) (Avise, 2004). There are several reasons why mtDNA markers have been used extensively in studies of animal population genetics. MtDNA is relatively easy to work with. Its small size, coupled with the conserved arrangement of genes, means that many pairs of universal primers will amplify regions of the mitochondria in a wide variety of vertebrates and invertebrates. This means that data often can be obtained without any *a priori* knowledge about a particular species

mitochondrial DNA sequence. Animal mtDNA has high copy number, and introns and pseudogenes are rare, which usually makes gene amplification fairly straight-forward and reduces the likelihood of analytical error (Saccone *et al*., 1999; Avise, 2004). Most importantly, unlike nuclear DNA (nDNA), it does not recombine and is transmitted as a single unit from parent to offspring through a maternal mode of inheritance (Avise, 2004). Also, the rate of nucleotide evolution of mtDNA is higher than nDNA as DNA repair mechanisms are slower, allowing for the build-up of neutral mutations (Brown *et al*., 1979). Hence, lineage sorting occurs faster in mtDNA, with the effective population size for achieving reciprocal monophyly being several times lower than nDNA where ancestral polymorphisms can persist (Moore, 1995; Hudson and Turelli, 2003; Rosenberg, 2003; Kubatko *et al*., 2011). This renders mtDNA sequence data suitable for systematic studies even at lower (inter-specific and intra-specific) taxonomic levels and mtDNA a highly efficient marker for use in phylogenetic methods to trace genealogical evolutionary histories and divergences in organisms (Avise, 1989).

Mitochondrial DNA data on their own, however, have some important limitations. Firstly, mtDNA represents only a single locus. The maternal mode of inheritance will result in phylogenies that reflect patterns of maternal gene flow and dispersal (Avise, 2004). This view reflects at best only the matrilineal history, which could well differ from the overall history of populations or species. Therefore, the inference made on species or population history may be biased. Secondly, the effective population size of mtDNA is only a quarter of that of nuclear autosomal sequences, therefore mtDNA lineages have a much faster sorting rate and higher allele extinction rate. The consequences of such features are that evolutionary relationships could be oversimplified by mtDNA data, and mtDNA markers can underestimate genetic diversity. The uncertainty in genealogical analysis may increase due to the increased probability 'missing' of haplotypes, and some population processes may not be detected correctly with mtDNA markers (Zhang and Hewitt, 2003). In some cases,

mtDNA could be heterozygous and heteroplasmic (Blaxter, 2004), and multiple sampling of the same individual may become necessary, while occasionally mitochondrial transfer between sister species may also cause problems for correct species diagnoses (Tautz *et al*., 2003).

1.5.2 MtDNA genes

Different parts of the mtDNA genome evolve at different rates and are useful for resolving divergences at different taxonomic levels (Moritz *et al*., 1987; Avise, 2004). In the past, cytochrome *b* (Cyt *b*) has been the most widely-used animal mtDNA marker, having been successfully employed for a variety of phylogenetic and phylogeographic studies in animals (Farias *et al*., 2001). A protein-coding gene containing overall conservative and variable regions, Cyt *b* has both slow and rapidly evolving codon positions, and the third codon is especially informative for studying closely-related species (Farias *et al*., 2001). NADH dehydrogenase 4 (ND4), also a protein-coding gene, has been found to have very high nucleotide and amino acid substitution rates even at intra-specific and population levels, and has been recommended as a useful gene for resolving cryptic species (Blouin *et al*., 1998). The cytochrome *c* oxidase I gene (*COI*) has base substitution rates similar to that of Cyt *b*, but the amino acid sequence evolution is slower, and it therefore provides a deeper and greater range of phylogenetic signal than any other mtDNA gene for assigning taxa to both higher and lower taxonomic groups (Hebert *et al*., 2003a; Hebert *et al*., 2003b). *COI* is thus rapidly becoming the mtDNA gene of choice for systematic studies as a result of the DNA barcoding project, which is further discussed in Section 1.5.3 (Hebert *et al*., 2003a).

1.5.3 DNA Barcoding

1.5.3.1 Concept and hypothesis

The numbers of eukaryotic species worldwide are estimated at 3.6 million to 100 million, with approximately only 1.5 to 1.8 million species having been described to date (Wilson, 2004). Multiple taxonomic experts are ordinarily required to identify specimens from even a single biotic survey, and the identification is dependent on the knowledge held by the taxonomists whose work cannot cover all taxon identification requested by non-specialists. Assembling teams of appropriate experts, or distributing specimens to them for identification, are both time consuming and expensive tasks. Moreover, accessing existing literature and assessing the validity and priority of various taxon names can be a challenge even for the expert taxonomist. For the non-specialist faced with an assemblage of suboptimal specimens that require species identifications in real time, no method currently exists to bring the sum total of taxonomic knowledge to bear on the problem. This fact is a major impediment to the assessment, conservation and management of global biodiversity (Hanner *et al*., 2005). Another problem is that many taxonomic protocols rely on phenotypic characters, and require lengthy and detailed inspection of the specimens (Costa and Carvalho, 2007). These traditional methods of identifying, naming and classifying organisms are largely based on visible morphology. There are limitations to this method when attempting to identify organisms during various stages of their development not considered in original treatments, or when examining fragmentary or processed remains (Hanner *et al*., 2005).

Therefore, to deal with these problems, Hebert *et al*. (2003a) introduced the concept of a DNA barcode and proposed a new approach to species identification. DNA barcoding offers several advantages compared to conventional taxonomic identification. One obvious advantage comes from the rapid acquisition of molecular

data. As a contrast, morphological data gathering can be time consuming and difficult. Furthermore, in three important situations, relevant species identification must necessarily be molecular based: 1. in determining the taxonomic identity of damaged organisms or fragments; 2. molecular-based identification is necessary when there are no obvious means to match adults with immature specimens such as fish larvae (Pegg *et al*., 2006); 3. when morphological traits do not clearly discriminate species, especially when size precludes visual identification or if species have polymorphic life cycles (Blaxter *et al*., 2005). The efficiency of DNA barcoding has also been reported in the detection and description of cryptic species (Pfenninger *et al*., 2007; Gomez *et al*., 2007; Zemlak *et al*., 2009; Smith *et al*., 2011; Hubert *et al*., 2012; Kadarusman *et al*., 2012; Puckridge *et al*., 2013), and of sibling species (Amaral *et al*. 2007 ; Van Velzen *et al*., 2007).

The main goals of DNA barcoding are to identify unknown specimens to species level, and enhance the discovery of new species and facilitate identification, particularly in cryptic, microscopic and other organisms with complex or inaccessible morphologies (Hebert *et al*., 2003a; Hebert *et al*., 2004a). The ultimate goal of the DNA barcoding movement is the development of comprehensive barcode libraries for all species on earth. The access to a public reference database of taxa allowing identification of a wide range of species will be beneficial whenever accurate taxonomic identification is required (Frezel and Leblois, 2008). Therefore, a project called the DNA Barcode of Life has developed a standardized, rapid and inexpensive identification method accessible to non-taxonomists. This project also aims to create a universal system for a eukaryotic species inventory based on a standard molecular approach. To this end, the Barcode of Life Data System (BOLD, http://www.boldsystems.org) enables the acquisition, storage, analysis and publication of DNA barcode records (Ratnasingham and Hebert, 2007).

The concept of DNA barcoding is the use of a sequence standard that corresponds to a single homologous gene region, amplified by the polymerase chain reaction (PCR) with universal primers, enabling distinguishing of species across a broad range of taxa. This is based on the premise that a short standardized sequence can distinguish individuals of a species because genetic variation between species exceeds that within species (Hajibabaei *et al*., 2007). For a barcoding approach to species identification to succeed, however, within-species DNA sequences need to be more similar to one another than to sequences in different species. This "matching hypothesis" (Costa and Carvalho, 2007) constitutes the key starting point for launching and implementing the new bioidentification system where a database linking a given species and respective DNA barcode array will be constructed.

Species identification through barcoding is usually achieved by the retrieval of a short DNA sequence, the 'barcode', from a standard part of the genome from the specimen under investigation. The barcode sequence from each unknown specimen is then compared with a library of reference barcode sequences derived from individuals of known identity. A specimen is identified if its sequence closely matches one in the barcode library. Otherwise, the new record can lead to a novel barcode sequence for a given species, or it can suggest the existence of a newly encountered species (Hajibabaei *et al*., 2007).

1.5.3.2 Barcode gene

The DNA barcode is a very short, standardized DNA sequence (400–800 bp) in a well-known gene. To be practical as a DNA barcode, a gene region must satisfy three criteria, which are: 1. contain significant species-level genetic variability and divergence; 2. possess conserved flanking sites for developing universal PCR primers for wide taxonomic application; and 3. have a short sequence length so as to facilitate current capabilities of DNA extraction and amplification. At present, only

mitochondrial sequences come close to fulfilling these requirements and cytochrome *c* oxidase I (*COI*) has been accepted as a practical, standardized species-level barcode for almost animal groups (Herbert *et al*., 2003b).

Various gene regions have been employed for species-level biosystematics (Table 1.1); however, DNA barcoding advocates the adoption of a 'global standard' by using a 650-base fragment of the 5' end of the *COI* (Hebert *et al*., 2003b). This fragment size has been selected so that a single sequence pass can obtain reliable sequence read in conventional Sanger sequencing platforms. Shorter fragments of *COI* have also been shown to be effective for the identification of specimens with degraded DNA, however, when a 650 base-pair sequence is not easily obtainable (Hajibabaei *et al*., 2006). In addition, the usability, robustness, and reliability of *COI* in a standard high-throughput barcoding analysis has been assessed extensively (Hajibabaei *et al*., 2005).

Table 1.1 Common species-level molecular markers (Hajibabaei *et al*., 2007).

Gene[a]	Genomic location	Number of sequences			
		Animals	Plants	Protists	Fungi
***COI*-barcode**[b]	Mitochondria	195 777	520	1931	410
16S-rDNA	Mitochondria	41 381	221	2059	285
cytb	Mitochondria	88 324	165	1920	1084
ITS1-rDNA	Nucleus	12 175	57 693	68 839	56 675
ITS2-rDNA	Nucleus	13 923	58 065	67 332	56 349
18S-rDNA	Nucleus	21 063	17 121	32 290	33 327
rbcl	Plastid	NA[c]	30 663	37 328	NA

[a]Gene abbreviations: *COI*, cytochrome *c* oxidase I; cyt*b*, cytochrome *b*; ITS, internal transcribed spacer; rbcL, large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase.
[b]*COI*-barcode statistics are retrieved from Barcode of Life Data systems (http://www.barcodinglife.org). Statistics for other loci are retrieved from GenBank.
[c]NA, not applicable.

Mitochondrial DNA (mtDNA) genes have long dominated the field of molecular systematics because of their maternal inheritance, limited recombination, rapid evolution, and the robustness of mtDNA against degradation (due to their high copy number compared with nuclear DNA), making them ideal markers for many species-level questions (Avise *et al*., 1987). These genes are widely accepted markers for molecular identification of various invertebrates and vertebrates. So far, the *COI* gene has proved to be suitable for the identification of a large range of vertebrates and invertebrates, including springtails (Hogg and Hebert, 2004), butterflies (Hebert *et al*., 2004a), crustaceans (Costa *et al*., 2007), birds (Hebert *et al*., 2004b) and fishes (Ward *et al*., 2005).

The availability of broad range primers for the amplification of this 5' region of *COI* from diverse phyla established this gene sequence as a particularly easily recovered segment of the mitochondrial genome (Folmer *et al*., 1994). In contrast to these conserved sequences, the overall rate of sequence evolution for *COI* is relatively high, especially at degenerate codon positions. With some important exceptions, the rate of evolution is high enough to result in sequence divergence between most species as well as varying levels of sequence polymorphism within species (Neigel *et al*., 2007). The well characterized *COI* gene has proved to be a robust evolutionary marker, enabling recovery of its 5' end. It third position nucleotides show a high incidence of base substitutions which lead to a rate of molecular evolution that is about three times greater than 12S or 16S rDNA (Lakra *et al*., 2009).

1.5.3.3 Fish DNA barcoding

Although fishes constitute the largest vertebrate group, they are still a manageable group for demonstrating the utility of DNA barcoding, with approximately 20,000 marine and 15,000 freshwater species (FishBase: www.fishbase.org). They are systematically diverse, ranging from ancient jawless species (Agnatha: lampreys and

hagfish) through to cartilaginous fishes (Chondrichthyes: sharks and rays) and to bony fish (Osteichthyes) (Ward *et al*., 2005).

Analyses from 2006 indicated that fisheries provided more than 2.9 billion people with at least 15% of their average per capita animal protein intake, and these products have become important contributors to human food security (FAO, 2008). DNA barcoding offers an accurate and unambiguous identification of not only whole fish, but fish eggs and larvae, fish fragments, fish fillets and processed fish (Costa and Carvalho, 2007). For example, identification of fish eggs and larvae is a challenge because it requires an experienced taxonomist, and involves lengthy examination of samples using microscopy to identify species-specific characteristics. A study by Webb *et al*. (2006) testing the application of molecular techniques in species identification of fish eggs revealed that over 60% of the eggs were misidentified. Some larvae can be particularly problematic if they have few morphologically distinguishable characteristics and show developmental variability (Webb *et al*., 2006). Phenotypic plasticity (Hebert, 2002) is a common phenomenon, and many larvae are easily damaged during collection, leading to a large degree of uncertainty in identification. Misidentification could mislead understanding on speciation, diversity, niche partitioning, and many other features of ecosystems. Webb *et al*., (2006) have shown that it is possible to identify larvae of fish using DNA barcoding techniques, but the resolution is currently limited by the availability of comparative adult sequences in the DNA sequence database.

Recently, a few studies have shown that barcoding can identify a large range of fish species (Ward *et al*., 2005; Pegg *et al*., 2006; Rock *et al*., 2008; Steinke *et al*., 2009; Hanner *et al*., 2011; Keskin and Atar, 2013; McCusker *et al*., 2013) In the Ward *et al*. (2005) proof-of-concept study, barcoding effectively discriminated between 207 species of Australian fish including 143 species of teleosts and 61 species of sharks and rays. Rock *et al*. (2008) analysed *COI* barcodes for 35 putative fish species collected in the Scotia Sea, and compared the resultant molecular data with field-based

morphological identifications, and additional sequence data obtained from GenBank and the Barcode of Life Data System (BOLD). They found that there was high congruence between morphological and molecular classification, and *COI* provided effective species-level discrimination for nearly all putative species. For two families, including the Liparidae and Zoarcidae, for which morphological field identification was unable to resolve taxonomy, DNA barcoding revealed significant species-level divergence (Rock *et al*., 2008).

1.5.4 Nuclear loci

Instead of mtDNA, nuclear markers can also provide sequence data for phylogenetic relationships (Friedlander *et al*., 1994) and phylogeographic studies (Zhang and Hewitt, 2003). Phylogeography is an approach to study phylogenetic within and among closely related species from different population of various geographic regions (Avise, 2004). For the first generation of phylogeographic studies, animal mtDNA has been used widely to see how individuals are genealogically linked through shared ancestors (Avise *et al*., 1987). Because mtDNA only shows the matrilineal history, data from nuclear loci are needed to obtain a full understanding of evolutionary history (Godinho *et al*., 2008). Analysis of nuclear markers provides both paternal and maternal information.

There are two main concerns have been raised regarding use of nuclear genes in phylogeography. First, recombination occurs frequently in the nuclear genome (Zhang and Hewitt, 2003). Through recombination, evolutionary history may be misrepresented, and can produce false inferred histories. Instead of a single tree, the sequences will split into a group of trees (Posada, 2001; Wiuf *et al*., 2001). This is because different segments within a haplotype, which has recombined, will have independent histories. Second, nuclear genes generally have low mutation rates, which may reduce information-content (Brown *et al*., 1979). Thus, nuclear sequences

often need to be longer compared to mtDNA to ensure adequate coverage of phylogenetically-informative characters. Nuclear genes are also often more difficult to work with, because they occur in low copy number, which may result in difficulties in amplification through PCR (Avise, 2000).

The number of studies employing both nuclear and mtDNA markers has increased in recent years (Daeman *et al*., 2001; Suarez *et al*., 2009; Schonhuth and Mayden, 2010; Seifertova *et al*., 2012). A study by Burg *et al*. (1999), which employed mtDNA sequence data and microsatellite analyses to examine the phylogeographic structure of harbour seal populations in British Columbia and parts of Alaska demonstrated a reciprocally monophyletic northern and southern split in harbour seal populations. However, neither of the markers alone revealed the multiple colonisations, resulting from Pleistocene glaciations, on population structure. Schonhuth and Mayden (2010) also performed a combined analysis of cytochrome *b* and Rag 1 among genus *Cyprinella* to determine phylogenetic relationships. Their study revealed that both genes showed high levels of genetic divergence between species. A study on the genetic structure of blacktip shark (*Carcharhinus limbatus*) continental nurseries using mitochondrial and microsatellite markers identified significant structuring at both markers among nine nurseries (Keeney *et al*., 2005). There are several other studies showing that greater information can be obtained by employing both mtDNA and nuclear DNA data (Nielsen *et al*., 1997; Lu *et al*., 2001; Near and Christina Cheng, 2008; Suarez *et al*., 2009; Ramirez *et al*., 2010).

1.6 TAXONOMIC METHODS

1.6.1 Pylogenetic Analyses

Molecular phylogenies present an estimate of evolutionary relationships between organisms based upon similarities and differences in their genetic/physical characteristics. Expansion of species concepts to include genetic dimensions, and advances in numerical taxonomic methods have now shifted the phylogenetic focus largely to molecular data (Avise, 2004). Molecular data are widely useful for producing molecular phylogenies for phylogenetic, phylogeographic, population genetic and species delimitation studies (Palumbi and Baker, 1994; Schneider *et al.,* 1998; Hebert *et al*., 2003a, Avise 2004). Phylogenetic trees have several uses such as inferring organismal phylogenies by combining it with analyses of other data sources, studying co-speciation, calibrating rates of molecular evolution, establishing the age of a taxa or lineage, analysis of gene duplication, estimating rates of diversification, extinction, polymorphism, recombination and population dynamics (Holder and Lewis, 2003). Numerous numbers of approaches have been used in order to map gene phylogenies or gene networks (Table 1.2).

The most traditional approaches to reconstruct phylogenies are the Neighbour-Joining (NJ) algorithm developed by Saitou and Nei, 1987. This is a popular distance-based method (Felsenstein, 2004) and often treated as the starting point for a computationally intensive search for the best phylogeny (Holder and Lewis, 2003). This method searches for pairs of Operational Taxonomic Units (OTU) that minimizes the total branch length at each stage of OTU´s, clustering beginning with a star tree. This star tree is produced under the assumption that there is no clustering of taxa. The first step is to find the first pair of OTU´s that show the lowest branch length. Once the first pair of neighbours is identified they are combined into one unit and this procedure is repeated until the final tree is produced. The NJ algorithm does not assume that all

lineages evolve at the same rate and produces only one unrooted tree that may minimize its total length. The NJ performance is dependent on the underlying model of molecular evolution selected to create the pairwise distance matrix between sequences used for tree construction. The tree obtained is additive, meaning that the distance between any two terminal nodes is the sum of the branch lengths connecting them. Backward and parallel mutations do occur in real data but these are not contemplated in the construction of the NJ tree. Although NJ is a fast and quite reliable method of producing the correct topology (Gascuel, 1997), statistical tests (e.g bootstrapping) to assess tree reliability are necessary. The NJ method is appropriate for large data sets, and is capable of conducting rapid bootstrap resampling tests, a nonparametric statistical analysis of support for phylogenetic trees (Holder and Lewis, 2003). As referred by Felsenstein (2004), computer simulations studies have shown that NJ performs quite well. However, its success is dependent on the sequence divergences being adequately corrected for multiple hits. A potentially serious weakness for distance method such as NJ is that the observed differences between sequences are not accurate reflections of the evolutionary distances between them. Multiple substitutions at the same site obscure the true distance and make sequences seem artificially close to each other. When the goal is to infer older relationships, it can be difficult to arrive at reliable values for the distance matrix that is the input for NJ; obviously, if the input into algorithm is poor, the algorithm has little chance of succeeding.

To perform a tree search, a standard must be used for comparing trees — an optimality criterion, in the terminology of phylogenetics (Holder and Lewis, 2003). The most popular criteria are parsimony, minimum evolution and Maximum Likelihood (ML) (see for more details Table 1.2). In contrast to distance-based method, ML map the history of gene sequences onto a tree. ML corrects for multiple mutational events at the same site to reconstruct the relationships between sequences that have been separated for a long time, or are evolving rapidly. The tree that has the highest

probability of producing the observed sequences is preferred (this probability is the likelihood of the tree). Likelihood-based approaches have proven especially powerful for inferring phylogenetic trees (Felsenstein, 2004), but are computer demanding. Likelihood methods for phylogenies were introduced for gene frequency data and are currently used for sequence data. Bayesian inference (BI) phylogenetics has been proposed more recently as a powerful method for the analysis of large phylogenetic trees and complex evolutionary models (Holder and Lewis, 2003). In this analysis the probability of a correct tree is the posterior probability of that tree. This posterior probability is dependent on how well represented are the data and the model of evolution proposed. It differs from the ML methods in that, BI analysis seek the tree that maximizes the probability of the tree, given the data and the model of evolution. In addition, Bayesian provides measuring of support faster than ML bootstrapping. Complex parameter-rich models present two problems for ML. When the ratio of data points to parameter is low, ML estimates of parameters can be unreliable. In Bayesian analysis, the final result does not depend on one specific value. Even if there is enough data to estimate many parameters, the hill-climbing algorithms that are used to find the ML point can be slow or unreliable as the number of parameter increases. In Bayesian, calculations can be approximated by the Markov Chain Monte Carlo (MCMC) procedure, lessening the computation time (Huelsenbeck and Rannala, 2004).

Table 1.2 Comparison of phylogenetic inference methods (Holder and Lewis, 2003).

Method	Advantages	Disadvantages	Software
Neighbour joining	Fast	Information is lost in compressing sequences into distances; reliable estimates of pairwise distances can be hard to obtain for divergent sequences	PAUP MEGA PHYLIP
Parsimony	Fast enough for the analysis of hundreds of sequences; robust if branches are short (closely	Can perform poorly if there is substantial variation in branch lengths (so-called 'Felsenstein zone')	PAUP MEGA PHYLIP

	related sequences or dense sampling)		
Minimum evolution	Uses models to correct for unseen changes	Distance corrections can break down when distances are large	PAUP MEGA PHYLIP
Maximum likelihood	The likelihood fully captures what the data tell us about the phylogeny under a given model	Can be prohibitively slow (depending on the thoroughness of the search and access to computational resources)	PAUP MEGA PHYLIP RaxML
Bayesian	Might be a faster way to assess support for trees than maximum likelihood boostrapping	The prior distributions for parameters must be specified: it can be difficult to determine whether the Markov chain Monte Carlo (MCMC) approximation has run for long enough	MrBayes BAMBE

1.7 THESIS AIMS

The overall aim of this study was to employ mitochondrial and nuclear markers to examine species and population diversity in the marine fish family, Carangidae. Several specific objectives were addressed in each chapter as follow:

1. To identify species-specific *COI* sequences in Indo-Malay Carangidae. These sequences will then be used as taxonomic tools to identify cryptic species and fish larvae in relation to dispersal and recruitment dynamics in the context of fisheries management. This objective will be met in Chapter 2 of this thesis.
2. To examine and compare population structuring of three species Carangidae (*Atule mate, Selar crumenophthalmus* and *Selaroides leptolepis*) with different life history (Chapter 3).
3. To examine the phylogeography of *Selar crumenophthalmus* within SE Asian region as a framework for exploring the impacts of historical and contemporary processes on the distribution of within and among population genetic diversity (Chapter 4).

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CHAPTER 2

DNA BARCODING REVEALS CRYPTIC DIVERSITY WITHIN COMMERCIALLY EXPLOITED INDO-MALAY CARANGIDAE (TELEOSTEII: PERCIFORMES)

Abstract

DNA barcodes, typically focusing on the cytochrome *c* oxidase I gene (*COI*) in many animals, have been used widely as a species-identification tool. Despite the wealth of DNA barcode data for fish from many temperate regions, there are relatively few available from the Southeast Asian region. Here, we target the marine fish Family Carangidae, one of the most commercially-important families from the Indo-Malay Archipelago (IMA), to produce an initial reference DNA barcode library. A 652 bp region of *COI* was sequenced for 723 individuals from 36 putative species of Family Carangidae distributed within IMA waters. Within the newly-generated dataset, three described species exhibited conspecific divergences up to ten times greater (4.32–4.82%) than mean estimates (0.24–0.39%), indicating a discrepancy with assigned morphological taxonomic identification, and the existence of cryptic species. Variability of the mitochondrial DNA *COI* region was compared within and among species to evaluate the *COI* region's suitability for species identification. The trend in range of mean K2P distances observed was generally in accordance with expectations based on taxonomic hierarchy: 0% to 4.82% between individuals within species, 0% to 16.4% between species within genera, and 8.64% to 25.39% between genera within families. The average Kimura 2-parameter (K2P) distance between individuals, between species within genera, and between genera within family were 0.37%, 10.53% and 16.56%, respectively. All described species formed monophyletic clusters in the Neighbour-joining phylogenetic tree, although three species representing complexes of six potential cryptic species were detected in Indo-Malay Carangidae. This study confirms that *COI* is an effective tool for species identification of Carangidae from the IMA. There were moderate levels of cryptic diversity among putative species within the central IMA. However, to explain the hypothesis of species richness in the IMA, it is necessary to sample the whole family across their broad geographic range. Such insights are helpful not only to document mechanisms driving diversification and

recruitment in Carangidae, but also to provide a scientific framework for management strategies and conservation of commercially-important fisheries resources.

2.1 Introduction

Spectacular biodiversity exists in tropical marine ecosystems. One mega-diverse tropical region, where the ranges of many tropical marine species overlap, is the centre of maximum marine biodiversity of the Indo-Malay Archipelago (IMA) (Lohman *et al*., 2011). Various hypotheses giving rise to this extraordinary species richness have been proposed (Reaka *et al*., 2008), though two in particular have been widely addressed (Carpenter and Springer, 2005; Santini and Winterbottom, 2002; Hubert *et al*., 2012): the Centre-of-Overlap and the Centre-of-Origin hypotheses, both of which postulate contrasting patterns of species ranges and distribution of species richness. The former proposes geographic isolation and allopatric speciation with midpoint ranges of species distributions falling on each side of the IMA, with overlap across the IMA. Large scale genetic structure is expected to result from geographic isolation and cryptic species may be expected to exhibit allopatric distribution ranges, potentially overlapping in the IMA. The Centre-of-Origin hypothesis proposes speciation centred in the IMA, with midpoint ranges of species distributions occurring within the IMA. Large-scale genetic structure is expected to be shallow as a consequence of high connectivity and larval dispersal across the IMA. Since the IMA encompassess the centre of the distributional range of the target taxa studied here, the Carangidae, we test whether there is any evidence of highly divergent cryptic lineages in sympatry, as predicted by the Centre-of-Origin hypothesis.

Given that only a small fraction of all global species have been formally described, between 1.5–1.8 million out of an estimated 10 million (Wilson, 2003), efforts to catalogue and understand drivers of biodiversity need to be prioritised. Research on

cryptic species has increased recently with studies (Ward *et al*., 2008; Carr *et al*., 2011; Hubert *et al*., 2012; Kadarusman *et al*., 2012; Puckridge *et al*., 2013) indicating the frequent occurrence of cryptic species occurring within and outside the IMA. One of the problems associated with identifying cryptic species is that many taxonomic protocols rely on phenotypic characters, and require lengthy and detailed inspection of the specimens (Costa and Carvalho, 2007). Such traditional methods of identifying, naming and classifying organisms are largely based on visible morphology. Misidentification of economically important species in cryptic species-complexes can result in inaccurate data collection potentially leading to the overexploitation of stocks (Fox *et al*., 2005). Therefore, in addition to disclosing potential drivers of diversification, accurate identification at the species-level is vital to ensure the successful management of commercially-important fish stocks in IMA waters, and here, a DNA barcoding database can play an important role.

The introduction of the DNA barcoding approach, which utilises a short, standardised gene region (Hebert *et al*., 2003a) to identify species (Hajibabaei *et al*., 2005; Smith *et al*., 2008; Ward, 2009; Huang *et al*., 2007; Aquilino *et al*., 2011; Hanner *et al*., 2011; Keskin and Atar, 2013; McCusker *et al*., 2013; Young *et al*., 2013) has been shown to be useful in solving taxonomic ambiguities. Hebert *et al*. (2003a) proposed that within species, DNA sequences would be more similar than that among different species, and that this 'barcoding gap' could be used to delimit species. To date, the cytochrome *c* oxidase I (*COI*) mitochondrial protein-coding gene has been accepted widely as a practical, standardized species-level barcode for the majority of the animal kingdom (Hebert *et al*., 2003b). The main goal of DNA barcoding is to facilitate rapid identification of potentially unidentified taxa in global biodiversity assessment and conservation, including cryptic and microscopic taxa, and organisms with morphologically ambiguous characters (Hebert *et al*., 2003a). DNA barcoding has also focused on the development of a global barcoding database (Ratnasingham and Hebert, 2007) as a species identification tool for large taxonomic assemblages of

animals, representing a quick and easy method for non-specialists to identify disparate specimens. The identification process through DNA barcoding is relatively straight-forward, and depends upon the quantifiable matching of *COI* sequences from unknown specimens with previously documented and archived voucher specimens. Where marked discordance is found in the *COI* sequences of test and reference specimens, additional taxonomic and related studies are undertaken to assess likelihood of discovering novel taxa (Hajibabaei *et al*., 2007).

To date, many barcoding projects involving various organisms from different geographic regions can be accessed from the public barcode library, the Barcode of Life Data Systems (www.barcodinglife.com) (Ratnasingham and Hebert, 2007). Despite the wealth of DNA barcode information for fish from many temperate regions (Ward *et al*., 2005; Hubert *et al*., 2008; Steinke *et al*., 2009; Zhang and Hanner, 2011; Costa *et al*., 2011; McCusker *et al*., 2013), there are relatively few data available from Southeast Asian waters, an area exceptionally rich in biodiversity. DNA barcoding should prove useful for rapid biodiversity assessment (Francis *et al*., 2010) in this region, where significant levels of biodiversity loss are escalating (Lohman *et al*., 2011). Our study provides the first barcode records for 723 specimens representing 36 putative species from Carangidae sampled from waters of the IMA. Variability of *COI* was compared both within and among species to evaluate its suitability for species identification. Samples for assaying the *COI* barcodes were analysed and compared with field-based morphological species identifications and additional molecular data from other geographical regions were obtained from GenBank and the BOLD System. Such analyses may identify hidden diversity in Carangidae, where such diversity exists.

The family Carangidae encompasses fishes whose body size ranges from small (TL = 16 cm) to large (TL =250 cm) and body shapes vary from elongate and fusiform to deep and strongly compressed (Randall, 1995). This diverse family of marine fishes are known variously by common names such as jacks, trevallies, amberjacks, pompanos,

scads, kingfish, pilotfish, queenfishes and rainbow runner (Mohsin and Ambak, 1996). Carangids represent an important food source and play a significant role in the commercial fisheries industry in Southeast Asia (Mohsin and Ambak, 1996). All members, small or large are considered as edible protein and can be caught in large numbers every year (ca.1,556,578 tonnes in 2010) (FAO). Despite their high economic value and ecological importance, the taxonomy of Carangids remains poorly understood (Laroche *et al*., 1984). FishBase citations include many synonyms, which indicate taxonomic ambiguities in Carangids (Froese and Pauly, 2012) due to morphological and meristic similarities across species, as well as plasticity in body shape, size and colour patterns (Ward *et al*., 2008; Lakra *et al*., 2009). In addition, Carangids typically display significant changes in morphology and pigmentation during growth (Bohlke and Chaplin, 1993), and such changes have likely lead to misidentification of specimens, and contributed to general taxonomic confusion. An interesting example of change with growth occurs in juveniles of African pompano (*Alectis ciliaris*), which are easily recognized by the presence of long filaments trailing from five to six dorsal and anal fins. As fish grow larger, these filaments shorten and eventually disappear (Randall *et al*., 1990). The exact biological mechanism behind such developmental change is unclear, as is the function of the filaments. Carangid eggs and newly hatched larvae are also difficult to distinguish from the eggs and larvae of many other families of marine fishes (Leis and Trnski, 1989), making it difficult to map spawning grounds and identify ichthyoplankton (Fox *et al*., 2008). Pigmentation changes during development in Carangid larvae and its diagnostic value is thereby of limited value for species identification (Miller *et al*., 1979). Unambiguous delineation of such apparent phenotypic plasticity is required not only for taxonomy and systematics, but also is of critical importance for fisheries management, trade and conservation purposes. cytochrome *c* oxidase I (*COI*) has been shown to accurately discriminate between closely related species of various animal groups (Hebert *et al*., 2004; Barret and Hebert, 2005; Hajibabaei *et al*., 2005; Huang *et al*., 2007; Smith *et al*., 2008), and is applied here to examine the integrity of species delineation in Carangids.

In addition to generating a reference DNA barcode data base, we examine patterns of genetic divergence in relation to habitat, body shape and size to test potential associations between species-specific characters, dispersal and connectivity. No study to date, to the best of our knowledge, has assessed biological characteristics in Carangidae in relation to patterns of spatial genetic structure.

2.2 Materials and Methods

2.2.1 Establishing a DNA barcode library

A standard protocol for establishing a DNA barcode library was utilised as a workflow for management and analysis of specimen data (BOLD, www.barcodinglife.org) (Figure 2.1). Establishing a project in BOLD requires several steps for each specimen to gain their own barcode status, namely (Ratnasingham and Hebert, 2007): 1. Species name; 2. Voucher data; 3. Collection record; 4. Identifier of the specimen; 5. *COI* sequence of at least 500 base pair (bp); 6. PCR primers used to generate the amplicon; 7. Trace files. The platform data records in BOLD consists of two main pages: a "specimen page" and a "sequence page" (Figure 2.1). The information found in the specimen page is illustrated in Figure 2.1 (A) where varied specimen data including Specimen Identifiers, Taxonomy, Specimen details, Collection Data, and Photography are deposited. Each specimen page is coupled to a sequence page (B in Figure 2.1) that records the barcode sequence (FASTA format), PCR primers and trace files, amino acid translation, and ultimately the GenBank accession number as well.

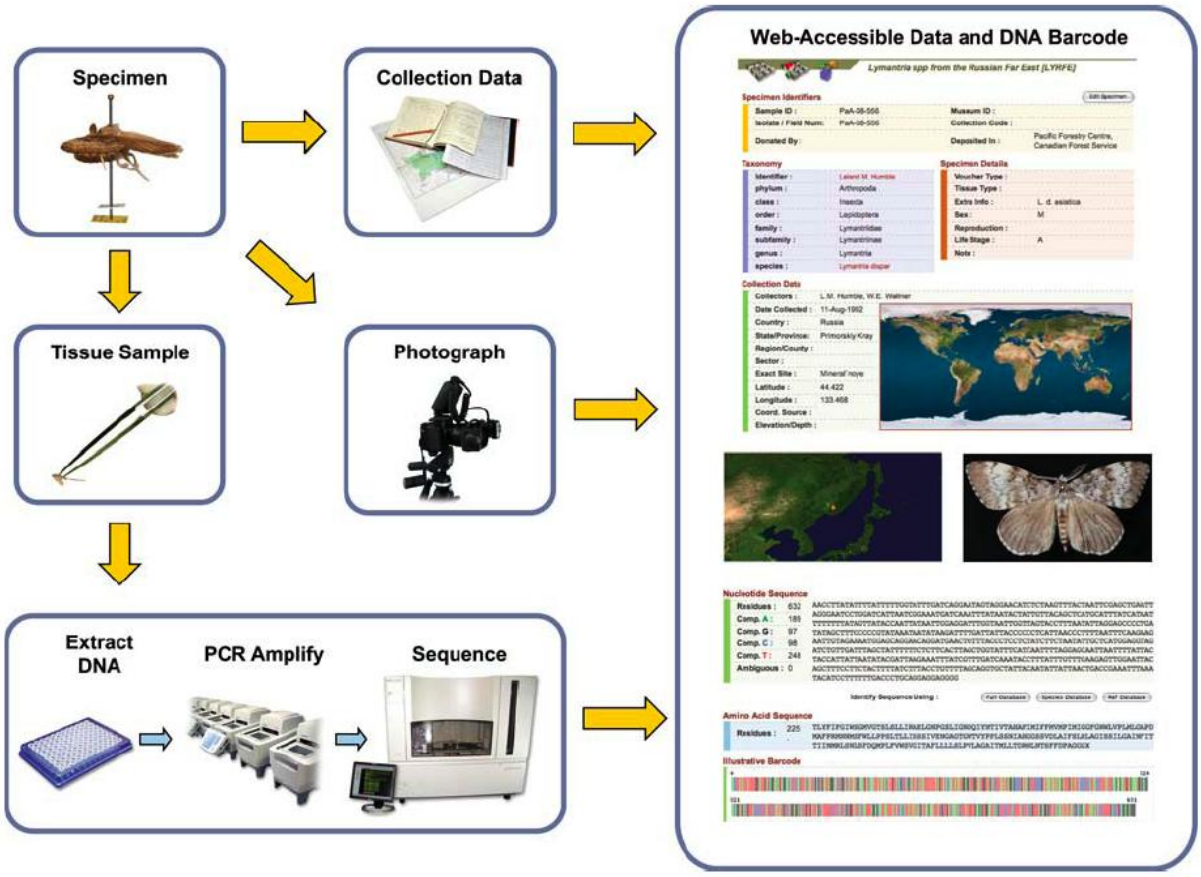

Figure 2.1 DNA barcode analytical chain: (A) Specimen page and (B) sequence page adapted from International Barcode of Life (www.ibol.org).

2.2.2 Sampling

We collected 845 Carangidae specimens from four geographic regions within the IMA: South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea. The samples were collected from several fish landing sites during two field trips; from October to November 2009, and from June to July 2010 (Figure 2.2). Specimens encompassed 39 putative species and 18 genera from the Family Carangidae. Sample collections included tissue sampling for genetic analysis, as well as collection of whole specimens (adult fish and larvae) for storage as barcode voucher specimens. All samples were preserved in 99% ethanol. Digital photographs of all fishes were taken immediately

and voucher specimens were tagged according to museum ID number and archived in the South China Sea Museum, Universiti Malaysia Terengganu (www.umt.edu.my). All details regarding collection dates, collection sites with geographical coordinates, taxonomy and vouchers can be found in the Barcode of Life Data System website (BOLD, www.barcodinglife.com) (Ratnasingham and Hebert, 2007) under project 'DNA Barcoding of Malaysian Fish' (DBMF). At least five individuals of each species were collected from each sampling site depending on their abundance. Few specimens were collected in some low abundance species (<5), while those that were abundant enabled the collection of more individuals (up to 75), with 29/36 species having sample sizes of >5 individuals. All fishes were identified based on morphology, with the help of expert local taxonomists in most cases, FAO-Fisheries Identification Sheets (Fischer and Whitehead 1974) and identification books published by the Department of Fisheries Malaysia (Annie and Albert, 2009; Mansor *et al*., 1998).

Fin clips were removed from the right pectoral fin of each fish and preserved in 99% ethanol. Fish specimens were then placed in ice, frozen on site and transported to South China Sea Museum, University Malaysia Terengganu. Fin clips were sent to the Canadian Centre for DNA Barcoding (CCDB), University of Guelph Ontario, Canada for further processing. Total genomic DNA was extracted from fin clips of 39 putative species and PCR amplifications performed using the procedure of (Ivanova *et al*., 2007). Following the CBOL standard practice, *COI* genes were sequenced in both directions. All *COI* sequences and trace files have been deposited in the Barcode of Life Data System (www.barcodinglife.com) under a project named 'DNA Barcoding of Malaysian Marine Fish' (DBMF). Sequences have also been deposited in GenBank (Appendix 1).

2.2.3 Data validation

For this study, we collected 845 individuals of Carangidae. However, a total of 110 individuals generated sequences of insufficient quality to be uploaded into the BOLD system, and were therefore not considered further. After exclusion of these 110 individuals, our *COI* data base encompasses a total of 735 sequences. Incorrect taxonomic classification may affect divergence assessment of our data set. Therefore, all 735 sequences were aligned and a Neighbour-joining tree produced using the BOLD platform. A small percentage (1.63%) of samples which did not cluster with their own taxa had their photographs reviewed and this revealed potential misidentification. The remaining three species (*Carangoides oblongus, Carangoides orthogrammus, Trachinotus blochii*) with one specimen each, failed to PCR amplify, leaving a total of 36 species in the data set. Subsequently, we analysed 723 sequences from 36 species and 18 genera from Family Carangidae.

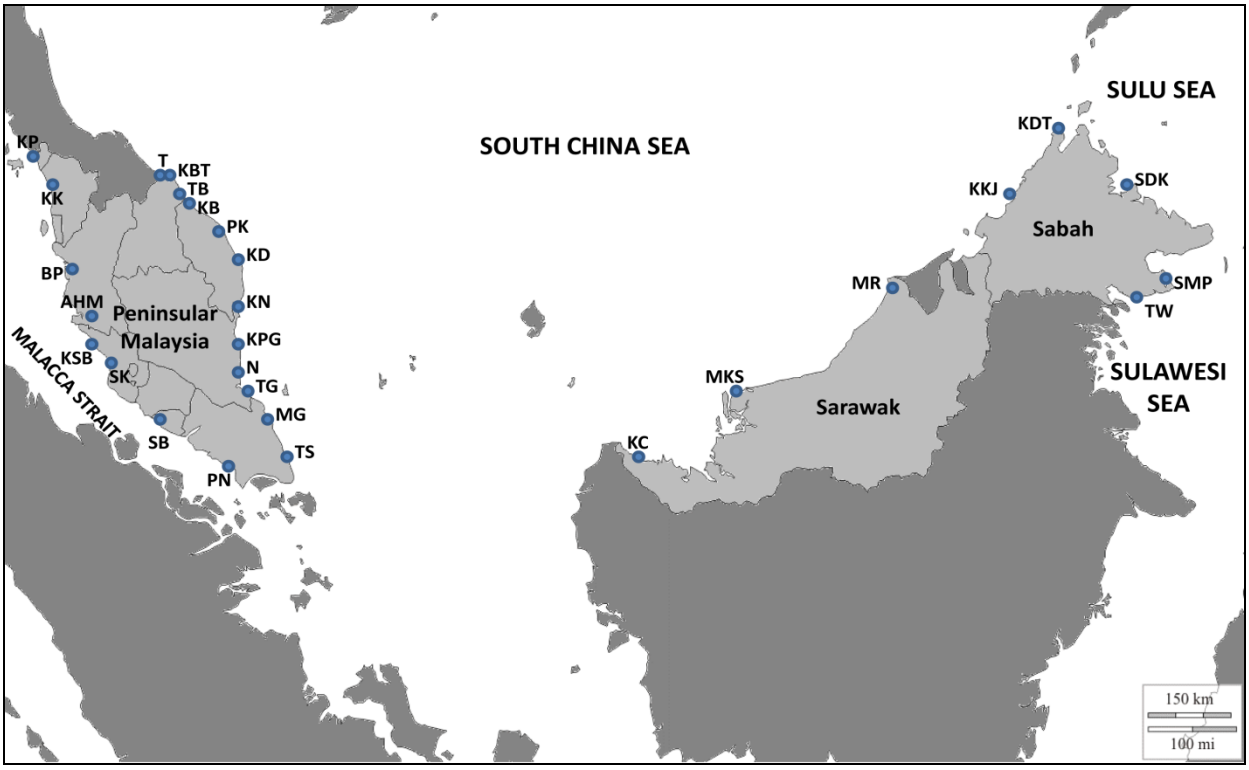

Figure 2.2 Distribution of locations for the 845 specimens sampled along the coast of Malaysia. See Appendix 1 for detailed sampling information.

2.2.4 *COI* divergence assessment

The diversity assessment for Carangidae were analysed from the data set with 723 sequences, 18 genera and 36 putative species. The Kimura 2-parameter (K2P) distance measure has become the most widely used in barcoding studies (Kimura, 1980) and was employed here. Genetic distances between specimens were calculated for each intraspecies, intragenus and intrafamily with the 'Distance Summary' command implemented by BOLD. K2P was also used for Neighbour-joining (NJ) analysis, using the BOLD Management and Analysis System. All sequences were aligned using the MUSCLE algorithm in the software programme MEGA5 (Kumar *et al*., 2004), and the amino acid translation was examined to ensure that no gaps or stop codons were present in the alignment. NJ analyses were conducted using 1000 bootstrap replicates. Nucleotide divergences of *COI* variation across 36 species of Carangidae were

analysed. Genetic distances among specimens were calculated for each intraspecies and intragenus pairwise comparison with the 'Distance Summary' analysis in BOLD. Other analytical tools in BOLD such as Nearest Neighbour, Identify Unknown and BOLD Identification System were also applied to the data. The Maximum Likelihood (ML) approach was also conducted by determining the highest likelihood tree bootstrapped 1000 times using RAxML 7.2.8 (Stamatakis *et al*., 2008). Bayesian phylogenetic analysis was conducted in Mr Bayes v3.2.1 (Ronquist *et al*., 2011), though outputs showed no convergence after 10 million generations. We thus discarded these analyses and present here only NJ and ML analyses.

We also employed the recently described bioinformatics tool, Automatic Barcode Gap Discovery (ABGD) (Puillandre *et al*., 2012) for species delimitation analysis. ABGD automatically detects the breaks in the distribution of genetic pairwise distances, referred to as the 'barcode gap' and uses it to partition the data. The method proposes a standard definition of the barcode gap and can be used even when the two distributions overlap to partition the data set into candidate species. The same species therefore should be grouped in the same partition. The outline of ABGD is the following: (i) It finds the first barcode gap that occurs at a distance larger than some value $dist_{limit}$, a limit under which distances are statistically more likely to be intraspecific. $dist_{limit}$ is a simple function of the population mutation rate, estimated from the data set. It is estimated on a preliminary partition of the data set with a threshold P given by the user (P is the prior maximum divergence of intraspecific diversity). (ii) Taking a threshold equal to the barcode gap computed in step (i), it computes a so-called primary partition, where groups are the first candidate species. (iii) To account for mutation rate variability across taxa and overlap of intra and interspecific diversities, ABGD is only completed after recursive application of these first two steps to each cluster of the primary partition. This recursion splits the primary partition into secondary partitions, and so on until no further splittings occur.

Additional *COI* sequences from GenBank and BOLD Systems were added to compare *COI* sequences of 23 selected species from this study with conspecifics from West (South Africa, Mozambique, Iran, India and Turkey) and East (Australia, Philippines, China, Japan, Hawaii, French Polynesia and Mexico) of the IMA. All species and GenBank accession numbers are listed in Appendix 1. According to Smith-Vaniz (1984), Carangidae belong to a monophyletic group that includes the Nematistiidae, Coryphaenidae, Rachycentridae, and Echeneidae. Johnson (1993) proposed that these five families be recognized as the suborder Carangoidei and hence the outgroups of the Carangidae are well defined. Therefore, *Echeneis naucrates* from family Echeneidae has been chosen to represent distantly related outgroup taxa (Reed *et al*., 2002).

2.2.5 Do *COI* divergence rates correspond with biological characteristics?

Our second line of enquiry was to test whether patterns of genetic divergence at *COI* correspond to specific biological characteristics. Family Carangidae is known to contain several body forms from deep-bodied species to slender planktivores and inhabit various habitats. Some are demersal species, feeding on benthic organisms near the sea bottom, while most are pelagic species. The family also display a wide range of body sizes from the smallest fish (*Alepes kleinii*) with a total length of 16cm, to the largest, the greater amberjack (*Seriola dumerili*) with a total length of 180cm recorded (Randall *et al*., 1990). Therefore, we tested several hypotheses; 1) fish with a fusiform body shape will move faster and potentially travel further and should therefore display less *COI* divergence within species compared to the deep-bodied species; 2) pelagic species will display less genetic divergence at *COI* compared to the demersal species, due to their potential to undertake long-distance migrations in oceanic waters; and 3) larger species will display less *COI* divergence compared to the smaller species, also due to their ability to travel further, thereby enhancing population connectivity. No study to date, to the best of our knowledge, has assessed these different biological

characteristics in Carangidae in relation to genetic divergence. Therefore, we classified 35 Carangidae species into several groups based on their biological characteristics and life histories (e.g., habitat, body shape, body size) (Appendix 2). We performed one-way ANOVAs to test whether levels of *COI* divergence between Carangidae species exhibited associations with habitat or biological characteristics.

2.3 Results

2.3.1 General findings

COI barcodes were recovered for a total of 36 species and 18 genera from the Family Carangidae, for the first time from the IMA. The number of sequences per species varied between 1 (*Carangoides gymnosthetus*) for species that were rare, to 75 (*Selar crumenophtalmus*) for species that were abundant in Malaysian waters. Thus a total of 723 *COI* barcodes with an average length of 652 bp were obtained for this commercially-important fish family. No insertions/deletions, heterozygous sites or stop codons were observed, supporting the view that all of the amplified sequences constitute functional mitochondrial *COI* sequences.

2.3.2 *COI* divergence assessment

COI nucleotide divergences were calculated for the dataset of 723 sequences of 36 species and 18 genera. Sample sizes and mean divergences at various taxonomic levels are given in Table 2.1.

Table 2.1 Kimura 2-parameter (K2P) distances between Indo-Malay Carangidae.

Comparison within	Taxa	Number of comparisons	Min (%)	Mean (%)	Max (%)	SE (%)
Species	36	13445	0	0.37	4.82	0.006
Genus	18	10680	0	10.53	16.4	0.028
Family	1	240503	8.64	16.56	25.39	0.006

As expected, genetic divergence increased progressively with higher taxonomic level: 0% to 4.82% between individuals within species, 0% to 16.4% between species within genera, and 8.64% to 25.39% between genera within family, which support a marked change in genetic divergence at the species boundary (Figure 2.3). The average within species K2P distance is 0.37% with far less, 0.00% for *Carangoides ferdau*, *Gnathanodon speciosus* and *Trachinotus baillonii*. The latter estimates were largely due to the low number of specimens collected, and all specimens were from the same landing site (n= 1–4). *Atropus atropos* (1.13%) and *Seriolina nigrofasciata* (1.79%) displayed slightly higher divergence rates than average (Table 2.2). The average congeneric distance was 10.53%, which was higher than the conspecific distance. The congeneric distances were lowest among queen fishes, *Scomberoides* (mean= 7.52%, 3 species), followed by *Caranx* (mean= 7.53%, 3 species); *Alepes* (mean= 8.84%, 4 species); *Decapterus* (mean= 8.89%, 3 species); *Alectis* (mean= 11.37%, 2 species); *Carangoides* (mean= 11.66%, 7 species) and the highest variation observed in the genus Selar (mean= 12.25%, 2 species) (Table 2.3).

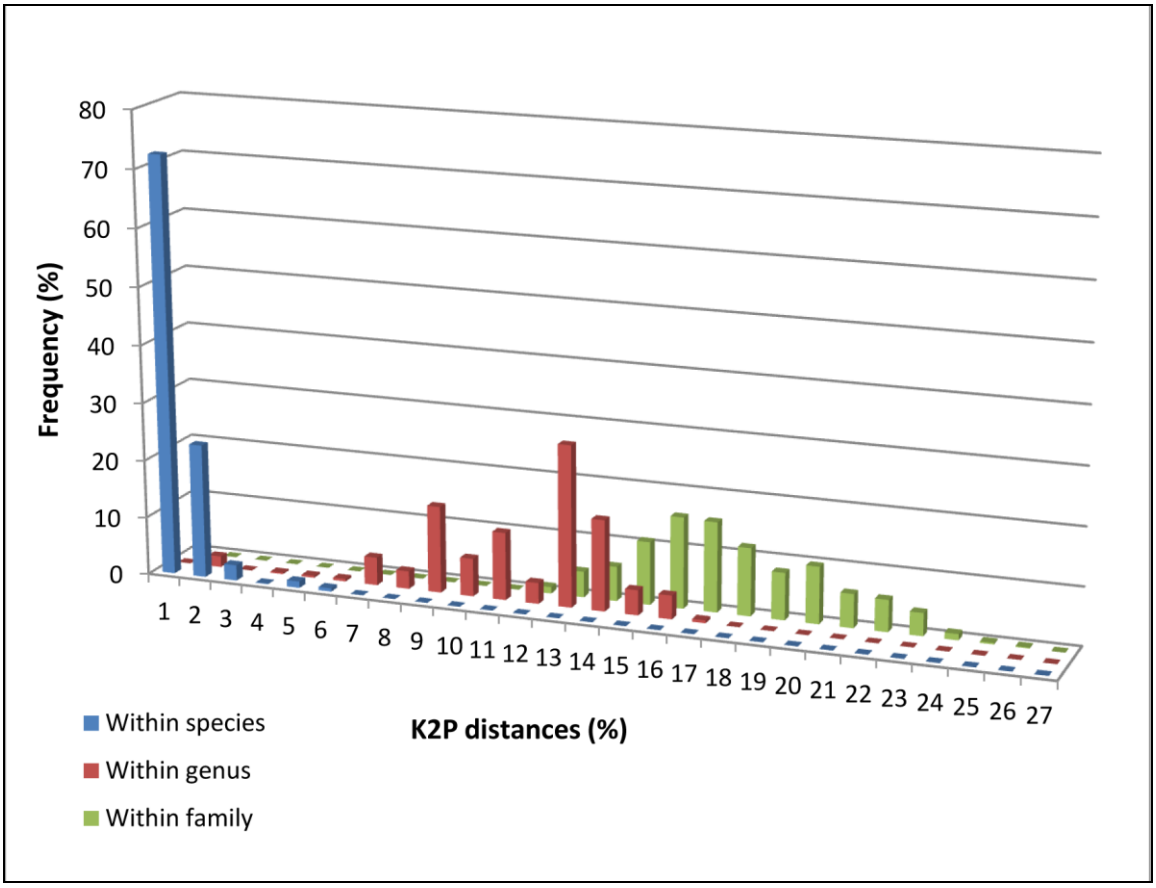

Figure 2.3 Frequency distributions of *COI* K2P distances (%) intraspecies, intragenus and intrafamily. 36 species, 18 genera and 1 family.

Table 2.2 Intraspecific nucleotide K2P distances for 36 species of Indo-Malay Carangidae

Species	No. of sequences (n)	Mean K2P distance (%)
Alectis ciliaris (Bloch, 1787)	8	0.16
Alectis indicus (Rüpell, 1830)	10	0.17
Alepes djedaba (Forsskål, 1775)	31	0.25
Alepes kleinii (Bloch, 1793)	11	0.16
Alepes melanoptera (Swainson, 1839)	15	0.40
Alepes vari (Cuvier, 1833)	13	0.16
Atropus atropos (Bloch & Schneider, 1801)	13	1.13
Atule mate (Cuvier, 1833)	67	0.34
Carangoides bajad (Forsskål, 1775)	26	0.39
Carangoides chrysophrys (Cuvier, 1833)	19	0.33
Carangoides dinema (Bleeker, 1851)	6	0.03
Carangoides ferdau (Forsskål, 1775)	2	0.00
Carangoides fulvoguttatus (Forsskål, 1775)	3	0.21

Carangoides gymnostethus (Cuvier, 1833)*	1	N/A
Carangoides hedlandensis (Whitley, 1934)	3	0.31
Carangoides malabaricus (Bloch & Schneider, 1801)	33	0.54
Caranx ignobilis (Forsskål, 1775)	6	0.51
Caranx sexfasciatus (Quoy & Gaimard, 1825)	8	0.16
Caranx tille (Cuvier, 1833)	9	0.07
Decapterus kurroides (Bleeker, 1855)	10	0.09
Decapterus macrosoma (Bleeker, 1851)	26	0.08
Decapterus maruadsi (Temminck & Schlegel, 1843)	24	0.15
Elagatis bipinnulata (Quoy & Gaimard, 1825)	8	0.22
Gnathanodon speciosus (Forsskål, 1775)	4	0.00
Megalaspis cordyla (Linnaeus, 1758)	63	0.53
Parastromateus niger (Bloch, 1795)	51	0.30
Scomberoides commersonnianus (Lacepède, 1801)	17	0.56
Scomberoides tala (Cuvier, 1832)	11	0.08
Scomberoides tol (Cuvier, 1832)	32	0.09
Selar boops (Cuvier, 1833)	40	0.37
Selar crumenophthalmus (Bloch, 1793)	75	0.39
Selaroides leptolepis (Cuvier, 1833)	39	0.18
Seriola dumerili (Risso, 1810)	4	0.31
Seriolina nigrofasciata (Rüppell, 1829)	9	1.79
Trachinotus baillonii (Lacepède, 1801)	4	0.00
Uraspis uraspis (Günther, 1860)	22	0.67

*only 1 sequence available

Table 2.3 Congeneric nucleotide K2P distances for seven genera in Indo-Malay Carangidae.

Genus	No. of sequences (n)	Mean K2P distance (%)
Alectis	18	11.37
Alepes	70	8.84
Carangoides	93	11.66
Caranx	23	7.53
Decapterus	60	8.89
Scomberoides	60	7.52
Selar	115	12.25

Mean intraspecific K2P divergence of Indo-Malay Carangidae was 0.37% (range 0–4.82%), while mean congeneric species K2P divergence was 10.53% (range 0–16.4%) (Table 2.1). In the NJ analyses, the majority of recognised species formed monophyletic clusters (Figure 2.4). Such patterns illustrate the utility of *COI* sequences to provide species-level resolution. All assemblages of conspecific individuals had bootstrap support of 98–100%. However, four species which have been identified as different species formed two monophyletic clusters in both NJ (species not shown in Figure 2.4) and ML (Figure 2.5) analyses; *Alepes vari* grouped together with *Alepes melanoptera*, while *Carangoides bajad* grouped in the same cluster as *Carangoides gymnosthetus*. These results were also supported by the ABGD analysis (Appendix 3).

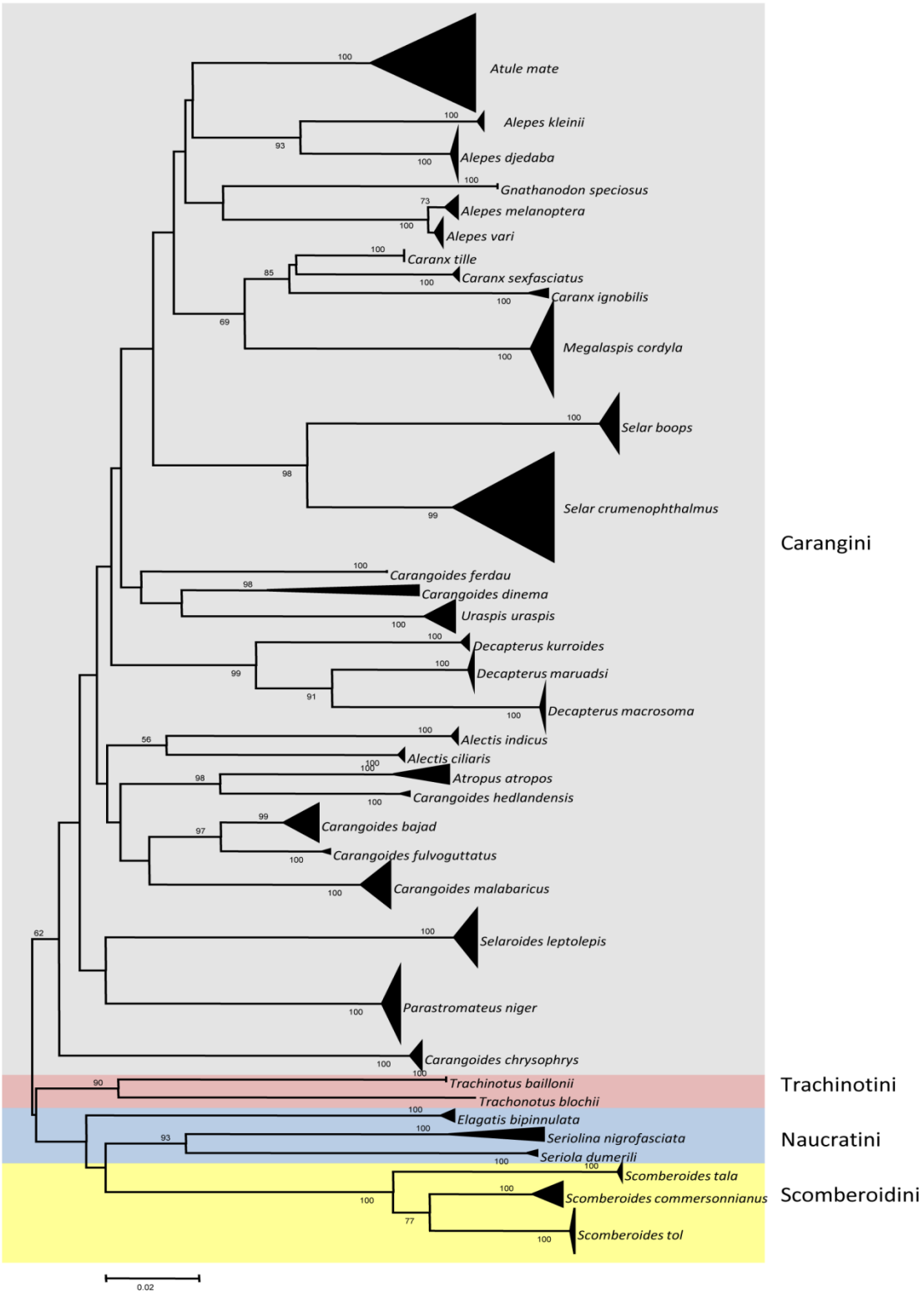

Figure 2.4 Neighbour-joining tree (K2P distance) of 36 Carangidae species. All species formed monophyletic clusters. Only bootstrap values greater than 50 are shown.

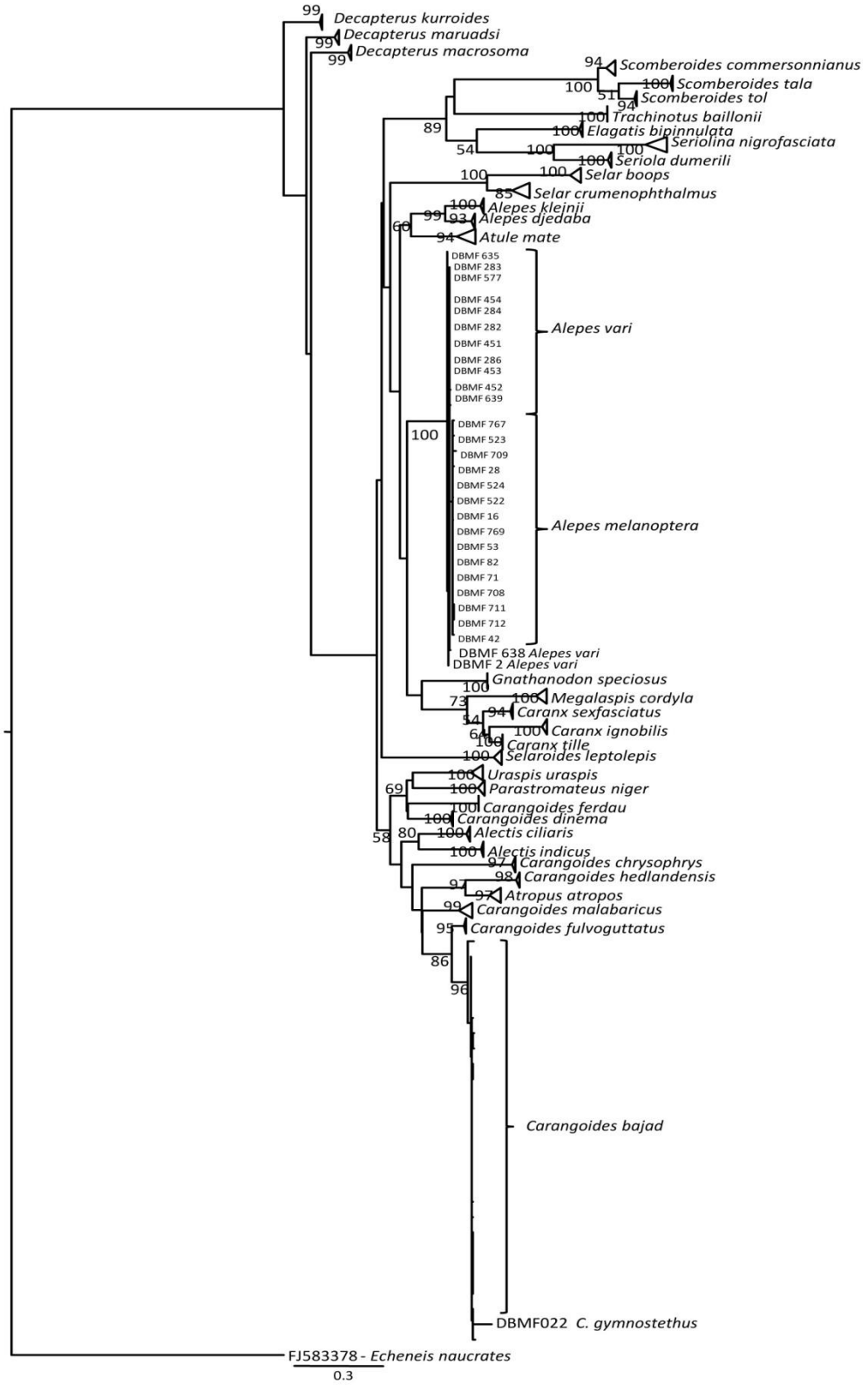

Figure 2.5 Phylogenetic tree from Maximum-likelihood analysis. Numbers above the branches represent bootstrap support based on 1000 replicates.

2.3.3 Cryptic diversity in the Indo-Malay Archipelago

In three species, we detected deep divergences among individuals that had been assigned to a single taxon. Closer observation of the data associated with *Atule mate*, *Selar crumenopthalmus* and *Seriolina nigrofasciata* showed maximum intraspecific divergences of 4.82%, 4.66% and 4.32% (Appendix 4) respectively, revealing that the specimens of each in fact formed two clusters in both NJ and ML analyses with 99–100% bootstrap support (Figures 2.6–2.11). Divergent as they were, members of the two clusters nonetheless were more similar to each other than to members of any other species in our data set.

2.3.3.1 *Atule mate*

Phylogenetic analyses also revealed two clusters generated from 67 *Atule mate* samples (Figures 2.6 and 2.7). Mean K2P distance within species was 0.34% with a maximum of 4.82% nucleotide divergence. These clusters were separated by a mean *COI* nucleotide divergence of 4%. Cluster I, the major lineage containing most specimens from all sampling regions exhibited no obvious geographic structuring, and was strongly supported with a bootstrap value of 100% in the NJ tree. In contrast, Cluster II is a minor lineage, containing only a single specimen from Tok Bali, Kelantan, eastern Peninsular Malaysia (TB). Phylogenetic trees constructed from control region and Rag 1 (nuclear DNA) data were consistent with the pattern observed at *COI* (see Chapter 3).

2.3.3.2 *Selar crumenophthalmus*

Seventy five specimens of *Selar crumenophthalmus* also formed two clusters in the *COI* NJ and ML trees (Figures 2.8 and 2.9). Mean K2P distance within species was 0.39% with a maximum of 4.66% nucleotide divergence. Cluster I comprised the majority of

the specimens with a high bootstrap value of 100%, while Cluster II comprised only two individuals from Kuala Kedah, western Peninsular Malaysia (KK) and Kuching, Sarawak (KC), also supported by a high bootstrap value of 100%. A mean pairwise distance of 4.5% separated these two clusters. No geographic pattern was apparent.

2.3.3.3 *Seriolina nigrofasciata*

Mean K2P distance within species of *Seriolina nigrofasciata* was 1.79% with a maximum nucleotide divergence of 4.32%. Nine specimens of this species formed two clusters with Cluster I comprising the specimens from Kota Kinabalu (KKJ) and Kudat (KDT), Sabah. Cluster II comprised only two individuals from Hutan Melintang (AHM) and Bagan Panchor (BP) from western Peninsular Malaysia, supported by a bootstrap value of 100% (Figures 2.10 and 2.11). A mean pairwise distance of 4.32% separated these two clusters.

COI sequences of 23 species examined here were compared with data available from conspecifics from other geographical regions (downloaded from BOLD and GenBank), and NJ trees were produced for each species (Appendix 5). From these 23 widespread species, 13 species (Appendix 5.11 - 5.23) exhibited shallow genetic structure with mixed *COI* lineages found on either side of the IMA. The other 10 species (Appendix 5.1 – 5.10) each formed two clusters with maximum nucleotide divergences ranging from 2.68–8.81%.

2.3.4 Patterns of *COI* divergence across species and habitats

Our second line of inquiry tested whether patterns of genetic divergence at *COI* corresponded to different types of habitat, body shape and size among Carangids. A one-way ANOVA revealed that there were no significant differences in levels of genetic

divergence among groups with different biological characteristics ($p>0.05$) (Appendix 6).

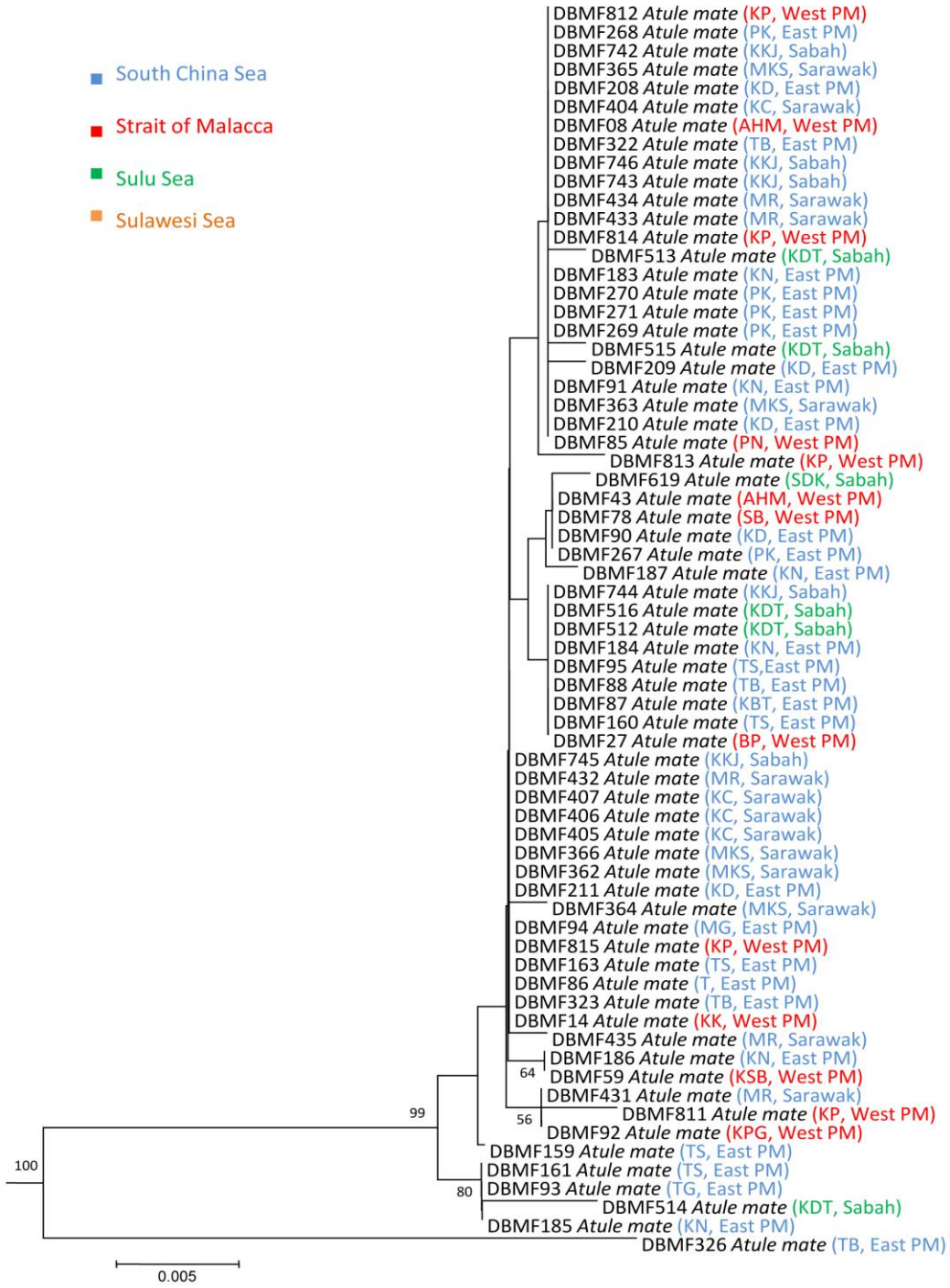

Figure 2.6 Neighbour-joining tree (K2P distance) of 67 *COI* sequences of *Atule mate*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

Figure 2.7 Maximum-likelihood tree of 67 *COI* sequences of *Atule mate*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

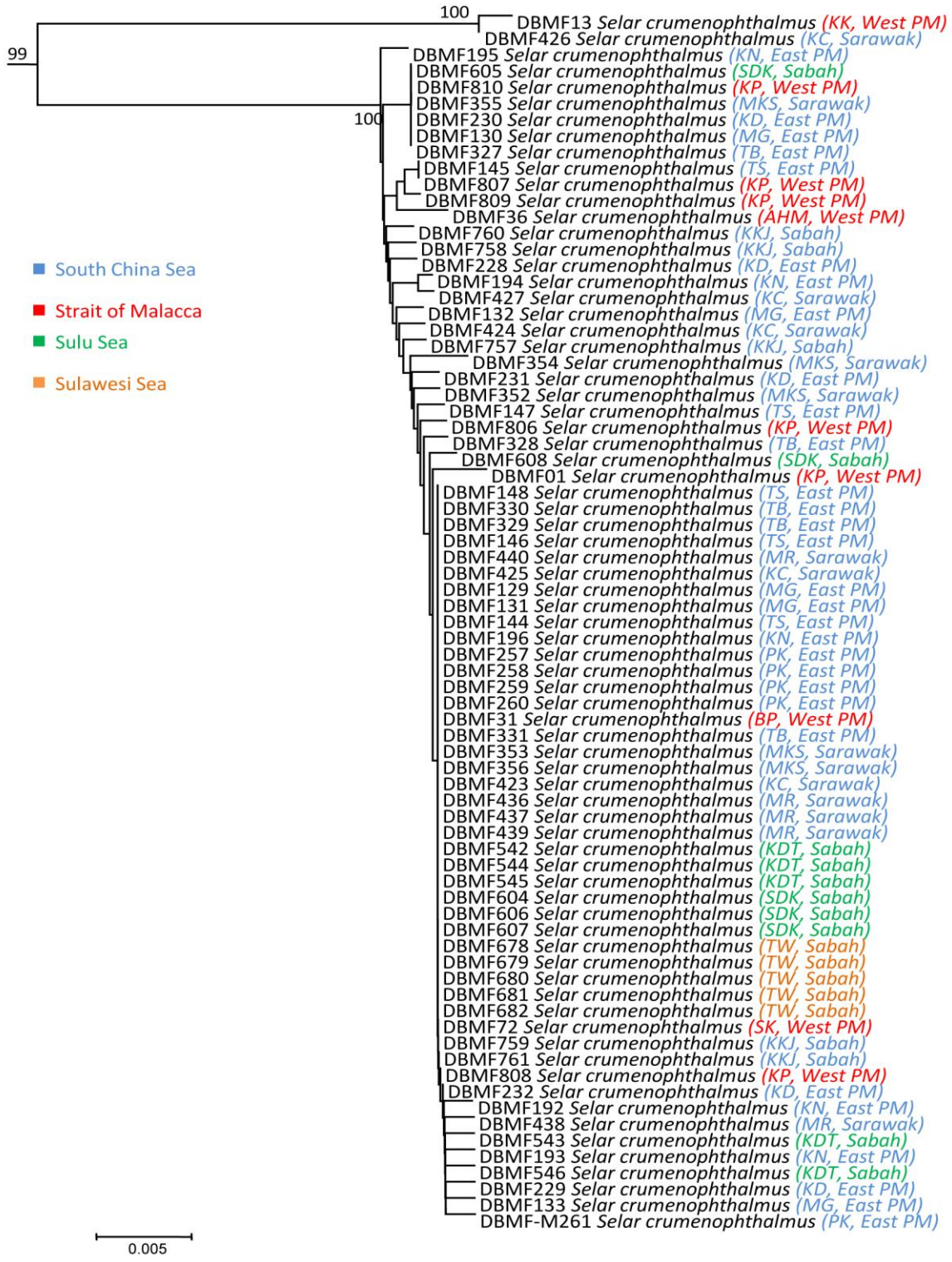

Figure 2.8 Neighbour-joining tree (K2P distance) of 75 *COI* sequences of *Selar crumenophthalmus*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

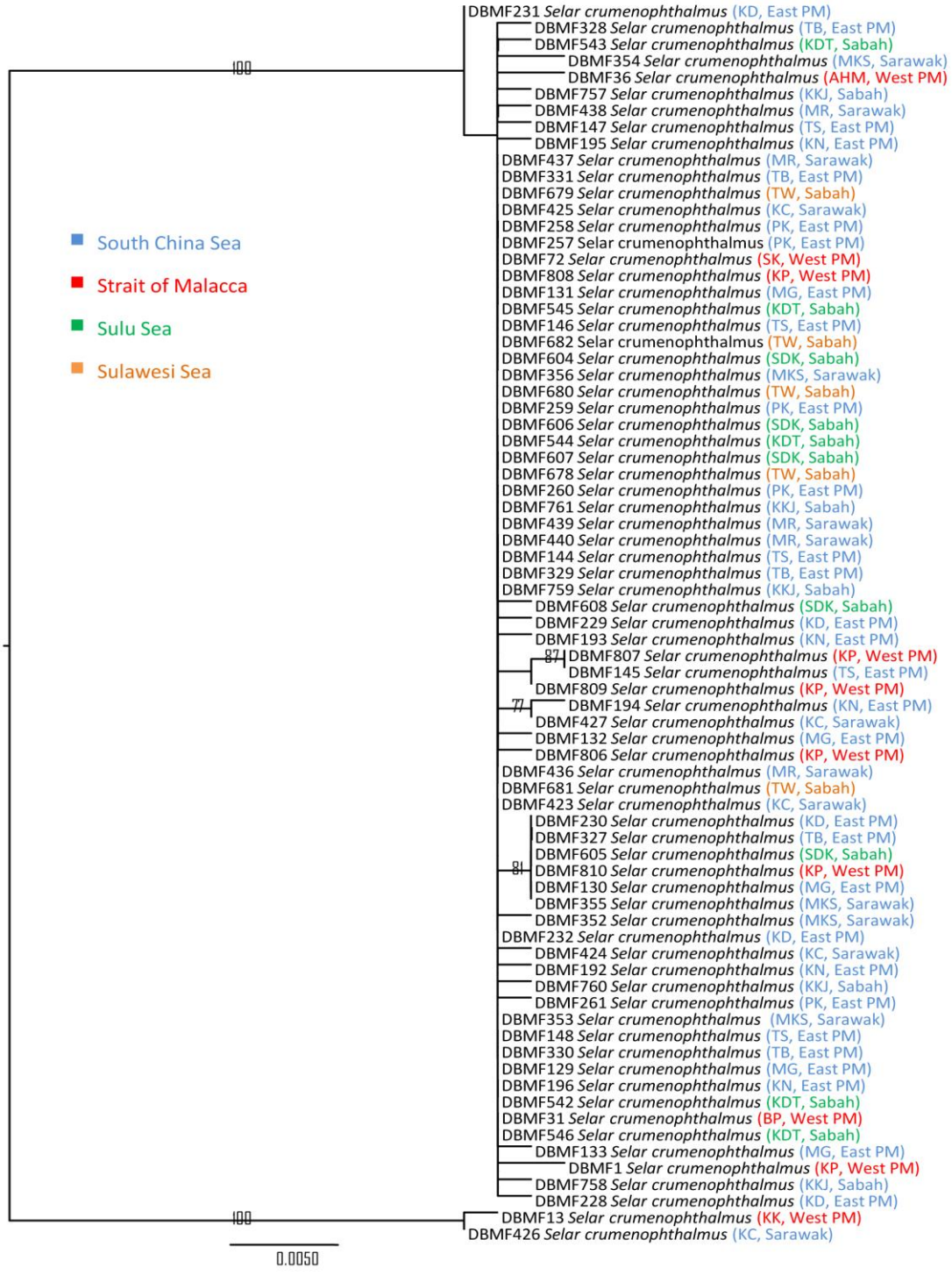

Figure 2.9 Maximum-likelihood tree of 75 *COI* sequences of *Selar crumenophthalmus*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

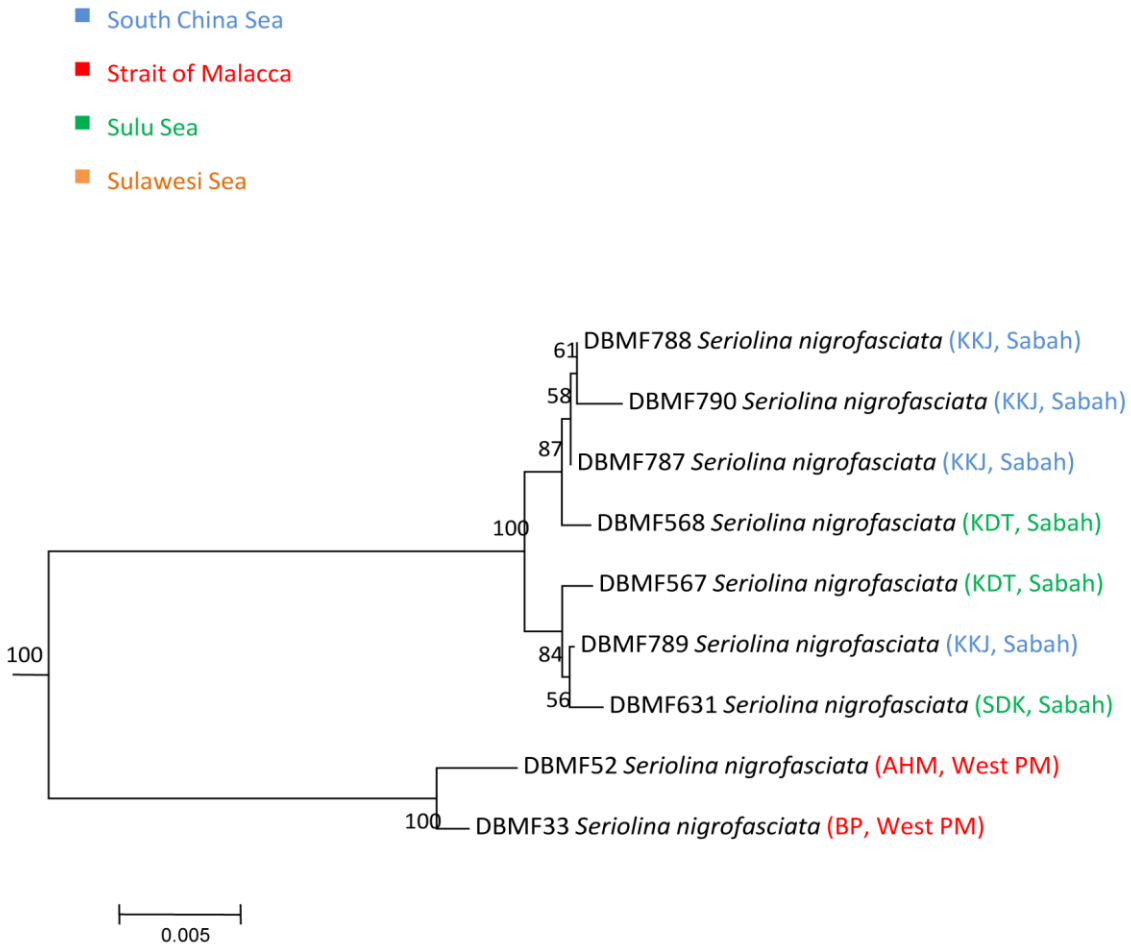

Figure 2.10 Neighbour-joining tree (K2P distance) of 9 *COI* sequences of *Seriolina nigrofasciata*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

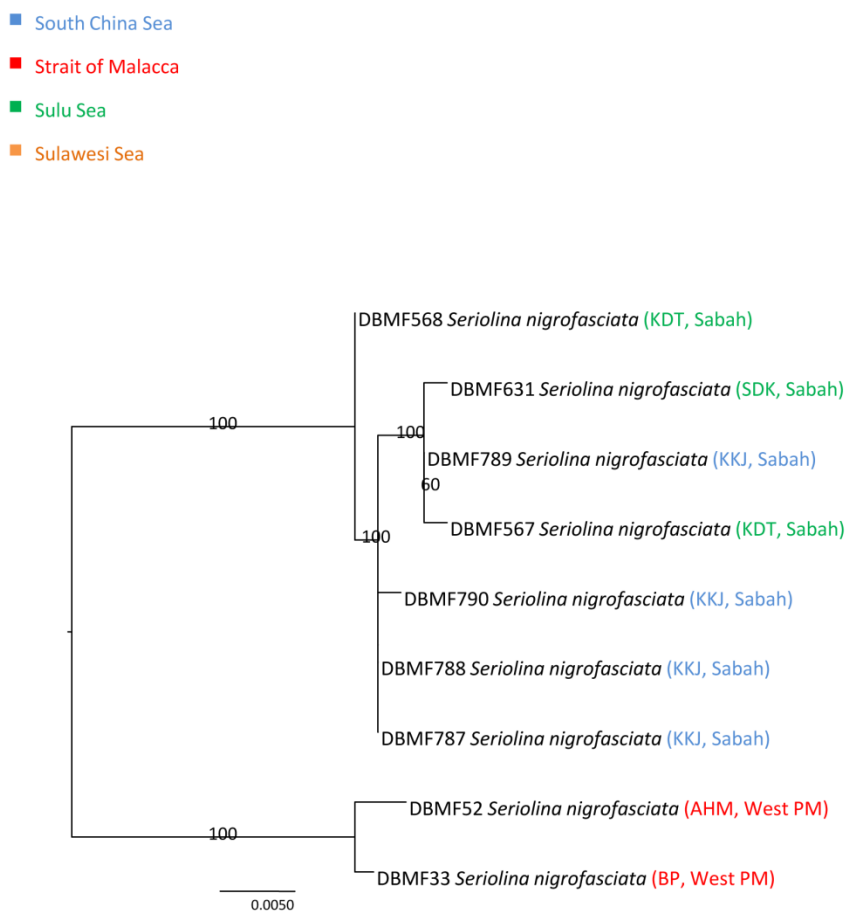

Figure 2.11 Maximum-likelihood tree of 9 *COI* sequences of *Seriolina nigrofasciata*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

2.4 Discussion

2.4.1 Species identification

According to the Fish Barcode of Life project database (www.fishbol.org), in 2009, 69% of species from Family Carangidae had been barcoded in Southeast Asia, but with some species represented by only a single sample. DNA barcodes had increased to 83% with 43 species having more than four barcodes in November 2011, including our data. We sequenced a total of 723 specimens from 18 genera and 36 species of Carangidae at the *COI* barcoding region. Thirty-three species could be accurately discriminated, illustrating the effectiveness of the *COI* gene for identifying commercial marine fish from Malaysian waters, and providing resolution at the species-level. However, the remaining three species showed deep divergences (4.32–4.82%) among individuals that had been assigned to a single taxon. Divergent as they were, members of the two clusters nonetheless were more similar to each other than members of any other species. These high sympatric divergences suggested that each may comprise cryptic species.

The average K2P distance of individuals within species was 0.37% compared with 10.53% for species within genera. Hence, congeneric species were approximately 28 times more divergent than conspecific individuals. The mean intraspecific K2P distance observed was similar to the intraspecific K2P distance reported for marine (0.24–0.39%) (Zhang and Hanner, 2011) and freshwater species (0.3–0.45%) (Hubert *et al*., 2008). The branch length among species tends to be much deeper than among conspecific individuals leading to a gap in the distribution of the pairwise distance among conspecific individuals and among species that has been referred to as the barcoding gap (Meyer and Paulay, 2005). Mean divergence among species within families increased to 16.56%. These data show that increasing genetic divergence was observed with increasing taxonomic level, supporting a marked difference in genetic

divergence at the species boundary. Such patterns in taxonomic distribution of nucleotide divergence supports observations obtained by Ward *et al*. (2005) with genetic distances of 0.39% for conspecifics, 9.93% for congenerics and 15.46% for confamilial species of 754 *COI* sequences representing 207 species of Australian fish. Data obtained in our study were also consistent with those obtained by Asgharian *et al*. (2011) for 187 individuals of Persian Gulf fish with values of 0.18%, 12% and 17.43% among conspecifics, congenerics and confamilial species respectively.

The NJ tree revealed that species identification and phylogenetic relationships based on morphological evidence and molecular methods are broadly consistent. However, the ML analyses suggested that four species might comprise only two taxonomic units, as these four species formed two reciprocally monophyletic clusters in the ML tree (*Alepes vari* and *Alepes melanoptera*; *Carangoides bajad* and *Carangoides gymnosthetus*). ABGD analysis supports such findings as the same pattern was evident. Further analyses should be undertaken by the inclusion of more genes and larger sample sizes to confirm the relationships across these four species. Phylogenetic relationships among species with NJ analysis were clearly established, and individuals from the same species were grouped in the same taxonomic cluster with 98–100% bootstrap support. According to Smith-Vaniz (1984), Carangidae can be categorized into four tribes based on morphological evidence; the Carangini, Trachinotini, Naucratini and Scomberoidini. All species of Carangidae in our study grouped according to Smith-Vaniz (1984) (Figure 2.3), with the larger clade consisting of specimens known as jacks, trevallies, scads and black pomfret (tribe Carangini). The second clade comprised the other three tribes; Trachinotini, Naucratini and Scomberoidini, representing pompano, amberjacks and queen fishes. The emergence of these four tribes in NJ analyses clearly demonstrates that there is deep phylogenetic signal in the relatively short *COI* sequence fragments, even though barcode analysis seeks only to delineate species boundaries. However, the phylogenetic relationships of these four tribes remain questionable (Kijima *et al*., 1986; Gushiken, 1988; Reed *et al*.,

2002), and our approach in isolation is not sufficient to explore such questions in depth. Additional gene regions, together with more comprehensive analytical methods including parsimony, ML and Bayesian approaches should be included to resolve such apparently deep phylogenetic relationships.

The main goals of DNA barcoding are to assign unknown specimens to a species category, and enhance the disclosure of new and cryptic species. DNA barcoding also facilitates identification, particularly in microscopic, diverse life history stages, and other organisms with complex or inaccessible morphology (Hebert *et al*., 2003a). Furthermore, the approach is also able to discriminate species of highly similar morphology. The Carangids, which are morphologically very similar, such as the three species (*Caranx ignobilis*, *Caranx sexfasciatus* and *Caranx tille*), formed a sister grouping (Figure 2.11). Because of such high similarity, they are sometimes misidentified. However, DNA barcoding discriminated these *Caranx* samples effectively on all occasions. Three distinct clusters were formed, separating the three species by an average interspecific distance of 7.53%, and average intraspecific distances of 0.51%, 0.16% and 0.07% for *Caranx ignobilis*, *Caranx sexfasciatus* and *Caranx tille*, respectively.

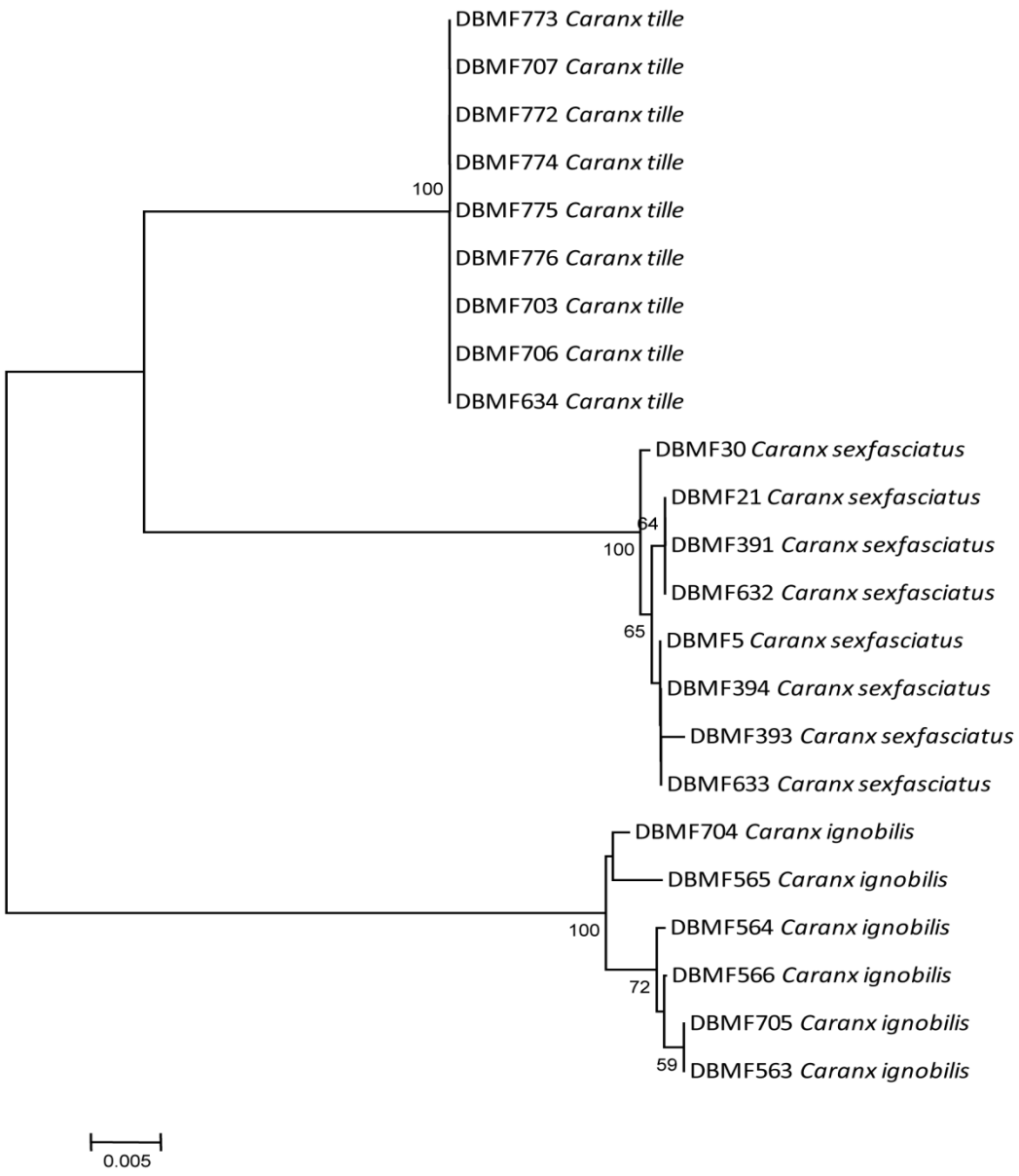

Figure 2.12 Neighbour-joining tree (K2P distance) of genus *Caranx*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.

2.4.2 Cryptic diversity in the IMA

The Indo-Malay Archipelago has long been considered as the centre of maximum marine biodiversity (Hall, 2002). A few studies based on the *COI* marker have discovered high cryptic diversity in coral reef fish around this region (Hubert *et al*., 2012; Ward *et al*., 2005). Several hypotheses have been proposed to explain the remarkably high diversity found in this region: 1) centre of origin (Briggs, 2005), 2) centre of accumulation (Jokiel and Martinelli, 1992), and 3) centre of overlap (Woodland, 1983). Hypotheses 1 and 2 have recently been raised (Hubert *et al*., 2012) to explain speciation and dispersal of marine species in the Indo-Malay Archipelago. It might either be the result of diversification within the region and subsequent species dispersed into peripheral areas (Centre of Origin), or the result of an overlap of the faunas from the Indian and Pacific Oceans (Centre of Overlap).

A few studies have identified high levels of cryptic species occurring within and outside the IMA (Ward *et al*., 2005; Zemlak *et al*., 2009; Hubert *et al*., 2012; Puckridge *et al*., 2013), though here, we detected only a moderate frequency of potentially cryptic species within commercially exploited Indo-Malay Carangidae. Small sample size, bias in range of species collected, and restricted geographic ranges may have lead to fewer cryptic species being identified compared to previous studies. By increasing the geographic sampling range, more cryptic diversity will likely be detected (Ward *et al*., 2005; Zemlak *et al*., 2009; Hubert *et al*., 2012). The majority of the species in Carangidae have a pelagic lifestyle. Interestingly, within marine ecosystems, most diversity is benthic, with such organisms including 98% of species diversity, while the remaining 2% are pelagic (Brunel, 2005). Three species representing complexes of six potential cryptic species were detected in Indo-Malay Carangidae*; Atule mate*, *Selar crumenophthalmus* and *Seriolina nigrofasciata*. All NJ and ML trees identified two separate lineages but only *Seriolina nigrofasciata* showed allopatric divergence, with the Sabah lineage separated from the West Peninsular Malaysia lineage by 4.32%. The

other two showed sympatric divergences with both clusters consisting of geographically mixed *COI* lineages.

Comparison of *COI* sequences of 23 species from this study with conspecific sequences available from other geographical regions (Asgharian *et al*., 2011; Lakra *et al*., 2011) revealed the existence of several more complexes of potentially cryptic species from outside the IMA. Using the ABGD analysis (Puillandre *et al*., 2012), 10 lineages were flagged as candidate cryptic species. Four recognised species; *Caranx sexfasciatus* (Appendix 5.4) , *Decapterus maruadsi* (Appendix 5.5), *Gnathanodon speciosus* (Appendix 5.6) and *Seriolina nigrofasciata* (Appendix 5.10) each comprised two lineages exhibiting allopatric divergences with a maximum nucleotide divergence of 7.1%, 2.7%, 3.8% and 4.35%, respectively. However, the remaining six species; *Atropus atropos* (Appendix 5.1), *Atule mate* (Appendix 5.2), *Carangoides chrysophrys* (Appendix 5.3), *Trachinotus blochii* (Appendix 5.7), *Scomberoides commersonnianus* (Appendix 5.8) and *Selar crumenophthalmus* (Appendix 5.9) showed sympatric divergences. As for *Seriolina nigrofasciata*, additional sequences from India and Iran clustered together, and samples from West Peninsular Malaysia were clearly separated from the western part of the IMA together with Sabah (Borneo), representing an additional complex of two potential cryptic species (Appendix 5.10). Such findings are consistent with large faunal discontinuities between Indian and Pacific Ocean ichthyofaunas as a consequence of geographic isolation on each side of IMA, as discussed by Springer and Williams (Springer and Williams, 1990). However, our data is not sufficient to explain the hypothesis of species richness in the IMA. To explore hypotheses of species diversification it is necessary to sample the whole family across their broad geographic range.

2.4.3 *COI* divergences in relation to different biological characteristics

Our study has examined only one family with different lifestyles, body shape and body size. We did not identify any significant association between genetic distances and these biological characteristics (pers. obs.). However, Zemlak *et al*. (2009) used *COI* to examine patterns of divergences between fish species representing different lifestyles from opposite sides of the Indian Ocean. They detected deep divergences between certain inshore taxa, with the inshore taxa (mean *COI* divergence =0.51%) exhibiting significantly higher levels of putative cryptic species than the offshore (mean *COI* divergence= 0.26%) fish. Such deep divergences were more representative of patterns in congeneric species than among populations of a single species, highlighting the possible genetic isolation of presumed cosmopolitan species. Out of the 35 species studied by Zemlak *et al*. (2009), the one member of Carangidae sampled, the needlescaled queenfish (*Scomberoides tol*), appears to represent a broadly distributed sibling species pair whose distribution spans the Indian Ocean. Such findings reinforce the need in such *COI* barcoding studies to sample throughout the extremes of the geographic range to investigate the extent of hidden diversity in marine fauna.

2.5 Conclusion

In conclusion, the establishment of an Indo-Malay Carangidae *COI* barcoding library presented here contributes to the global DNA barcoding effort to document and catalogue the diversity of life, particularly with regard to conservation and management applications. We anticipate that the accumulation of biodiversity data will help drive and inform effective planning and monitoring of conservation and fisheries programmes in the Indo-Malay region. Intensification of industrial and commercial activities in Malaysian waters renders the biodiversity of the region highly vulnerable to threats and degradation. Therefore, such data are helpful not only to document mechanisms driving population structuring and recruitment in Carangidae,

but also provide a scientific framework in support of effective management strategies and the conservation of commercially-important fisheries resources.

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CHAPTER 3

STOCK STRUCTURE OF COMMERCIALLY EXPLOITED INDO-MALAY CARANGIDAE (TELEOSTEII: PERCIFORMES)

Abstract

Selar crumenophthalmus (pelagic)*, Atule mate* (moderately pelagic) and *Selaroides leptolepis* (demersal) are commercially-important Carangidae with contrasting habitat use. In this chapter, I tested the hypothesis that pelagic species will display less genetic divergence compared to demersal species, due to their potential to undertake long-distance migrations in oceanic waters. To evaluate population genetic structure of these three species, 650bp of *COI*, 450bp of control region (mtDNA), and 910bp of Rag1 (nuclear DNA) were sequenced in each species. Population structure of *Atule mate* and *Selar crumenophthalmus* was lower than *Selaroides leptolepis*, consistent with my hypothesis. The neighbor-joining trees of *Atule mate* were split into three distinct clades with bootstrap values of 98-99% in *COI*, 100% in control region and 65-70% in Rag1. The neighbor-joining trees of *Atule mate* were split into three distinct clades with bootstrap values of 98-99% in COI, 100% in control region and 65-70% in Rag1. However in *Selar crumenophthalmus*, only one cluster appeared in *COI* data with maximum nucleotide divergence of 0.78%, and two clades in both control region and Rag1 with 99% and 60-65% bootstrap values respectively. In *Selaroides leptolepis*, all trees were split into three closely related clades, which did not appear to have any geographic structure with bootstrap values of 62-98% in *COI*, 73% in control region and 56% in Rag1. Hierarchical molecular variance analysis (AMOVA), pair wise F_{ST} comparisons and the nearest-neighbor statistic (*S*nn) showed significant genetic differences among Kuwait and IMA populations for *Atule mate*. Within IMA itself, two distinct mitochondrial lineages were detected in *Atule mate* suggesting potential cryptic species. However, lack of significant genetic differentiation in *Selar crumenophthalmus* suggesting a panmictic population of this species in the IMA. The present data suggest that samples size should be increased, and additional more rapidly evolving genetic markers should be used to detect population structuring in Indo-Malay Carangidae.

3.1 Introduction

The Indo-Malay Archipelago (IMA) is one of the most important commercial fishery areas in the world, and thus plays a critical role in providing food resources. According to the Department of Statistics Malaysia (2012), international comparisons in 2010 for production of fish capture showed that Malaysia is ranked 17th in the world and 11th in Asia, with total marine fish landings of 1.4 million metric tonnes (mt). There are 100 fishing districts around Peninsular Malaysia and Malaysian Borneo, making the fisheries sector an important economic sub-sector, playings a significant role in the national economy (Annual Fisheries Statistics, 2011). Various marine ecosystems can be found in the IMA, which due to complex geological features enhancing habitat diversity, leading to a productive and successful fishing industry. Located between the Indian and Pacific Ocean, the archipelago consists of over 25, 000 islands, providing a coral reef areas of approximately 4,006 km^2 (Burke *et al*., 2002) and coastal mangroves of approximately 5,669 km^2 (Wong, 2004). Such ecological heterogeneity provides as important resources for feeding and nursery grounds for a plethora of marine taxa. The South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea also offer opportunities for exploitation of fish. Therefore, this mega-diverse tropical region (Lohman *et al*., 2011) harbours many species of commercially high-value marine fish for exploitation.

However, due to its high levels of biodiversity, this region has experienced unsustainable exploitation of fisheries, and significant habitat destruction (Chong *et al*., 2010). Almost half (48%) of the total of freshwater and marine fishes in Malaysia are currently threatened to some degree, while nearly one third (27%), mostly from marine and coral habitats, require urgent scientific data to evaluate their status (Chong *et al*., 2010). Fish extinctions in Malaysia are increasingly likely due to habitat loss or modification (76%), overfishing (27%) and by-catch (23%) (Chong *et al*., 2010). For strictly coral-reef fishes, the most important threats identified are habitat degradation

(63%), pollution including sedimentation (34%), by-catch (28%) and overfishing (22%) (Chong *et al*., 2010). Coral reefs area in Sabah, for example, have experienced degradation in the past due to dynamite fishing (Pilcher and Cabanban, 2000), though the establishment of marine parks since 1994 has restricted blast-fishing activities to protect coral reef communities.

Limited data on the genetic basis of stock structure for any pelagic fish adds to the complexity in managing these marine resources. Indeed, it is now well established that an understanding of the spatial and temporal dynamics of fish stock structure is fundamental to effective stock management practices (Carvalho and Hauser, 1994; Begg *et al*., 1999; Hauser and Carvalho, 2008). Determining stock or population structure of any fish species is a challenging task as many fish populations vary in distribution and abundance, sometimes across small spatial and temporal scales. Additionally, many fisheries comprise poorly defined mixtures of multiple stocks. Fisheries stocks that differ to varying degrees in their biological and/or genetic integrity need to be monitored, assessed and managed separately. Failure to recognize stock structure may lead to erosion of some spawning components, over-exploitation, and depletion of less productive stocks.

Various techniques such as meristics and morphometrics (Garcia-Rodriguez *et al*., 2011), traditional tags, parasites as natural tags, otolith chemistry (Suzanne *et al*., 2004), molecular genetics and electronic tags (Thorsteinsson, 2002) have been used to determine stock or population structure and dynamics of fish populations (Begg and Waldman, 1999; Kerr *et al*., 2005). Among these, a genetic approach to fish stock assessment has been widely used in determining stock due to its cost-effectiveness and robustness of results obtained (Carvalho and Hauser, 1994, Dudgeon *et al.*, 2012). Genetic approaches provides information on levels of genetic diversity in fish populations, degree of genetic differentiation among fish populations, and hence population genetic structure. Additional inferences can thereby be made on levels of

gene flow among fish populations, such as the effective number of gene migrants that are exchanged among populations. Where significant genetic divergence exists, the information typically indicates demographically independent entities: valuable information in the quest to predict response to harvesting. Some examples include the mitochondrial DNA analysis of the Indian scad mackerel *Decapterus russelli* (Carangidae) in the Indo-Malay Archipelago by (Perrin and Borsa, 2001). Based on their results, cytochrome *b* gene sequence data revealed two major genetic lineages. The distinction of two clades within *D. russelli* could be explained by historical isolation of the Sulawesi Sea region from other areas in the IMA during Pleistocene climatic cycling. However, Borsa (2003) conducted a study on the genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) in the IMA using mitochondrial and nuclear DNA markers. Here, no significant heterogeneity in cytochrome *b* haplotype frequencies or in aldolase B-1 allele frequencies was detected across populations, suggesting the presence of a single stock of the species in the region. There thus remains discordance in the extent of structuring across species and regions. Additional data are required.

The degree and distribution of genetic diversity in marine biota is determined not only by contemporary levels of gene flow, but also by demographic processes, population history, and selection (Hewitt, 2000; D'Amato and Carvalho, 2005). Insight into historical processes can enhance the understanding of population structure underlying evolutionary processes (Grant and Bowen, 1998). The magnitude and pattern of polymorphism in DNA sequences are informative for the inference of the history of a population as well as the mechanisms responsible for generating and maintaining the polymorphism (Li, 1997). Such information could provide insight into the spatial components of phylogeographic lineages and explain the evolutionary process of geographically related populations (Avise, 2000). Therefore, in the present study, the population structure of three commercially-important species of Indo-Malay Carangidae was analysed from mitochondrial DNA (cytochrome *c* oxidase I (*COI*) and

control region), and nuclear DNA (Recombination Activating Gene (Rag1)). Being commercially and ecologically important, Carangidae are one of the main capture targets for fisheries in the IMA. Carangidae comprises fishes whose body shapes vary from elongate and fusiform to deeply ovate and strongly compressed. Their habitats range from pelagic to demersal; many are semi-pelagic (Laroche *et al*., 1984). Several preliminary studies of Carangidae mainly examined their ecology and fishery biology (Blaber and Cyrus, 1983; Dalzell and Penaflor, 1989; Ditty *et al*., 2004; Honebrink, 2000; Roos *et al*., 2007; Smith and Parrish, 2002; von Westernhagen, 1973; Wetherbee *et al*., 2004), but little information concerning the assessment of population genetic structure and genetic diversity in high commercial-value species such as *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* hitherto exists. It is unknown whether these three species in Malaysian waters form single respective stocks, or are genetically subdivided into distinct separate populations. Such core information is required for their effective conservation and management, since rates of harvesting continue to decrease (Abu-Talib *et al*., 2000, Department of Statistics Malaysia, 2012).

3.2 Materials and Methods

3.2.1 Sampling

Three species of Carangidae with contrasting habitat use, which are highly pelagic (*Selar crumenophthalmus*), moderately pelagic (*Atule mate*) and demersal (*Selaroides leptolepis*) were examined in this study (refer Chapter 2, Section 2.2.5). Multiple samples were collected from four geographic regions within the IMA: South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea (Figure 3.1). Population samples were collected from several fish landing sites during two collecting trips; from October to November 2009, and from June to July 2010. Digital photographs of individuals were taken immediately, and voucher specimens were tagged according to museum ID

number and archived in the South China Sea Museum, Universiti Malaysia Terengganu (www.umt.edu.my). All details regarding collection dates, collection sites with geographical coordinates, taxonomy and vouchers can be found in the Barcode of Life Data System website (BOLD, www.boldsystems.org) (Ratnasingham and Hebert, 2007) under project 'DNA Barcoding of Malaysian Fish' (DBMF). All fishes were identified based on morphology, with support from expert local taxonomists in most cases. Where expert's advice was not available, guidance was obtained from FAO-Fisheries Identification Sheets (Fisher and Whitehead, 1974) and identification books published by the Department of Fisheries Malaysia (Annie and Albert, 2009; Mansor *et al*., 1998).

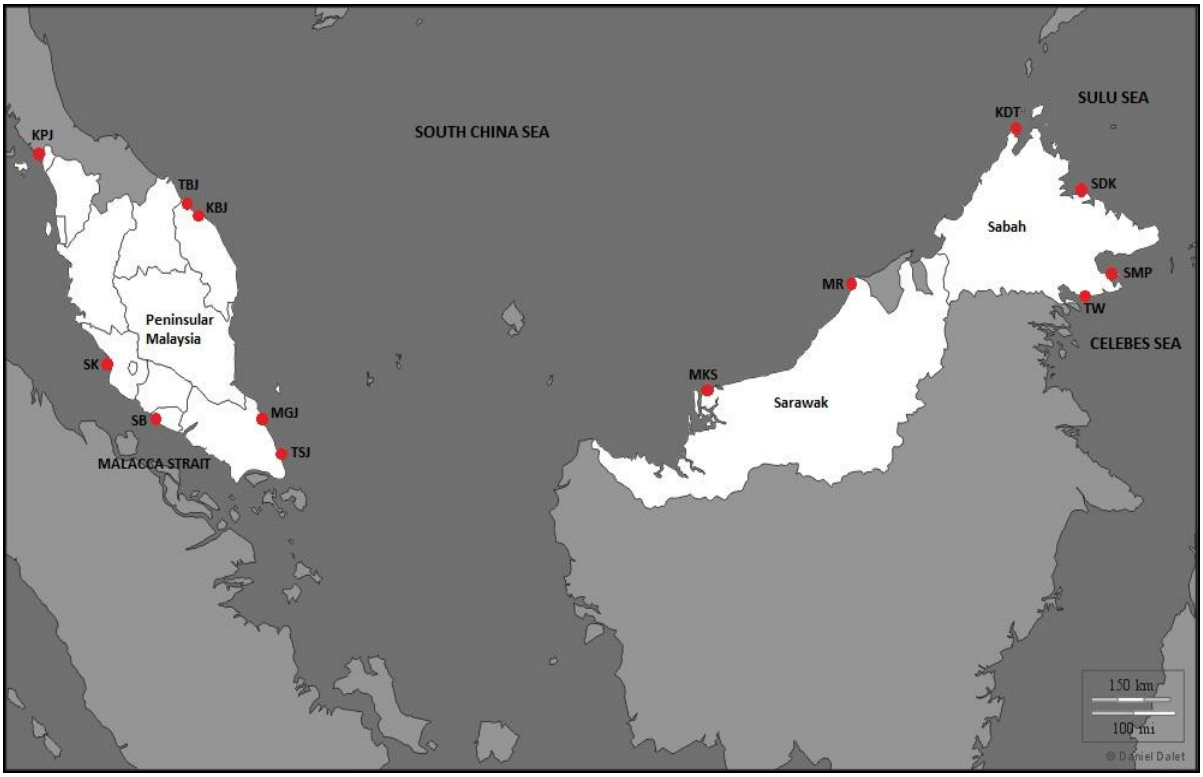


Figure 3.1 Distribution locations for 180 specimens sampled along the coast of Malaysia. Samples were collected from respective landing sites (in red) in four geographical regions of IMA; South China Sea, Strait of Malacca, Sulu Sea and Celebes Sea. Sample sizes for each species and sample code are given in Table 3.1.

Table 3.1 List of samples from different locations.

Species	Sampling site	Geographical location	Geographic region*	Sample size	Sample code
Atule mate	Kuala Perlis, Perlis	West Peninsular Malaysia (North)	SM	10	KPJ
	Tok Bali, Kelantan	East coast Peninsular Malaysia (North)	SCS	10	TBJ
	Tanjung Sedili, Johor	East coast Peninsular Malaysia (South)	SCS	10	TSJ
	Mukah, Sarawak	Borneo	SCS	10	MKS
	Kudat, Sabah	Borneo	SS	5	KDT
	Sandakan, Sabah	Borneo	SS	8	SDK
	Semporna, Sabah	Borneo	CS	10	SMP
	Kuwait	Persian/Arabian Gulf	AG	5	KWT
Selar crumenophthalmus	Kuala Perlis, Perlis	West Peninsular Malaysia (North)	SM	10	KPJ
	Sekinchan, Selangor	West Peninsular Malaysia (South)	SM	5	SK
	Tok Bali, Kelantan	East coast Peninsular Malaysia (North)	SCS	5	TBJ
	Tanjung Sedili, Johor	East coast Peninsular Malaysia (South)	SCS	10	TSJ
	Mukah, Sarawak	Borneo	SCS	5	MKS
	Kudat, Sabah	Borneo	SS	5	KDT
	Sandakan, Sabah	Borneo	SS	5	SDK
	Semporna, Sabah	Borneo	CS	5	SMP
	Tawau, Sabah	Borneo	CS	5	TW
Selaroides leptolepis	Kuala Perlis, Perlis	West Peninsular Malaysia (North)	SM	7	KPJ
	Kuala Sungai Baru, Melaka	West Peninsular Malaysia (South)	SM	5	SB

	Kuala Besut, Terengganu	East coast Peninsular Malaysia (North)	SCS	5	KBJ
	Mersing, Johor	East coast Peninsular Malaysia (South)	SCS	10	MGJ
	Miri, Sarawak	Borneo	SCS	5	MR
	Kudat, Sabah	Borneo	SS	5	KDT
	Sandakan, Sabah	Borneo	SS	10	SDK
	Semporna, Sabah	Borneo	CS	5	SMP
	Tawau, Sabah	Borneo	CS	5	TW
			Total	180	

*Symbols equal: AG, Arabian Gulf; CS, Celebes Sea; SCS, South China Sea; SM, Strait of Malacca; SS, Sulu Sea.

3.2.2 DNA extraction

Fin clips were taken from the right pectoral fin of each fish and preserved in 99% ethanol. Fish specimens were then placed in ice, frozen on site and transported to South China Sea Museum, University Malaysia Terengganu. Total genomic DNA was extracted from fin clips of 180 specimens using the "salting out" method (Miller *et al*., 1988). Isolated DNA was resuspended in 100µl deionized water. A fragment of 650 base pairs (bp) of *COI*, 450 bp of the control region and 950 bp of nuclear gene (Rag1) were amplified using the list of primers in Table 3.2.

Table 3.2 List of sequencing primers used in this study.

Gene	Name	Primer sequences	References
COI	Fish F2	5' TCGACTAATCATAAAGATATCGGCAC 3'	Ward *et al*.,
	Fish R2	5' ACTTCAGGGTGACCGAAGAATCAGAA 3'	2005
Control	Fish CR_F	5' CCTGAAGTAGGAACCAGATG 3'	
region	Fish CR_R	5' AACTCTCACCCCTAGCTCCCAAAG 3'	
	JUR R1_F	5' CAGAAAAAGGAGACTCTAACTCCTG 3'	Cardenas *et*
	JUR R2_R	5' TGCTTGCGGGGCTTTCTA 3'	*al*., 2009
Nuclear	Fish Rag 1_F1	5' CGGCTTTCACCAGTTTGAAT 3'	Newly
gene	Fish Rag1_F2	5' GGATCTGGAGGAGGACATCA 3'	designed
(Rag1)	Fish Rag1_R1	5' TGCTGGGAGTTGAAGCTGTA 3'	primers
	Fish Rag1_R2	5' TGCTGGGAGTTRAAGCTGTA 3'	
	Fish Rag1_R3	5' CCTATATTTGAAGGTAGAGGACAGG 3'	
	Fish Rag1_R4	5' ATATTTGAAGGTAGAGGACAGGAG 3'	

3.2.3 PCR amplification and sequencing

Polymerase reactions were prepared in 11µl reaction volumes including 1µl DNA, 6.6µl ultra pure water, 1.0µl 10X PCR buffer, 0.2µl $MgCl_2$ (25mM), 0.5 µl of each primer (10 µM), 1.0µl dNTPs (2mM), 0.2µl *Taq* polymerase (500U). The thermal regime for *COI* consisted of an initial step of 5 min at 94^{O}C followed by 35 cycles of 1 min at 94^{O}C, 1 min 30s at 58.2^{O}C, and 1 min at 72^{O}C, followed in turn by 10 min at 72^{O}C. For the control region, the amplification started with an initial step of 2 min at 95^{O}C followed by 35 cycles of 30s at 94^{O}C, 30s at 48.3^{O}C, and 1 min at 72^{O}C, followed in turn by 10 min at 72^{O}C. The amplification programme for Rag 1 was carried out initially at 95^{O}C for 3 min followed by 35 cycles with: 94^{O}C for 30s, 52^{O}C for 45s, 72^{O}C for 1 min 30s and finally 10min of final extension at 72^{O}C. DNA amplification products were separated in 1.2% (w/v) agarose gels at 100v with 1X Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and visualized under UV illumination. Prior to sequencing, 10µl PCR products were cleaned with 1U shrimp alkaline phophatase (Promega) to dephosphorylate residual deoxynucleotides and 0.5U Exonuclease I (Promega) to

degrade excess primers [Werle *et al*., 1994]. The purification thermal conditions consisted of 37^{O}C for 1 hour and 80^{O}C for 15 min. Bidirectional sequencing was performed using BigDye Termation chemistry on an Applied Biosystems 3730 sequencer by Macrogen Inc, (www.macrogen.com, South Korea). Once sequencing was completed, the raw nucleotide sequences were checked by eye to ensure sequence information was consistent in both directions. In addition, all the sequences were verified by making comparison with the known specimens from GenBank.

3.2.4 Data analysis

Initial editing of ambiguous bases was undertaken with MEGA5 software (Kumar *et al*., 2004). The edited sequences of each locus were aligned using Clustal W implemented in the same software. The alignments obtained were further visually cross-checked. Amino acid sequence translation (vertebrate mitochondrial code) was applied and checked for stop codons, to ensure the amplification of mtDNA rather than nuclear copies of *COI* sequences, and then translated back for subsequent analysis. Prior to analysis of the data, the program PHASE (Stephens *et al*., 2001) was used to resolve the heterozygous sites in RAG1 sequences to reconstruct haplotypes. PHASE uses a statistical method to infer linkage phase of polymorphic sites from a population sample of genotypic data. It was unnecessary to use PHASE on the mitochondrial sequences because mitochondrial DNA is haploid and therefore contained no heterozygous sites.

3.2.5 Population genetic analysis

DnaSP5.0 (Rozas *et al*., 2003) was used to calculate sequence diversity statistics as well as determination of identical haplotypes. The Arlequin software package version 3.5.1.2 (Excoffier and Lischer, 2010) was used to perform an analysis of molecular variance (AMOVA) to examine population structure of each species. AMOVA examines

the variance in gene frequencies between different groupings while also taking into account the number of mutations between the haplotypes (Excoffier *et al.*, 1992). AMOVA can group individuals hierarchically by their region and source population/locality, and evaluates the proportion of overall genetic variation that is attributable to that grouping. It examines the structure of the genetic variation among regions (F_{CT}), among-localities within regions (F_{SC}), and among individuals within localities (F_{ST}) (Weir and Cockerham, 1984). AMOVA categorizes the distribution of genetic variation across geographic space by examining the degree of genetic differentiation within and among the different hierarchical groupings. For the regional comparison here, the localities were divided into four regions in the IMA, South China Sea (SCS), Strait of Malacca (SM), Sulu Sea (SS) and Celebes Sea (CS), and additional Arabian Gulf (AG) for *Atule mate* analysis. The nearest-neighbor statistic (*S*nn) (Hudson, 2000) was estimated using DnaSP5.0 software. This statistic measures population differentiation by testing whether low divergent sequences are from the same location, and it is particularly useful when populations show high levels of haplotype diversity (Hudson, 2000). A minimum spanning network was constructed with Network 4.6.1.1, based on haplotype frequencies to search for phylogeographic structure.

3.2.6 Phylogenetic analysis

A neighbour-joining phylogeny of the mitochondrial and nuclear loci were reconstructed in MEGA5 using the K2P distance model (MEGA5; Kumar *et al*., 2001). One thousand bootstraps were performed to estimate support. The program MEGA5 was also used for a maximum likelihood analysis, using the GTRGAMMA model of nucleotide evolution. Nucleotide sequence evolution models were evaluated using likelihood-ratio test implemented by Modeltest3.06 (Posada and Crandall, 1998). Support for inferred relationships was estimated by conducting 1000 bootstrap

replicates. Bayesian phylogenetic analysis was conducted in BEAST v1.7.3 (Drummond *et al*., 2012).

3.3 Results

3.3.1 Mitochondrial DNA analysis

3.3.1.1 *COI*

A total of 68, 53 and 57 individuals were assayed from nine localities of *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* respectively, for 650 base pairs (bp) of *COI* gene. Twenty-four, 23 and 13 unique haplotypes were identified, with seven, four and two haplotypes recovered more than once in each species respectively (Table 3.3). To further depict the phylogenetic and geographical relationships among the identified *COI* sequences, haplotype networks were constructed using the median-joining method in Network 4.6.1.1 software (Figure 3.2). For *Atule mate*, the resulting networks exhibited a star-like pattern surrounding haplotype H_8, which was found in every localities of *Atule mate* from the IMA. Haplotype H_1 and H_2 were only found in individuals from Kuwait (KWT). The other two haplotypes, H_3 and H_7, were shared by Tok Bali (TBJ) and Semporna (SMP). Two haplotypes (H_14 and H_17) were shared by two localities, while H_4 and H9 were shared by four and five localities respectively. The other 15 haplotypes were singletons, and were restricted to a single locality (Table 3.3a). For *Selar crumenophthalmus*, haplotype H_3 was found in every locality. Two haplotypes (H_2 and H_10) were shared by two localities, while H_5 were shared by four localities. The other 19 haplotypes were only found once and restricted to a single locality (Table 3.3b). *Selaroides leptolepis* also showed a star-like pattern in haplotype network, with haplotype H_4 was found in every locality. Only one haplotype (H_12) was shared by Semporna (SMP) and Tawau (TW). Finally, the other

11 haplotypes were found in only one locality, most of them being singleton haplotypes (Table 3.3c). The haplotype (h) and nucleotide (π) diversity were markedly higher in *Atule mate* (h= 0.893, π= 0.01424) compared to *Selar crumenophthalmus* (h= 0.758, π= 0.00215) and *Selaroides leptolepis (*h=0.458, π=0.00279).

Table 3.3 Distribution of haplotype frequencies in *COI* by species.

a) *Atule mate*

Localities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	n	h	π
Kuala Perlis (KPJ)								2	4	1	1	1	1												10	0.844	0.00378
Tok Bali (TBJ)			2	3	1	1	1	1	1																10	0.911	0.01958
Tanjung Sedili (TSJ)				1				4	2								1			1	1				10	0.844	0.00245
Mukah (MKS)				1				5	3					1											10	0.711	0.00151
Semporna (SMP)			1				2	1							1	1	2	1	1						10	0.956	0.02188
Kudat (KDT)				1				1						1			1					1			5	1.000	0.00422
Sandakan (SDK)								4	1					1									1	1	8	0.786	0.00271
Kuwait (KWT)	4	1																							5	0.400	0.00065
Total	4	1	3	6	1	1	3	18	11	1	1	1	1	3	1	1	4	1	1	1	1	1	1	1	68		

n, sample sizes; h, haplotype diversity; π, nucleotide diversity

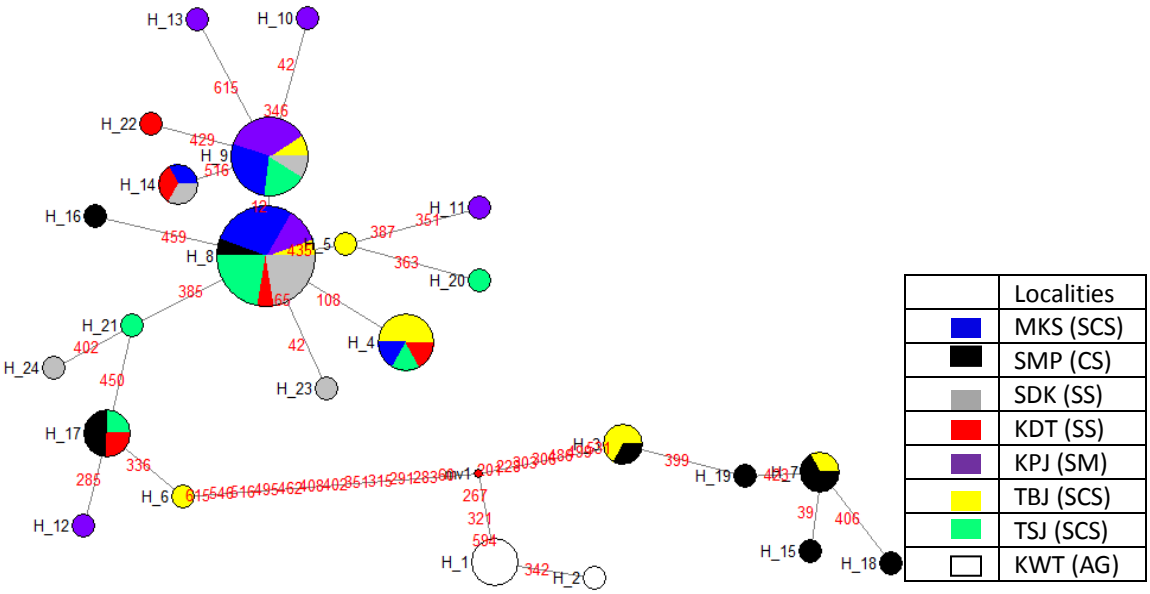
b) *Selar crumenophthalmus*

Localities	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	n	h	π
Kuala Perlis (KPJ)	1	1	2	1	1	1	1	1																9	0.9722	0.11099
Tanjung Sedili (TSJ)		1	4							1			1	1	1	1								10	0.8667	0.06850
Sekinchan (SK)			2						1	1	1													5	0.9000	0.07027
Tok Bali (TBJ)			3		1							1												5	0.7000	0.03373
Mukah (MKS)			2		1													1	1					5	0.9000	0.07027
Kudat (KDT)			3																	1	1			5	0.7000	0.03373
Sandakan (SDK)			3		1																	1		5	0.7000	0.03373
Semporna (SMP)			2																				1	4	0.8333	0.09026
Tawau (TW)			5																					5	0	0
Total	1	2	26	1	4	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	53		

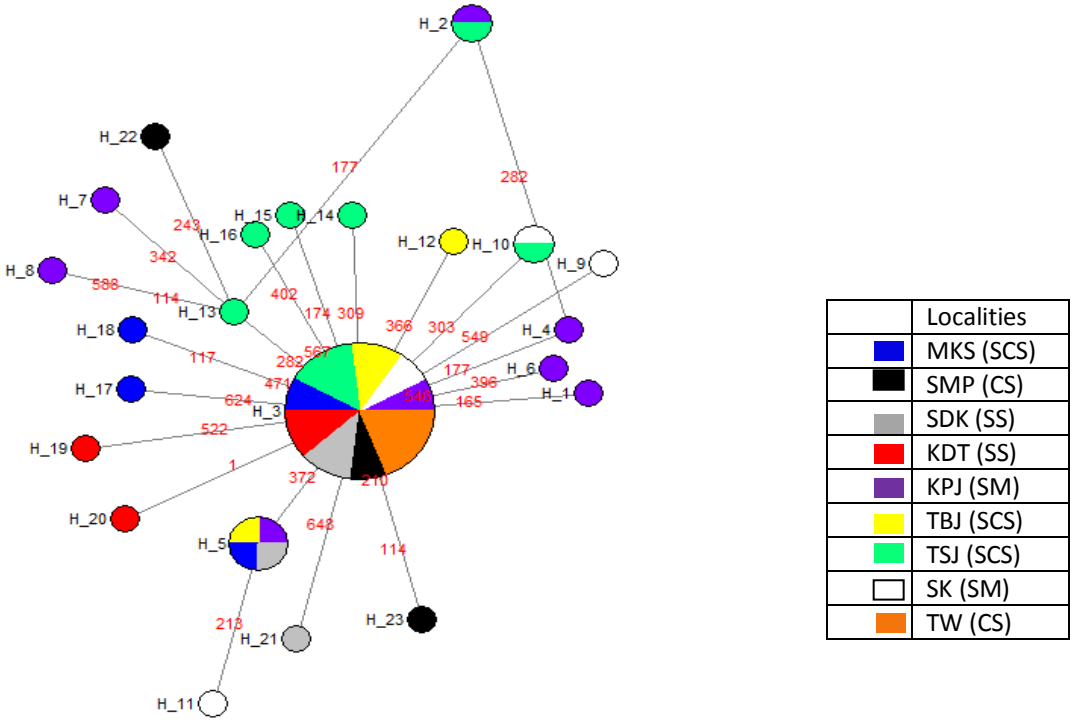
c) Selaroides leptolepis

Localities	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**n**	**h**	**π**
Kuala Perlis (KPJ)	3	1	1	1	1									7	0.8571	0.19298
Kuala Besut (KBJ)				4		1								5	0.4000	0.02503
Kudat (KDT)				5										5	0	0
Mersing (MGJ)				7			1	1	1					10	0.5333	0.03774
Miri (MR)				5										5	0	0
Kuala Sungai Baru (SB)				6										6	0	0
Sandakan (SDK)				8						1	1			10	0.3778	0.02493
Semporna (SMP)				3								1		4	0.5000	0.25536
Tawau (TW)				3								1	1	5	0.7000	0.23989
Total	3	1	1	42	1	1	1	1	1	1	1	2	1	57		

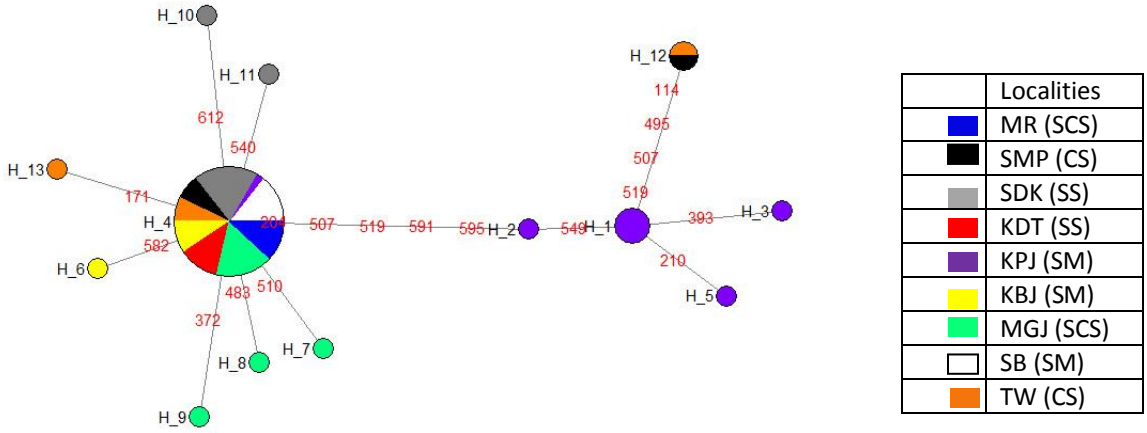
n, sample sizes; h, haplotype diversity; π, nucleotide diversity



a) Atule mate



b) Selar crumenophthalmus



c) Selaroides leptolepis

Figure 3.2 Median-joining networks constructed for the *COI* haplotypes of a*) Atule mate*, b) *Selar crumenophthalmus* and c) *Selaroides leptolepis* populations. Each circle represents one unique haplotype, with the area being proportional to the frequency of the haplotype in all localities. See Table 3.1 for detailed geographical location and Table 3.3 for haplotype frequencies distribution.

F_{ST} values were low for *Selar crumenophthalmus* (F_{ST}= -0.0054) compared to *Atule mate* (F_{ST}=0.03387) and *Selaroides leptolepis* (F_{ST}=0.1355) (Table 3.4 a), as expected. Additional samples of *Atule mate* from Kuwait (KWT) were also analysed for comparison with the IMA samples (F_{ST}= 0.09813) (Table 3.4 b). For *Atule mate,* the pairwise F_{ST} between Kuwait (KWT) and all IMA sites (pairwise F_{ST}= 0.27143- 0.41003, P-values= 0-0.04785) was significantly different. There were also significant differentiation among MKS and SMP (pairwise F_{ST}= 0.12281, P-value= 0.00977), as well as between KPJ and SMP (pairwise F_{ST}= 0.08163, P-value= 0.04297) (Table 3.5 a). The pairwise F_{ST} for *Selar crumenophthalmus* data primarily demonstrates no significant differentiation among localities (Table 3.5 b). While for *Selaroides leptolepis* data, significant differentiation was detected between the KPJ and six other sites; KBJ (F_{ST} = 0.26473, P-value=0.04883), MGJ (F_{ST} = 0.24247, P-value=0.01367), MR (F_{ST} = 0.4385, P-value=0.0586), SB (F_{ST} = 0.4717, P- value=0.00391), KDT (F_{ST} = 0.4385, P-value=0.01270) and SDK (F_{ST} =0.32897, P-value= 0.00293) (Table 3.5 c). High pairwise

F_{ST} values between KPJ and the rest of the IMA samples inflate the *Selaroides leptolepis* average F_{ST}. When AMOVA was repeated with the KPJ samples excluded, overall F_{ST} value of *Selaroides leptolepis* was low but non-significant (F_{ST}= -0.01360, P> 0.05).

Table 3.4 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing F-statistics analysis for *COI*.

a) between IMA localities

Hierarchical level	*Atule mate*		*Selar crumenophthalmus*		*Selaroides leptolepis*	
	F-statistics	**P-value**	**F-statistics**	**P-value**	**F-statistics**	**P-value**
Among all regions (F_{CT})	0.02055	0.28152	0.02830	0.26491	-0.01543	0.55523
Among localities within regions (F_{SC})	0.01359	0.27761	-0.03472	0.80059	0.14864	0.02151
Among individuals within localities (F_{ST})	0.03387	0.10166	-0.00544	0.53959	0.13550	0.00587

b) between IMA and Kuwait (KWT) for *Atule mate*

Hierarchical level	*Atule mate*	
	F-statistics	**P-value**
Among all regions (F_{CT})	0.08144	0.09971
Among localities within regions (F_{SC})	0.01817	0.31183
Among individuals within localities (F_{ST})	0.09813	0.00196

Table 3.5 Population pairwise F_{ST} (below) for *COI* and corresponding P values (above) by species.

a) *Atule mate*

Loc.	KWT	TBJ	TSJ	MKS	SMP	KPJ	KDT	SDK
KWT		**0.00293**	**0.00195**	**0.00000**	**0.00098**	**0.00488**	**0.04785**	**0.00586**
TBJ	0.29598		0.24414	0.10352	0.42090	0.11328	0.82520	0.05371
TSJ	0.33333	0.03541		0.99902	0.17383	0.47754	0.75586	0.99902
MKS	0.41003	0.08864	-0.06545		**0.00977**	0.51660	0.42285	0.90234
SMP	0.27143	0.01754	0.04255	0.12281		**0.04297**	0.89160	0.05664
KPJ	0.33333	0.06619	-0.00529	0.00285	0.08163		0.23242	0.24805
KDT	0.30000	-0.03390	-0.03943	0.02439	-0.03789	0.04918		0.53418
SDK	0.37747	0.09264	-0.05253	-0.06810	0.08046	0.04003	-0.01064	

Symbols equal: KWT, Kuwait; TBJ, Tok Bali; MKS, Mukah; SMP, Semporna; KPJ, Kuala Perlis; KDT, Kudat; SDK, Sandakan. Significant values appear in bold.

b) ***Selar crumenophthalmus***

Loc.	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK
TBJ		0.99902	0.99902	0.99902	0.42188	0.42238	0.99902	0.99902	0.99902
TSJ	-0.04126		0.99902	0.99902	0.08691	0.79395	0.99902	0.99902	0.99902
MKS	-0.11111	-0.04972		0.99902	0.16113	0.99902	0.99902	0.99902	0.99902
SMP	-0.09053	-0.06543	-0.08541		0.16309	0.52148	0.99902	0.99902	0.99902
TW	0.12500	0.16667	0.25000	0.23077		0.16600	0.20117	0.45508	0.43262
KPJ	-0.00544	-0.02097	-0.05649	-0.02683	0.27419		0.99902	0.26562	0.42578
SK	-0.05263	-0.07547	-0.07143	-0.08541	0.25000	-0.03096		0.99902	0.99902
KDT	-0.09375	-0.04126	-0.05263	-0.09053	0.12500	0.01941	-0.05263		0.99902
SDK	-0.16667	-0.04126	-0.11111	-0.09053	0.12500	-0.00544	-0.05263	-0.09375	

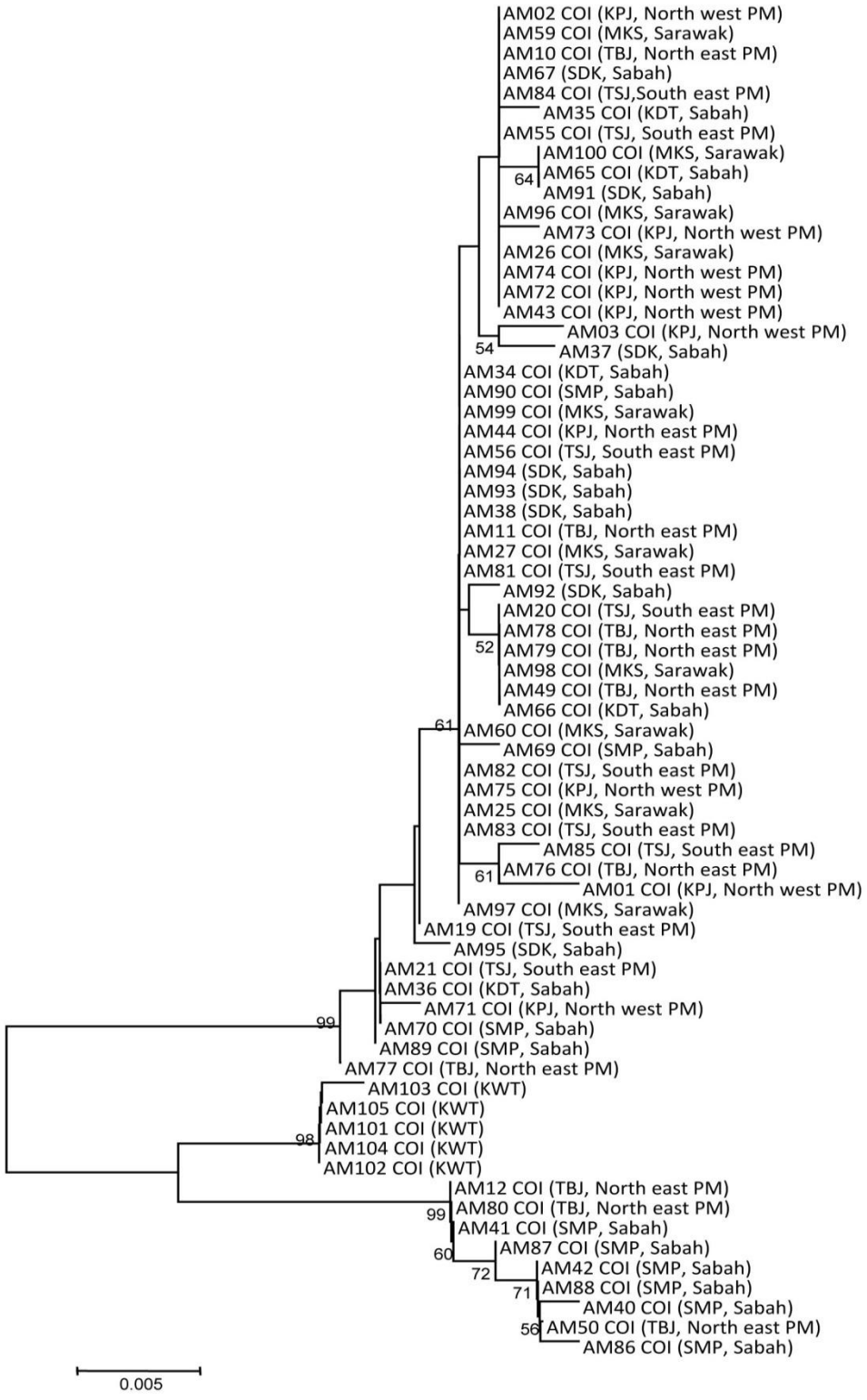
Symbols equal: TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan; KDT, Kudat; SDK, Sandakan.
Significant values appear in bold.

c) ***Selaroides leptolepis***

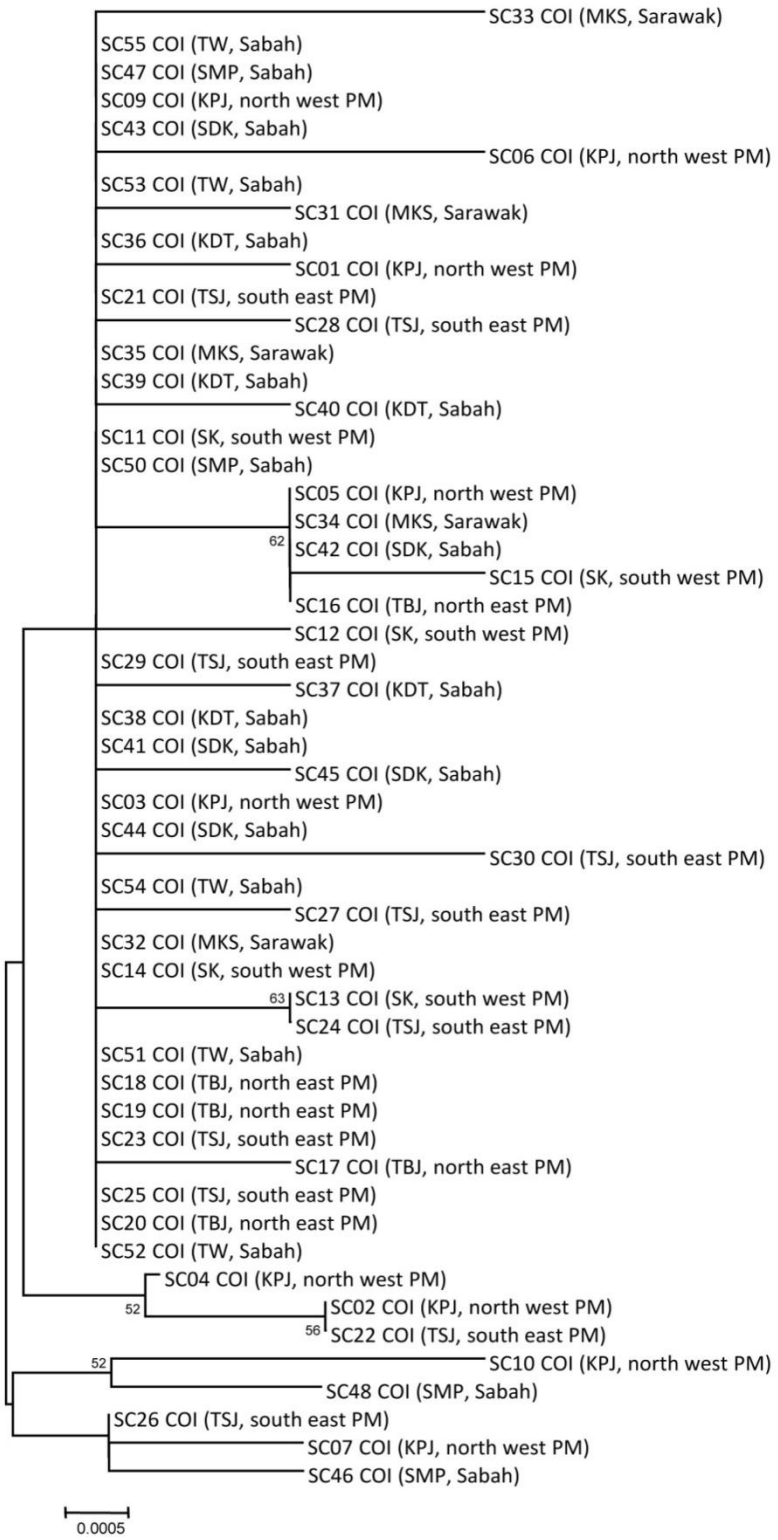
Loc.	KBJ	MGJ	MR	KPJ	SB	KDT	SDK	TW	SMP
KBJ		0.99902	0.99902	**0.04883**	0.44824	0.99902	0.99902	0.99902	0.99902
MGJ	-0.07383		0.52734	**0.01367**	0.51855	0.50684	0.99902	0.99902	0.99902
MR	-0.00000	0.01235		**0.00586**	0.99902	0.99902	0.99902	0.44238	0.42773
KPJ	0.26473	0.24247	0.43850		**0.00391**	**0.01270**	**0.00293**	0.12598	0.07227
SB	0.04000	0.04000	0.00000	0.47170		0.99902	0.51855	0.18945	0.38867
KDT	-0.00000	0.01235	0.00000	0.43850	0.00000		0.99902	0.45605	0.43555
SDK	-0.07759	-0.03535	-0.03659	0.32897	-0.00990	-0.03659		0.55957	0.99902
TW	-0.05769	-0.04972	0.12500	0.14137	0.16832	0.12500	-0.00324		0.99902
SMP	-0.11913	-0.09233	0.06250	0.20731	0.11111	0.06250	-0.07633	-0.20787	

Symbols equal: KBJ, Kuala Besut; MGJ, Mersing; MR, Miri; KPJ, Kuala Perlis; SB, Kuala Sungai Baru; KDT, Kudat; SDK, Sandakan; TW, Tawau; SMP, Semporna.
Significant values appear in bold.

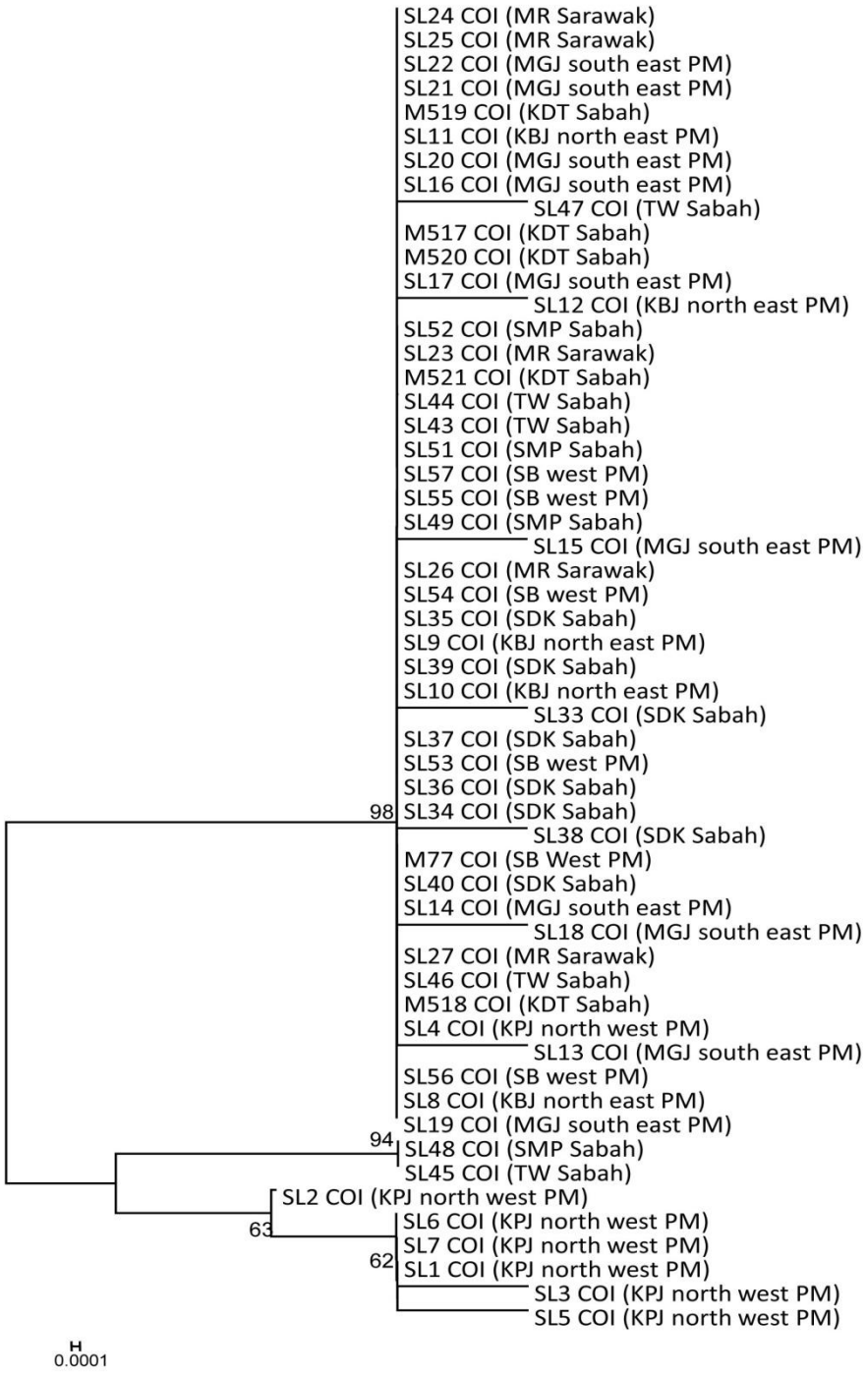
The neighbor-joining (NJ) phylogenetic trees (Figure 3.3) generated from the matrix of nucleotide distances in *COI* indicated three distinct clusters in *Atule mate* (Figure 3.3 a) and *Selaroides leptolepis* (Figure 3.3 c) with mean K2P distances within species for *Atule mate* and *Selaroides leptolepis* were 1.5% (max. of 4.6%) and 0.3% (max. of 1.2%) nucleotide divergence respectively. However, in *Selar crumenophthalmus*, only one cluster appeared with a mean of 0.2% K2P distance within species (max. of 0.8%) nucleotide divergence. For *Atule mate*, Cluster I, the major lineage including most specimens from all sampling sites exhibited no obvious geographic structuring, and was strongly supported with a bootstrap value of 99-100% in NJ trees (Figure 3.3 a). In contrast, Cluster II is a minor lineage, including all individuals from Kuwait (Arabian Gulf), while the third Cluster including three individuals from Tok Bali (TBJ) and six individuals from Semporna (SMP). However, for *Selar crumenophthalmus,* data included most specimens from all sampling sites in one cluster. For *Selaroides leptolepis*, Cluster I including all specimens from all sampling sites, and was strongly supported with a bootstrap value of 98%, while Cluster II included two individuals from Semporna (SMP) and Tawau (TW) with 94% bootstrap value. The third Cluster included six individuals from Kuala Perlis (KPJ) supported by 63% bootstrap value (Figure 3.3 c). However, no geographic pattern was apparent in both *Selar crumenophthalmus* and *Selaroides leptolepis*. The Maximum Likelihood (Appendix 7.1 a, 8.1 a, 9.1 a) and the Bayesian (Appendix 7.1 b, 8.1 b, 9.1 b) trees also had identical topologies for each species.


AM02 COI (KPJ, North west PM)
AM59 COI (MKS, Sarawak)
AM10 COI (TBJ, North east PM)
AM67 (SDK, Sabah)
AM84 COI (TSJ,South east PM)
AM35 COI (KDT, Sabah)
AM55 COI (TSJ, South east PM)
AM100 COI (MKS, Sarawak)
64
AM65 COI (KDT, Sabah)
AM91 (SDK, Sabah)
AM96 COI (MKS, Sarawak)
AM73 COI (KPJ, North west PM)
AM26 COI (MKS, Sarawak)
AM74 COI (KPJ, North west PM)
AM72 COI (KPJ, North west PM)
AM43 COI (KPJ, North west PM)
AM03 COI (KPJ, North west PM)
54
AM37 (SDK, Sabah)
AM34 COI (KDT, Sabah)
AM90 COI (SMP, Sabah)
AM99 COI (MKS, Sarawak)
AM44 COI (KPJ, North east PM)
AM56 COI (TSJ, South east PM)
AM94 (SDK, Sabah)
AM93 (SDK, Sabah)
AM38 (SDK, Sabah)
AM11 COI (TBJ, North east PM)
AM27 COI (MKS, Sarawak)
AM81 COI (TSJ, South east PM)
AM92 (SDK, Sabah)
AM20 COI (TSJ, South east PM)
AM78 COI (TBJ, North east PM)
52
AM79 COI (TBJ, North east PM)
AM98 COI (MKS, Sarawak)
AM49 COI (TBJ, North east PM)
AM66 COI (KDT, Sabah)
61
AM60 COI (MKS, Sarawak)
AM69 COI (SMP, Sabah)
AM82 COI (TSJ, South east PM)
AM75 COI (KPJ, North west PM)
AM25 COI (MKS, Sarawak)
AM83 COI (TSJ, South east PM)
AM85 COI (TSJ, South east PM)
61
AM76 COI (TBJ, North east PM)
AM01 COI (KPJ, North west PM)
AM97 COI (MKS, Sarawak)
AM19 COI (TSJ, South east PM)
AM95 (SDK, Sabah)
AM21 COI (TSJ, South east PM)
AM36 COI (KDT, Sabah)
AM71 COI (KPJ, North west PM)
99
AM70 COI (SMP, Sabah)
AM89 COI (SMP, Sabah)
AM77 COI (TBJ, North east PM)
AM103 COI (KWT)
AM105 COI (KWT)
98
AM101 COI (KWT)
AM104 COI (KWT)
AM102 COI (KWT)
AM12 COI (TBJ, North east PM)
99
AM80 COI (TBJ, North east PM)
AM41 COI (SMP, Sabah)
60
AM87 COI (SMP, Sabah)
72
AM42 COI (SMP, Sabah)
71
AM88 COI (SMP, Sabah)
AM40 COI (SMP, Sabah)
56
AM50 COI (TBJ, North east PM)
AM86 COI (SMP, Sabah)
0.005

a) Atule mate



b) Selar crumenophthalmus



c) Selaroides leptolepis

Figure 3.3 Neighbour-joining tree (K2P distance) of 68, 53 and 57 *COI* sequences of a) *Atule mate,* b) *Selar crumenophthalmus* and c) *Selaroides leptolepis*, respectively.

3.3.1.2 Control region

A total of 450bp of control region was sequenced in 65, 55 and 56 individuals of *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis*, respectively. A total of 64, 36 and 43 haplotypes were identified from eight localities of *Atule mate* and nine localities of each, *Selar crumenophthalmus* and *Selaroides leptolepis* (Table 3.6). In *Atule mate*, only one haplotype (H_19) was shared between two individuals of SMP, the other 63 haplotypes were singletons (Table 3.6 a). However, for *Selar crumenophthalmus*, H_18 was found in three localities, while four haplotypes (H_9, H_13, H_19, H_20) were shared by two localities. The remaining haplotypes were only found once and restricted to a single locality (Table 3.6 b). As in *Selaroides leptolepis*, H_14 was found in four localities (KDT, MGJ, MR and SDK) and two haplotypes were shared by two localities (H_20 and H_24) (Table 3.6 c). As for the other species, the remaining haplotypes were singletons. The haplotype (h) and nucleotide (π) diversity were markedly higher in *Atule mate* (h= 1.0, π= 0.08747) compared to *Selar crumenophthalmus* (h= 0.961, π= 0.01014) and *Selaroides leptolepis* (h= 0.968, π= 0.01763).

Table 3.6 Distribution of haplotype frequencies in control region by species

a) *Atule mate*

Loc.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
KWT	1	1	1	1	1																																														
KPJ						1	1	1	1	1	1	1	1	1	1																																						
SMP																1	1	1	2	1	1	1	1	1																														
TBJ																									1	1	1	1	1	1	1	1	1	1																				
TSJ																																			1	1	1	1	1	1	1	1	1	1								
KDT																																														1	1	1	1	1		
MKS																																																		1	1
SDK																																																		
Total	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

cont.

Loc.	52	53	54	55	56	57	58	59	60	61	62	63	64	n	h	π
KWT														5	1.0	0.0405
KPJ														10	1.0	0.1361
SMP														10	0.9778	0.5705
TBJ														10	1.0	0.5317
TSJ														10	1.0	0.1643
KDT														5	1.0	0.1605
MKS	1	1	1	1	1	1								8	1.0	0.1561
SDK							1	1	1	1	1	1	1	7	1.0	0.1333
Total	1	1	1	1	1	1	1	1	1	1	1	1	1	65		

b) *Selar crumenophthalmus*

Loc.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	n	h	π
KPJ	1	1	1	1	1	1	1	2	1																												10	0.9778	0.43158
KDT									1	1	1	1	1																								5	1.0	0.18702
MKS													1	2	1	1																					5	0.9	0.06331
SDK																	1	3	1																		5	0.7	0.02692
SMP																		2		1				1	1												5	0.9	0.04073
TW																		5																			5	0	0
SK																			1	1	1	1	1														5	1.0	0.09336
TBJ																										1	4										5	0.4	0.01334
TSJ																												1	1	1	2	1	1	1	1	1	10	0.9778	0.09427
Total	1	1	1	1	1	1	1	2	2	1	1	1	2	2	1	1	1	10	2	2	1	1	1	1	1	1	4	1	1	1	2	1	1	1	1	1	55		

c) *Selaroides leptolepis*

Loc.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	n	h	π
KPJ	1	1	1	1	1	1	1																																					7	1.0	0.01834
KBJ								1	1	1	1	1																																	5	1.0	0.01047
KDT													1	1	1	1	1																											5	1.0	0.00827
MGJ														3				1	1	1	1	1	1	1																					10	0.933	0.00651
MR														1										1	1	1	1																	5	1.0	0.00571
SDK														5														2	1	1	1													10	0.756	0.00494
SB																		1														1	1	1	1									5	1.0	0.01176
SMP																																				1	1	1	1						4	1.0	0.02984
TW																																								2	1	1	1	5	0.9	0.02268
Total	1	1	1	1	1	1	1	1	1	1	1	1	1	10	1	1	1	1	1	2	1	1	1	2	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	56		

F_{ST} values were low (F_{ST}= 0.00448) and non-significant (P>0.05) for Indo-Malay *Atule mate* compared to *Selar crumenophthalmus* (F_{ST}=0.17983, P<0.05) and *Selaroides leptolepis* (F_{ST}=0.0466, P<0.05) (Table 3.7 a). AMOVA also revealed low F_{ST} value (F_{ST}=0.00402) when additional samples from Kuwait (KWT) were included in the analysis for *Atule mate* (Table 3.7 b). The pairwise F_{ST} for *Atule mate* data demonstrates no significant differentiation among all localities, even between Kuwait (KWT) and the IMA (Table 3.8a). For *Selar crumenophthalmus*, significant differentiation was detected between TBJ and all other localities (pairwise F_{ST}= 0.25926-0.8 0.41003, P-values= 0.004-0.037). There were also significant differentiation among TW and five other localities; TSJ (F_{ST} = 0.40379, P-value=0), SMP (F_{ST} = 0.25, P-value=0.01172), KPJ (F_{ST} = 0.40379, P-value=0), SK (F_{ST} = 0.5, P-value=0.00781) and KDT (F_{ST} = 0.5, P-value=0.00781), and between KPJ and SDK (F_{ST} = 0.14074, P-value=0.00977) (Table 3.8 b). To test whether high pairwise F_{ST} values between TBJ and TW, with the rest of the IMA samples may inflate *Selar crumenophthalmus* average F_{ST}, AMOVA was repeated with TBJ and TW population excluded. The overall F_{ST} value was low and significant (F_{ST}= 0.05221, P< 0.05). While for *Selaroides leptolepis* data, significant differentiation was detected between SDK and three other localities: KBJ (F_{ST} = 0.14013, P-value=0.04492), KPJ (F_{ST} = 0.13027, P-value=0.0166) and TW (F_{ST} = 0.18367, P-value=0.03613) (Table 3.8 c).

*S*nn value was 1.0 and significant (P=0.0) between Kuwait (KWT) and the IMA *Atule mate* sequences, suggesting individuals from these two localities are highly differentiated. This result supports the *COI* data for *Atule mate* where KWT populations are significantly different from the rest of IMA samples. The analysis was repeated with the Kuwait (KWT) population excluded to test whether genetic differentiation was evident at finer spatial scales in the seas surrounding Malaysia. The Snn value was not significant (Snn= 0.21833, P=0.067), indicating that no genetic differentiation was evident among *Atule mate* populations within IMA. The Snn tests were also significant for *Selaroides leptolepis* (Snn=0.31392, P=0) suggesting at least

two localities are differentiated. However, non-significant Snn values were evident in *Selar crumenophthalmus* (Snn=0.0543, P>0.01).

The neighbor-joining (NJ) phylogenetic tree (Figure 3.4) generated from the matrix of nucleotide distances for control region data suggested three distinct clusters in *Atule mate* and *Selaroides leptolepis*, strongly supported by bootstrap values of 100% and 73% respectively (Figure 3.4 a, c). The mean K2P distances within species were 9.8% (max. of 21.6%) and 2% (max. of 6.8%) nucleotide divergence in *Atule mate* and *Selaroides leptolepis* respectively. The patterns observed for *Atule mate* and *Selaroides leptolepis* was consistent with the *COI* data (Figure 3.3 a, c). However, in *Selar crumenophthalmus* two clusters were detected with mean K2P distance within species of 1% (max. of 4.8%) nucleotide divergence. Cluster I included most specimens from all sampling sites, while Cluster II included two individuals from Kuala Perlis (KPJ) (Figure 3.4 b). However, no geographic pattern was apparent in both *Selar crumenophthalmus* and *Selaroides leptolepis*. The Maximum Likelihood (Appendix 7.2 a, 8.2 a, 9.2 a) and the Bayesian (Appendix 7.2 b, 8.2 b, 9.2 b) trees also had identical topologies for each species.

Table 3.7 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing F-statistics analysis for control region.

a) between IMA localities

Hierarchical level	*Atule mate*		*Selar crumenophthalmus*		*Selaroides leptolepis*	
	F-statistics	**P-value**	**F-statistics**	**P-value**	**F-statistics**	**P-value**
Among all regions (F_{CT})	0.00402	0.07820	0.03994	0.17498	0.02975	0.08895
Among localities within regions (F_{SC})	0.00047	1.0	0.14571	0	0.01736	0.21310
Among individuals within localities (F_{ST})	0.00448	0.12610	0.17983	0	0.04660	0.01173

b) between IMA localities and Kuwait (KWT) for *Atule mate*

Hierarchical level	*Atule mate*	
	F-statistics	**P-value**
Among all regions (F_{CT})	0.00358	0.04301
Among localities within regions (F_{SC})	0.00043	1.0
Among individuals within localities (F_{ST})	0.00402	0.10264

Table 3.8 Population pairwise F_{ST} (below) for control region and corresponding P values (above) by species.

a) *Atule mate*

Localities	KWT	TBJ	TSJ	MKS	SMP	KPJ	KDT	SDK
KWT		0.99902	0.99902	0.99902	0.51660	0.99902	0.99902	0.99902
TBJ	0.00000		0.99902	0.99902	0.50293	0.99902	0.99902	0.99902
TSJ	0.00000	0.00000		0.99902	0.46582	0.99902	0.99902	0.99902
MKS	0.00000	0.00000	0.00000		0.48535	0.99902	0.99902	0.99902
SMP	0.01235	0.01111	0.01111	0.01142		0.46484	0.49512	0.48438
KPJ	0.00000	0.00000	0.00000	0.00000	0.01111		0.99902	0.99902
KDT	0.00000	0.00000	0.00000	0.00000	0.01235	0.00000		0.99902
SDK	0.00000	0.00000	0.00000	0.00000	0.01164	0.00000	0.00000	

Symbols equal: KWT, Kuwait; TBJ, Tok Bali; MKS, Mukah; SMP, Semporna; KPJ, Kuala Perlis; KDT, Kudat; SDK, Sandakan. Significant values appear in bold.

b) *Selar crumenophthalmus*

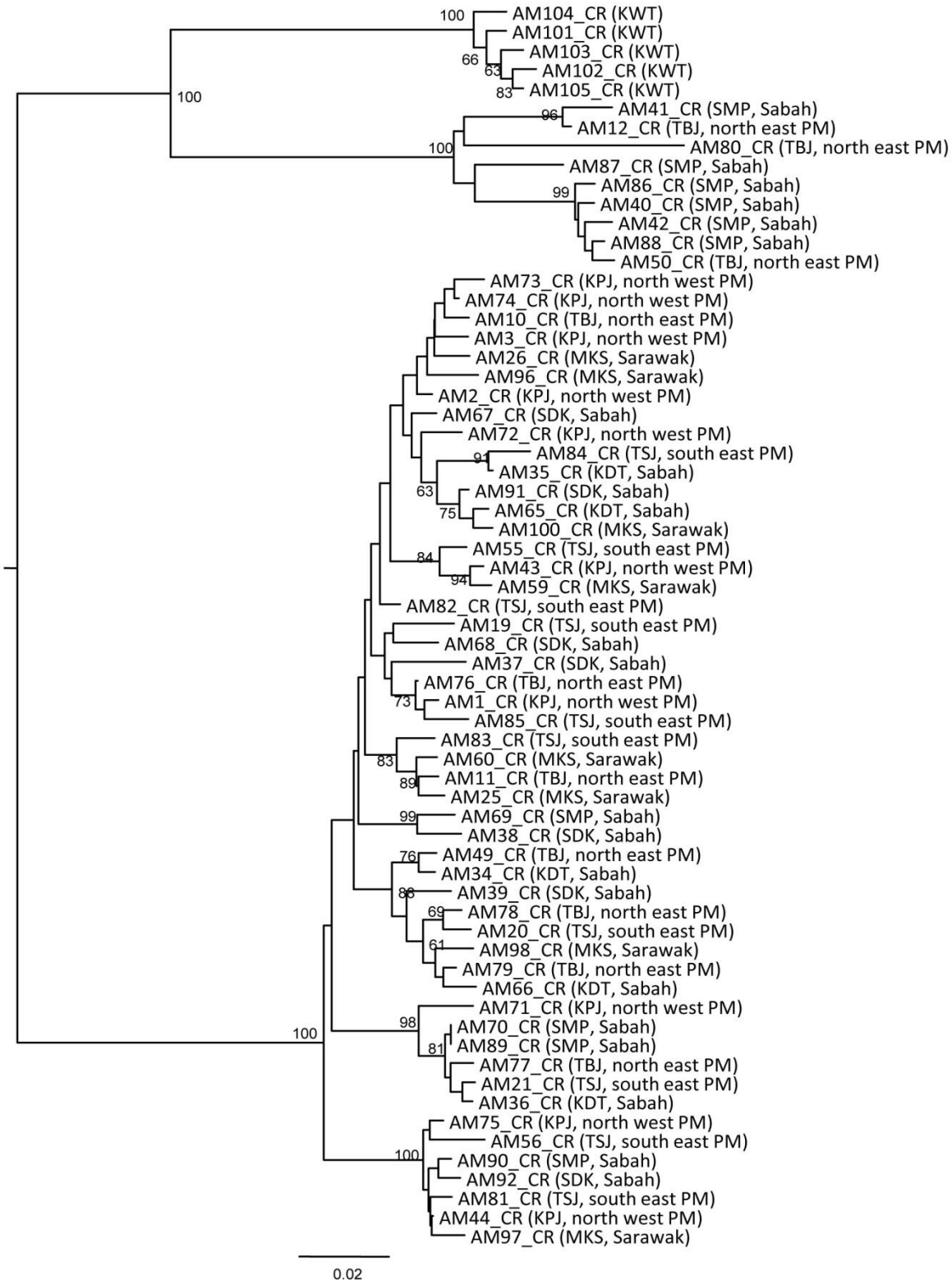
Localities	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK
TBJ		**0.00488**	**0.02930**	**0.03711**	**0.01172**	**0.00781**	**0.04297**	**0.03613**	**0.02734**
TSJ	0.25926		0.11230	0.09668	**0.00000**	0.22363	0.49805	0.53613	**0.01172**
MKS	0.35000	0.05632		0.22266	**0.01172**	0.12695	0.45605	0.68164	0.07812
SMP	0.35000	0.05632	0.10000		0.15723	0.11523	0.69141	0.44922	0.99902
TW	0.80000	0.40379	0.55000	0.25000		**0.00000**	**0.00781**	**0.00781**	0.47461
KPJ	0.25926	0.02222	0.05632	0.05632	0.40379		0.53320	0.77344	**0.00977**
SK	0.30000	0.01235	0.05000	0.01042	0.50000	0.01235		0.99902	0.16895
KDT	0.30000	0.01235	0.01042	0.05000	0.50000	-0.00787	0.00000		0.17285
SDK	0.45000	0.14074	0.20000	-0.05263	0.12500	0.14074	0.11458	0.15000	

Symbols equal: TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan; KDT, Kudat; SDK, Sandakan. Significant values appear in bold.

c) ***Selaroides leptolepis***

Localities	KBJ	MGJ	MR	KPJ	SB	KDT	SDK	TW	SMP
KBJ		0.27246	0.99902	0.99902	0.99902	0.99902	**0.04492**	0.44043	0.99902
MGJ	0.03727		0.99902	0.22949	0.45801	0.99902	0.40527	0.07227	0.36230
MR	0.00000	-0.04730		0.99902	0.99902	0.99902	0.20410	0.42090	0.99902
KPJ	0.00000	0.03504	0.00000		0.99902	0.99902	**0.01660**	0.12988	0.99902
SB	0.00000	0.01743	0.00000	0.00000		0.99902	0.05566	0.43848	0.99902
KDT	0.00000	-0.0279	-0.04167	0.00000	0.00000		0.20703	0.44336	0.99902
SDK	0.14013	0.00654	0.04085	0.13027	0.14013	0.04085		**0.03613**	0.05273
TW	0.05000	0.08116	0.05000	0.04654	0.05000	0.05000	0.18367		0.42188
SMP	0.00000	0.03919	0.00000	0.00000	0.00000	0.00000	0.14820	0.05308	

Symbols equal: KBJ, Kuala Besut; MGJ, Mersing; MR, Miri; KPJ, Kuala Perlis; SB, Kuala Sungai Baru; KDT, Kudat; SDK, Sandakan; TW, Tawau; SMP, Semporna. Significant values appear in bold.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AM75_CR (KPJ, north west PM)
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AM92_CR (SDK, Sabah)
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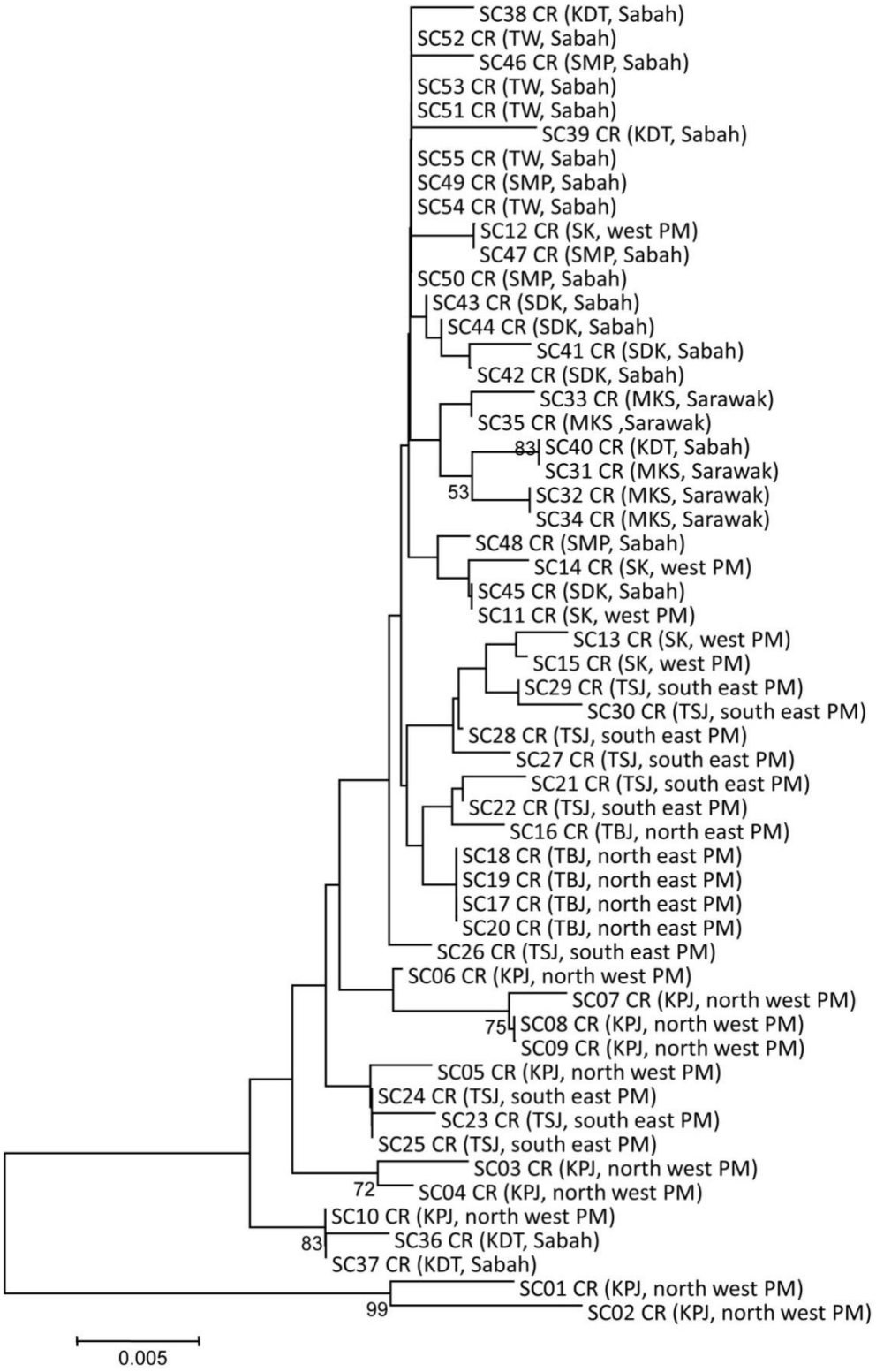
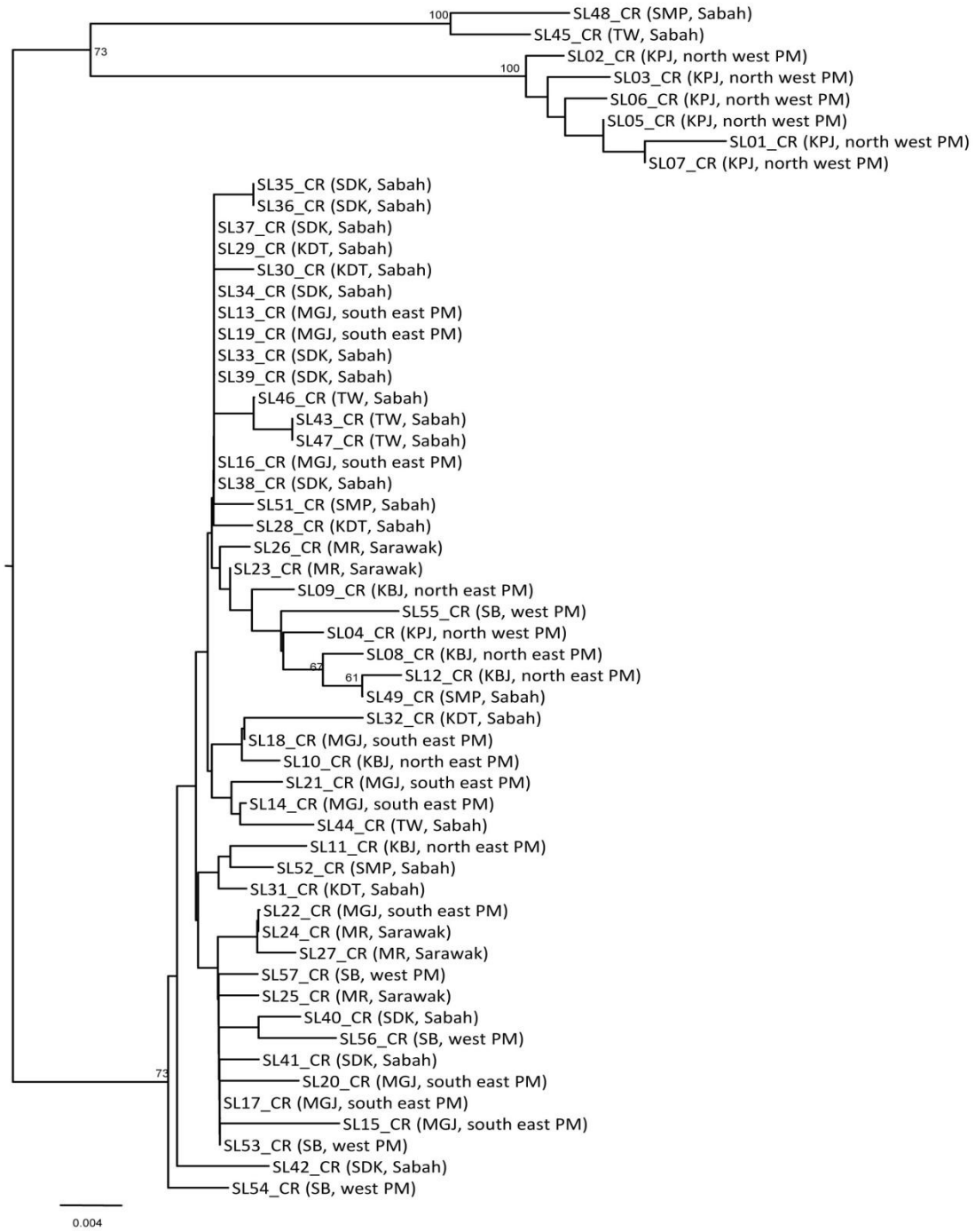
a) Atule mate



b) Selar crumenophthalmus



c) Selaroides leptolepis

Figure 3.4 Neighbour-joining tree (K2P distance) of 65, 55 and 56 control region sequences of a) *Atule mate,* b) *Selar crumenophthalmus and* c) *Selaroides leptolepis*, respectively.

3.3.2 Nuclear DNA analysis

A total of 67, 54 and 45 individuals of *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* were assayed for 910 bp of Rag1 gene, respectively. There were four, three and two unique alleles identified in each species respectively (Table 3.9). F_{ST} values were high for *Atule mate* (F_{ST}=0.40156) compared to *Selar crumenophtalmus* (F_{ST}= -0.03958) and *Selaroides leptolepis* (F_{ST}= -0.07324) (Table 3.10 a). Additional samples from Kuwait (KWT) were also analysed for comparison with *Atule mate* samples from IMA (F_{ST}= 0.61104, P-vaue= 0) (Table 3.10 b). The pairwise F_{ST} analysis revealed a lack of genetic structure among sampled areas and non-significant for *Selar crumenophthalmus* and *Selaroides leptolepis* (Table 3.11 b, c). However, for *Atule mate*, comparison between Kuwait (KWT) and other sampled sites in IMA revealed high pairwise F_{ST} values (0.61648-1.0000) and significant P-values (0-0.00781) (Table 3.11 a). There were also significant differentiations between SMP and four other sites (TSJ, MKS, KPJ and SDK). Similar to *COI* and control region data, the haplotype (h) and nucleotide (π) diversity were markedly higher in *Atule mate* (h= 0.379, π= 0.00092) compared to *Selar crumenophthalmus* (h= 0.0734, π= 0.0001) and *Selaroides leptolepis* (h= 0.0444, π= 0.00006). Neighbour-joining phylogenetic tree for Rag1 also suggested three distinct clusters in *Atule mate* strongly supported with 65-70% bootstrap values. However, two clusters were detected in *Selar crumenophthalmus* and *Selaroides leptolepis* with 60-65% and 56% bootstrap values respectively. Mean K2P distances within species were 0.1%, 0.2% and 0.1% with 0.6%, 0.5% and 0.6% maximum nucleotide divergence for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* respectively. The pattern observed for *Atule mate* clusters were consistent with the pattern observed at *COI* (Figure 3.3 a) and control region (Figure 3.4 a) data. The Maximum Likelihood (Appendix 7.3 a, 8.3 a, 9.3 a) and the Bayesian (Appendix 7.3 b, 8.3 b, 9.3 b) trees also had identical topologies for each species.

Table 3.9 Distribution of allele frequencies in Rag1 by species.

a) *Atule mate*

Localities	1	2	3	4	n
Kuala Perlis (KPJ)		10			10
Tok Bali (TBJ)		6	3	1	10
Tanjung Sedili (TSJ)		9			9
Mukah (MKS)		10			10
Semporna (SMP)		4	6		10
Kudat (KDT)		5			5
Sandakan (SDK)		8			8
Kuwait (KWT)	5				5
Total	5	52	9	1	67

b) *Selar crumenophthalmus*

Localities	1	2	3	n
Kuala Perlis (KPJ)	10			10
Tanjung Sedili (TSJ)	9		1	10
Sekinchan (SK)	4	1		5
Tok Bali (TBJ)	5			5
Mukah (MKS)	5			5
Kudat (KDT)	5			5
Sandakan (SDK)	5			5
Semporna (SMP)	5			5
Tawau (TW)	4			4
Total	52	1	1	54

c) *Selaroides leptolepis*

Localities	1	2	n
Kuala Perlis (KPJ)	1	6	7
Kuala Besut (KBJ)		4	4
Kudat (KDT)		5	5
Mersing (MGJ)		4	4
Miri (MR)		5	5
Kuala Sungai Baru (SB)		5	5
Sandakan (SDK)		6	6
Semporna (SMP)		4	4
Tawau (TW)		5	5
Total	1	44	45

Table 3.10 Results of the analysis of molecular variance (AMOVA) for *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* showing F-statistics analysis for Rag1.

a) between IMA localities

Hierarchical level	*Atule mate*		*Selar crumenophthalmus*		*Selaroides leptolepis*	
	F-statistics	**P-value**	**F-statistics**	**P-value**	**F-statistics**	**P-value**
Among all regions (F_{CT})	0.25620	0.30596	-0.03423	0.96872	0.03696	0.40274
Among localities within regions (F_{SC})	0.19543	0.01662	-0.05170	0.68231	-0.11443	1.00000
Among individuals within localities (F_{ST})	0.40156	0	-0.03958	0.93157	-0.07324	1.00000

b) between IMA localities and Kuwait (KWT) for *Atule mate*

Hierarchical level	*Atule mate*	
	F-statistics	**P-value**
Among all regions (F_{CT})	0.50635	0.10068
Among localities within regions (F_{SC})	0.21206	0.02053
Among individuals within localities (F_{ST})	0.61104	0.00000

Table 3.11 Population pairwise F_{ST} (below) for Rag1 and corresponding P values (above) by species.

a) ***Atule mate***

Localities	KWT	TBJ	TSJ	MKS	SMP	KPJ	KDT	SDK
KWT		**0.00098**	**0.00000**	**0.00000**	**0.00000**	**0.00098**	**0.00781**	**0.00195**
TBJ	0.61648		0.09180	0.08789	0.38477	0.09180	0.23535	0.11035
TSJ	1.00000	0.23295		0.99902	**0.01465**	0.99902	0.99902	0.99902
MKS	1.00000	0.25000	0.00000		**0.00879**	0.99902	0.99902	0.99902
SMP	0.65616	0.02299	0.53905	0.55556		**0.00684**	0.09766	**0.01074**
KPJ	1.00000	0.25000	0.00000	0.00000	0.55556		0.99902	0.99902
KDT	1.00000	0.14013	0.00000	0.00000	0.45205	0.00000		0.99902
SDK	1.00000	0.21426	0.00000	0.00000	0.52096	0.00000	0.00000	

Symbols equal: KWT, Kuwait; TBJ, Tok Bali; MKS, Mukah; SMP, Semporna; KPJ, Kuala Perlis; KDT, Kudat; SDK, Sandakan. Significant values appear in bold.

b) ***Selar crumenophthalmus***

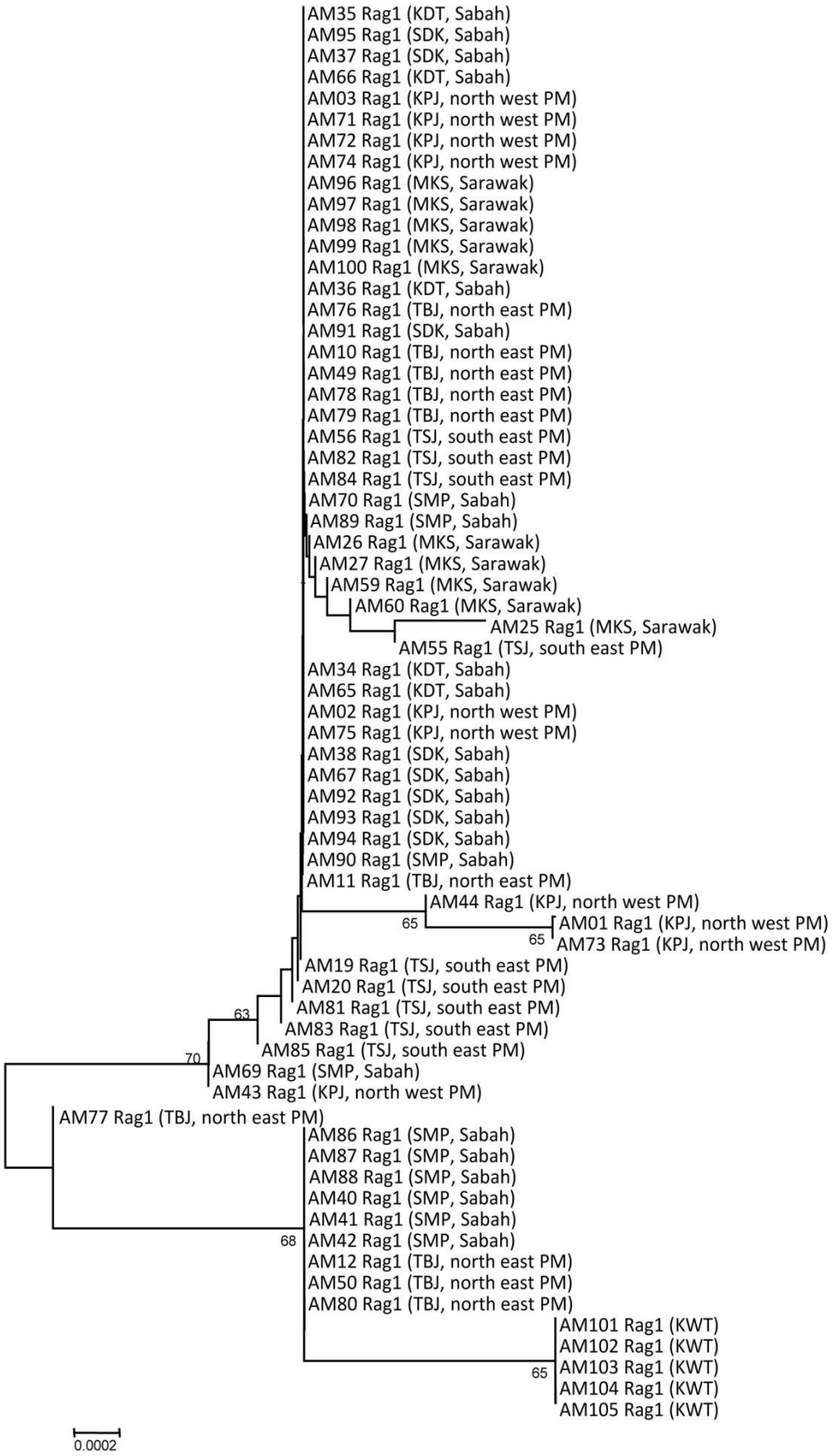
Localities	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK
TBJ		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
TSJ	-0.08434		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
MKS	0.00000	-0.08434		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
SMP	0.00000	-0.08434	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902
TW	0.00000	-0.12150	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902
KPJ	0.00000	-0.00000	0.00000	0.00000	0.00000		0.33398	0.99902	0.99902
SK	-0.00000	-0.03659	-0.00000	-0.00000	-0.05263	0.14894		0.99902	0.99902
KDT	0.00000	-0.08434	0.00000	0.00000	0.00000	0.00000	0.00000		0.99902
SDK	0.00000	-0.08434	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

Symbols equal: TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan; KDT, Kudat; SDK, Sandakan. Significant values appear in bold.

c) ***Selaroides leptolepis***

Localities	KPJ	SB	SMP	TW	KBJ	MGJ	MR	KDT	SDK
KPJ		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
SB	-0.05528		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
SMP	-0.09804	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
TW	-0.05528	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902
KBJ	-0.09804	0.00000	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902
MGJ	-0.09804	0.00000	0.00000	0.00000	0.00000		0.99902	0.99902	0.99902
MR	-0.05528	0.00000	0.00000	0.00000	0.00000	0.00000		0.99902	0.99902
KDT	-0.05528	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000		0.99902
SDK	-0.02439	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	

Symbols equal: KBJ, Kuala Besut; MGJ, Mersing; MR, Miri; KPJ, Kuala Perlis; SB, Kuala Sungai Baru; KDT, Kudat; SDK, Sandakan; TW, Tawau; SMP, Semporna. Significant values appear in bold.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AM74 Rag1 (KPJ, north west PM)
AM96 Rag1 (MKS, Sarawak)
AM97 Rag1 (MKS, Sarawak)
AM98 Rag1 (MKS, Sarawak)
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AM100 Rag1 (MKS, Sarawak)
AM36 Rag1 (KDT, Sabah)
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AM55 Rag1 (TSJ, south east PM)
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AM94 Rag1 (SDK, Sabah)
AM90 Rag1 (SMP, Sabah)
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AM43 Rag1 (KPJ, north west PM)
AM77 Rag1 (TBJ, north east PM)
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AM88 Rag1 (SMP, Sabah)
AM40 Rag1 (SMP, Sabah)
AM41 Rag1 (SMP, Sabah)
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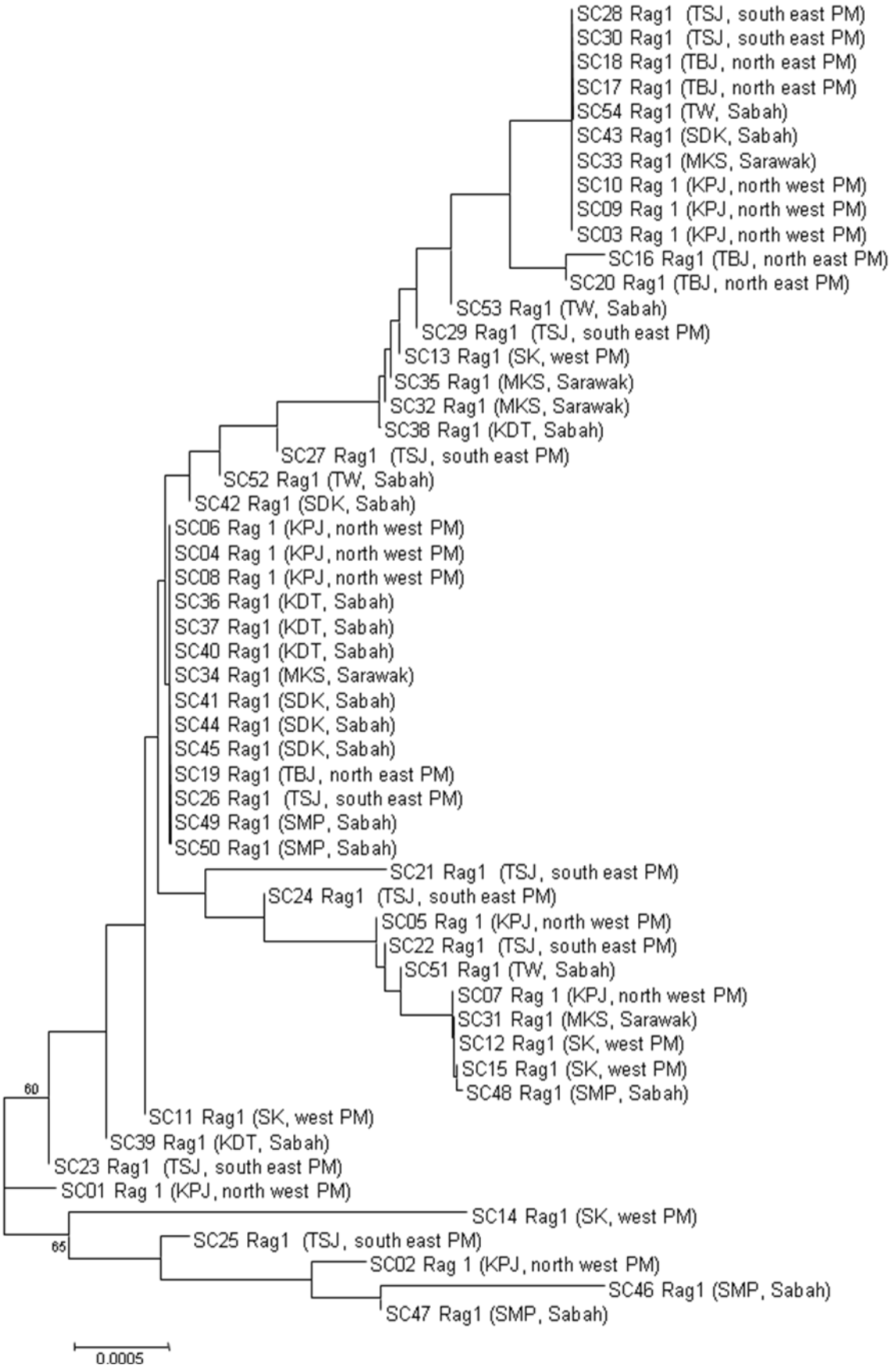
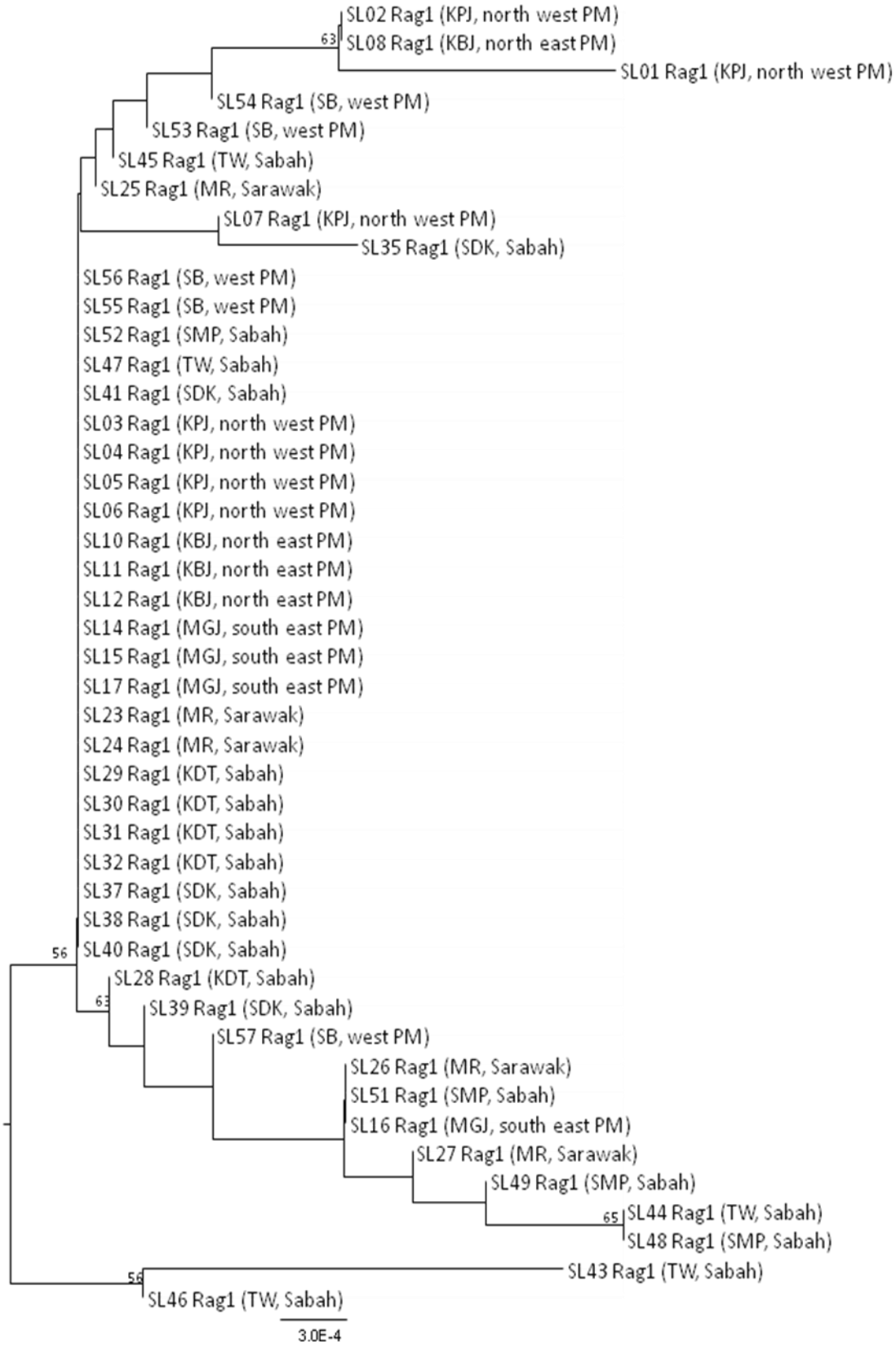
a) Atule mate



b) Selar crumenophthalmus



c) Selaroides leptolepis

Figure 3.5 Neighbour-joining tree (K2P distance) of 67, 54 and 45 Rag1 sequences of a) *Atule mate,* b) *Selar crumenophthalmus and* c) *Selaroides leptolepis*, respectively.

3.4 Discussion

3.4.1 General findings

Population structure inferred from nuclear as well as mtDNA markers was lower in the pelagic species *Atule mate* and *Selar crumenophthalmus* than in the demersal species, *Selaroides leptolepis*, which is consistent with the hypothesis that pelagic and semi-pelagic species will display less genetic divergence due to their potential to undertake long-distance migrations in oceanic waters. However, before proceeding with the discussion, I should address several caveats with the interpretation of the data. Due to the high haplotype diversity and the small sample sizes, most haplotypes in the control region data appeared in the sample only once and thus the F_{ST} analysis will not reflect true levels of population structure (Hudson, 2000). This issue with the diversity of control region haplotypes has been identified before in other marine taxa (Hauser *et al*., 2001; Ely *et al*., 2005; Wu *et al*., 2012) and can, in theory be overcome by much larger sample sizes than were used in this study. In an attempt to generate meaningful differentiation measures from the control region data we utilised an alternative measure of genetic differentiation - the nearest-neighbor statistic (*S*nn) (Hudson *et al*., 1992). This statistic measures population differentiation by testing whether low divergent sequences are from the same location, and it is particularly useful when populations show high levels of haplotype diversity (Hudson, 2000). The *S*nn values were high and significant between Kuwait (KWT) and the IMA *Atule mate* sequences, suggesting individuals from these two localities are differentiated. Such findings support the *COI* data for *Atule mate* where Kuwait (KWT) populations are significantly different from the rest of the IMA samples. However, when the analysis was repeated with the Kuwait (KWT) population excluded, to test whether genetic differentiation was evident at finer spatial scales in the seas surrounding Malaysia, the *S*nn value was low and non-significant indicating that no genetic differentiation was evident among *Atule mate* populations within the IMA. The latter result contradicts the *COI* data which showed significant differentiation among the IMA samples. A combination of slightly higher mutation rates provide

more opportunity for drift to vary allele frequencies, combined with insufficient sample sizes may account for slight pairwise genetic differences using the control region marker for *Atule mate* specimens.

Overall, the Neighbor-joining analyses defined three clades (both nuclear and mtDNA markers) in *Atule mate* with bootstrap values of 98-99% in *COI*, 100% in control region and 65-70% in Rag1. However in *Selar crumenophthalmus*, only one cluster emerged in *COI* data with maximum nucleotide divergence of 0.78%, and two clades in both control region and Rag1 with 99% and 60-65% bootstrap values respectively. In *Selaroides leptolepis*, all trees were split into three closely related clades, which did not appear to have any geographic structure with bootstrap values of 62-98% in *COI*, 73% in control region and 56% in Rag1. The higher mutation rate for the control region than for *COI* and Rag1 was suggested to explain their different pattern of phylogenetic relationships (Theisen *et al*., 2008). The same discrepancy was also reported between control region and Cyt*b* in wahoo (Theisen *et al*., 2008) and three *Trachurus* species (Karaiskou *et al*., 2004).

3.4.2 Population genetic structure

The results of pairwise F_{ST} comparisons, AMOVA tests and *S*nn statistics showed there was significant population genetic subdivision among localities in *Atule mate*. Three differentiated mitochondrial lineages were present in *Atule mate*. Two lineages comprise haplotypes formerly identified by Mat Jaafar *et al*. (2012), with the major lineage including specimens from all sampling regions across the IMA. However, no geographic structuring was observed in this mitochondrial lineage of *Atule mate*. The second lineage in Mat Jaafar *et al*.'s (2012) study consisted of only a single specimen from Tok Bali (TBJ). In the present study, we included more specimens from Tok Bali (TBJ) and Semporna (SMP), and three of the Tok Bali (TBJ) specimens (AM12, AM50, AM80) and six Semporna (SMP) specimens (AM40, AM41, AM42, AM86. AM87, AM88) grouped together, with the formerly identified (Mat Jaafar *et al*., 2012) potential cryptic species. The third lineage included only specimens from Kuwait (KWT). The same pattern was also evident in data from the

control region and Rag1. Following these observations, we hypothesize that cryptic species may be present in Indo-Malay *Atule mate*. The F_{ST} P-values among IMA populations and the Kuwait population in *COI* and Rag1 data were all significant ($P<0.05$), indicating limited gene flow among these two regions in the absence of obvious dispersal barriers.

In contrast, the results obtained here indicated no significant heterogeneity in *COI*, control region or in Rag1 across *Selar crumenophthalmus* populations within the IMA. This result is also consistent with a study by Pedrosa-Geramsio *et al*. (2011). Their data indicates a homogenous population of *Selar crumenophthalmus* in the Sulu Sea. Panmixia of the species in the region is likely because of the high mobility of the species and high dispersal potential of larvae resulting in no, or very weak population structuring. Pelagic marine fishes usually have high fecundity, very large population sizes, and high dispersal potential at egg, larval and adult stages. These life-history features and the continuity of the pelagic environment in theory suggest little genetic divergence over large spatial scales. For a more comprehensive analysis of genetic variation in *Selar crumenophthalmus*, the sampling design of future surveys should address a much broader geographic scale, and utilise more rapidly evolving nuclear markers, such as microsatellites or SNPs.

For *Selaroides leptolepis* there was significant differentiation in *COI* and control region data. The significant differentiation between KPJ with six other localities within the IMA suggested *Selaroides leptolepis* are genetically subdivided into distinct populations although additional studies, possibly with additional nuclear markers (e.g. SNPs), are required to assess patterns more widely. However, no geographical structure was observed in the NJ tree. Overall, low F_{ST} values were detected in the Indo-Malay *Atule mate* and *Selar crumenophthalmus* compared to *Selaroides leptolepis*, indicating extensive gene flow among the former species within the IMA. Seventy percent of marine organisms have a planktonic stage during the larval phase when larvae may actively disperse (Bonhomme and Planes, 2000; Thorrold *et al*., 2007). In general, fish with longer larval duration display less genetic differentiation than those with shorter larval duration (Waples, 1987; Bay

et al., 2006; Bradbury *et al*., 2008; Hauser and Carvalho, 2008). However, a growing body of evidence suggests the importance of other factors, such as currents and larval retention (Rohfritsch and Borsa, 2005; Froukh and Kochzius, 2007; Carreras-Carbonell *et al*., 2006), that may cause strong differentiation even in species with a long larval phase (Taylor and Hellberg, 2003; Planes *et al*., 1998). Unfortunately, little is known regarding the reproductive biology of *Atule mate*, *Selar crumenophthalmus* and *Selaroides leptolepis* (e.g., Leis *et al*., 2004). Further potential isolating mechanisms in the marine environment are discussed in Chapter 4 (section 4.5.2) and Chapter 5 (section 5.4) of this thesis.

3.4.3 Fisheries management in the IMA

In 2010, the marine fish landing in Malaysia was *c.* 1.4 million mt (Annual Fisheries Statistics, 2011), a figure that has increased approximately five times since fisheries statistics were first documented in 1961. The contribution of fish caught from the inshore sector is *c.* 65%, the remainder 17% comes from the deep-sea sector. However, this figure decreased in the year 2011 by 3.9%, which amounted to 1,373,105 mt as compared with 1,428,881 mt in 2010. The inshore landings decreased by 2.07% from 1,108,897 mt in 2010 to 1,085,965 mt in 2011. Landings from the deep-sea fisheries sector also recorded a decrease of 10.26% from 319,984 mt in 2010 to 287,140 mt in 2011. Overfishing is the leading threat to the world's marine fishes (Reynolds *et al*., 2002; Dulvy *et al*., 2003), including Malaysia. The coastal demersal fishes in Sarawak and Sabah were reported in 1998 to be overfished and heavily overfished, respectively, while the offshore demersal fishes as well as coral reef fishes in Sabah were heavily overfished (Oakley *et al*., 2000). In addition, habitat degradation or modification is a major threat to fish species survival in Malaysia. Therefore, determination of population genetic structure provides essential information to underpin resource recovery and to aid in delineating and monitoring populations for fisheries management (Han *et al*., 2008).

Genetics and fisheries management can interact in several ways. When the population genetic structure of a species is known, the distribution of subpopulations in mixed fisheries can be estimated (Hauser and Carvalho, 2008; Waples *et al*., 2008; Waples and Naish, 2009). Regulation of harvests to protect weaker populations can be made based on these distributions, in order to develop effective fish stock management practices. The emergence of two separate lineages (max. *COI* nucleotide divergences of 4.6%) in *Atule mate,* suggests that at least two different stocks of this species occur in the IMA waters, although no obvious geographical structure was detected. These stocks should be managed separately because different stocks are likely to require different conservation strategies (Schonrogge *et al*., 2002). For *Selar crumenophthalmus*, the observed homogeneity was interpreted as supporting the view that this species should be managed in the IMA as one stock, though it is always important to confirm such assertions through temporal analysis of samples to assess stability (Waples, 1998). However, there is some evidence for second lineage was detected in Chapter 2, but this was only based on two individuals, and therefore requires further sampling effort at these localities for confirmation of this pattern. Even though *Selaroides leptolepis* also showed significant differentiation between IMA localities, the data presented here is still preliminary based on small sample sizes. Additional samples should be collected and more powerful genetic markers should be used to further investigate stock structure and boundaries in Indo-Malay Carangidae.

In Malaysia, various management plans have been developed to promote sustainability of fisheries resources. For inshore fisheries, a restructuring programme was implemented by reducing the number of fishermen (Hj Mohamed, 1991). This reduction will allow for greater opportunities and profit maximization through larger catches per capita. To ensure this programme is conducted successfully, fishermen will be granted licenses and only those with a license will be allowed to fish. Through this scheme, effective limited entry into the fishing area can be monitored. In addition, programmes to restore the fisheries resource were also implemented by establishing networks of marine protected areas (MPA) to maintain fish recruitment to heavily fished areas (Chong *et al*., 2010). Research in

biology and population fisheries resources has also intensified and here, molecular studies can play a crucial role. Biologically important characteristics of populations, including their size and productive efficiency, are determined by the historically established gene pools (Altukhov and Salmenkova, 1987). Therefore, the population genetic analysis of wild exploited taxa is of primary importance in developing an optimal strategy for effective management. Such a strategy should provide not only for maximum economic benefits, but also for long-term maintenance of natural populations. The current population genetic study of three Carangidae species (*Atule mate*, *Selar crumenophthalmus*, *Selaroides leptolepis*) provides a base-line of reference data upon which additional more detailed studies can be conducted. Importantly also, data presented here indicates a complex scenario of genetic structuring that endorses the need to better define the dynamics and putative stock boundaries of exploited stocks.

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CHAPTER 4

PHYLOGEOGRAPHY OF *SELAR CRUMENOPHTHALMUS* ACROSS THE INDO-MALAY ARCHIPELAGO

Abstract

The emergence of land-bridges and sea-barriers in the recent geological past has been reported to influence genetic and geographic structuring of marine taxa. In this study, I investigate phylogeographic pattern of *Selar crumenophthalmus* in the Indo-Malay Archipelago. Samples were collected from six geographic regions; the South China Sea, Strait of Malacca, Sulu Sea, Celebes Sea, Andaman Sea and the Bismarck Sea. Fragments of 650 bp of *COI*, 450 bp of the control region and 950 bp of nuclear gene (Rag1) were analysed. Both mtDNA and nuclear DNA data revealed low genetic differentiation among localities with low F_{ST} values indicating extensive gene flow within regions. The results did not support the existence of different management units of *Selar crumenophthalmus* across the Indo-Malay Archipelago, though additional studies, possibly with additional nuclear markers (e.g. SNPs) are required to assess patterns more widely.

4.1 Introduction

A wealth of biodiversity exists in tropical marine ecosystems. The ranges of many tropical marine species overlap in a centre of maximum marine biodiversity, which is located in the Indo-Malayan region. The Indo-Malay Archipelago (IMA) houses one of the highest levels of species richness and endemism in the world (Briggs, 2005; Hoeksema, 2007). Pleistocene glaciations and sea level changes over the last few million years have dramatically influenced the geography of the central Indo-Malay Archipelago, where the Sunda and Sahul Shelves emerged as broad geographic barriers partly isolating the Indian Ocean from the West Pacific, and enclosing the South China Sea, the Sulu Sea, and the Sulawesi Sea, respectively (Voris, 2000). For example, the Sulu Sea was isolated from the South China Sea until 10,000 years ago, thereby isolating many marine species. Even small changes in sea level are expected to have restricted movement of pelagic species, such as in the Strait of Malacca between Malaysia and Sumatra, which are both narrow and shallow. Allopatric speciation would be expected only when the populations have long been separated by such geographical and hydrological barriers.

Marine connectivity between the Pacific and Indian Oceans would have been drastically reduced and possibly completely interrupted when sea levels were much lower than at present, during the Pleistocene (up to 120m below current levels; Voris 2000). Numerous molecular phylogenetic and population genetic studies on various marine fishes and invertebrates have revealed a genetic discontinuity between the Indian and Pacific Oceans (Barber *et al*., 2000; Lourie and Vincent, 2004; Rohfritsch and Borsa, 2005; Crandall *et al*., 2008). One example is the phylogeographic disjunction of barramundi (*Lates calcarifer*) on either side of the Torres Straits (Chenoweth *et al*., 1998), which formed a land barrier connecting Australia and New Guinea during the Pleistocene (Voris, 2000). The phylogeographic structure of false clown anemonefish (*Amphiprion ocellaris*) was also shaped by sea level changes during the Pleistocene, rather than by contemporary geography. Significant differences in cytochrome *b* haplotype

frequencies were found between *A.ocellaris* populations from the western edge of the Sunda Shelf, and those from the rest of the IMA, including the South China Sea, Sunda Strait, Bali Strait, Sulu and Celebes Seas, respectively (Nelson *et al*., 2000).

In contrast with inshore-sedentary fishes and invertebrates, little genetic heterogeneity has been reported for pelagic fishes from the IMA. Family Carangidae is one of the most commercially-important fish families in this region. They are highly mobile pelagic species that exhibit geographic structure across the IMA. This family is abundant and widely distributed in the Indo-West Pacific and is a major fishery resource in Southeast Asia. However, the full diversity of this assemblage is also yet to be described, and the factors driving these radiations remain largely unknown. Molecular phylogeography can contribute greatly to our understanding of Carangidae evolution by providing a robust theoretical framework to elucidate the historical processes that have shaped fish diversity and distributions. At a broader scale, fishes provide a useful phylogeographic model system with which to explore the historical biogeography of the IMA, by revealing processes that may have affected the regions biota as a whole.

The third objective of my PhD thesis is therefore to investigate the phylogeographic history and population genetic structuring of *Selar crumenophthalmus* (Bloch, 1793), a highly mobile, pelagic Carangidae that is broadly distributed across the IMA. The bigeye scad, *Selar crumenophthalmus* is an oceanic species, which is abundant and widely distributed in the tropical and subtropical belt of all oceans, and is a major fisheries resource in Southeast Asia (Mansor and Abdullah, 1995; Smith-Vaniz, 1999). However, knowledge of the biology, ecology, distribution and stock structure of this and all other tropical Carangidae is still preliminary. In the present study, I expand the study area to include Indonesia, to address questions regarding the origins and maintenance of population differentiation in this highly vagile species. Analyses at the population-level examined population structuring and phylogeography of this species using mitochondrial (*COI* and control region) and nuclear (Rag 1) markers. For fisheries, knowing whether a population consists of one homogeneous (genetically identical) population, or many discrete

populations associated with different geographic areas, can assist fisheries managers in designing suitable management plans.

4.2 Materials and Methods

4.2.1 Data sampling

For this study, *Selar crumenophthalmus* was chosen based on its extensive geographical distribution, and sample availability. DNA sequences were generated for approximately five to ten individuals from each location, from multiple localities spread as evenly as possible throughout the Indo-Malay Archipelago (Table 4.1). Samples were collected from 12 localities comprising six geographic regions: South China Sea, Strait of Malacca, Sulu Sea, Celebes Sea, Andaman Sea and Bismarck Sea (Figure 4.1).

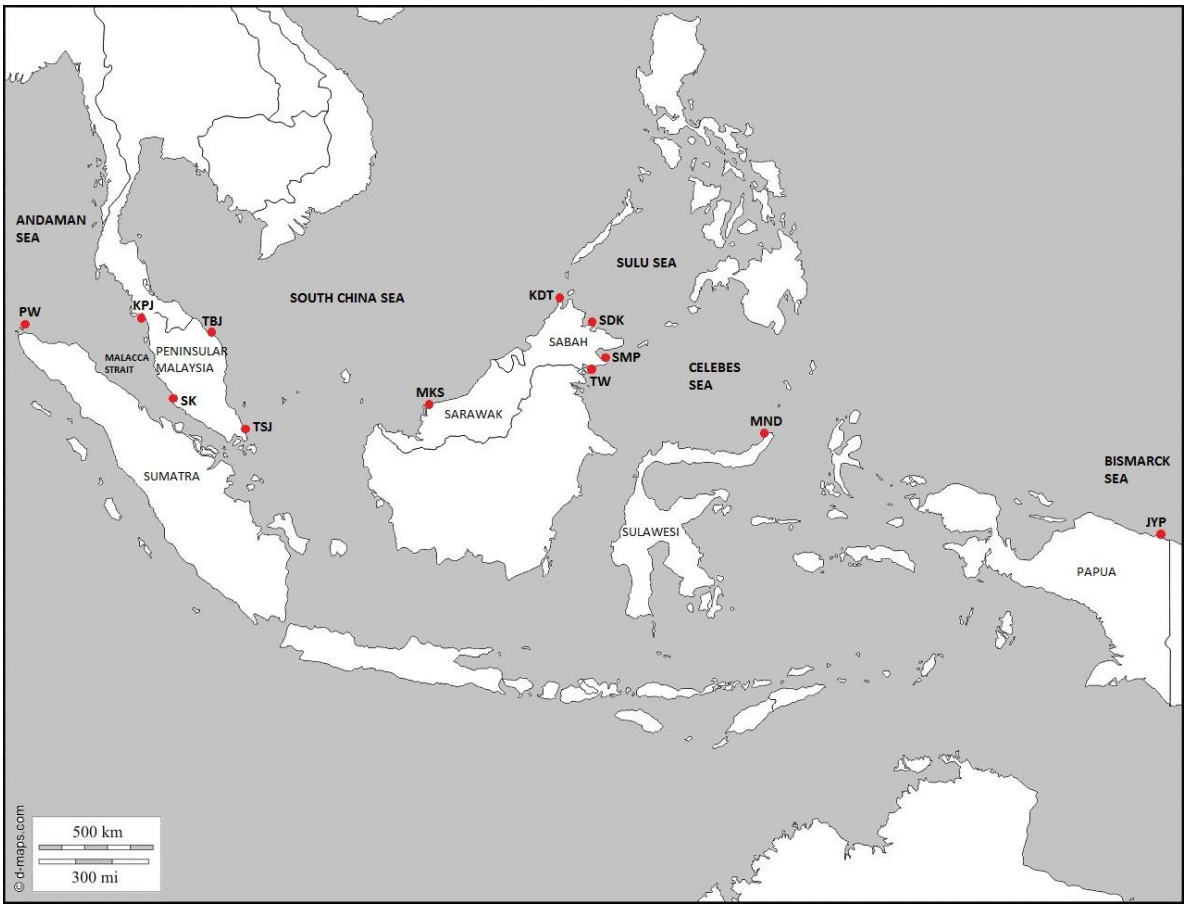

Figure 4.1 Distribution of locations for the 80 specimens sampled along the coast of Malaysia and Indonesia. Samples were collected from respective landing sites (in red) in five geographical regions of the IMA, including Indonesia; Andaman Sea, South China Sea, Strait of Malacca, Sulu Sea, Celebes Sea and Bismarck Sea. Sample sizes for each species and sample codes are given in Table 4.1.

4.2.2 DNA extraction and Polymerase Chain Reaction (PCR)

Fin clips were removed from the right pectoral fin of each fish and preserved in 99% ethanol. Fish specimens were then placed in ice, frozen on site and transported to South China Sea Museum, University Malaysia Terengganu. Total genomic DNA was extracted from fin clips of 80 specimens using the "salting out" method (Miller *et al*., 1988). Isolated DNA was resuspended in 100µl deionized water. A fragment of 650 base pairs (bp) of *COI*, 450 bp of the control region and 950 bp of nuclear gene (Rag1) were amplified using the list of primers in Table 4.2.

Table 4.1 *Selar crumenophthalmus* sampling regime, illustrating geographic regions, sample sites and sample sizes analysed for mtDNA and nuclear DNA.

Geographic regions (code)	Sampling sites (code)	Sample size (n)	Number of sequences		
			COI	**Control region**	**Rag1**
Strait of Malacca (SM)	Kuala Perlis (KPJ)	10	9	10	10
	Sekinchan (SK)	5	5	5	5
South China Sea (SCS)	Tok Bali (TBJ)	5	5	5	5
	Tanjung Sedili (TSJ)	10	10	10	10
	Mukah (MKS)	5	5	5	5
Sulu Sea (SS)	Kudat (KDT)	5	5	5	5
	Sandakan (SDK)	5	5	5	5
Celebes Sea (CS)	Semporna (SMP)	5	4	5	5
	Tawau (TW)	5	5	5	4
	Manado (MND)	10	10	10	9
Andaman Sea (AS)	Pulau Weh (PW)	10	9	9	9
Bismarck Sea (BS)	Jayapura (JYP)	8	8	8	8
Total		83	80	82	80

Table 4.2 List of sequencing primers used in this study.

Gene	Name	Primer sequences	References
Cytochrome c oxidase I	Fish F2	5' TCGACTAATCATAAAGATATCGGCAC 3'	Ward *et al.*, 2005
	Fish R2	5' ACTTCAGGGTGACCGAAGAATCAGAA 3'	
Control region	Fish CR_F	5' CCTGAAGTAGGAACCAGATG 3'	Cardenas *et al.*, 2009
	Fish CR_R	5' AACTCTCACCCCTAGCTCCCAAAG 3'	
	JUR R1_F	5' CAGAAAAAGGAGACTCTAACTCCTG 3'	
	JUR R2_R	5' TGCTTGCGGGGCTTTCTA 3'	
Nuclear gene	Fish Rag 1_F1	5' CGGCTTTCACCAGTTTGAAT 3'	Newly designed primers
	Fish Rag1_F2	5' GGATCTGGAGGAGGACATCA 3'	
	Fish Rag1_R1	5' TGCTGGGAGTTGAAGCTGTA 3'	
	Fish Rag1_R2	5' TGCTGGGAGTTRAAGCTGTA 3'	
	Fish Rag1_R3	5' CCTATATTTGAAGGTAGAGGACAGG 3'	
	Fish Rag1_R4	5' ATATTTGAAGGTAGAGGACAGGAG 3'	

4.2.3 PCR amplification and sequencing

Polymerase reactions were prepared in 11µl reaction volumes including 1µl DNA, 6.6µl ultra pure water, 1.0µl 10X PCR buffer, 0.2µl $MgCl_2$ (25mM), 0.5 µl of each primer (10 µM), 1.0µl dNTPs (2mM), 0.2µl *Taq* polymerase (500U). The thermal regime for *COI* consisted of an initial step of 5 min at 94^{O}C followed by 35 cycles of 1 min at 94^{O}C, 1 min 30s at 58.2^{O}C, and 1 min at 72^{O}C, followed in turn by 10 min at 72^{O}C. For the control region, the amplification started with an initial step of 2 min at 95^{O}C followed by 35 cycles of 30s at 94^{O}C, 30s at 48.3^{O}C, and 1 min at 72^{O}C, followed in turn by 10 min at 72^{O}C. The reaction programme for Rag 1 was carried out initially at 95^{O}C for 3 min followed by 35 cycles: 94^{O}C for 30s, 52^{O}C for 45s, 72^{O}C for 1 min 30s, and finally 10min of final extension at 72^{O}C. DNA amplification products were separated in 1.2% (w/v) agarose gels at 100v with 1X Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide and visualized under UV illumination. The most intense products were selected for sequencing. Prior to sequencing, 10µl PCR products were cleaned with 1U shrimp alkaline phosphatase (Promega) to dephosphorylate residual deoxynucleotides and 0.5U Exonuclease I (Promega) to degrade excess primers (Werle *et al*., 1994). The purification thermal conditions consisted of 37^{O}C for 1 hour and 80^{O}C for 15 min. Bidirectional sequencing was performed using BigDye Termation chemistry on an Applied Biosystems 3730 sequencer by Macrogen Inc, (www.macrogen.com, South Korea). Once sequencing was completed, the raw nucleotide sequences were checked by eye to ensure sequence information was consistent in both directions.

4.2.4 Data analysis

Initial editing of ambiguous bases was undertaken with MEGA5 software (Kumar *et al*., 2004). The edited sequences of each locus were aligned using Clustal W implemented in the same software. The alignments obtained were further visually cross-checked. Amino acid sequence translation (vertebrate mitochondrial code) was applied and checked for stop codons to ensure the amplification of mtDNA rather than nuclear copies of *COI* sequences, and then translated back for

subsequent analysis. Prior to analysis of the data, the program PHASE (Stephens *et al*., 2001) was used to resolve the heterozygous sites in RAG1 sequences to reconstruct haplotypes. PHASE uses a statistical method to infer linkage phase of polymorphic sites from a population sample of genotypic data. It was unnecessary to use PHASE on the mitochondrial sequences because mitochondrial DNA is haploid and therefore contained no heterozygous sites.

4.2.5 Population genetic analysis

DnaSP version 4.0 (Rozas *et al*., 2003) was used to calculate sequence diversity statistics as well as determination of identical haplotypes. The Arlequin software package version 3.5.1.2 (Excoffier and Lischer, 2010) was used to perform an analysis of molecular variance (AMOVA) to examine population structure of each species. AMOVA examines the variance in gene frequencies between different groupings while also taking into account the number of mutations between the haplotypes (Excoffier *et al.*, 1992). AMOVA can group individuals hierarchically by their region and source population, and evaluates the proportion of overall genetic variation that is attributable to that grouping. It examines the structure of the genetic variation among regions (F_{CT}), among-populations within regions (F_{SC}), and among individuals within populations (FST) (Weir and Cockerham, 1984). AMOVA categorizes the distribution of genetic variation across geographic space by examining the degree of genetic differentiation within and among the different hierarchical groupings. For the regional comparison here, the populations were divided into six regions, South China Sea, Strait of Malacca, Sulu Sea, Sulawesi Sea, Andaman Sea and Bismarck Sea. A minimum spanning network was constructed with Network 4.6.1.1, based on haplotype frequencies to search for phylogeographic structure.

Tajima's (1989) *D* statistic and Fu's (1997) *F*s were performed to calculate the conformity of DNA sequence evolution to expectations based on neutrality. Although these tests were developed as tests of selective neutrality, they are also useful for detecting departures from population size equilibrium caused by

population expansions or bottlenecks (Aris-Brosou and Excoffier, 1996; Tajima, 1989; Fu, 1997). Fu's *F*s (1997) is more sensitive than Tajima's *D* (1989), where a significant negative value indicates an excess of singleton haplotypes, which results following population expansions. Mantel (1967) tests were used to examine significant relationships between genetic distance and geographic distance both for the nuclear and the mitochondrial markers. All the above methods were analysed in the software package Arlequin v3.5.1.2.

4.3 Results

4.3.1 Mitochondrial DNA analysis

A total of 80 and 82 individuals were assayed from 12 populations (six geographic regions) for a 650 base pairs (bp) region of the *COI* gene, and 394 bp of control region, respectively. Thirty-one unique haplotypes were identified, with six haplotypes recovered more than once in *COI* data (Table 4.3a), while 64 haplotypes were identified from control region data (Table 4.3b). To further depict the phylogenetic and geographical relationships among the identified *COI* sequences, haplotype networks were constructed using the median-joining method in Network 4.6.1.1 software (Figure 4.2). The resulting networks exhibited a star-like pattern surrounding haplotype H_3, which was found in every population of *Selar crumenophthalmus* from the IMA. Haplotype H_2 was shared by samples from the Strait of Malacca (KPJ), the Andaman Sea (PW), and one locality from the South China Sea region (TSJ). PW and TSJ also shared haplotype H_13. Haplotype H_9 was shared at a Strait of Malacca location (SK), and MND from the Celebes Sea. SK also shared haplotype H_10 with samples from the South China Sea (TSJ). Haplotype H_5 was shared by samples from the northern Strait of Malacca (KPJ), two locations from the South China Sea (TBJ and MKS), and one location from the Sulu Sea (SDK). The other 25 haplotypes were singletons, and were restricted to a single location (Table 4.3a). In control region data, haplotype H_1 occurred in all regions except the Sulu Sea. Eleven haplotypes were identified from more than one

location, and the other 35 haplotypes were restricted to a single sampling site (Table 4.3b). Haplotype (h) and nucleotide (π) diversity from discrete locations were markedly higher in the control region than *COI*, as expected (control region; h= 0.945, π= 0.01074; *COI*; h=0.735, π= 0.0031).

The AMOVA indicated non-significant variation among localities within regions in *COI* data. This result seems to be largely due to all locations sharing at least one haplotype (H_3). F_{ST} values were low in both mtDNA data (*COI*, F_{ST}= -0.00686; control region, F_{ST}= 0.04592). For *COI* data, AMOVA indicated non-significant variation among individuals among localities (P>0.05). The pairwise F_{ST} analysis revealed a lack of genetic structure among the sampled localities. The pairwise F_{ST} values were low in both mtDNA markers, and non-significant, except for the comparison between KPJ and TW (pairwise F_{ST} = 0.27419 and P-value= 0.026) for *COI* data (Table 4.5a). Control region data showed significant F_{ST} values between KPJ and PW (pairwise F_{ST} = 0.056 and P-value= 0.034), SK and KPJ (pairwise F_{ST} = 0.14 and P-value= 0.0195), JYP and four other populations: TBJ (pairwise F_{ST} = 0.2 and P-value= 0.044); MKS (pairwise F_{ST} = 0.2 and P-value= 0.0498); TW (pairwise F_{ST} = 0.245 and P-value= 0.017) and KPJ (pairwise F_{ST} = 0.182 and P-value= 0.006) (Table 4.5b). Generally, low F_{ST} values indicated extensive gene flow among regions. In addition, a Mantel test on mtDNA revealed no correlation between geographic and genetic distance (r= 0.058, p> 0.05).

Tajima's D statistic for *COI* data was negative for all populations but non-significant, except for the MND population. Fu's Fs was significantly negative for KPJ, PW and SDK, while the rest of the populations were not significant. For control region data, both Tajima's D and Fu's Fs statistics were not significant (Table 4.6). For selectively neutral markers, negative D-values indicate excesses of low-frequency haplotypes, relative to mutation/drift equilibrium, with one or very few alleles at high frequency, and rare alleles that derive from the latter by very few mutations. This is generally ascribed to rapid population expansion following a severe reduction in effective size (bottleneck). While negative Fs-values indicate excess number of allele, as would be expected from a recent population expansion.

4.3.2 Nuclear DNA analysis

A total of 80 individuals were assayed for 786 bp of the Rag1 gene. There were three unique alleles identified where '1' allele was most common and shared by all populations. Allele '2' was restricted to an individual from KPJ, and allele '3' restricted to KDT (Table 4.3c). The genetic divergences were also low at all levels of the hierarchy (Table 4.4). When each region was considered separately, populations did not show significant genetic differentiation between locations. A Mantel test also showed no correlation between genetic and geographic distances, consistent with mtDNA data ($r= 0.039$, $p>0.05$).

Table 4.3 Distribution of haplotype frequencies in each population of *Selar crumenophthalmus* by locus.

a) COI

Population	Geographic region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	n
Kuala Perlis (KPJ)	SM	1	1	2	1	1	1	1	1																								9
Sekinchan (SK)	SM			2						1	1	1																					5
Pulau Weh (PW)	AS		1	4		1								1																	1	1	9
Tanjung Sedili (TSJ)	SCS		1	4							1			1	1	1	1																10
Tok Bali (TBJ)	SCS			3		1							1																				5
Mukah (MKS)	SCS			2		1												1	1														5
Kudat (KDT)	SS			3																1	1												5
Sandakan (SDK)	SS			3		1																1											5
Semporna (SMP)	CS			2																			1	1									4
Tawau (TW)	CS			5																													5
Manado (MND)	CS			5						1																	1	1	1	1			10
Jayapura (JYP)	BS			6																					1	1							8
Total		1	3	41	1	5	1	1	1	2	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	80

Symbols equal: SM, Strait of Malacca; AS, Andaman Sea; SCS, South China Sea; SS, Sulu Sea; CS, Celebes Sea; BS, Bismarck Sea

b) **Control region**

Population	Geographic region	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	n
Kuala Perlis (KPJ)	SM						1									1	2	1	1	1	1	1	1																											10
Sekinchan (SK)	SM	3																																1	1															5
Pulau Weh (PW)	AS	3	1	2	1	1	1																																											9
Tanjung Sedili (TSJ)	SCS	3			1				1		1																																				1	1	1	1	10
Tok Bali (TBJ)	SCS																	1																				1					1	1	1					5
Mukah (MKS)	SCS																	1										1	1	1	1																			5
Kudat (KDT)	SS										1	1	1	1	1																																		5
Sandakan (SDK)	SS								1				1					1														1	1																5
Semporna (SMP)	CS	1																							1		1									1	1												5
Tawau (TW)	CS																										1												1	1	2									5
Manado (MND)	CS	3										2						1						1	1	1	1																						10
Jayapura (JYP)	BS	5						1	1	1																																								8
Total		18	1	2	2	1	2	1	3	1	2	3	2	1	1	1	2	5	1	1	1	1	1	1	2	1	3	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	1	1	1	1	82

Symbols equal: SM, Strait of Malacca; AS, Andaman Sea; SCS, South China Sea; SS, Sulu Sea; CS, Celebes Sea; BS, Bismarck Sea

c) Rag1

Population	Geographic region	1	2	3	n
Kuala Perlis (KPJ)	**SM**	**9**	**1**		**10**
Sekinchan (SK)	**SM**	**5**			**5**
Pulau Weh (PW)	**AS**	**9**			**9**
Tanjung Sedili (TSJ)	**SCS**	**10**			**10**
Tok Bali (TBJ)	**SCS**	**5**			**5**
Mukah (MKS)	**SCS**	**5**			**5**
Kudat (KDT)	**SS**	**4**		**1**	**5**
Sandakan (SDK)	**SS**	**5**			**5**
Semporna (SMP)	**CS**	**5**			**5**
Tawau (TW)	**CS**	**4**			**4**
Manado (MND)	**CS**	**10**			**10**
Jayapura (JYP)	**BS**	**8**			**8**
Total		**78**	**1**	**1**	**80**

Symbols equal: SM, Strait of Malacca; AS, Andaman Sea; SCS, South China Sea; SS, Sulu Sea; CS, Celebes Sea; BS, Bismarck Sea

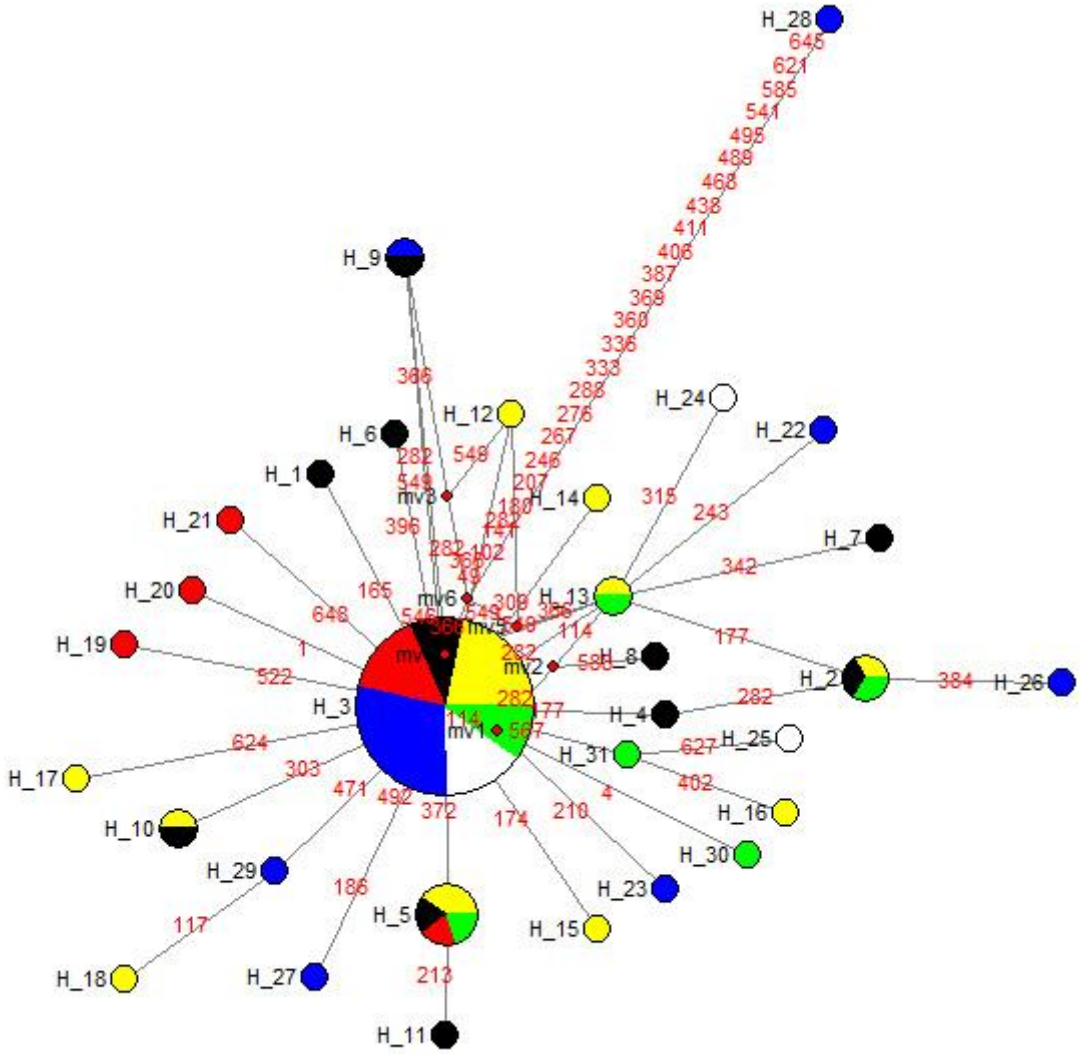

Figure 4.2 Median-joining network constructed for *COI* haplotypes of *Selar crumenophthalmus*. Each circle represents one unique haplotype, with the area being proportional to the frequency of the haplotype in all populations. Colour equal to geographic regions: black, Strait of Malacca; yellow, South China Sea; red, Sulu Sea; blue, Celebes Sea; white, Bismarck Sea; green, Andaman Sea.

Table 4.4 Results of the analysis of molecular variance (AMOVA) for *Selar crumenophthalmus* showing F-statistics analysis for mtDNA and nuclear DNA.

Hierarchical level	*COI*		CR		Rag1	
	F-statistics	P-value	F-statistics	P-value	F-statistics	P-value
Among all regions (F_{CT})	0.01234	0.40078	0.00481	0.36266	0.01345	0.27957
Among localities within regions (F_{SC})	-0.01945	0.61975	0.04131	0.06061	-0.01673	1.00000
Among individuals within localities (F_{ST})	-0.00686	0.57967	0.04592	0.0391	-0.00306	0.72825

Table 4.5 Population pairwise F_{ST} for sampled *Selar crumenophthalmus* populations (below) and corresponding P values (above) by locus.

a) *COI*

	PW	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK	JYP	MND
PW		0.99902	0.60156	0.99902	0.99902	0.10352	0.87891	0.99902	0.99902	0.99902	0.32910	0.99902
TBJ	-0.08526		0.99902	0.99902	0.99902	0.46582	0.43457	0.99902	0.99902	0.99902	0.57324	0.99902
TSJ	-0.02273	-0.05769		0.52539	0.50586	0.99902	0.14551	0.51562	0.99902	0.99902	0.99902	0.58301
MKS	-0.08011	-0.11111	0.04412		0.99902	0.16016	0.99902	0.99902	0.99902	0.99902	0.29102	0.99902
SMP	-0.07143	-0.09053	-0.00737	-0.08541		0.16406	0.52930	0.99902	0.99902	0.99902	0.53418	0.99902
TW	0.15094	0.12500	-0.00000	0.25000	0.23077		**0.02637**	0.16016	0.46191	0.41992	0.50488	0.26074
KPJ	-0.02993	-0.00544	0.099949	-0.05649	-0.02683	0.27419		0.99902	0.26758	0.43066	0.05176	0.35645
SK	-0.05064	-0.05263	0.04412	-0.07143	-0.08541	0.25000	-0.03096		0.99902	0.99902	0.27734	0.99902
KDT	-0.05307	-0.09375	-0.05769	-0.05263	-0.09053	0.12500	0.01941	-0.05263		0.99902	0.58984	0.99902
SDK	-0.08526	-0.16667	-0.05769	-0.11111	-0.09053	0.12500	-0.00544	-0.05263	-0.09375		0.61426	0.99902
JYP	0.02236	-0.04265	-0.08562	0.05419	0.00271	-0.00386	0.13150	0.05419	-0.04265	-0.04265		0.37012
MND	-0.03535	-0.06061	-0.01415	-0.04126	-0.06892	0.11392	0.01700	-0.06855	-0.06061	-0.06061	-0.00030	

Symbols equal: PW, Pulau Weh; TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan, KDT, Kudat; SDK, Sandakan; JYP, Jayapura; MND, Manado. Significant values appear in bold.

b) control region

	PW	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK	JYP	MND
PW		0.12988	0.68262	0.14453	0.45020	0.06934	**0.03418**	0.48730	0.13965	0.12793	0.23047	0.40234
TBJ	0.06172		0.26367	0.99902	0.99902	0.46875	0.76367	0.15234	0.99902	0.99902	**0.04395**	0.35059
TSJ	-0.02526	0.03727		0.28906	0.99902	0.06543	0.10547	0.56543	0.45898	0.44238	0.34082	0.79590
MKS	0.06172	-0.04167	0.03727		0.99902	0.43750	0.78418	0.15527	0.99902	0.99902	**0.04980**	0.32129
SMP	-0.00626	-0.04167	-0.02479	0.00000		0.68652	0.51758	0.51074	0.99902	0.99902	0.25684	0.94141
TW	0.10623	0.05000	0.08116	0.05000	0.01042		0.10938	0.08691	0.44336	0.46582	**0.01660**	0.11621
KPJ	0.05553	-0.00787	0.04444	-0.00787	0.01235	0.05632		**0.01953**	0.50391	0.75195	**0.00586**	0.08105
SK	-0.00430	0.15000	-0.01083	0.15000	0.03409	0.20000	0.14074		0.18457	0.17578	0.99902	0.49121
KDT	0.06172	0.00000	0.01743	0.00000	0.00000	0.05000	0.01235	0.15000		0.99902	0.05273	0.52051
SDK	0.06172	-0.04167	0.01743	-0.04167	0.00000	0.05000	-0.00787	0.15000	-0.04167		0.06250	0.34961
JYP	0.03004	0.20000	0.00988	0.20000	0.08108	0.24511	0.18233	-0.07117	0.20000	0.17874		0.18750
MND	-0.00016	0.03021	-0.01343	0.03021	-0.05719	0.07524	0.04602	0.00362	0.00974	0.03021	0.0387	

Symbols equal: PW, Pulau Weh; TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan, KDT, Kudat; SDK, Sandakan; JYP, Jayapura; MND, Manado. Significant values appear in bold.

c) Rag1

	PW	TBJ	TSJ	MKS	SMP	TW	KPJ	SK	KDT	SDK	JYP	MND
PW		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.38770	0.99902	0.99902	0.99902
TBJ	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
TSJ	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.31348	0.99902	0.99902	0.99902
MKS	0.00000	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
SMP	0.00000	0.00000	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
TW	0.00000	0.00000	0.00000	0.00000	0.00000		0.99902	0.99902	0.99902	0.99902	0.99902	0.99902
KPJ	-0.01124	-0.08434	0.00000	-0.08434	-0.08434	-0.12150		0.99902	0.99902	0.99902	0.99902	0.99902
SK	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-0.08434		0.99902	0.99902	0.99902	0.99902
KDT	0.12621	0.00000	0.14894	0.00000	0.00000	-0.05263	-0.03659	0.00000		0.99902	0.38770	0.31934
SDK	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-0.08434	0.00000	0.00000		0.99902	0.99902
JYP	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	-0.02418	0.00000	0.10112	0.00000		0.99902
MND	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.14894	0.00000	0.00000	

Symbols equal: PW, Pulau Weh; TBJ, Tok Bali; TSJ, Tanjung Sedili; MKS, Mukah; SMP, Semporna; TW, Tawau; KPJ, Kuala Perlis; SK, Sekinchan, KDT, Kudat; SDK, Sandakan; JYP, Jayapura; MND, Manado. Significant values appear in bold.

Table 4.6 Tajima's D and Fu's Fs statistical neutrality tests for mtDNA data in *Selar crumenophthalmus.*

Population	*COI*		CR	
	Tajima's D	**Fu's Fs**	**Tajima's D**	**Fu's Fs**
KPJ	-1.20498	-5.10673*	-1.04232	-2.54428
SK	-1.0938	-1.40478	-1.04849	-0.18585
PW	-1.39844	-2.97753*	0.74673	0.04579
TSJ	-0.97256	1.04042	-0.84906	-3.09515
TBJ	-0.97256	-0.8292	-0.8554	-1.63258
MKS	-1.0938	-1.40478	0.08298	-1.80529
KDT	-0.97256	-0.8292	-1.17432	-1.55426
SDK	-0.97256	-0.8292 *	-1.17432	-1.90106
MND	-1.9582 *	1.09681	-0.755	-0.6739
SMP	-0.78012	0.13353	-0.84004	-0.7916
TW	0	N/A	-0.74682	-0.33158
JYP	-1.5347	0.20428	-0.81246	-1.38724

*Significant values

4.4 Discussion

4.4.1 Genetic population structure and gene flow

Evidence from the current study showed that levels of genetic differentiation using F_{ST} values for both mtDNA and nuclear DNA markers were low in Indo-Malay *Selar crumenophthalmus*, indicating extensive gene flow among populations. Although only a few studies have examined the population genetic structure of species in the family Carangidae to date, similar results from mitochondrial and nuclear sequence data have been reported from the carangid genus *Decapterus* in Southeast Asia and the IMA (Arnaud *et al*., 1999; Borsa, 2003). The current finding is also consistent with a study by Pedrosa-Geramsio *et al*. (2011), which showed a homogeneous population of *Selar crumenophthalmus* in the Sulu Sea using mtDNA control region. However, in a preliminary survey based on the sequence polymorphism of the cytochrome *b* gene, Perrin and Borsa (2001) distinguished two putative mitochondrial lineages in *Decapterus russelli* from the Indo-Malay Archipelago, which were separated by 2.2% average nucleotide divergence. These two mitochondrial lineages had a very heterogeneous geographic distribution and co-occurred at high frequencies in the Celebes Sea alone. The distinction of two clades within *D. russelli* was compatible with Pleistocene events that isolated the Celebes Sea region from other areas in the Indo-Malay Archipelago. Another study by the same authors (Borsa, 2003) on the genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) found no significant heterogeneity in cytochrome *b* haplotype frequencies or in aldolase B-1 allele frequencies across populations from the Indo-Malay Archipelago, suggesting the presence of a single panmictic population in this region. Both species, *Decapterus macrosoma* and *Selar crumenophthalmus* have the same life-history features which explain the similarities of no/little genetic structure between populations. Pelagic marine fishes usually have high fecundity and high dispersal potential of egg, larval and adult stages. *D. macrosoma* travel large distance as eggs, larvae (Delsman, 1926) and

adults (Hardenberg, 1937). A study by Roos *et al*. (2007) on the biology of *Selar crumenophthalmus* around Reunion Island, southwest India Ocean showed that soon after spawning, larvae and post-larvae of *Selar crumenophthalmus* would have been displaced by coastal currents to other more distant sites. This suggests that the absence of population genetic structure could be a consequence of the life history and reproductive characteristics of both, *D.macrosoma* and *Selar crumenophthlmus*.

The presence of shared haplotypes at distant locations also suggests gene flow across large spatial scales. Because the mitochondrial control region is one of the fastest evolving and hyper variable gene regions known (Moritz *et al*., 1987), the presence of shared haplotypes may indicate that gene flow between distant populations has occurred on a relatively recent evolutionary time scale. Alternatively, the most frequently occurring haplotypes, as well as those found at the largest geographic scales, can also be interpreted as being the oldest (Posada and Crandall, 2001). In the present study, the shared haplotypes in *Selar crumenophtalmus*, separated by vast distances, may actually be much older than other non-shared haplotypes in our samples and may have had considerably more time to traverse long distances through many successive generations. For a more comprehensive analysis of genetic variation in *Selar crumenophthalmus*, the sampling design in future should address a much broader geographic scale, so as to include samples from the Indian Ocean in particular. Also, it may be advantageous to use faster evolving genetic markers (e.g. microsatellites or SNPs) to further investigate stock structure for fisheries management purposes.

4.4.2 Potential isolating mechanisms in the IMA

Connectivity among marine populations is determined predominantly by the dispersal capabilities of adults as well as their eggs and larvae and the extent to which adults share a common gene pool. Dispersal distances and directions have a profound effect

on gene flow and genetic differentiation within species. Genetic homogeneity over large areas is a common feature of marine pelagic fishes and can reflect high dispersal capability resulting in high levels of gene flow (Doherty *et al.,* 1995; Shulman and Bermingham, 1995; Bernardi *et al.,* 2001). Panmixia of *Selar crumenophthalmus* in the region is likely because of the high mobility of the species, which would facilitate mixing among populations within and between regions. Along with migration, the transportation of eggs and/or larvae via oceanic currents may result in very weak or no apparent population structuring (Caley *et al*., 1996). Unfortunately, little is known regarding the reproductive biology of most Carangidae species (e.g., Leis *et al*., 2004). For the carangid *Gnathanodon speciosus*, hatching has been observed 18h after spawning (Watson and Leis, 1974). Assuming a comparable duration for *Selar crumenophthalmus*, this would allow some amount of passive dispersal prior to hatching. Further, carangid fish do not settle (Leis, 1991) and early juveniles (e.g., 21 mm standard length) often associate with floating or drifting objects (Honebrink, 2000). Such behavior would also facilitate population mixing in these species because juveniles have an opportunity to disperse for longer durations and/or distances. Taken together, we conclude that the absence of geographic structure reported here for *Selar crumenophthalmus* is due to the active movement of adult individuals and/or the passive dispersal of eggs and juveniles at frequencies sufficient to homogenize populations across the IMA.

Although the current study indicated no genetic differentiation between *Selar crumenophthalmus* populations, a few studies have shown that population subdivision does occur in marine fishes even across small spatial scales, ranging from tens to a few hundred kilometres (Knutsen *et al*., 2003; Nielsen *et al*., 2004; Bremer *et al*., 2005; Jorgensen *et al*., 2005; Rohfritsch and Borsa, 2005; Knutsen *et al*., 2007; Shui *et al*., 2008; Fauvelot and Borsa, 2011; Nielsen *et al*., 2012). Some marine fishes exhibit significant genetic structure, often attributed to the presence of geographic barriers. Indo-Pacific physical barriers, such as the Sunda Shelf, and the physical oceanography

of the IMA provide potential mechanisms for non-geographical genetic differences (Barber *et al*., 2006; Rocha *et al*., 2007). For example, the Indonesian Archipelago itself is a biogeographic barrier, separating the Indian Ocean from Malayan provinces (Schopf, 1979). This complex of islands represents a barrier to gene flow within species (Benzie and Stoddart, 1992), as well as separating closely related species (McMillan, 1994). Leray *et al*. (2010) also demonstrated a broad geographic break consistent with a Sunda Shelf barrier for *D. trimaculatus*. Drew and Barber (2009) demonstrated a strong genetic break consistent with the western Sunda Shelf Barrier in the Lemon Damsel *Pomacentrus moluccensis*. Lourie *et al*., (2005) studied four species of seahorse around Southeast Asia and found population discontinuities within the Philippines, north−south and east−west across the Coral Triangle, and corresponding to the western Sunda Shelf Barrier. Timm *et al*., (2008) tested connectivity with the false clown anemonefish, *Amphiprion ocellaris*, and found population differentiation that corresponded to the southern Sunda Shelf Barrier, easternmost Indonesia, and a broad north−south break. However, due to the highly mobile pelagic lifestyle of *Selar crumenophthalmus*, there is no evidence of a Sunda Shelf break within the IMA. Extending the sampling area to include samples from peripheral regions in the Indian and western Pacific Oceans will help provide a more comprehensive picture of the importance of the Sunda Shelf barrier within the region.

In addition, genetic differentiation in some pelagic marine fishes might be linked to circulation patterns and water exchanges between seas or oceans. Surface currents in the western part of the IMA vary seasonally according to typical monsoon cycles. During the wet monsoon (November to March), currents flow towards the East with speeds of ca. 1 to 2 knots. During the dry monsoon (May–September), the circulation is completely reversed with currents flowing towards the West. During the inter-monsoon (April–October), the winds and the currents are weak and variable. The monsoon cycle induces shifts in water circulation and changes in salinity, which influence the dispersion of the larvae of pelagic fishes (Hardenberg, 1937). According

to a preliminary report by Hardenberg (1937), two or perhaps three stocks of *Decapterus* are present in the periphery of the Java Sea, which follow different migration routes and timings in relation to monsoons. Rohfritsch and Borsa (2005) also detected at least three geographically distinct populations of *Decapterus ruselli* in the IMA, which should be managed as distinct management units.

4.4.3 Future works

The use of mitochondrial DNA (mtDNA) markers is now an established technique for elucidating population genetic structure and phylogeography (Niwa *et al*., 2003; Martinez and Zadoya, 2005; Santos *et al*., 2010). MtDNA is maternally inherited making it valuable for population studies for it allows the reconstruction of maternal lineages that often are correlated with geography. It has become widely used for genetic studies for fish populations as it is a rapidly evolving marker, and is likely to be more sensitive to gene flow (Ward *et al.*, 2001). Rapidly evolving mtDNA sequences, such as the control region that evolves about 4-5 times faster than the rest of the mtDNA molecule (Taberlet, 1996) provide more opportunity for drift to vary allele frequencies. This is probably the reason why nucleotide diversity estimated from the control region in this study appeared to be slightly higher than nucleotide diversity estimates derived from *COI* (control region; h= 0.945, π= 0.01074; *COI*; h=0.735, π= 0.0031). Several studies also showed lack of genetic structure detected among highly mobile marine fish using mtDNA alone (Chow *et al*., 2000; Bremer *et al*., 2005; Cardenas *et al*., 2009; Horne *et al*., 2008). Further study should involve faster evolving genetic markers (e.g. microsatellites, SNPs), which are potentially capable of detecting subtle signals of population subdivision (Martinsohn and Ogden, 2009; Habicht *et al*., 2010; Nielsen *et al*., 2012; Kruck *et al*., 2013). Single nucleotide polymorphisms (SNPs) are an attractive alternative for genetic markers used in the current study, primarily because they sometimes are under selection and therefore might exhibit orders of magnitude higher levels of genetic differentiation among populations, as well

as providing a framework for exploring the functional significance of genetic differentiation (Limborg *et al*., 2012; Nielsen *et al*., 2012). SNPs are also applicable for analysing both neutral and adaptive genetic variation, which promises exciting opportunities for fishery management and conservation (Nielsen *et al*., 2009; Stapley *et al*., 2010). Therefore, these markers should be used in the future to detect population genetic structure of highly mobile commercial fish species, as well as for tackling Illegal, Unreported and Unregulated (IUU) fishing in the IMA region (Nielsen *et al*., 2012).

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CHAPTER 5

GENERAL DISCUSSION

5.1 General summary

Using the Carangidae, a commercially-important fish family in the Indo-Malay Archipelago (IMA) as the target group, the effectiveness of molecular methods in species identification, delimitation and detecting population structure for management and conservation of fisheries resources was examined. Molecular analyses were undertaken at two levels: species- and population-level studies investigated speciation and dispersal hypotheses regarding patterns of biodiversity in the IMA, and population genetic structure of Carangidae within the region. The species-level work included phylogenetic analyses and DNA barcoding studies to quantify diversity at the cytochrome *c* oxidase I (*COI*) gene at both intra- and inter-species levels, while analyses at the population-level examined population structuring and phylogeography utilising additional genetic markers; the mtDNA control region (D-loop) and a nuclear gene (Rag 1).

In Chapter 2, as expected, the *COI* gene accurately discriminated thirty-species of Family Carangidae. However, there were three species (*Atule mate*, *Selar crumenophthalmus* and *Seriolina nigrofasciata*) that exhibited deep divergences (4.32-4.82%) among individuals that were previously assigned to a single taxon. These highly divergent sympatric lineages suggest that each may comprise more than one cryptic species. Despite high levels of cryptic species known to occur within and outside the IMA (Ward *et al*., 2005; Zemlak *et al*., 2009; Hubert *et al*., 2012), we detected only a moderate level of cryptic diversity among putative species within the central IMA, at least with the tools employed here.

The population genetic structure of three species of Carangidae encompassing a range of life history traits (highly pelagic, semi-pelagic/demersal, demersal) was compared in Chapter 3. We predicted a relatively homogeneous population structure in pelagic species compared to demersal species, due to their potential to undertake long-

distance migrations in oceanic waters. However, no significant geographic structuring was detected across all three species. *Atule mate*, in which potential cryptic species were identified in Chapter 2, showed the same pattern in phylogenetic trees constructed from control region and Rag 1 data. Two mitochondrial lineages were present in the Indo-Malay *Atule mate*.

In Chapter 4, we focussed on a highly mobile pelagic species, *Selar crumenophthalmus,* and expanded the study area to include Indonesia to investigate phylogeographic patterns and population genetic structuring. It is important to know whether a population consists of one homogeneous population, or many discrete populations associated with different geographic areas. Such data can assist fisheries managers in designing suitable management plans. Low levels of genetic differentiation in both mtDNA and nuclear DNA suggested there was extensive gene flow among populations of *Selar crumenophthalmus* in the IMA. Similar results have also been reported from other carangid genera in the region (Arnaud *et al*., 1999; Borsa, 2003; Pedrosa-Geramsio *et al*., 2011). The absence of geographic structuring reported here for *Selar crumenophthalmus* may be due to the active movement of adult individuals and/or the passive dispersal of eggs and juveniles at frequencies sufficient to homogenize populations in the IMA.

5.2 Hotspots of biodiversity in SE Asia

The greatest diversity of species is most likely to occur where the greatest diversity of habitats are present (Hiscock and Smirthwaite, 2004). The oceans cover more than 70% of the planet's surface area. Within coastal areas there are a wide variety of habitats with known high species diversity such as sea grass beds (McRoy, 1981), coastal sedimentary habitats (Gray, 1994), mangrove forests (MacNae, 1968; Walsh, 1974) and coral reefs (Loya, 1972; Huston, 1985; Sheppard, 1980). Therefore, much of

the global biodiversity is found in highly diverse marine and coastal habitats. Marine biodiversity is higher in benthic rather than pelagic systems (Angel, 1993). In the pelagic realm, diversity is higher in coastal areas rather than the open ocean (Angel, 1993), since there is a greater range of habitats nearer to the coast. Angel (1993) estimates that there are probably only 1,200 oceanic fish species against 13,000 coastal species.

Marine biodiversity hotspots are areas with high numbers of species and habitat richness. In the marine environment, the greatest diversity is seen on coral reefs. Within the Indo-Pacific region, coral reef biodiversity increases, both latitudinally and longitudinally, as one moves towards a hotspot in the IMA (Rosen, 1981; Briggs, 2000, 2005; Roberts *et al*., 2002; Mora *et al*., 2003). Stehli and Wells (1971) showed that diversity of bivalve molluscs at species, genus and family levels increased towards the tropics in the Indo-Pacific. Similar patterns have been shown for mangroves, and gastropod snails (Huston, 1994). Using rRNA techniques Palumbi (1995) showed that species have indeed radiated out into the Indo-Pacific region from the centre: the IMA. It appears that the IMA is the `epicentre' for evolution of marine tropical biodiversity (Veron, 1995), and known as one mega-diverse biodiversity hotspot in the region (Lohman *et al*., 2011), likely due to its high proportion of tropical coral reefs and coastal ecosystems. The IMA consists of over 25,000 islands, providing coral reef areas (approx. 4,006 km^2) (Burke *et al*., 2002) and coastal mangroves (approx. 5,669 km^2) (Wong, 2004). Such habitat heterogeneity provides resources such as feeding and nursery grounds for a plethora of marine taxa. This extraordinary diversity has built up, and likely moved into the region (Renema *et al*., 2008), over geological timescales, but it is maintained through the wide array of physical conditions (salinity, wave exposure, depth, temperature, and turbidity) found across SE Asia that fulfil the requirements of a broad range of species.

The reason for such high levels of diversity in the Indo-Pacific region is thought not to be solely the result of a long period of evolutionary stability, but rather due to the fact that there is a large diversity of types of islands and archipelagos which differ in size, in their geological history, and in distance from sources of colonising species. There have been periods of isolation over evolutionary time, which have given rise to allopatric speciation (speciation caused by the erection of physical boundaries between populations). Throughout geological time there have been massive extinctions followed by rapid evolution and speciation (Huston, 1994).

The emergence of this extraordinary species richness in the IMA has led to a number of hypotheses that attempt to explain this diversity. These are: (1) the result of diversification within the region and subsequent species dispersion to marginal locations ('Centre of Origin'; Briggs, 2005); (2) as a result of speciation in several areas peripheral to the central region, and these species subsequently extending their ranges into the region by way of prevailing currents, for example ('Centre of Accumulation'; Jokiel and Martinelli, 1992); or finally (3) the IMA as a 'Centre of Overlap' in which geographic isolation and allopatric speciation with midpoint ranges of species distributions falling on each side of the IMA, with overlap across the region (Woodland, 1983). Recently, Hubert *et al*. (2012) detected high levels of cryptic diversity in coral reef fishes within the Indo-Malay-Philippines Archipelago (IMPA), which had allopatric distributions. Their findings indicated that the IMPA represented an overlapping area for species distributions from each side of the Pacific and Indian Oceans. This is due to the regions geological history, its location on the junction between the two main tropical oceans, and the presence of a land bridge during glacial times in the region, which fostered allopatric divergence and secondary contact of taxa between the two oceans. In addition, one of the Indo-Malay Carangidae species in the current study (*Seriolina nigrofasciata*) also showed allopatric divergence, with one lineage consisting of samples from Sabah separated from the other lineage consisting of samples from West Peninsular Malaysia, Iran and India (Appendix 5.10) (Mat Jaafar

et al., 2012). The other three Carangidae species (*Caranx sexfasciatus*, *Decapterus maruadsi*, *Gnathanodon speciosus*) also showed allopatric divergence when additional conspecific sequences available from other geographical regions were compared (Appendix 5). Such findings are consistent with large faunal discontinuities between the Indian and Pacific Ocean ichthyofaunas as a consequence of geographic isolation on each side of the IMA (Springer and Williams, 1990). However, our data is not sufficient to support alternate species richness hypotheses in the IMA. Further study should sample the entire family across their broad geographic range in order to explore hypotheses of species diversification within a robust phylogenetic framework.

5.3 Applications of fish DNA barcoding

The so-called "taxonomic impediment" and ambiguities in species identification was a major driver for Hebert *et al*. (2003) to introduce DNA barcoding as a taxonomic tool and global bio-identification system for all animal species. The approach employs DNA sequences as taxon 'barcodes', based on the mtDNA gene, *COI,* to generate unique, globally-applicable genetic identification tags for each species. DNA barcoding reveals only a tiny segment of each species' genome, but because it examines the same core region, such target sequences can be compared across all species, revealing how given sequences have changed from species to species and over evolutionary time (Costa and Carvalho, 2010). *COI* was chosen as the target gene as it amplifies readily in most animal phyla, with robust universal primers (Folmer *et al*., 1994; Zhang and Hewitt 1997; Hebert *et al*., 2003). It also provides a greater range of phylogenetic signal for both higher- and lower taxonomic levels.

According to the barcoding approach, species could be identified based on a 'barcoding gap' between intra- and inter-specific genetic distances by using a threshold value of 2−3% (Hebert *et al*., 2003), or a 10-fold value (Hebert *et al*., 2004)

for species delimitation. In current study, all described species formed monophyletic clusters in NJ phylogenetic tree, confirming the effectiveness of *COI* as species identification tool for Indo-Malay Carangidae. However, the ML analyses suggested that four species might comprise only two taxonomic units, as these four species formed two reciprocally monophyletic clusters in the ML tree. ABGD analysis also supported this findings, suggested a problem with the taxonomy at the generic level in Carangidae. Further analyses should be undertaken by the inclusion of more genes and larger sample sizes to confirm the relationships across these four species.

Despite using widely applicable primers in DNA barcoding approach, examination of the DNA barcoding literature reveals that the majority of projects actually rely on taxon-specific primers, rather than universal primers, in order to optimize PCR performance (Barrett and Hebert, 2005; Costa *et al.*, 2007; Hebert and Gregory, 2005; Hebert *et al.*, 2004a; Hebert *et al*., 2004b; Ivanova *et al.*, 2005; Ivanova *et al.*, 2006), particularly with degraded material (Lambert *et al.*, 2005). In addition, some DNA barcoding projects have used even small fragments (<400 bp) of *COI* (Hajibabaei et al., 2007; Hajibabaei et al., 2006; Page *et al.*, 2005; Whiteman *et al.*, 2004), so-called "mini-barcodes".

So far, *COI* has been widely accepted as a marker for molecular identification of various invertebrates and vertebrates, including springtails (Hogg and Hebert, 2004), butterflies (Hebert *et al*., 2004a), crustaceans (Costa *et al*., 2007), birds (Hebert *et al*., 2004b) and a large range of fish species (Ward *et al*., 2005; Pegg *et al*., 2006 ; Rock *et al*., 2008; Steinke *et al*., 2009; Hanner *et al*., 2011; Keskin and Atar, 2013; McCusker *et al*., 2013; Young *et al*., 2013). Furthermore, DNA barcoding not only successfully discriminated potential cryptic species within Indo-Malay Carangidae (Chapter 2), but also in Indo-Australian Melanotaeniidae (Kadarusman *et al*., 2012) and Indo-Malay-Philippines coral reef fishes (Hubert *et al*., 2012). Thus, DNA barcoding was not only effective for the identification of species, but it proved to be effective for the discovery

of cryptic diversity in this biodiversity hotspot (Hebert *et al*., 2004a; Zemlak *et al*., 2009; Hubert *et al*., 2012). Cryptic diversity is particularly challenging for management, but once recognized using molecular tools, reliable diagnostic morphological characters may subsequently be identified (Smith *et al*., 2011).

In addition to the use of DNA barcodes for species identification and clarification of taxonomic uncertainties (e.g, cryptic species) (Hebert *et al*., 2004a; Ward *et al*., 2008; Carr *et al*., 2011; Hubert *et al*., 2012; Mat Jaafar *et al*., 2012), the approach also facilitates numerous related applications in fisheries, including identification of ambiguous life history stages (Webb *et al*., 2006; Costa and Carvalho 2007; Hubert *et al*., 2010; Taylor *et al*., 2002; Fox, Taylor *et al*., 2005; Fox *et al*., 2008). DNA barcoding has accurately identified not only the whole fish, but also fish eggs and larvae, fish fragments, fish fillets and processed fish (Costa and Carvalho, 2007). Through such applications, more extensive data on larval recruitment and ecology and geographic ranges of fisheries resources can be obtained to improve knowledge on nursery areas and spawning grounds for fisheries management and conservation. For example, a study by Webb *et al*. (2006), testing the application of molecular techniques in species identification of fish eggs, revealed that over 60% of the eggs were misidentified when phenotypic characters were used. Misidentification could obscure understanding on speciation, diversity, niche partitioning, and many other features of ecosystems. Webb *et al*. (2006) have shown that it is possible to identify larvae of fish using DNA barcoding techniques, but the resolution is currently limited by the availability of comparative adult sequences in the DNA sequence database.

Another valuable application of fish DNA barcoding is the analysis of dietary habits of predators (Albaina *et al*., 2010; Budarf *et al*., 2011; Carreon-Martinez *et al*., 2011; Fox *et al*., 2012; Valdez-Moreno *et al*., 2012). Identification of prey-remains from predator's stomach contents could provide more information about their trophic relationships within ecosystems, and play a key part of their conservation

management. Moreover, DNA barcoding could also be used in forensics applications, including the monitoring of illegal trade of wildlife, especially protected and endangered species (Holmes *et al*., 2009; Dawney *et al*., 2007; Nielsen *et al*., 2012), the monitoring of fisheries quotas and by-catch, inspection of fisheries markets and products (Smith *et al*., 2008; Filonzi *et al*., 2010), and improvements in the traceability of seafood products (Wong and Hanner 2008; Keskin and Atar, 2012; Nicole *et al*., 2012).

5.4 Population differentiation and stock structure in marine fishes

Marine fishes with high migration and dispersal potential at egg, larval and adult stages generally have typically very low F_{ST} values (defined as the fraction of the total genetic variation attributable to differences among populations) indicating high connectivity among widely distributed populations (Palumbi, 1992, 2003; Martinsohn and Ogden, 2009; Habicht *et al.,* 2010; Nielsen *et al*., 2012; Hemmer-Hanse, 2013). Local adaptation may be constrained due to high levels of gene flow (Hauser and Carvalho 2009), leading to a close association between populations at a large spatial scale (Palumbi 1992; Ward *et al*., 1994). Accordingly, a lack of genetic differentiation has been detected among populations in many marine fishes (Chiang *et al*., 2006; Cassista and Hart 2007; Cardenas *et al*., 2009; Wu *et al*., 2010). The present results support this paradigm indicating little genetic differentiation in big-eye scad, *Selar crumenophthalmus* across the IMA (Chapter 4). However, this paradigm has shifted recently, with an increasing number of studies that have detected population subdivision in marine fishes even across small spatial scales, ranging from tens to a few hundred kilometres (Knutsen *et al*., 2003; Nielsen *et al*., 2004; Bremer *et al*., 2005; Jørgensen *et al*., 2005; Rohfritsch and Borsa 2005; Knutsen *et al*., 2007; Shui *et al*., 2008; Fauvelot and Borsa 2011, Limborg *et al*., 2012; Hemmer-Hansen *et al*., 2013).

Significant genetic differentiation between populations is usually driven by physical barriers (e.g. continents) and to a lesser extent by ocean currents, temperature and salinity (Palumbi 1994; Borsa *et al*., 1997; Graves 1998), resulting in marine species with high dispersal capabilities being at least partially isolated. Several studies have shown that genetic structure is associated with circulation patterns and the water exchange between oceans (Froukh and Kochzius, 2007; Shui *et al*., 2008). For example, the ecological differences between the northern and southern Red Sea lead to differences in fish communities in the Red Sea at about 20N°, congruent with genetic differentiation between northern and southern populations of *Larabicus quadrilineatus* (Froukh and Kochzius, 2007). Geographic features also influence ocean circulation and probably gene flow as well. For example, the Indonesian Archipelago itself is a biogeographic barrier, separating the Indian Ocean from Malayan provinces (Schopf, 1979). This complex of islands represents a barrier to gene flow within species (Benzie and Stoddart, 1992; Lourie *et al*., 2005b; Timm *et al*., 2008; Drew and Barber, 2009; Leray *et al*., 2010), as well as separating closely related species (McMillan, 1994). This is also consistent with present findings, when two mtDNA lineages were detected in three species of Carangidae, respectively, separating the Indian Ocean specimens from the IMA's (Chapter 2). Genetic differentiation between populations may also arise from species life history traits such as spawning asynchrony among populations, retention of eggs and larvae, and adult homing behaviour (Doherty *et al*., 1995; Hellberg, 1996; Borsa *et al*., 1997; Taylor and Hellberg, 2003).

Another mechanism that may explain genetic differentiation within marine systems is the consequences of historical events. Pleistocene glaciations and sea level changes over the last few million years have had a dramatic influence on the geography of the central Indo-Malay Archipelago, where the Sunda and Sahul shelves emerged as temporary land barriers partly isolating the Indian Ocean from the West Pacific and enclosing the South China Sea, the Sulu Sea, and the Celebes Sea (Voris, 2000). Thus, the repeated lowering of sea levels (as low as 120m below present levels) in this region

drove the geographic isolation of the enclosing seas during the Last Glacial Maximum and earlier glacial cycles, leading to allopatric speciation within marine populations. Numerous studies on various marine fishes and invertebrates have revealed a genetic discontinuity between the Indian and Pacific Oceans, explained by sea-level changes and associated vicariant events (Chenoweth *et al*., 1998; Williams and Benzie, 1998; Planes and Fauvulot, 2002; Bay *et al*., 2004; Lu *et al*., 2006; Menezes *et al*., 2006; Crandall *et al*., 2008), even within the IMA (Barber *et al*., 2002; Lourie *et al*., 2005a; Rohfritsch and Borsa, 2005; Timm *et al*., 2008). One example is the phylogeographic disjunction of barramundi (*Lates calcarifer*) on either side of the Torres Strait (Chenoweth *et al*., 1998), which formed a land barrier connecting Australia and New Guinea during the Pleistocene (Voris, 2000). Admixture of haplotypes from two mtDNA lineages was interpreted as evidence for recent secondary introgression in barramundi (Chenoweth *et al*., 1998). Isolation-by-distance also accounts for genetic divergence in high-dispersal species (Beacham *et al*., 2002).

5.5 Management implications

Many aspects of fisheries management and conservation rely on the accurate identification of populations and/or stocks (Ovenden *et al*., 2013) to maintain accurate records to assist with fisheries management. The advantages of DNA as a data source for species identification have been incorporated into the 'DNA barcoding' approach (e.g. Hebert *et al*., 2003). The accuracy of DNA barcoding depends largely on the validity of reference sequences. Well-established quality assurance processes exist to ensure the accuracy of reference data, such as linking DNA sequences to museum voucher specimens and documenting biological and collection data associated with specimens (Ratnasingham and Hebert, 2007). Meta-analyses of the accuracy of DNA barcoding for numerous taxa have demonstrated it to generally be >90% (e.g. April *et al*., 2011). Therefore, it is important to enhance the effort to document the diversity of

life. There are a few public reference databases that have been developed as a species identification tool for large taxonomic assemblages of animals, representing a quick and easy method for non-specialists to identify disparate specimens: FISH-BOL (www.fishbol.org) (Ward *et al*., 2009), GenBank (www.ncbi.nlm.nih.gov/genbank) and Barcode of Life Data Systems (www.boldsystems.org) (Ratnasingham and Hebert, 2007). The main challenge to the greater use of DNA barcoding in fisheries management, however, is the incompleteness of reference databases. In the current study, I produced an initial reference DNA barcode library for Indo-Malay Carangidae to contribute in developing an integrated taxonomic framework, for subsequently informing management strategies for conservation and management of Carangidae (Chapter 2).

In marine species it is difficult to identify populations and migration among them directly by mark recapture methods using tags, due to the high mortality in their early life stages and the large dispersal distances, limiting successful recapture (Thorrold *et al.,* 2002; Bolle *et al*., 2005). Morphometrics, meristics and life history characteristics have been used successfully for stock identification at a range of different scales (Elliott *et al*., 1995; Cadrin and Friedland, 1999), but are often limited by their possible alteration by environmental variation (Lindsey, 1964; Todd *et al*., 1981). Indirect methods using the techniques of molecular genetics are applied to discriminate among marine species and populations, analyse migration patterns among populations, and to estimate respective effective population sizes (Abaunza *et al*., 2008; Waples *et al*., 2008). Molecular techniques use the variation of distinct alleles at a defined locus, to understand the genetic structure of populations. It is based on the premise that migration and mating patterns among populations will determine the extent to which individuals share a common gene pool, and that a comparison of samples taken from each can be used to estimate their integrity (Carvalho and Hauser, 1998). Thus, where populations exchange few individuals, opportunities for genetic differentiation arising from local adaptation and random genetic change will be high, resulting in a discrete

population structure. Many types of molecular markers are used for this purpose (Park and Moran, 1994; O'connell and Wright, 1997; Parker *et al.,* 1998; Chiang *et al*., 2008; Hauser and Carvalho 2009; Nielsen *et al*., 2011).

A major challenge in implementing genetic data into fisheries management is the mixing of distinct populations of migratory fish species at specific fishing grounds. Many fisheries in SE Asia do not exploit a single population, but a mixture of different populations depending on the time of the year and the fishing area. Therefore, in order to ensure sustainable management, biological processes and management actions must be matched (Reiss *et al*., 2009). The studied species are among the most commercially-important fishes in the IMA. The lack of basic knowledge on these species might suggest that the stocks have no boundaries. Therefore, population decline remains undetected and hence, stocks are not regulated in response to overfishing. For *Selar crumenophthalmus*, the observed genetic homogeneity was interpreted as supporting the view that this species should be managed in the IMA as one stock, though it is always important to confirm such assertions through temporal analysis of samples to assess stability (Waples, 1998). However, even though low levels of genetic differentiation were detected in Chapter 4, the additional samples from Kuala Kedah (KK) and Kuching (KC), included in the study in Chapter 2, suggests a second lineage may be present in this species. However, this result was based only on two individuals, and therefore requires further sampling effort for confirmation of this pattern. For *Atule mate*, the presence of two lineages within the IMA suggests the possible existence of cryptic species, and that this species may therefore be better managed as two different stocks, which may require different conservation strategies (Schonrogge *et al*., 2002). Since a large proportion of the IMA fisheries occur as mixed-stocks, appropriate stock assessment models will be of particular importance, since current models are based mainly on single-species stock assessment, even in areas where mixed stocks are targeted.

Illegal, unreported and unregulated (IUU) fishing is one of the factors in overexploitation that increasingly places fisheries stocks at risk. It is estimated that 3.4 – 8.1 million t of fish is taken by IUU fishing each year in the Asia-Pacific region (Marine Resources Assessment Group and University of British Columbia, 2008). This represents between 8 and 16% of the reported 51 million t of catch from the Pacific Ocean in recent years. A global assessment of IUU fishing found that SE Asia experiences a high level of detected IUU fishing, specifically in the Celebes Sea and East coast peninsular Malaysia (Palma and Tsamenyi, 2008; Poh and Fanning, 2012). In Malaysia, cases of IUU consist of otter trawling, pair trawling, push net, fish bombing and cyanide fishing (Burke *et al*., 2002; Sea Resources Management 2008; Poh and Fanning, 2012). These types of destructive fishing have been regarded as illegal since the Fisheries Act of 1985. However, the Malaysian Department of Fisheries has reported nearly 6000 cases of otter trawling, pair trawling and push net offences in Malaysia from 1990-1999. This region is also facing IUU fishing by foreign vessels from neighbouring countries such as Thailand, Indonesia and Vietnam. In 2007, the head of the Malaysian International Tuna Port has estimated that the annual economic losses from IUU fishing in the Asia-Pacific are over RM15 billion (approximately US$4.5b) (Datuk Annuar Zaini Binyamin, 2007). Therefore, genetic data has great potential for investigating IUU fishing and fish fraud. For example, by using gene-associated markers (e.g. SNPs), individual marine fish can be assigned to a single source, while being excluded from all other candidate regions (Withler *et al*., 2004; Martinsohn and Ogden, 2009; Nielsen *et al*., 2011). However, genetic divergence is often low in marine fishes, which typically exhibit considerable gene flow among populations, requiring a large number of neutral markers or the inclusion of markers under selection (e.g. gene-associated SNPs) to local environmental conditions to distinguish among regions with sufficient power.

5.6 Future work and recommendations

Data from the current study provides the basis for a reference DNA barcoding library for marine fishes from the Indo-Malay Archipelago. These data contribute to the global DNA barcoding effort to document the diversity of life, particularly with regard to conservation and management applications. Data are further presented that support the detection of a few potential cryptic species within the IMA. However, in order to explore the hypotheses of drivers of species diversification in the region, additional studies employing *COI* barcoding, or additional more rapidly-evolving markers, should involve the sampling of the whole family across a broader geographic range. One example, a study by Zemlak *et al*. (2009), used *COI* to examine patterns of divergences between fish species representing different lifestyles from opposite sides of the Indian Ocean. They detected deep divergences between certain inshore taxa, with the inshore taxa (mean *COI* divergence =0.51%) exhibiting significantly higher levels of putative cryptic species than the offshore (mean *COI* divergence= 0.26%) taxa. Such deep divergences were more representative of patterns in congeneric species than among populations of a single species, highlighting the possible genetic isolation of presumed cosmopolitan species. Such findings reinforce the need for such *COI* barcoding studies to sample throughout the extremes of the geographic range to investigate the extent of hidden diversity in marine fauna.

Genetic approaches to detect stock structure will continue to be developed for fisheries management and conservation. Some additional changes in this field are necessary, involving increases in analytical power by increasing sample sizes and numbers of DNA markers (Waples and Naish, 2009). New types of genetic markers (e.g. SNP) and the inclusion of markers under directional selection have the potential to increase the ability to discriminate between component stocks and hence to increase the number of species that can be analysed as mixed stocks (Martinsohn and Ogden, 2009; Habicht *et al*., 2010; Nielsen *et al*., 2012; Kruck *et al*., 2013). Analyses are

increasingly likely to rely on models of population structure focusing on the behaviour of individuals on ecological time frames rather than on the long-term average behaviours of entire populations (Christie *et al*., 2010; Harrison *et al.,* 2012). Analyses to identify the number of discrete genetic stocks and map their distributions (Pritchard *et al*., 2000; Guillot *et al*., 2005) will be most successful when individuals are sampled evenly throughout their geographic range. This approach lends itself well to combining genetic information with geographical, oceanographic or other environmental information to increase the explanatory power of the analysis (Fontaine *et al.,* 2007; Galarza *et al*., 2009). The lack of population structuring detected among the three target species of Carangidae suggested additional multidisciplinary approaches should be used together with temporal genetic data (Purcell and Edmands, 2011; Saenz-Agudelo *et al*., 2012; Ruggeri *et al*., 2012; McCairns *et al*., 2012; Ciannelli *et al*., 2013; Varela *et al*., 2013; Therkildsen *et al*., 2013; Kovach *et al*., 2013) to tease apart any genetic subdivision, or alternatively, that these taxa are indeed panmictic.

The data and interpretation presented in this thesis provide the first steps in better understanding the genetic make-up of commercially exploited Carangidae in the IMA. It is now dependent upon fisheries managers to decide the ways in which genetic data can improve fisheries management and conservation. Moreover, Governments and funding agencies are required to provide increased resources necessary to protect fisheries for future generations. Improved communication between fisheries managers and geneticists (e.g. Ovenden *et al*., 2013), as well as other scientists, is a fundamental requirement to further enhance such integration of data and approaches. Such communication needs to focus on the nature and scope of key questions to be addressed by managers and policy makers, and the matching of questions to cost-effective and robust genetic tools that can be readily deployed.

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APPENDICES

Appendix 1

Specimen data and GenBank accession numbers used in this study.

Voucher number/ Museum ID	Locality	Year of collection	Species	GenBank accession number	BOLD sample ID
UMTF03430	Kuantan, Peninsular Malaysia (KN)	2010	*Alectis ciliaris*	JX261576	DBMF-M182
AHM12-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Alectis ciliaris*	HQ560980	DBMF-M47
UMTF03977	Tawau, Sabah (TW)	2010	*Alectis ciliaris*	JX261130	DBMF-M729
UMTF03429	Kuantan, Peninsular Malaysia (KN)	2010	*Alectis ciliaris*	JX261128	DBMF-M181
UMTF03976	Tawau, Sabah (TW)	2010	*Alectis ciliaris*	JX261420	DBMF-M728
UMTF03736	Miri, Sarawak (MR)	2010	*Alectis ciliaris*	JX261045	DBMF-M488
UMTF03978	Tawau, Sabah (TW)	2010	*Alectis ciliaris*	JX261555	DBMF-M730
UMTF03866	Sandakan, Sabah (SDK)	2010	*Alectis ciliaris*	JX261543	DBMF-M618
NPPF1084	Nayband National Park Coast, Iran	2009	*Alectis ciliaris*	HQ149787	NPPF1084
ITHJ1	Nagasaki, Japan	2005	*Alectis ciliaris*	JF952663	ABFJ200-07.COI-5P
ITHJ2	Nagasaki, Japan	2005	*Alectis ciliaris*	JF952664	ABFJ201-07.COI-5P
BW-A1472	Queensland, Australia	1998	*Alectis ciliaris*	EF609280	FOAC473-05
N/A	India	2006	*Alectis ciliaris*	EU514500	N/A
KK09-01	Kuala Kedah, Peninsular Malaysia (KK)	2009	*Alectis indicus*	HQ560959	DBMF-M19
UMTF03975	Tawau, Sabah (TW)	2010	*Alectis indicus*	JX261640	DBMF-M727
UMTF03806	Kudat, Sabah (KDT)	2010	*Alectis indicus*	JX261288	DBMF-M560
UMTF03733	Miri, Sarawak (MR)	2010	*Alectis indicus*	JX261127	DBMF-M485
AHM10-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Alectis indicus*	HQ560978	DBMF-M45
UMTF03734	Miri, Sarawak (MR)	2010	*Alectis indicus*	JX261178	DBMF-M486
UMTF03735	Miri, Sarawak (MR)	2010	*Alectis indicus*	JX261217	DBMF-M487
UMTF03974	Tawau, Sabah (TW)	2010	*Alectis indicus*	JX261340	DBMF-M726

UMTF03807	Kudat, Sabah (KDT)	2010	*Alectis indicus*	JX261350	DBMF-M561
SK02-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Alectis indicus*	HQ560997	DBMF-M68
NPPF1062	Nayband National Park Coast, Iran	2009	*Alectis indicus*	HQ149788	NPPF1062
NPPF1046	Nayband National Park Coast, Iran	2009	*Alectis indicus*	HQ149789	NPPF1046
NPPF1012	Nayband National Park Coast, Iran	2009	*Alectis indicus*	HQ149790	NPPF1012
UMTF03607	Mukah, Sarawak (MKS)	2010	*Alepes djedaba*	JX261639	DBMF-M359
PN01-01	Pontian, Peninsular Malaysia (PN)	2009	*Alepes djedaba*	HQ561009	DBMF-M81
UMTF03605	Mukah, Sarawak (MKS)	2010	*Alepes djedaba*	JX261253	DBMF-M357
UMTF03822	Kudat, Sabah (KDT)	2010	*Alepes djedaba*	JX261582	DBMF-M574
UMTF03823	Kudat, Sabah (KDT)	2010	*Alepes djedaba*	JX261382	DBMF-M575
UMTF03824	Kudat, Sabah (KDT)	2010	*Alepes djedaba*	JX261385	DBMF-M576
UMTF04091	Kuala Perlis, Peninsular Malaysia (KP)		*Alepes djedaba*	JX261610	DBMF-M843
UMTF04090	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes djedaba*	JX261246	DBMF-M842
UMTF03826	Kudat, Sabah (KDT)	2010	*Alepes djedaba*	JX261567	DBMF-M578
UMTF04089	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes djedaba*	JX261428	DBMF-M841
UMTF03606	Mukah, Sarawak (MKS)	2010	*Alepes djedaba*	JX261206	DBMF-M358
SK04-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Alepes djedaba*	HQ560999	DBMF-M70
BP10-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Alepes djedaba*	HQ560965	DBMF-M29
HM04-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Alepes djedaba*	HQ560972	DBMF-M39
UMTF04092	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes djedaba*	JX261018	DBMF-M844
UMTF03943	Tawau, Sabah (TW)	2010	*Alepes djedaba*	JX261550	DBMF-M695
UMTF03942	Tawau, Sabah (TW)	2010	*Alepes djedaba*	JX261148	DBMF-M694
UMTF03941	Tawau, Sabah (TW)	2010	*Alepes djedaba*	JX261607	DBMF-M693
UMTF03703	Miri, Sarawak (MR)	2010	*Alepes djedaba*	JX261642	DBMF-M455
UMTF03704	Miri, Sarawak (MR)	2010	*Alepes djedaba*	JX261023	DBMF-M456
UMTF03705	Miri, Sarawak (MR)	2010	*Alepes djedaba*	JX261067	DBMF-M457
UMTF03707	Miri, Sarawak (MR)	2010	*Alepes djedaba*	JX261500	DBMF-M459
UMTF03669	Kuching, Sarawak (KC)	2010	*Alepes djedaba*	JX261362	DBMF-M421
UMTF03609	Mukah, Sarawak (MKS)	2010	*Alepes djedaba*	JX261351	DBMF-M361

UMTF03668	Kuching, Sarawak (KC)	2010	*Alepes djedaba*	JX261029	DBMF-M420
UMTF03608	Mukah, Sarawak (MKS)	2010	*Alepes djedaba*	JX261156	DBMF-M360
UMTF03667	Kuching, Sarawak (KC)	2010	*Alepes djedaba*	JX261293	DBMF-M419
UMTF03944	Tawau, Sabah (TW)	2010	*Alepes djedaba*	JX261588	DBMF-M696
UMTF03945	Tawau, Sabah (TW)	2010	*Alepes djedaba*	JX261122	DBMF-M697
UMTF03666	Kuching, Sarawak (KC)	2010	*Alepes djedaba*	JX261077	DBMF-M418
ADC210.3-2	Tugela Banks, KwaZulu-Natal, South Africa	2004	*Alepes djedaba*	JF492804	TZMSB089-04.COI-5P
ADC210.3-1	Tugela Banks, KwaZulu-Natal, South Africa	2003	*Alepes djedaba*	JF492805	TZMSB088-04.COI-5P
ADC210.3-1	Tugela Banks, KwaZulu-Natal, South Africa	2003	*Alepes djedaba*	JF492806	TZMSA390-04.COI-5P
WL-M36	Maharashtra, India	2006	*Alepes djedaba*	EF609497	WLIND036-07
WL-M35	Maharashtra, India	2006	*Alepes djedaba*	EF609498	WLIND035-07
WL-M34	Maharashtra, India	2006	*Alepes djedaba*	EF609499	WLIND034-07
WL-M33	Maharashtra, India	2006	*Alepes djedaba*	EF609500	WLIND033-07
WL-M32	Maharashtra, India	2006	*Alepes djedaba*	EF609501	WLIND032-07
UMTF03819	Kudat, Sabah (KDT)	2010	*Alepes kleinii*	JX261594	DBMF-M571
UMTF04093	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261530	DBMF-M845
UMTF04094	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261344	DBMF-M846
UMTF03820	Kudat, Sabah (KDT)	2010	*Alepes kleinii*	JX261396	DBMF-M572
UMTF03818	Kudat, Sabah (KDT)	2010	*Alepes kleinii*	JX261049	DBMF-M570
UMTF03817	Kudat, Sabah (KDT)	2010	*Alepes kleinii*	JX261103	DBMF-M569
UMTF03821	Kudat, Sabah (KDT)	2010	*Alepes kleinii*	JX261284	DBMF-M573
UMTF04095	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261076	DBMF-M847
UMTF04097	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261039	DBMF-M849
UMTF04098	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261090	DBMF-M850
UMTF04096	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Alepes kleinii*	JX261086	DBMF-M848
UMTF03772	Kudat, Sabah (KDT)	2010	*Alepes melanoptera*	JX261407	DBMF-M524
KK06-01	Kota Kinabalu, Sabah (KKJ)	2009	*Alepes melanoptera*	HQ560956	DBMF-M16
BP09-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Alepes melanoptera*	HQ560964	DBMF-M28
HM07-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Alepes melanoptera*	HQ560975	DBMF-M42

KSB01-01	Kuala Sungai Besar, Peninsular Malaysia (KSB)	2009	*Alepes melanoptera*	HQ560986	DBMF-M53
SK05-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Alepes melanoptera*	HQ561000	DBMF-M71
PN02-01	Pontian, Peninsular Malaysia (PN)	2009	*Alepes melanoptera*	HQ561010	DBMF-M82
UMTF03770	Kudat, Sabah (KDT)	2010	*Alepes melanoptera*	JX261624	DBMF-M522
UMTF03771	Kudat, Sabah (KDT)	2010	*Alepes melanoptera*	JX261457	DBMF-M523
UMTF03956	Tawau, Sabah (TW)	2010	*Alepes melanoptera*	JX261561	DBMF-M708
UMTF03957	Tawau, Sabah (TW)	2010	*Alepes melanoptera*	JX261267	DBMF-M709
UMTF03959	Tawau, Sabah (TW)	2010	*Alepes melanoptera*	JX261647	DBMF-M711
UMTF03960	Tawau, Sabah (TW)	2010	*Alepes melanoptera*	JX261161	DBMF-M712
UMTF04015	Kota Kinabalu, Sabah (KKJ)	2010	*Alepes melanoptera*	JX261047	DBMF-M767
UMTF04017	Kota Kinabalu, Sabah (KKJ)	2010	*Alepes melanoptera*	JX261188	DBMF-M769
UMTF03887	Sandakan, Sabah (SDK)	2010	*Alepes vari*	JX261282	DBMF-M639
UMTF03825	Kudat, Sabah (KDT)	2010	*Alepes vari*	JX261228	DBMF-M577
UMTF03531	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Alepes vari*	JX261520	DBMF-M283
UMTF03532	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Alepes vari*	JX261010	DBMF-M284
UMTF03534	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Alepes vari*	JX261494	DBMF-M286
UMTF03700	Miri, Sarawak (MR)	2010	*Alepes vari*	JX261192	DBMF-M452
UMTF03886	Sandakan, Sabah (SDK)	2010	*Alepes vari*	JX261379	DBMF-M638
UMTF03699	Miri, Sarawak (MR)	2010	*Alepes vari*	JX261475	DBMF-M451
UMTF03883	Sandakan, Sabah (SDK)	2010	*Alepes vari*	JX261234	DBMF-M635
UMTF03702	Miri, Sabah (MR)	2010	*Alepes vari*	JX261434	DBMF-M454
KP02-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Alepes vari*	HQ560946	DBMF-M2
UMTF03701	Miri, Sarawak (MR)	2010	*Alepes vari*	JX261644	DBMF-M453
UMTF03949	Tawau, Sabah (TW)	2010	*Atropus atropos*	JX261095	DBMF-M701
UMTF03948	Tawau, Sabah (TW)	2010	*Atropus atropos*	JX261368	DBMF-M700
UMTF03946	Tawau, Sabah (TW)	2010	*Atropus atropos*	JX261411	DBMF-M698
UMTF03632	Mukah, Sarawak (MKS)	2010	*Atropus atropos*	JX261181	DBMF-M384
UMTF03631	Mukah, Sarawak (MKS)	2010	*Atropus atropos*	JX261436	DBMF-M383
UMTF03630	Mukah, Sarawak (MKS)	2010	*Atropus atropos*	JX261352	DBMF-M382

UMTF03629	Mukah, Sarawak (MKS)	2010	*Atropus atropos*	JX261490	DBMF-M381
UMTF03947	Tawau, Sabah (TW)	2010	*Atropus atropos*	JX261357	DBMF-M699
KSB05-01	Kuala Sg. Besar, Peninsular Malaysia (KSB)	2009	*Atropus atropos*	HQ560989	DBMF-M57
SK03-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Atropus atropos*	HQ560998	DBMF-M69
UMTF03950	Tawau, Sabah (TW)	2010	*Atropus atropos*	JX261172	DBMF-M702
UMTF03633	Mukah, Sarawak (MKS)	2010	*Atropus atropos*	JX261287	DBMF-M385
HM05-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Atropus atropos*	HQ560973	DBMF-M40
WL-M24	Maharashtra, India	2006	*Atropus atropos*	EF609502	WLIND024-07
WL-M23	Maharashtra, India	2006	*Atropus atropos*	EF609503	WLIND023-07
WL-M22	Maharashtra, India	2006	*Atropus atropos*	EF609504	WLIND022-07
WL-M21	Maharashtra, India	2006	*Atropus atropos*	EF609505	WLIND021-07
WL-M20	Maharashtra, India	2006	*Atropus atropos*	EF609506	WLIND020-07
UMTF03518	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Atule mate*	JX261063	DBMF-M270
UMTF03610	Mukah, Sarawak (MKS)	2010	*Atule mate*	JX261194	DBMF-M362
UMTF03611	Mukah, Sarawak (MKS	2010	*Atule mate*	JX261349	DBMF-M363
UMTF03612	Mukah, Sarawak (MKS)	2010	*Atule mate*	JX261612	DBMF-M364
UMTF03613	Mukah, Sarawak (MKS)	2010	*Atule mate*	JX261429	DBMF-M365
UMTF03614	Mukah, Sarawak (MKS)	2010	*Atule mate*	JX261546	DBMF-M366
UMTF03763	Kudat, Sabah (KDT)	2010	*Atule mate*	JX261535	DBMF-M515
UMTF03762	Kudat, Sabah (KDT)	2010	*Atule mate*	JX261507	DBMF-M514
UMTF03761	Kudat, Sabah (KDT)	2010	*Atule mate*	JX261484	DBMF-M513
UMTF03760	Kudat, Sabah (KDT)	2010	*Atule mate*	JX261056	DBMF-M512
HM08-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Atule mate*	HQ560976	DBMF-M43
KSB07-01	Kuala Sungai Besar, Peninsular Malaysia (KSB)	2009	*Atule mate*	HQ560990	DBMF-M59
SB02-01	Kuala Sungai Baru, Peninsular Malaysia (SB)	2009	*Atule mate*	HQ561006	DBMF-M78
PN05-01	Pontian, Peninsular Malaysia (PN)	2009	*Atule mate*	HQ561013	DBMF-M85
T02-01	Tumpat, Peninsular Malaysia (T)	2009	*Atule mate*	HQ561014	DBMF-M86

KBT06-01	Kuala Besar, Peninsular Malaysia (KBT)	2009	*Atule mate*	HQ561015	DBMF-M87
TB05-01	Tok Bali, Peninsular Malaysia (TB)	2009	*Atule mate*	HQ561016	DBMF-M88
KD03-01	Kuala Dungun , Peninsular Malaysia (KD)	2009	*Atule mate*	HQ561017	DBMF-M90
KN04-01	Kuantan, Peninsular Malaysia (KN)	2009	*Atule mate*	HQ561018	DBMF-M91
KPG06-01	Kuala Pahang, Peninsular Malaysia (KPG)	2009	*Atule mate*	HQ561019	DBMF-M92
TG06-01	Tanjung Gemuk, Peninsular Malaysia (TG)	2009	*Atule mate*	HQ561020	DBMF-M93
MG06-01	Mersing, Peninsular Malaysia (MG)	2009	*Atule mate*	HQ561021	DBMF-M94
TS05-01	Tanjung Sedili, Peninsular Malaysia (TS)	2009	*Atule mate*	HQ561022	DBMF-M95
UMTF04063	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Atule mate*	JX261597	DBMF-M815
UMTF04062	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Atule mate*	JX261075	DBMF-M814
UMTF04061	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Atule mate*	JX261226	DBMF-M813
UMTF04060	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Atule mate*	JX261474	DBMF-M812
UMTF04059	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Atule mate*	JX261289	DBMF-M811
UMTF03407	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Atule mate*	JX261378	DBMF-M159
UMTF03408	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Atule mate*	JX261302	DBMF-M160
UMTF03409	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Atule mate*	JX261254	DBMF-M161
UMTF03431	Kuantan, Peninsular Malaysia (KN)	2010	*Atule mate*	JX261512	DBMF-M183
UMTF03411	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Atule mate*	JX261413	DBMF-M163
UMTF03432	Kuantan, Peninsular Malaysia (KN)	2010	*Atule mate*	JX261370	DBMF-M184
UMTF03433	Kuantan, Peninsular Malaysia (KN)	2010	*Atule mate*	JX261012	DBMF-M185
UMTF03434	Kuantan, Peninsular Malaysia (KN)	2010	*Atule mate*	JX261405	DBMF-M186
UMTF03435	Kuantan, Peninsular Malaysia (KN)	2010	*Atule mate*	JX261233	DBMF-M187
KP08-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Atule mate*	HQ560949	DBMF-M8
UMTF03994	Kota Kinabalu, Sabah (KKJ)	2010	*Atule mate*	JX261261	DBMF-M746
UMTF03683	Miri, Sarawak (MR)	2010	*Atule mate*	JX261446	DBMF-M435
UMTF03682	Miri, Sarawak (MR)	2010	*Atule mate*	JX261548	DBMF-M434
UMTF03681	Miri, Sarawak (MR)	2010	*Atule mate*	JX261222	DBMF-M433
UMTF03680	Miri, Sarawak (MR)	2010	*Atule mate*	JX261094	DBMF-M432
UMTF03679	Miri, Sarawak (MR)	2010	*Atule mate*	JX261035	DBMF-M431
UMTF03456	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Atule mate*	JX261533	DBMF-M208

UMTF03457	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Atule mate*	JX261249	DBMF-M209
UMTF03458	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Atule mate*	JX261545	DBMF-M210
UMTF03459	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Atule mate*	JX261437	DBMF-M211
UMTF03993	Kota Kinabalu, Sabah (KKJ)	2010	*Atule mate*	JX261501	DBMF-M745
UMTF03992	Kota Kinabalu, Sabah (KKJ)	2010	*Atule mate*	JX261410	DBMF-M744
UMTF03991	Kota Kinabalu, Sabah (KKJ)	2010	*Atule mate*	JX261200	DBMF-M743
UMTF03990	Kota Kinabalu, Sabah (KKJ)	2010	*Atule mate*	JX261614	DBMF-M742
UMTF03867	Sandakan, Sabah (SDK)	2010	*Atule mate*	JX261412	DBMF-M619
UMTF03764	Kudat, Sabah (KDT)	2010	*Atule mate*	JX261401	DBMF-M516
UMTF03515	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Atule mate*	JX261635	DBMF-M267
UMTF03516	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Atule mate*	JX261280	DBMF-M268
UMTF03517	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Atule mate*	JX261578	DBMF-M269
UMTF03519	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Atule mate*	JX261633	DBMF-M271
KK04-01	Kuala Kedah, Peninsular Malaysia (KK)	2009	*Atule mate*	HQ560955	DBMF-M14
BP08-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Atule mate*	HQ560963	DBMF-M27
UMTF03655	Kuching, Sarawak (KC)	2010	*Atule mate*	JX261531	DBMF-M407
UMTF03654	Kuching, Sarawak (KC)	2010	*Atule mate*	JX261445	DBMF-M406
UMTF03653	Kuching, Sarawak (KC)	2010	*Atule mate*	JX261218	DBMF-M405
UMTF03652	Kuching, Sarawak (KC)	2010	*Atule mate*	JX261374	DBMF-M404
UMTF03570	Tok Bali, Peninsular Malaysia (TB)	2010	*Atule mate*	JX261505	DBMF-M322
UMTF03571	Tok Bali, Peninsular Malaysia (TB)	2010	*Atule mate*	JX261419	DBMF-M323
UMTF03574	Tok Bali, Peninsular Malaysia (TB)	2010	*Atule mate*	JX261557	DBMF-M326
MBCSC:Z711217	China: South China Sea	2007	*Atule mate*	EU595060	FSCS551-07
MBCSC:Z711007	China: South China Sea	2007	*Atule mate*	EU595063	FSCS337-07
MBCSC:Z711003	China: South China Sea	2007	*Atule mate*	EU595067	FSCS333-07
MBCSC:Z711004	China: South China Sea	2007	*Atule mate*	EU595066	FSCS334-07
MBCSC:Z711005	China: South China Sea	2007	*Atule mate*	EU595065	FSCS335-07
MBCSC:Z711006	China: South China Sea	2007	*Atule mate*	EU595064	FSCS336-07
MBCSC:Z711008	China: South China Sea	2007	*Atule mate*	EU595062	FSCS338-07
MBCSC:Z711219	China: South China Sea	2007	*Atule mate*	EU595058	FSCS553-07

MBCSC:Z711216	China: South China Sea	2007	*Atule mate*	EU595061	FSCS550-07
MBCSC:Z711218	China: South China Sea	2007	*Atule mate*	EU595059	FSCS552-07
GD 9083018	China	2006	*Atule mate*	EF607335	FSCS257-06
BW-A1465	Queensland, Australia	1998	*Atule mate*	EF609293	FOAC466-05
NPPF1180	Nayband National Park Coast, Iran	2009	*Atule mate*	HQ149797	NPPF1180
NPPF1073	Nayband National Park Coast, Iran	2009	*Atule mate*	HQ149798	NPPF1073
NPPF1072	Nayband National Park Coast, Iran	2009	*Atule mate*	HQ149799	NPPF1072
NPPF1071	Nayband National Park Coast, Iran	2009	*Atule mate*	HQ149800	NPPF1071
NPPF1038	Nayband National Park Coast, Iran	2009	*Atule mate*	HQ149801	NPPF1038
MBCSC:ZC I07339	China: South China Sea	2007	*Atule mate*	FJ237967	FSCS764-08
MBCSC:ZC I07332	China: South China Sea	2007	*Atule mate*	FJ237968	FSCS757-08
MBCSC:ZC I07329	China: South China Sea	2007	*Atule mate*	FJ237969	FSCS754-08
UMTF03420	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides bajad*	JX261394	DBMF-M172
UMTF03585	Tok Bali, Peninsular Malaysia (TB)	2010	*Carangoides bajad*	JX261219	DBMF-M337
UMTF03586	Tok Bali, Peninsular Malaysia (TB)	2010	*Carangoides bajad*	JX261131	DBMF-M338
UMTF03419	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides bajad*	JX261171	DBMF-M171
UMTF04011	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides bajad*	JX261098	DBMF-M763
UMTF04012	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides bajad*	JX261124	DBMF-M764
UMTF04013	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides bajad*	JX261109	DBMF-M765
UMTF03713	Miri, Sarawak (MR)	2010	*Carangoides bajad*	JX261526	DBMF-M465
UMTF03714	Miri, Sarawak (MR)	2010	*Carangoides bajad*	JX261416	DBMF-M466
UMTF03715	Miri, Sarawak (MR)	2010	*Carangoides bajad*	JX261021	DBMF-M467
UMTF03775	Kudat, Sabah (KDT)	2010	*Carangoides bajad*	JX261641	DBMF-M527
UMTF03777	Kudat, Sabah (KDT)	2010	*Carangoides bajad*	JX261263	DBMF-M529
UMTF03776	Kudat, Sabah (KDT)	2010	*Carangoides bajad*	JX261510	DBMF-M528
UMTF03677	Kuching, Sarawak (KC)	2010	*Carangoides bajad*	JX261273	DBMF-M429
UMTF04010	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides bajad*	JX261159	DBMF-M762
UMTF03587	Tok Bali, Peninsular Malaysia (TB)	2010	*Carangoides bajad*	JX261136	DBMF-M339
UMTF03588	Tok Bali, Peninsular Malaysia (TB)	2010	*Carangoides bajad*	JX261424	DBMF-M340
UMTF03589	Tok Bali, Peninsular Malaysia (TB)	2010	*Carangoides bajad*	JX261108	DBMF-M341

UMTF03779	Kudat, Sabah (KDT)	2010	*Carangoides bajad*	JX261266	DBMF-M531
UMTF03778	Kudat, Sabah (KDT)	2010	*Carangoides bajad*	JX261345	DBMF-M530
UMTF03717	Miri, Sarawak (MR)	2010	*Carangoides bajad*	JX261144	DBMF-M469
UMTF03423	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides bajad*	JX261460	DBMF-M175
UMTF03422	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides bajad*	JX261225	DBMF-M174
UMTF03828	Sandakan, Sabah (SDK)	2010	*Carangoides bajad*	JX261489	DBMF-M580
UMTF04014	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides bajad*	JX261593	DBMF-M766
UMTF03676	Kuching, Sarawak (KC)	2010	*Carangoides bajad*	JX261276	DBMF-M428
UMTF04047	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides chrysophrys*	JX261153	DBMF-M799
UMTF03636	Mukah, Sarawak (MKS)	2010	*Carangoides chrysophrys*	JX261573	DBMF-M388
KK07-01	Kuala Kedah, Peninsular Malaysia (KK)	2009	*Carangoides chrysophrys*	HQ560957	DBMF-M17
UMTF04044	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides chrysophrys*	JX261517	DBMF-M796
UMTF04045	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides chrysophrys*	JX261622	DBMF-M797
UMTF04046	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides chrysophrys*	JX261118	DBMF-M798
UMTF04048	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides chrysophrys*	JX261560	DBMF-M800
UMTF03635	Mukah, Sarawak (MKS)	2010	*Carangoides chrysophrys*	JX261146	DBMF-M387
UMTF03841	Sandakan, Sabah (SDK)	2010	*Carangoides chrysophrys*	JX261229	DBMF-M593
UMTF03840	Sandakan, Sabah (SDK)	2010	*Carangoides chrysophrys*	JX261142	DBMF-M592
UMTF03839	Sandakan, Sabah (SDK)	2010	*Carangoides chrysophrys*	JX261024	DBMF-M591
UMTF03838	Sandakan, Sabah (SDK)	2010	*Carangoides chrysophrys*	JX261608	DBMF-M590
UMTF03837	Sandakan, Sabah (SDK)	2010	*Carangoides chrysophrys*	JX261575	DBMF-M589
UMTF03634	Mukah, Sarawak (MKS)	2010	*Carangoides chrysophrys*	JX261158	DBMF-M386
UMTF03802	Kudat, Sabah (KDT)	2010	*Carangoides chrysophrys*	JX261570	DBMF-M554
UMTF03637	Mukah, Sarawak (MKS)	2010	*Carangoides chrysophrys*	JX261341	DBMF-M389
UMTF03966	Tawau, Sabah (TW)	2010	*Carangoides chrysophrys*	JX261034	DBMF-M718
UMTF03801	Kudat, Sabah (KDT)	2010	*Carangoides chrysophrys*	JX261595	DBMF-M553
UMTF03800	Kudat, Sabah (KDT)	2010	*Carangoides chrysophrys*	JX261399	DBMF-M552
UMTF03972	Tawau, Sabah (TW)	2010	*Carangoides dinema*	JX261552	DBMF-M724
UMTF03971	Tawau, Sabah (TW)	2010	*Carangoides dinema*	JX261041	DBMF-M723
UMTF03903	Semporna, Sabah (SMP)	2010	*Carangoides dinema*	JX261235	DBMF-M655

UMTF03902	Semporna, Sabah (SMP)	2010	*Carangoides dinema*	JX261508	DBMF-M654
UMTF03904	Semporna, Sabah (SMP)	2010	*Carangoides dinema*	JX261007	DBMF-M656
UMTF03889	Sandakan, Sabah (SDK)	2010	*Carangoides dinema*	JX261328	DBMF-M641
AHM14-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Carangoides ferdau*	HQ560982	DBMF-M49
BP01-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Carangoides ferdau*	HQ560960	DBMF-M20
MBIO1835.4	French Polynesia: Society Islands, Moorea	2006	*Carangoides ferdau*	JQ431538	MBFB018-07.COI-5P
UMTF03493	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides fulvoguttatus*	JX261364	DBMF-M245
UMTF03492	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides fulvoguttatus*	JX261174	DBMF-M244
UMTF03491	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides fulvoguttatus*	JX261084	DBMF-M243
BW-A1474	Queensland, Australia	1998	*Carangoides fulvoguttatus*	EF609302	FOAC475-05
ADC08 Smith 210.11 #6	Mozambique: Pomene	2008	*Carangoides fulvoguttatus*	JF493025	DSFSE764-08.COI-5P
ADC08 Smith 210.11 #5	Mozambique: Pomene	2008	*Carangoides fulvoguttatus*	JF493026	DSFSE765-08.COI-5P
ADC08 Smith 210.11 #2	Mozambique: Pomene	2008	*Carangoides fulvoguttatus*	JF493027	DSFSE783-08.COI-5P
ADC08 Smith 210.11 #3	Mozambique: Pomene	2008	*Carangoides fulvoguttatus*	JF493028	DSFSE782-08.COI-5P
ADC08 Smith 210.11 #4	Mozambique: Pomene	2008	*Carangoides fulvoguttatus*	JF493029	DSFSE781-08.COI-5P
BP03-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Carangoides gymnostethus*	HQ560962	DBMF-M22
UMTF03369	Mersing, Peninsular Malaysia (MG)	2010	*Carangoides hedlandensis*	JX261275	DBMF-M121
UMTF03370	Mersing, Peninsular Malaysia (MG)	2010	*Carangoides hedlandensis*	JX261305	DBMF-M122
UMTF03371	Mersing, Peninsular Malaysia (MG)	2010	*Carangoides hedlandensis*	JX261372	DBMF-M123
UMTF03845	Sandakan, Sabah (SDK)	2010	*Carangoides malabaricus*	JX261632	DBMF-M597
UMTF04041	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides malabaricus*	JX261329	DBMF-M793
UMTF03788	Kudat, Sabah (KDT)	2010	*Carangoides malabaricus*	JX261061	DBMF-M540
UMTF03786	Kudat, Sabah (KDT)	2010	*Carangoides malabaricus*	JX261092	DBMF-M538

UMTF03969	Tawau, Sabah (TW)	2010	*Carangoides malabaricus*	JX261190	DBMF-M721
UMTF03486	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides malabaricus*	JX261643	DBMF-M238
UMTF03487	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides malabaricus*	JX261011	DBMF-M239
UMTF03488	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides malabaricus*	JX261637	DBMF-M240
UMTF03489	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides malabaricus*	JX261618	DBMF-M241
UMTF03490	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Carangoides malabaricus*	JX261111	DBMF-M242
UMTF04043	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides malabaricus*	JX261046	DBMF-M795
UMTF03842	Sandakan, Sabah (SDK)	2010	*Carangoides malabaricus*	JX261369	DBMF-M594
UMTF03785	Kudat, Sabah (KDT)	2010	*Carangoides malabaricus*	JX261346	DBMF-M537
AHM13-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Carangoides malabaricus*	HQ560981	DBMF-M48
UMTF03550	Kuala Besut, Peninsular Malaysia (KB)	2010	*Carangoides malabaricus*	JX261409	DBMF-M302
UMTF03553	Kuala Besut, Peninsular Malaysia (KB)	2010	*Carangoides malabaricus*	JX261471	DBMF-M305
UMTF03554	Kuala Besut, Peninsular Malaysia (KB)	2010	*Carangoides malabaricus*	JX261581	DBMF-M306
UMTF03647	Mukah, Sarawak (MKS)	2010	*Carangoides malabaricus*	JX261290	DBMF-M399
UMTF03646	Mukah, Sarawak (MKS)	2010	*Carangoides malabaricus*	JX261551	DBMF-M398
UMTF03708	Miri, Sarawak (MR)	2010	*Carangoides malabaricus*	JX261406	DBMF-M460
UMTF03710	Miri, Sarawak (MR)	2010	*Carangoides malabaricus*	JX260999	DBMF-M462
UMTF03711	Miri, Sarawak (MR)	2010	*Carangoides malabaricus*	JX261135	DBMF-M463
UMTF03712	Miri, Sarawak (MR)	2010	*Carangoides malabaricus*	JX261600	DBMF-M464
UMTF03424	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides malabaricus*	JX261270	DBMF-M176
UMTF03425	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides malabaricus*	JX261149	DBMF-M177
UMTF03426	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides malabaricus*	JX261155	DBMF-M178
UMTF03427	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides malabaricus*	JX261113	DBMF-M179
UMTF03428	Kuantan, Peninsular Malaysia (KN)	2010	*Carangoides malabaricus*	JX261173	DBMF-M180
UMTF03843	Sandakan, Sabah (SDK)	2010	*Carangoides malabaricus*	JX261133	DBMF-M595
UMTF04040	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides malabaricus*	JX261469	DBMF-M792
UMTF03844	Sandakan, Sabah (SDK)	2010	*Carangoides malabaricus*	JX261473	DBMF-M596
UMTF03846	Sandakan, Sabah (SDK)	2010	*Carangoides malabaricus*	JX261466	DBMF-M598
UMTF 04039	Kota Kinabalu, Sabah (KKJ)	2010	*Carangoides malabaricus*	JX261004	DBMF-M791

UMTF03953	Tawau, Sabah (TW)	2010	*Caranx ignobilis*	JX261044	DBMF-M705
UMTF03812	Kudat, Sabah (KDT)	2010	*Caranx ignobilis*	JX261366	DBMF-M566
UMTF03810	Kudat, Sabah (KDT)	2010	*Caranx ignobilis*	JX261065	DBMF-M564
UMTF03811	Kudat, Sabah (KDT)	2010	*Caranx ignobilis*	JX261360	DBMF-M565
UMTF03809	Kudat, Sabah (KDT)	2010	*Caranx ignobilis*	JX261433	DBMF-M563
UMTF03952	Tawau, Sabah (TW)	2010	*Caranx ignobilis*	JX261496	DBMF-M704
Cign5	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx ignobilis*	HQ654676	BTL066-10.COI-5P
Cign4	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx ignobilis*	HQ654677	BTL065-10.COI-5P
Cign3	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx ignobilis*	HQ654678	BTL064-10.COI-5P
Cign2	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx ignobilis*	HQ654679	BTL063-10.COI-5P
Cign1	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx ignobilis*	HQ654680	BTL062-10.COI-5P
ADC 210.17-1	South Africa: Kwazulu Natal	2004	*Caranx ignobilis*	DQ884975	TZMSB094-04
ADC 210.17-3	South Africa: Kwazulu Natal	2004	*Caranx ignobilis*	DQ884976	TZMSC059-05
ADC 210.17-4	South Africa: Kwazulu Natal	2004	*Caranx ignobilis*	DQ884977	TZMSC060-05
BIOUG<CAN>:BW-A1440	Australia: Queensland	1998	*Caranx ignobilis*	DQ885071	FOAC441-05
BIOUG<CAN>:BW-A1436	Australia: Queensland	1998	*Caranx ignobilis*	DQ885072	FOAC437-05
BIOUG<CAN>:BW-A1437	Australia: Queensland	1998	*Caranx ignobilis*	DQ885073	FOAC438-05
BIOUG<CAN>:BW-A1438	Australia: Queensland	1998	*Caranx ignobilis*	DQ885074	FOAC439-05
BPBM 39590; PCMB B415	USA: Hawaii, Oahu, off Kahuku	2004	*Caranx ignobilis*	DQ427060	N/A

MBIO1858.4	French Polynesia: Society Islands, Moorea, Cook bays	2006	*Caranx ignobilis*	JQ431540	MBFB037-07.COI-5P
Smith 210.17 #6_05	South Africa: Cape Videl	2006	*Caranx ignobilis*	JF493038	DSFSE302-07.COI-5P
NBFGR:11803D	India	N/A	*Caranx ignobilis*	FJ347936	N/A
WL-M53	India	N/A	*Caranx ignobilis*	EU014220	N/A
WL-M54	India	N/A	*Caranx ignobilis*	EU014221	N/A
BP02-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Caranx sexfasciatus*	HQ560961	DBMF-M21
BP11-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Caranx sexfasciatus*	HQ560966	DBMF-M30
KP05-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Caranx sexfasciatus*	HQ560947	DBMF-M5
UMTF03881	Sandakan, Sabah (SDK)	2010	*Caranx sexfasciatus*	JX261315	DBMF-M633
UMTF03639	Mukah, Sarawak (MKS)	2010	*Caranx sexfasciatus*	JX261569	DBMF-M391
UMTF03880	Sandakan, Sabah (SDK)	2010	*Caranx sexfasciatus*	JX261414	DBMF-M632
UMTF03641	Mukah, Sarawak (MKS)	2010	*Caranx sexfasciatus*	JX261464	DBMF-M393
UMTF03642	Mukah, Sarawak (MKS)	2010	*Caranx sexfasciatus*	JX261259	DBMF-M394
ADC09_210.22#4	Mozambique: Pomene	2008	*Caranx sexfasciatus*	JF493042	DSFSF026-09.COI-5P
ADC 210.22-2	South Africa: KwaZulu-Natal, Mpenjati Estuary	2004	*Caranx sexfasciatus*	JF493043	TZMSB099-04.COI-5P
Smith 210.22 #3_05	South Africa: Park Rynie	2007	*Caranx sexfasciatus*	JF493044	DSFSE215-07.COI-5P
MBIO836.4	French Polynesia: Society Islands, Moorea, Haapiti	2006	*Caranx sexfasciatus*	JQ431548	MBFA507-07.COI-5P
MBIO835.4	French Polynesia: Society Islands, Moorea, Haapiti	2006	*Caranx sexfasciatus*	JQ431549	MBFA506-07.COI-5P
MBIO1860.4	French Polynesia: Society Islands, Moorea, Cook bays	2006	*Caranx sexfasciatus*	JQ431550	MBFB039-07.COI-5P
BPBM 39581; PCMB B416	USA: Hawaii	2004	*Caranx sexfasciatus*	DQ427061	N/A
BIOUG<CAN>:BW-A1446	Australia: Queensland	1995	*Caranx sexfasciatus*	EF609305	FOAC447-05

GGAJ2	Japan: Nagasaki, Nagasaki, Teguma	2005	*Caranx sexfasciatus*	JF952696	ABFJ208-07.COI-5P
GGAJ1	Japan: Nagasaki, Nagasaki, Teguma	2005	*Caranx sexfasciatus*	JF952695	ABFJ207-07.COI-5P
MBIO1861.4	French Polynesia: Society Islands, Moorea, Cook bays	2006	*Caranx sexfasciatus*	JQ431547	MBFB040-07.COI-5P
NPPF1158	Nayband National Park Coast, Iran	2009	*Caranx sexfasciatus*	HQ149821	NPPF1158
MBIO1191.4	French Polynesia: Society Islands, Moorea, SE Opunohu Bay	2006	*Caranx sexfasciatus*	JQ431546	MBFA701-07.COI-5P
Csex5	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	2010	*Caranx sexfasciatus*	HQ654682	BTL075-10.COI-5P
Csex3	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx sexfasciatus*	HQ654684	BTL073-10.COI-5P
Csex4	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx sexfasciatus*	HQ654683	BTL074-10.COI-5P
Csex2	Philippines: Batangas, Calabarzon, Taal Lake, Butong	2010	*Caranx sexfasciatus*	HQ654685	BTL072-10.COI-5P
Csex8	Philippines: Batangas, Calabarzon, Taal Lake, Talisay		*Carans sexfasciatus*	HQ654681	BTL076-10.COI-5P
UMTF03951	Tawau, Sabah (TW)	2010	*Caranx tille*	JX261631	DBMF-M703
UMTF04024	Kota Kinabalu, Sabah (KKJ)	2010	*Caranx tille*	JX261373	DBMF-M776
UMTF04023	Kota Kinabalu, Sabah (KKJ)	2010	*Caranx tille*	JX261220	DBMF-M775
UMTF04022	Kota Kinabalu, Sabah (KKJ)	2010	*Caranx tille*	JX261324	DBMF-M774
UMTF04021	Kota Kinabalu, Sabah (KKJ)	2010	*Caranx tille*	JX261587	DBMF-M773
UMTF04020	Kota Kinabalu, Sabah (KKJ)	2010	*Caranx tille*	JX261563	DBMF-M772
UMTF03955	Tawau, Sabah (TW)	2010	*Caranx tille*	JX261205	DBMF-M707
UMTF03954	Tawau, Sabah (TW)	2010	*Caranx tille*	JX261272	DBMF-M706
UMTF03882	Sandakan, Sabah (SDK)	2010	*Caranx tille*	JX261274	DBMF-M634
ADC09_210.23#1	South Africa: Tugela Banks	2009	*Caranx tille*	GU805027	DSFSF433-09.COI-5P
UMTF03900	Semporna, Sabah (SMP)	2010	*Decapterus kurroides*	JX261421	DBMF-M652
UMTF03901	Semporna, Sabah (SMP)	2010	*Decapterus kurroides*	JX261337	DBMF-M653
UMTF03898	Semporna, Sabah (SMP)	2010	*Decapterus kurroides*	JX261066	DBMF-M650

UMTF03897	Semporna, Sabah (SMP)	2010	*Decapterus kurroides*	JX261377	DBMF-M649
UMTF03920	Tawau, Sabah (TW)	2010	*Decapterus kurroides*	JX261617	DBMF-M672
UMTF03919	Tawau, Sabah (TW)	2010	*Decapterus kurroides*	JX261180	DBMF-M671
UMTF03916	Tawau, Sabah (TW)	2010	*Decapterus kurroides*	JX261572	DBMF-M668
UMTF03917	Tawau, Sabah (TW)	2010	*Decapterus kurroides*	JX261123	DBMF-M669
UMTF03899	Semporna, Sabah (SMP)	2010	*Decapterus kurroides*	JX261107	DBMF-M651
UMTF04003	Kota Kinabalu, Sabah (KKJ)	2010	*Decapterus macrosoma*	JX261016	DBMF-M755
UMTF04004	Kota Kinabalu, Sabah (KKJ)	2010	*Decapterus macrosoma*	JX261160	DBMF-M756
UMTF03529	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Decapterus macrosoma*	JX261441	DBMF-M281
UMTF03528	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Decapterus macrosoma*	JX261499	DBMF-M280
UMTF03527	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Decapterus macrosoma*	JX261515	DBMF-M279
UMTF03526	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Decapterus macrosoma*	JX261215	DBMF-M278
UMTF03525	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Decapterus macrosoma*	JX261389	DBMF-M277
UMTF03921	Tawau, Sabah (TW)	2010	*Decapterus macrosoma*	JX261134	DBMF-M673
UMTF03922	Tawau, Sabah (TW)	2010	*Decapterus macrosoma*	JX261629	DBMF-M674
UMTF03796	Kudat, Sabah (KDT)	2010	*Decapterus macrosoma*	JX261203	DBMF-M548
UMTF03797	Kudat, Sabah (KDT)	2010	*Decapterus macrosoma*	JX261033	DBMF-M549
UMTF03798	Kudat, Sabah (KDT)	2010	*Decapterus macrosoma*	JX261596	DBMF-M550
UMTF03923	Tawau, Sabah (TW)	2010	*Decapterus macrosoma*	JX261514	DBMF-M675
KP06-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Decapterus macrosoma*	HQ560948	DBMF-M6
UMTF03799	Kudat, Sabah (KDT)	2010	*Decapterus macrosoma*	JX261243	DBMF-M551
UMTF03924	Tawau, Sabah (TW)	2010	*Decapterus macrosoma*	JX260997	DBMF-M676
UMTF03925	Tawau, Sabah (TW)	2010	*Decapterus macrosoma*	JX261534	DBMF-M677
UMTF04000	Kota Kinabalu, Sabah (KKJ)	2010	*Decapterus macrosoma*	JX261216	DBMF-M752
UMTF03861	Sandakan, Sabah (SDK)	2010	*Decapterus macrosoma*	JX261442	DBMF-M613
UMTF03860	Sandakan, Sabah (SDK)	2010	*Decapterus macrosoma*	JX261121	DBMF-M612
UMTF03859	Sandakan, Sabah (SDK)	2010	*Decapterus macrosoma*	JX261269	DBMF-M611
UMTF03858	Sandakan, Sabah (SDK)	2010	*Decapterus macrosoma*	JX261248	DBMF-M610
UMTF03857	Sandakan, Sabah (SDK)	2010	*Decapterus macrosoma*	JX261126	DBMF-M609
UMTF04001	Kota Kinabalu, Sabah (KKJ)	2010	*Decapterus macrosoma*	JX261449	DBMF-M753

UMTF04002	Kota Kinabalu, Sabah (KKJ)	2010	*Decapterus macrosoma*	JX261170	DBMF-M754
UMTF03795	Kudat, Sabah (KDT)	2010	*Decapterus macrosoma*	JX261519	DBMF-M547
ADC 210.27-3	South Africa: KwaZulu-Natal, Durban	2004	*Decapterus macrosoma*	JF493342	TZMSB169-04.COI-5P
ADC 210.27-2	South Africa: KwaZulu-Natal, Durban	2004	*Decapterus macrosoma*	JF493343	TZMSB168-04.COI-5P
ADC09_210.27#8	Mozambique: Pomene	2008	*Decapterus macrosoma*	JF493341	DSFSF018-09.COI-5P
ADC09_210.27#6	Mozambique: Pomene	2008	*Decapterus macrosoma*	JF493340	DSFSF114-09.COI-5P
Smith 210.27-5	South Africa: KwaZulu-Natal, Park Rynie	2005	*Decapterus macrosoma*	JF493346	TZMSC462-05.COI-5P
ADC210.27-1	South Africa: KwaZulu-Natal, Park Rynie	2003	*Decapterus macrosoma*	JF493344	TZMSA181-04.COI-5P
Smith 210.27-4	South Africa: KwaZulu-Natal, Park Rynie	2005	*Decapterus macrosoma*	JF493345	TZMSC461-05.COI-5P
UMTF03386	Mersing, Peninsular Malaysia (MG)	2010	*Decapterus maruadsi*	JX261074	DBMF-M138
UMTF03385	Mersing, Peninsular Malaysia (MG)	2010	*Decapterus maruadsi*	JX261320	DBMF-M137
UMTF03384	Mersing, Peninsular Malaysia (MG)	2010	*Decapterus maruadsi*	JX261589	DBMF-M136
UMTF03382	Mersing, Peninsular Malaysia (MG)	2010	*Decapterus maruadsi*	JX261479	DBMF-M134
UMTF04087	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Decapterus maruadsi*	JX261196	DBMF-M839
UMTF03661	Kuching, Sarawak (KC)	2010	*Decapterus maruadsi*	JX261400	DBMF-M413
UMTF03662	Kuching, Sarawak (KC)	2010	*Decapterus maruadsi*	JX261183	DBMF-M414
UMTF03663	Kuching, Sarawak (KC)	2010	*Decapterus maruadsi*	JX261283	DBMF-M415
UMTF03664	Kuching, Sarawak (KC)	2010	*Decapterus maruadsi*	JX261177	DBMF-M416
UMTF03665	Kuching, Sarawak (KC)	2010	*Decapterus maruadsi*	JX261197	DBMF-M417
UMTF03389	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Decapterus maruadsi*	JX261013	DBMF-M141
UMTF03390	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Decapterus maruadsi*	JX261141	DBMF-M142
UMTF04088	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Decapterus maruadsi*	JX261278	DBMF-M840
UMTF03452	Kuantan, Peninsular Malaysia (KN)	2010	*Decapterus maruadsi*	JX261048	DBMF-M204
UMTF03391	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Decapterus maruadsi*	JX261150	DBMF-M143
UMTF03451	Kuantan, Peninsular Malaysia (KN)	2010	*Decapterus maruadsi*	JX261053	DBMF-M203
UMTF03455	Kuantan, Peninsular Malaysia (KN)	2010	*Decapterus maruadsi*	JX261444	DBMF-M207
UMTF03454	Kuantan, Peninsular Malaysia (KN)	2010	*Decapterus maruadsi*	JX261553	DBMF-M206
UMTF04084	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Decapterus maruadsi*	JX261425	DBMF-M836
KSB11-01	Kuala Sg. Besar, Peninsular Malaysia (KSB)	2009	*Decapterus maruadsi*	HQ560993	DBMF-M63
UMTF04085	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Decapterus maruadsi*	JX261260	DBMF-M837

UMTF04086	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Decapterus maruadsi*	JX261140	DBMF-M838
UMTF03388	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Decapterus maruadsi*	JX261169	DBMF-M140
UMTF03387	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Decapterus maruadsi*	JX261397	DBMF-M139
UMTF04078	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Elagatis bipinnulata*	JX261166	DBMF-M830
UMTF03892	Semporna, Sabah (SMP)	2010	*Elagatis bipinnulata*	JX261525	DBMF-M644
UMTF03895	Semporna, Sabah (SMP)	2009	*Elagatis bipinnulata*	JX261619	DBMF-M647
UMTF04074	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Elagatis bipinnulata*	JX261544	DBMF-M826
UMTF03896	Semporna, Sabah (SMP)	2010	*Elagatis bipinnulata*	JX261002	DBMF-M648
UMTF04076	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Elagatis bipinnulata*	JX261521	DBMF-M828
UMTF04077	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Elagatis bipinnulata*	JX261189	DBMF-M829
UMTF03894	Semporna, Sabah (SMP)	2010	*Elagatis bipinnulata*	JX261506	DBMF-M646
ADC210.31-1	South Africa: KwaZulu-Natal, Park Rynie	2004	*Elagatis bipinnulata*	JF493407	TZMSC069-05.COI-5P
Smith 210.31 #3_05	South Africa: Park Rynie	2007	*Elagatis bipinnulata*	JF493408	DSFSE202-07.COI-5P
Smith 210.31-2	South Africa: KwaZulu-Natal, Park Rynie	2005	*Elagatis bipinnulata*	JF493409	TZMSC469-05.COI-5P
MBIO1297.4	French Polynesia: Society Islands, Moorea	2006	*Elagatis bipinnulata*	JQ431698	MBFA781-07.COI-5P
WL-M82	India	N/A	*Elagatis bipinnulata*	EU014211	N/A
WL-M83	India	N/A	*Elagatis bipinnulata*	EU014212	N/A
WL-M84	India	N/A	*Elagatis bipinnulata*	EU014213	N/A
WL-M85	India	N/A	*Elagatis bipinnulata*	EU014214	N/A
WL-M86	India	N/A	*Elagatis bipinnulata*	EU014215	N/A
MFL882	Mexico: Quintana Roo, Xcalak	2005	*Elagatis bipinnulata*	GU224776	MFLII562-07.COI-5P
BIOUG<CAN>:BW-A1417	Australia: Queensland	2000	*Elagatis bipinnulata*	EF609345	FOAC418-05
UMTF03758	Kudat, Sabah (KDT)	2010	*Gnathanodon speciosus*	JX261245	DBMF-M510
UMTF03759	Kudat, Sabah (KDT)	2010	*Gnathanodon speciosus*	JX261536	DBMF-M511
UMTF03756	Kudat, Sabah (KDT)	2010	*Gnathanodon speciosus*	JX261186	DBMF-M508
UMTF03757	Kudat, Sabah (KDT)	2010	*Gnathanodon speciosus*	JX261431	DBMF-M509
NPPF1121	Iran: Bushehr, Nayband National Park Coast	2009	*Gnathanodon speciosus*	HQ149855	NPPF1121

NPPF1032	Iran: Bushehr, Nayband National Park Coast	2009	*Gnathanodon speciosus*	HQ149856	NPPF1032
ADC08 Smith 210.32 #1	Mozambique: Pomene	2008	*Gnathanodon speciosus*	JF493544	DSFSE776-08.COI-5P
WL-M73	India	N/A	*Gnathanodon speciosus*	EU148563	N/A
WL-M71	India	N/A	*Gnathanodon speciosus*	EU148562	N/A
WL-M70	India	N/A	*Gnathanodon speciosus*	EU148561	N/A
BIOUG<CAN>:BW-A1412	Australia: Queensland	1995	*Gnathanodon speciosus*	EF609362	FOAC413-05
UMTF03615	Mukah, Sarawak (MKS)	2010	*Megalaspis cordyla*	JX261591	DBMF-M367
UMTF03617	Mukah, Sarawak (MKS)	2010	*Megalaspis cordyla*	JX261057	DBMF-M369
UMTF03618	Mukah, Sarawak (MKS)	2010	*Megalaspis cordyla*	JX261574	DBMF-M370
UMTF03564	Tok Bali, Peninsular Malaysia (TB)	2010	*Megalaspis cordyla*	JX261078	DBMF-M316
UMTF03563	Tok Bali, Peninsular Malaysia (TB)	2010	*Megalaspis cordyla*	JX261628	DBMF-M315
UMTF03562	Tok Bali, Peninsular Malaysia (TB)	2010	*Megalaspis cordyla*	JX261430	DBMF-M314
UMTF03561	Tok Bali, Peninsular Malaysia (TB)	2010	*Megalaspis cordyla*	JX261050	DBMF-M313
UMTF03560	Tok Bali, Peninsular Malaysia (TB)	2010	*Megalaspis cordyla*	JX261069	DBMF-M312
UMTF03931	Tawau, Sabah (TW)	2010	*Megalaspis cordyla*	JX261361	DBMF-M683
UMTF03932	Tawau, Sabah (TW)	2010	*Megalaspis cordyla*	JX261006	DBMF-M684
UMTF03549	Kuala Besut, Peninsular Malaysia (KB)	2010	*Megalaspis cordyla*	JX261483	DBMF-M301
UMTF03548	Kuala Besut, Peninsular Malaysia (KB)	2010	*Megalaspis cordyla*	JX261502	DBMF-M300
UMTF03547	Kuala Besut, Peninsular Malaysia (KB)	2010	*Megalaspis cordyla*	JX261435	DBMF-M299
UMTF03546	Kuala Besut, Peninsular Malaysia (KB)	2010	*Megalaspis cordyla*	JX261417	DBMF-M298
UMTF03545	Kuala Besut, Peninsular Malaysia (KB)	2010	*Megalaspis cordyla*	JX261201	DBMF-M297
UMTF03933	Tawau, Sabah (TW)	2010	*Megalaspis cordyla*	JX261003	DBMF-M685
UMTF03934	Tawau, Sabah (TW)	2010	*Megalaspis cordyla*	JX261210	DBMF-M686
UMTF03935	Tawau, Sabah (TW)	2010	*Megalaspis cordyla*	JX261522	DBMF-M687
UMTF03514	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Megalaspis cordyla*	JX261244	DBMF-M266
UMTF03513	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Megalaspis cordyla*	JX261027	DBMF-M265
UMTF03512	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Megalaspis cordyla*	JX261312	DBMF-M264

UMTF03511	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Megalaspis cordyla*	JX261319	DBMF-M263
UMTF03510	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Megalaspis cordyla*	JX261359	DBMF-M262
UMTF03470	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Megalaspis cordyla*	JX261488	DBMF-M222
UMTF03469	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Megalaspis cordyla*	JX261580	DBMF-M221
UMTF03468	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Megalaspis cordyla*	JX261584	DBMF-M220
UMTF03467	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Megalaspis cordyla*	JX261015	DBMF-M219
UMTF03466	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Megalaspis cordyla*	JX261071	DBMF-M218
UMTF03450	Kuantan, Peninsular Malaysia (KN)	2010	*Megalaspis cordyla*	JX261459	DBMF-M202
UMTF03449	Kuantan, Peninsular Malaysia (KN)	2010	*Megalaspis cordyla*	JX261601	DBMF-M201
UMTF03448	Kuantan, Peninsular Malaysia (KN)	2010	*Megalaspis cordyla*	JX261356	DBMF-M200
UMTF03447	Kuantan, Peninsular Malaysia (KN)	2010	*Megalaspis cordyla*	JX261418	DBMF-M199
UMTF03694	Miri, Sarawak (MR)	2010	*Megalaspis cordyla*	JX261279	DBMF-M446
UMTF03695	Miri, Sarawak (MR)	2010	*Megalaspis cordyla*	JX261208	DBMF-M447
UMTF03696	Miri, Sarawak (MR)	2010	*Megalaspis cordyla*	JX261119	DBMF-M448
UMTF03697	Miri, Sarawak (MR)	2010	*Megalaspis cordyla*	JX261613	DBMF-M449
UMTF03698	Miri, Sarawak (MR)	2010	*Megalaspis cordyla*	JX261117	DBMF-M450
KK01-01	Kuala Kedah, Peninsular Malaysia (KK)	2009	*Megalaspis cordyla*	HQ560952	DBMF-M11
KP10-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Megalaspis cordyla*	HQ560951	DBMF-M10
UMTF03406	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Megalaspis cordyla*	JX261026	DBMF-M158
UMTF03405	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Megalaspis cordyla*	JX261310	DBMF-M157
UMTF03404	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Megalaspis cordyla*	JX261334	DBMF-M156
UMTF03403	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Megalaspis cordyla*	JX261427	DBMF-M155
UMTF03402	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Megalaspis cordyla*	JX261549	DBMF-M154
UMTF04025	Kota Kinabalu, Sabah (KKJ)	2010	*Megalaspis cordyla*	JX261586	DBMF-M777
UMTF04027	Kota Kinabalu, Sabah (KKJ)	2010	*Megalaspis cordyla*	JX261625	DBMF-M779
UMTF04028	Kota Kinabalu, Sabah (KKJ)	2010	*Megalaspis cordyla*	JX261616	DBMF-M780
UMTF04029	Kota Kinabalu, Sabah (KKJ)	2010	*Megalaspis cordyla*	JX261145	DBMF-M781
UMTF04064	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Megalaspis cordyla*	JX261358	DBMF-M816
UMTF04065	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Megalaspis cordyla*	JX261187	DBMF-M817
UMTF04066	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Megalaspis cordyla*	JX261470	DBMF-M818

UMTF04067	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Megalaspis cordyla*	JX261455	DBMF-M819
UMTF04068	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Megalaspis cordyla*	JX261068	DBMF-M820
PN03-01	Pontian, Peninsular Malaysia (PN)	2009	*Megalaspis cordyla*	HQ561011	DBMF-M83
SB03-01	Kuala Sg. Baru, Peninsular Malaysia (SB)	2009	*Megalaspis cordyla*	HQ561007	DBMF-M79
UMTF03738	Kudat, Sabah (KDT)	2010	*Megalaspis cordyla*	JX261472	DBMF-M490
UMTF03739	Kudat, Sabah (KDT)	2010	*Megalaspis cordyla*	JX261202	DBMF-M491
UMTF03740	Kudat, Sabah (KDT)	2010	*Megalaspis cordyla*	JX261325	DBMF-M492
UMTF03741	Kudat, Sabah (KDT)	2010	*Megalaspis cordyla*	JX261562	DBMF-M493
UMTF03742	Kudat, Sabah (KDT)	2010	*Megalaspis cordyla*	JX261211	DBMF-M494
KSB13-01	Kuala Sg. Besar, Peninsular Malaysia (KSB)	2009	*Megalaspis cordyla*	HQ560994	DBMF-M65
AHM15-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Megalaspis cordyla*	HQ560983	DBMF-M50
UMTF03616	Mukah, Sarawak (MKS)	2010	*Megalaspis cordyla*	JX261590	DBMF-M368
WL-M19	India: Maharashtra	2006	*Megalaspis cordyla*	EF609548	WLIND019-07
WL-M18	India: Maharashtra	2006	*Megalaspis cordyla*	EF609549	WLIND018-07
WL-M17	India: Maharashtra	2006	*Megalaspis cordyla*	EF609550	WLIND017-07
WL-M16	India: Maharashtra	2006	*Megalaspis cordyla*	EF609551	WLIND016-07
WL-M15	India: Maharashtra	2006	*Megalaspis cordyla*	EF609552	WLIND015-07
NPPF1056	Iran: Bushehr, Nayband National Park Coast	2009	*Megalaspis cordyla*	HQ149881	NPPF1056
NPPF1053	Iran: Bushehr, Nayband National Park Coast	2009	*Megalaspis cordyla*	HQ149882	NPPF1053
NPPF1051	Iran: Bushehr, Nayband National Park Coast	2009	*Megalaspis cordyla*	HQ149883	NPPF1051
NPPF1016	Iran: Bushehr, Nayband National Park Coast	2009	*Megalaspis cordyla*	HQ149884	NPPF1016
ON8	Japan: Yokohama, Yokosuka, Arasaki	2006	*Megalaspis cordyla*	JF952790	ABFJ246-07.COI-5P
Smith 210.34 #3	South Africa: KwaZulu-Natal	2005	*Megalaspis cordyla*	JF493867	TZMSC616-06.COI-5P
Smith 210.34 #2	South Africa: KwaZulu-Natal	2005	*Megalaspis cordyla*	JF493868	TZMSC615-06.COI-5P
Smith 210.34 #1	South Africa: KwaZulu-Natal	2005	*Megalaspis cordyla*	JF493869	TZMSC614-06.COI-5P

ADC10_210.34 #4	South Africa: Tugela Banks	2009	*Megalaspis cordyla*	HQ945872	DSFSG233-10.COI-5P
ADC10_210.17 #7	South Africa: Scottburgh	2010	*Megalaspis cordyla*	HQ561501	DSFSG166-10.COI-5P
ADC09_210.34#5	South Africa: Tugela Banks	2009	*Megalaspis cordyla*	GU804934	DSFSF706-09.COI-5P
UMTF03568	Tok Bali, Peninsular Malaysia (TB)	2010	*Parastromateus niger*	JX261342	DBMF-M320
HM09-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Parastromateus niger*	HQ560977	DBMF-M44
KSB02-01	Kuala Sg. Besar, Peninsular Malaysia (KSB)	2009	*Parastromateus niger*	HQ560987	DBMF-M54
SK07-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Parastromateus niger*	HQ561002	DBMF-M73
PN04-01	Pontian, Peninsular Malaysia (PN)	2009	*Parastromateus niger*	HQ561012	DBMF-M84
UMTF03436	Kuantan, Peninsular Malaysia (KN)	2010	*Parastromateus niger*	JX261395	DBMF-M188
UMTF03437	Kuantan, Peninsular Malaysia (KN)	2010	*Parastromateus niger*	JX261073	DBMF-M189
UMTF03438	Kuantan, Peninsular Malaysia (KN)	2010	*Parastromateus niger*	JX261184	DBMF-M190
UMTF03439	Kuantan, Peninsular Malaysia (KN)	2010	*Parastromateus niger*	JX261318	DBMF-M191
UMTF03520	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Parastromateus niger*	JX261592	DBMF-M272
UMTF03521	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Parastromateus niger*	JX261125	DBMF-M273
UMTF03523	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Parastromateus niger*	JX261403	DBMF-M275
UMTF03524	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Parastromateus niger*	JX261527	DBMF-M276
UMTF03565	Tok Bali, Peninsular Malaysia (TB)	2010	*Parastromateus niger*	JX261380	DBMF-M317
UMTF03567	Tok Bali, Peninsular Malaysia (TB)	2010	*Parastromateus niger*	JX261492	DBMF-M319
UMTF03569	Tok Bali, Peninsular Malaysia (TB)	2010	*Parastromateus niger*	JX261579	DBMF-M321
UMTF03619	Mukah, Sarawak (MKS)	2010	*Parastromateus niger*	JX261448	DBMF-M371
UMTF03620	Mukah, Sarawak (MKS)	2010	*Parastromateus niger*	JX261239	DBMF-M372
UMTF03621	Mukah, Sarawak (MKS)	2010	*Parastromateus niger*	JX261365	DBMF-M373
UMTF03622	Mukah, Sarawak (MKS)	2010	*Parastromateus niger*	JX261331	DBMF-M374
UMTF03623	Mukah, Sarawak (MKS)	2010	*Parastromateus niger*	JX261332	DBMF-M375
UMTF03656	Kuching, Sarawak (KC)	2010	*Parastromateus niger*	JX261537	DBMF-M408
UMTF03657	Kuching, Sarawak (KC)	2010	*Parastromateus niger*	JX261509	DBMF-M409
UMTF03658	Kuching, Sarawak (KC)	2010	*Parastromateus niger*	JX261513	DBMF-M410
UMTF03659	Kuching, Sarawak (KC)	2010	*Parastromateus niger*	JX261101	DBMF-M411
UMTF03660	Kuching, Sarawak (KC)	2010	*Parastromateus niger*	JX261621	DBMF-M412

UMTF03718	Miri, Sarawak (MR)	2010	*Parastromateus niger*	JX261030	DBMF-M470
UMTF03719	Miri, Sarawak (MR)	2010	*Parastromateus niger*	JX261301	DBMF-M471
UMTF03720	Miri, Sarawak (MR)	2010	*Parastromateus niger*	JX261627	DBMF-M472
UMTF03721	Miri, Sarawak (MR)	2010	*Parastromateus niger*	JX261043	DBMF-M473
UMTF03722	Miri, Sarawak (MR)	2010	*Parastromateus niger*	JX261195	DBMF-M474
UMTF03743	Kudat, Sabah (KDT)	2010	*Parastromateus niger*	JX261353	DBMF-M495
UMTF03744	Kudat, Sabah (KDT)	2010	*Parastromateus niger*	JX261540	DBMF-M496
UMTF03745	Kudat, Sabah (KDT)	2010	*Parastromateus niger*	JX261120	DBMF-M497
UMTF03746	Kudat, Sabah (KDT)	2010	*Parastromateus niger*	JX261271	DBMF-M498
UMTF03747	Kudat, Sabah (KDT)	2010	*Parastromateus niger*	JX261051	DBMF-M499
UMTF03847	Sandakan, Sabah (SDK)	2010	*Parastromateus niger*	JX261511	DBMF-M599
UMTF03848	Sandakan, Sabah (SDK)	2010	*Parastromateus niger*	JX261326	DBMF-M600
UMTF03849	Sandakan, Sabah (SDK)	2010	*Parastromateus niger*	JX261415	DBMF-M601
UMTF03850	Sandakan, Sabah (SDK)	2010	*Parastromateus niger*	JX261191	DBMF-M602
UMTF03851	Sandakan, Sabah (SDK)	2010	*Parastromateus niger*	JX261055	DBMF-M603
UMTF03911	Tawau, Sabah (TW)	2010	*Parastromateus niger*	JX261602	DBMF-M663
UMTF03912	Tawau, Sabah (TW)	2010	*Parastromateus niger*	JX261462	DBMF-M664
UMTF03913	Tawau, Sabah (TW)	2010	*Parastromateus niger*	JX261493	DBMF-M665
UMTF03914	Tawau, Sabah (TW)	2010	*Parastromateus niger*	JX261224	DBMF-M666
UMTF03915	Tawau, Sabah (TW)	2010	*Parastromateus niger*	JX261615	DBMF-M667
UMTF04079	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Parastromateus niger*	JX261558	DBMF-M831
UMTF04080	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Parastromateus niger*	JX261463	DBMF-M832
UMTF04081	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Parastromateus niger*	JX261468	DBMF-M833
UMTF04082	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Parastromateus niger*	JX261478	DBMF-M834
UMTF04083	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Parastromateus niger*	JX261147	DBMF-M835
WL-M51	India: Maharashtra	2006	*Parastromateus niger*	EF609567	WLIND051-07
WL-M50	India: Maharashtra	2006	*Parastromateus niger*	EF609568	WLIND050-07
WL-M49	India: Maharashtra	2006	*Parastromateus niger*	EF609569	WLIND049-07
WL-M48	India: Maharashtra	2006	*Parastromateus niger*	EF609570	WLIND048-07
WL-M47	India: Maharashtra	2006	*Parastromateus niger*	EF609571	WLIND047-07

Smith 210.36 #1	South Africa: KwaZulu-Natal	2005	*Parastromateus niger*	JF494092	TZMSC617-06.COI-5P
ADC09_210.36#2	South Africa: Tugela Banks	2009	*Parastromateus niger*	GU804931	DSFSF710-09.COI-5P
BW-A1422	Australia: Queensland	1997	*Parastromateus niger*	EF609429	FOAC423-05
UMTF03983	Tawau, Sabah (TW)	2010	*Scomberoides commersonnianus*	JX261298	DBMF-M735
UMTF03593	Mukah, Sarawak (MKS)	2010	*Scomberoides commersonnianus*	JX261179	DBMF-M345
UMTF03592	Mukah, Sarawak (MKS)	2010	*Scomberoides commersonnianus*	JX261037	DBMF-M344
UMTF03928	Tawau, Sabah (TW)	2010	*Scomberoides commersonnianus*	JX261381	DBMF-M734
UMTF03981	Tawau, Sabah (TW)	2010	*Scomberoides commersonnianus*	JX261255	DBMF-M733
UMTF03980	Tawau, Sabah (TW)	2010	*Scomberoides commersonnianus*	JX261634	DBMF-M732
UMTF03979	Tawau, Sabah (TW)	2010	*Scomberoides commersonnianus*	JX261603	DBMF-M731
UMTF03890	Sandakan, Sabah (SDK)	2010	*Scomberoides commersonnianus*	JX261451	DBMF-M642
KSB12-01	Kuala Sg. Besar, Peninsular Malaysia (KSB)	2009	*Scomberoides commersonnianus*	JX261439	DBMF-M64
UMTF03580	Tok Bali, Peninsular Malaysia (TB)	2010	*Scomberoides commersonnianus*	JX261423	DBMF-M332
UMTF03581	Tok Bali, Peninsular Malaysia (TB)	2010	*Scomberoides commersonnianus*	JX261017	DBMF-M333
UMTF03591	Mukah, Sarawak (MKS)	2010	*Scomberoides commersonnianus*	JX261020	DBMF-M343
UMTF03594	Mukah, Sarawak (MKS)	2010	*Scomberoides commersonnianus*	JX261487	DBMF-M346
UMTF03582	Tok Bali, Peninsular Malaysia (TB)	2010	*Scomberoides commersonnianus*	JX261209	DBMF-M334
UMTF03583	Tok Bali, Peninsular Malaysia (TB)	2010	*Scomberoides commersonnianus*	JX261258	DBMF-M335
UMTF03584	Tok Bali, Peninsular Malaysia (TB)	2010	*Scomberoides commersonnianus*	JX261564	DBMF-M336
UMTF03590	Mukah, Sarawak (MKS)	2010	*Scomberoides commersonnianus*	JX261031	DBMF-M342
NPPF1127	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides commersonnianus*	HQ149937	NPPF1127
NPPF1058	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides commersonnianus*	HQ149938	NPPF1058
NPPF1047	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides commersonnianus*	HQ149939	NPPF1047
NPPF1015	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides commersonnianus*	HQ149940	NPPF1015
ADC09_210.38#1	Mozambique: Pomene	2008	*Scomberoides commersonnianus*	JF494451	DSFSF584-09.COI-5P
ADC09_210.38#2	Mozambique: Pomene	2008	*Scomberoides commersonnianus*	GU805100	DSFSF518-09.COI-5P

BIOUG<CAN>:BW-A1487	Australia: Western Australia	1995	*Scomberoides commersonnianus*	EF609456	FOAC488-05
UMTF03539	Kuala Besut, Peninsular Malaysia (KB)	2010	*Scomberoides tala*	JX261223	DBMF-M291
UMTF03748	Kudat, Sabah (KDT)	2010	*Scomberoides tala*	JX261518	DBMF-M500
UMTF03984	Tawau, Sabah (TW)	2010	*Scomberoides tala*	JX261091	DBMF-M736
UMTF03344	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tala*	JX261606	DBMF-M96
UMTF03345	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tala*	JX261456	DBMF-M97
UMTF03535	Kuala Besut, Peninsular Malaysia (KB)	2010	*Scomberoides tala*	JX261447	DBMF-M287
UMTF03346	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tala*	JX261316	DBMF-M98
UMTF03347	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tala*	JX261096	DBMF-M99
UMTF03348	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tala*	JX261626	DBMF-M100
UMTF03537	Kuala Besut, Peninsular Malaysia (KB)	2010	*Scomberoides tala*	JX261100	DBMF-M289
UMTF03538	Kuala Besut, Peninsular Malaysia (KB)	2010	*Scomberoides tala*	JX261566	DBMF-M290
UMTF03627	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261638	DBMF-M379
UMTF03626	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261598	DBMF-M378
UMTF03625	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261060	DBMF-M377
UMTF03628	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261556	DBMF-M380
UMTF03751	Kudat, Sabah (KDT)	2010	*Scomberoides tol*	JX261295	DBMF-M503
UMTF03732	Miri, Sarawak (MR)	2010	*Scomberoides tol*	JX261453	DBMF-M484
UMTF03731	Miri, Sarawak (MR)	2010	*Scomberoides tol*	JX261112	DBMF-M483
UMTF03624	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261371	DBMF-M376
UMTF03729	Miri, Sarawak (MR)	2010	*Scomberoides tol*	JX261477	DBMF-M481
UMTF03728	Miri, Sarawak (MR)	2010	*Scomberoides tol*	JX261354	DBMF-M480
UMTF03349	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tol*	JX261236	DBMF-M101
UMTF03350	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tol*	JX261001	DBMF-M102
UMTF03351	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tol*	JX261296	DBMF-M103
UMTF03352	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tol*	JX261154	DBMF-M104
UMTF03353	Mersing, Peninsular Malaysia (MG)	2010	*Scomberoides tol*	JX261321	DBMF-M105
UMTF03755	Kudat, Sabah (KDT)	2010	*Scomberoides tol*	JX261387	DBMF-M507
UMTF03754	Kudat, Sabah (KDT)	2010	*Scomberoides tol*	JX261609	DBMF-M506

UMTF04053	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Scomberoides tol*	JX261164	DBMF-M805
UMTF04051	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Scomberoides tol*	JX261604	DBMF-M803
UMTF04050	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Scomberoides tol*	JX261058	DBMF-M802
UMTF04049	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Scomberoides tol*	JX261231	DBMF-M801
UMTF03413	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Scomberoides tol*	JX261238	DBMF-M165
UMTF03753	Kudat, Sabah (KDT)	2010	*Scomberoides tol*	JX261199	DBMF-M505
UMTF03752	Kudat, Sabah (KDT)	2010	*Scomberoides tol*	JX261250	DBMF-M504
UMTF03481	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Scomberoides tol*	JX261070	DBMF-M233
UMTF03482	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Scomberoides tol*	JX261559	DBMF-M234
UMTF03483	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Scomberoides tol*	JX261539	DBMF-M235
UMTF03484	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Scomberoides tol*	JX261227	DBMF-M236
UMTF03485	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Scomberoides tol*	JX261005	DBMF-M237
UMTF03648	Mukah, Sarawak (MKS)	2010	*Scomberoides tol*	JX261450	DBMF-M400
UMTF03536	Kuala Besut, Peninsular Malaysia (KB)	2010	*Scomberoides tol*	JX261285	DBMF-M288
UMTF 03730	Miri, Sarawak (MR)	2010	*Scomberoides tol*	JX261165	DBMF-M482
NPPF1054	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides tol*	HQ149941	NPPF1054
NPPF1005	Iran: Bushehr, Nayband National Park Coast	2009	*Scomberoides tol*	HQ149942	NPPF1005
GD 9086042	China	2006	*Scomberoides tol*	EF607527	FSCS067-06
GD 9086041	China	2006	*Scomberoides tol*	EF607528	FSCS066-06
GD 9086040	China	2006	*Scomberoides tol*	EF607529	FSCS065-06
GD 9086039	China	2006	*Scomberoides tol*	EF607530	FSCS064-06
GD 9086038	China	2006	*Scomberoides tol*	EF607531	FSCS063-06
ADC 210.40-2	South Africa: Kwazulu Natal	2004	*Scomberoides tol*	DQ885050	TZMSB200-04
ADC09_210.40#7	South Africa: Tugela Banks	2009	*Scomberoides tol*	GU804963	DSFSF667-09.COI-5P
BIOUG<CAN>:BW-A1484	Australia: Western Australia	1995	*Scomberoides tol*	DQ885124	FOAC485-05
BIOUG<CAN>:BW-	Australia: Queensland	1996	*Scomberoides tol*	DQ885123	FOAC486-05

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ADC09_210.40 #8	South Africa: Tugela Banks	2009	*Scomberoides tol*	GU804962	DSFSF669-09.COI-5P
ADC09_210.40#6	South Africa: Tugela Banks	2009	*Scomberoides tol*	GU804999	DSFSF464-09.COI-5P
KK02-01	Kuala Kedah, Peninsular Malaysia (KK)	2009	*Selar boops*	HQ560953	DBMF-M12
UMTF03376	Mersing, Peninsular Malaysia (MG)	2010	*Selar boops*	JX261523	DBMF-M128
UMTF03693	Miri, Sarawak (MR)	2010	*Selar boops*	JX261516	DBMF-M445
UMTF03691	Miri, Sarawak (MR)	2010	*Selar boops*	JX261102	DBMF-M443
UMTF03690	Miri, Sarawak (MR)	2010	*Selar boops*	JX261104	DBMF-M442
UMTF03689	Miri, Sarawak (MR)	2010	*Selar boops*	JX261299	DBMF-M441
UMTF03599	Mukah, Sarawak (MKS)	2010	*Selar boops*	JX261083	DBMF-M351
UMTF03598	Mukah, Sarawak (MKS)	2010	*Selar boops*	JX261309	DBMF-M350
UMTF03597	Mukah, Sarawak (MKS)	2010	*Selar boops*	JX261355	DBMF-M349
UMTF03596	Mukah, Sarawak (MKS)	2010	*Selar boops*	JX261542	DBMF-M348
UMTF03595	Mukah, Sarawak (MKS)	2010	*Selar boops*	JX261503	DBMF-M347
UMTF03906	Semporna, Sabah (SMP)	2010	*Selar boops*	JX261262	DBMF-M658
UMTF03907	Semporna, Sabah (SMP)	2010	*Selar boops*	JX261398	DBMF-M659
UMTF03908	Semporna, Sabah (SMP)	2010	*Selar boops*	JX261198	DBMF-M660
UMTF03909	Semporna, Sabah (SMP)	2010	*Selar boops*	JX261392	DBMF-M661
UMTF03910	Semporna, Sabah (SMP)	2010	*Selar boops*	JX261114	DBMF-M662
UMTF03559	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selar boops*	JX261264	DBMF-M311
UMTF03558	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selar boops*	JX261085	DBMF-M310
UMTF03557	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selar boops*	JX261605	DBMF-M309
UMTF03556	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selar boops*	JX261252	DBMF-M308
UMTF03499	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar boops*	JX261375	DBMF-M251
UMTF03498	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar boops*	JX261532	DBMF-M250
UMTF03497	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar boops*	JX261393	DBMF-M249
UMTF03496	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar boops*	JX261214	DBMF-M248
UMTF03495	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar boops*	JX261314	DBMF-M247
UMTF03474	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar boops*	JX261467	DBMF-M226
UMTF03473	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar boops*	JX261059	DBMF-M225

UMTF03472	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar boops*	JX261062	DBMF-M224
UMTF03471	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar boops*	JX261291	DBMF-M223
UMTF03446	Kuantan, Peninsular Malaysia (KN)	2010	*Selar boops*	JX261476	DBMF-M198
UMTF03995	Kota Kinabalu, Sabah (KKJ)	2010	*Selar boops*	JX261343	DBMF-M747
UMTF03996	Kota Kinabalu, Sabah (KKJ)	2010	*Selar boops*	JX261297	DBMF-M748
UMTF03997	Kota Kinabalu, Sabah (KKJ)	2010	*Selar boops*	JX261093	DBMF-M749
UMTF03998	Kota Kinabalu, Sabah (KKJ)	2010	*Selar boops*	JX261504	DBMF-M750
UMTF03999	Kota Kinabalu, Sabah (KKJ)	2010	*Selar boops*	JX261363	DBMF-M751
UMTF03445	Kuantan, Peninsular Malaysia (KN)	2010	*Selar boops*	JX261461	DBMF-M197
UMTF03375	Mersing, Peninsular Malaysia (MG)	2010	*Selar boops*	JX261064	DBMF-M127
UMTF03374	Mersing, Peninsular Malaysia (MG)	2010	*Selar boops*	JX261105	DBMF-M126
UMTF03373	Mersing, Peninsular Malaysia (MG)	2010	*Selar boops*	JX261481	DBMF-M125
UMTF03372	Mersing, Peninsular Malaysia (MG)	2010	*Selar boops*	JX261008	DBMF-M124
UMTF03930	Tawau, Sabah (TW)	2010	*Selar crumenophthalmus*	JX261185	DBMF-M682
UMTF03602	Mukah, Sarawak (MKS)	2010	*Selar crumenophthalmus*	JX261323	DBMF-M354
UMTF03601	Mukah, Sarawak (MKS)	2010	*Selar crumenophthalmus*	JX261482	DBMF-M353
KK03-01	Kuala Kedah, Peninsular Malaysia (KK)	2010	*Selar crumenophthalmus*	HQ560954	DBMF-M13
UMTF03929	Tawau, Sabah (TW)	2010	*Selar crumenophthalmus*	JX261486	DBMF-M681
UMTF03928	Tawau, Sabah (TW)	2010	*Selar crumenophthalmus*	JX261327	DBMF-M680
UMTF03927	Tawau, Sabah (TW)	2010	*Selar crumenophthalmus*	JX261386	DBMF-M679
UMTF03685	Miri, Sarawak (MR)	2010	*Selar crumenophthalmus*	JX261524	DBMF-M437
UMTF03686	Miri, Sarawak (MR)	2010	*Selar crumenophthalmus*	JX261336	DBMF-M438
UMTF03687	Miri, Sarawak (MR)	2010	*Selar crumenophthalmus*	JX261307	DBMF-M439
UMTF03688	Miri, Sarawak (MR)	2010	*Selar crumenophthalmus*	JX261143	DBMF-M440
UMTF03856	Sandakan, Sabah (SDK)	2010	*Selar crumenophthalmus*	JX261565	DBMF-M608
UMTF03855	Sandakan, Sabah (SDK)	2010	*Selar crumenophthalmus*	JX261313	DBMF-M607
UMTF03926	Tawau, Sabah (TW)	2010	*Selar crumenophthalmus*	JX261247	DBMF-M678
KP01-01	Kuala Perlis, Peninsular Malaysia (KP)	2009	*Selar crumenophthalmus*	HQ560945	DBMF-M1
UMTF03575	Tok Bali, Peninsular Malaysia (TB)	2010	*Selar crumenophthalmus*	JX261304	DBMF-M327
UMTF03576	Tok Bali, Peninsular Malaysia (TB)	2010	*Selar crumenophthalmus*	JX261242	DBMF-M328

UMTF03577	Tok Bali, Peninsular Malaysia (TB)	2010	*Selar crumenophthalmus*	JX261129	DBMF-M329
UMTF03578	Tok Bali, Peninsular Malaysia (TB)	2010	*Selar crumenophthalmus*	JX261458	DBMF-M330
UMTF03579	Tok Bali, Peninsular Malaysia (TB)	2010	*Selar crumenophthalmus*	JX261115	DBMF-M331
UMTF03794	Kudat, Sabah (KDT)	2010	*Selar crumenophthalmus*	JX261138	DBMF-M546
UMTF03793	Kudat, Sabah (KDT)	2010	*Selar crumenophthalmus*	JX261080	DBMF-M545
UMTF03792	Kudat, Sabah (KDT)	2010	*Selar crumenophthalmus*	JX261157	DBMF-M544
UMTF03791	Kudat, Sabah (KDT)	2010	*Selar crumenophthalmus*	JX261000	DBMF-M543
UMTF03790	Kudat, Sabah (KDT)	2010	*Selar crumenophthalmus*	JX261529	DBMF-M542
UMTF03444	Kuantan, Peninsular Malaysia (KN)	2010	*Selar crumenophthalmus*	JX261193	DBMF-M196
UMTF03443	Kuantan, Peninsular Malaysia (KN)	2010	*Selar crumenophthalmus*	JX261339	DBMF-M195
UMTF03442	Kuantan, Peninsular Malaysia (KN)	2010	*Selar crumenophthalmus*	JX261204	DBMF-M194
UMTF03441	Kuantan, Peninsular Malaysia (KN)	2010	*Selar crumenophthalmus*	JX261292	DBMF-M193
UMTF04005	Kota Kinabalu, Sabah (KKJ)	2010	*Selar crumenophthalmus*	JX261082	DBMF-M757
UMTF04006	Kota Kinabalu, Sabah (KKJ)	2010	*Selar crumenophthalmus*	JX261306	DBMF-M758
UMTF04007	Kota Kinabalu, Sabah (KKJ)	2010	*Selar crumenophthalmus*	JX261182	DBMF-M759
UMTF04008	Kota Kinabalu, Sabah (KKJ)	2010	*Selar crumenophthalmus*	JX261480	DBMF-M760
UMTF04009	Kota Kinabalu, Sabah (KKJ)	2010	*Selar crumenophthalmus*	JX261079	DBMF-M761
UMTF03440	Kuantan, Peninsular Malaysia (KN)	2010	*Selar crumenophthalmus*	JX261052	DBMF-M192
UMTF03854	Sandakan, Sabah (SDK)	2010	*Selar crumenophthalmus*	JX261213	DBMF-M606
UMTF03853	Sandakan, Sabah (SDK)	2010	*Selar crumenophthalmus*	JX261338	DBMF-M605
UMTF03396	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Selar crumenophthalmus*	JX261294	DBMF-M148
UMTF03395	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Selar crumenophthalmus*	JX261599	DBMF-M147
UMTF03394	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Selar crumenophthalmus*	JX261348	DBMF-M146
UMTF03393	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Selar crumenophthalmus*	JX261443	DBMF-M145
UMTF03392	Tanjung Sedili, Peninsular Malaysia (TS)	2010	*Selar crumenophthalmus*	JX261630	DBMF-M144
UMTF03381	Mersing, Peninsular Malaysia (MG)	2010	*Selar crumenophthalmus*	JX261322	DBMF-M133
UMTF03380	Mersing, Peninsular Malaysia (MG)	2010	*Selar crumenophthalmus*	JX261571	DBMF-M132
UMTF03379	Mersing, Peninsular Malaysia (MG)	2010	*Selar crumenophthalmus*	JX261232	DBMF-M131
UMTF03378	Mersing, Peninsular Malaysia (MG)	2010	*Selar crumenophthalmus*	JX261452	DBMF-M130
UMTF03377	Mersing, Peninsular Malaysia (MG)	2010	*Selar crumenophthalmus*	JX261554	DBMF-M129

UMTF03852	Sandakan, Sabah (SDK)	2010	*Selar crumenophthalmus*	JX261438	DBMF-M604
HM01-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Selar crumenophthalmus*	HQ560970	DBMF-M36
UMTF03604	Mukah, Sarawak (MKS)	2010	*Selar crumenophthalmus*	JX261645	DBMF-M356
UMTF04054	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Selar crumenophthalmus*	JX261237	DBMF-M806
UMTF04055	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Selar crumenophthalmus*	JX260998	DBMF-M807
UMTF04056	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Selar crumenophthalmus*	JX261465	DBMF-M808
UMTF04057	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Selar crumenophthalmus*	JX261402	DBMF-M809
UMTF04058	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Selar crumenophthalmus*	JX261347	DBMF-M810
UMTF03600	Mukah, Sarawak (MKS)	2010	*Selar crumenophthalmus*	JX261116	DBMF-M352
UMTF03671	Kuching, Sarawak (KC)	2010	*Selar crumenophthalmus*	JX261303	DBMF-M423
UMTF03509	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar crumenophthalmus*	JX261022	DBMF-M261
UMTF03508	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar crumenophthalmus*	JX261087	DBMF-M260
UMTF03507	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar crumenophthalmus*	JX261311	DBMF-M259
UMTF03506	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar crumenophthalmus*	JX261422	DBMF-M258
UMTF03505	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selar crumenophthalmus*	JX261277	DBMF-M257
SK06-01	Sekinchan, Peninsular Malaysia (SK)	2009	*Selar crumenophthalmus*	HQ561001	DBMF-M72
BP12-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Selar crumenophthalmus*	HQ560967	DBMF-M31
UMTF03603	Mukah, Sarawak (MKS)	2010	*Selar crumenophthalmus*	JX261139	DBMF-M355
UMTF03672	Kuching, Sarawak (KC)	2010	*Selar crumenophthalmus*	JX261268	DBMF-M424
UMTF03673	Kuching, Sarawak (KC)	2010	*Selar crumenophthalmus*	JX261230	DBMF-M425
UMTF03674	Kuching, Sarawak (KC)	2010	*Selar crumenophthalmus*	JX261009	DBMF-M426
UMTF03675	Kuching, Sarawak (KC)	2010	*Selar crumenophthalmus*	JX261376	DBMF-M427
UMTF03684	Miri, Sarawak (MR)	2010	*Selar crumenophthalmus*	JX261547	DBMF-M436
UMTF03480	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar crumenophthalmus*	JX261036	DBMF-M232
UMTF03479	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar crumenophthalmus*	JX261568	DBMF-M231
UMTF03478	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar crumenophthalmus*	JX261391	DBMF-M230
UMTF03477	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar crumenophthalmus*	JX261384	DBMF-M229
UMTF03476	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selar crumenophthalmus*	JX261611	DBMF-M228
ADC08 Smith	Mozambique: Pomene	2008	*Selar crumenophthalmus*	JF494491	DSFSE546-08.COI-5P

210.41 #5					
ADC08 Smith 210.41 #4	Mozambique: Pomene	2008	*Selar crumenophthalmus*	JF494492	DSFSE551-08.COI-5P
ADC08 Smith 210.41 #1	Mozambique: Pomene	2008	*Selar crumenophthalmus*	JF494493	DSFSE554-08.COI-5P
ADC08 Smith 210.41 #2	Mozambique: Pomene	2008	*Selar crumenophthalmus*	JF494494	DSFSE563-08.COI-5P
NBFGR:SC187	India	N/A	*Selar crumenophthalmus*	FJ347941	N/A
NBFGR:SC188	India	N/A	*Selar crumenophthalmus*	FJ347942	N/A
N/A	Japan	N/A	*Selar crumenophthalmus*	AY541647	N/A
NPPF1153	Iran: Bushehr, Nayband National Park Coast	2009	*Selar crumenophthalmus*	HQ149944	NPPF1153
NPPF1149	Iran: Bushehr, Nayband National Park Coast	2009	*Selar crumenophthalmus*	HQ149945	NPPF1149
NPPF1147	Iran: Bushehr, Nayband National Park Coast	2009	*Selar crumenophthalmus*	HQ149946	NPPF1147
NPPF1142	Iran: Bushehr, Nayband National Park Coast	2009	*Selar crumenophthalmus*	HQ149947	NPPF1142
NPPF1017	Iran: Bushehr, Nayband National Park Coast	2009	*Selar crumenophthalmus*	HQ149948	NPPF1017
UMTF03768	Kudat, Sabah (KDT)	2010	*Selaroides leptolepis*	JX261054	DBMF-M520
UMTF03724	Miri, Sarawak (MR)	2010	*Selaroides leptolepis*	JX261333	DBMF-M476
UMTF03725	Miri, Sarawak (MR)	2010	*Selaroides leptolepis*	JX261281	DBMF-M477
UMTF03726	Miri, Sarawak (MR)	2010	*Selaroides leptolepis*	JX261498	DBMF-M478
UMTF03727	Miri, Sarawak (MR)	2010	*Selaroides leptolepis*	JX261152	DBMF-M479
UMTF03363	Mersing, Peninsular Malaysia (MG)	2010	*Selaroides leptolepis*	JX261646	DBMF-M115
UMTF03541	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selaroides leptolepis*	JX261330	DBMF-M293
UMTF03540	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selaroides leptolepis*	JX261388	DBMF-M292
UMTF03875	Sandakan, Sabah (SDK)	2010	*Selaroides leptolepis*	JX261176	DBMF-M627
UMTF03873	Sandakan, Sabah (SDK)	2010	*Selaroides leptolepis*	JX261491	DBMF-M625

UMTF03362	Mersing, Peninsular Malaysia (MG)	2010	*Selaroides leptolepis*	JX261221	DBMF-M114
UMTF03361	Mersing, Peninsular Malaysia (MG)	2010	*Selaroides leptolepis*	JX261585	DBMF-M113
UMTF03462	Kuala Dungun, Peninsular Malaysia (KD)	2010	*Selaroides leptolepis*	JX261583	DBMF-M214
UMTF03504	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selaroides leptolepis*	JX261110	DBMF-M256
UMTF03503	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selaroides leptolepis*	JX261089	DBMF-M255
UMTF03502	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selaroides leptolepis*	JX261167	DBMF-M254
UMTF03501	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selaroides leptolepis*	JX261432	DBMF-M253
UMTF03500	Pulau Kambing, Peninsular Malaysia (PK)	2010	*Selaroides leptolepis*	JX261623	DBMF-M252
SB01-01	Kuala Sg. Baru, Peninsular Malaysia (SB)	2009	*Selaroides leptolepis*	HQ561005	DBMF-M77
UMTF03360	Mersing, Peninsular Malaysia (MG)	2010	*Selaroides leptolepis*	JX261286	DBMF-M112
UMTF03359	Mersing, Peninsular Malaysia (MG)	2010	*Selaroides leptolepis*	JX261137	DBMF-M111
UMTF03765	Kudat, Sabah (KDT)	2010	*Selaroides leptolepis*	JX261308	DBMF-M517
BP15-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Selaroides leptolepis*	HQ560969	DBMF-M34
UMTF03940	Tawau, Sabah (TW)	2010	*Selaroides leptolepis*	JX261440	DBMF-M692
UMTF03766	Kudat, Sabah (KDT)	2010	*Selaroides leptolepis*	JX261649	DBMF-M518
UMTF03769	Kudat, Sabah (KDT)	2010	*Selaroides leptolepis*	JX261099	DBMF-M521
UMTF03415	Kuantan, Peninsular Malaysia (KN)	2010	*Selaroides leptolepis*	JX261038	DBMF-M167
UMTF03414	Kuantan, Peninsular Malaysia (KN)	2010	*Selaroides leptolepis*	JX261032	DBMF-M166
UMTF03937	Tawau, Sabah (TW)	2010	*Selaroides leptolepis*	JX261454	DBMF-M689
UMTF03938	Tawau, Sabah (TW)	2010	*Selaroides leptolepis*	JX261390	DBMF-M690
UMTF03723	Miri, Sarawak (MR)	2010	*Selaroides leptolepis*	JX261265	DBMF-M475
UMTF03544	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selaroides leptolepis*	JX261014	DBMF-M296
UMTF03543	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selaroides leptolepis*	JX261241	DBMF-M295
UMTF03542	Kuala Besut, Peninsular Malaysia (KB)	2010	*Selaroides leptolepis*	JX261019	DBMF-M294
UMTF03936	Tawau, Sabah (TW)	2010	*Selaroides leptolepis*	JX261367	DBMF-M688
UMTF03418	Kuantan, Peninsular Malaysia (KN)	2010	*Selaroides leptolepis*	JX261163	DBMF-M170
UMTF03417	Kuantan, Peninsular Malaysia (KN)	2010	*Selaroides leptolepis*	JX261528	DBMF-M169
UMTF03416	Kuantan, Peninsular Malaysia (KN)	2010	*Selaroides leptolepis*	JX261620	DBMF-M168
GD 9086061	China	2006	*Selaroides leptolepis*	EF607545	FSCS086-06
GD 9086060	China	2006	*Selaroides leptolepis*	EF607546	FSCS085-06

GD 9086059	China	2006	*Selaroides leptolepis*	EF607547	FSCS084-06
GD 9086058	China	2006	*Selaroides leptolepis*	EF607548	FSCS083-06
GD 9081027	China	2006	*Selaroides leptolepis*	EF607549	FSCS179-06
GD 9086062	China	2006	*Selaroides leptolepis*	EF607550	FSCS087-06
UMTF04030	Kota Kinabalu, Sabah (KKJ)	2010	*Seriola dumerili*	JX261106	DBMF-M782
UMTF04031	Kota Kinabalu, Sabah (KKJ)	2010	*Seriola dumerili*	JX261426	DBMF-M783
UMTF04032	Kota Kinabalu, Sabah (KKJ)	2010	*Seriola dumerili*	JX261404	DBMF-M784
UMTF04033	Kota Kinabalu, Sabah (KKJ)	2010	*Seriola dumerili*	JX261257	DBMF-M785
ADC09_210.43#1	South Africa: Pumula	2009	*Seriola dumerili*	JF494498	DSFSF133-09.COI-5P
N/A	Japan: Nagasaki	2006	*Seriola dumerili*	NC016870	N/A
N/A	Japan: Kouchi	2006	*Seriola dumerili*	AB517559	N/A
N/A	Japan: Nagasaki	2006	*Seriola dumerili*	AB517558	N/A
ADC10_210.43 #6	South Africa:Park Rynie	2010	*Seriola dumerili*	HQ945927	DSFSG343-10.COI-5P
N/A	Turkey	N/A	*Seriola dumerili*	JQ623993	N/A
MBCSC:HN SY08340	China: South China Sea	2008	*Seriola dumerili*	FJ237927	CFCS024-08
BIOUG<CAN>:BW-A1492	Australia: Western Australia	1995	*Seriola dumerili*	EF609458	FOAC493-05
ADC09_210.43#4	South Africa: Pumula	2009	*Seriola dumerili*	JF494496	DSFSF136-09.COI-5P
ADC09_210.43#3	South Africa: Pumula	2009	*Seriola dumerili*	JF494495	BOLD:DSFSF135-09.COI-5P
ADC09_210.43#2	South Africa: Pumula	2009	*Seriola dumerili*	JF494497	DSFSF134-09.COI-5P
MBCSC:HN SY08576	China: South China Sea	2008	*Seriola dumerili*	FJ237923	CFCS260-08
MBCSC:HN SY08575	China: South China Sea	2008	*Seriola dumerili*	FJ237924	CFCS259-08
MBCSC:HN SY08574	China: South China Sea	2008	*Seriola dumerili*	FJ237925	CFCS258-08
MBCSC:HN SY08578	China: South China Sea	2008	*Seriola dumerili*	FJ237921	CFCS262-08

MBCSC:HN SY08358	China: South China Sea	2008	*Seriola dumerili*	FJ237926	CFCS042-08
MBCSC:HN SY08577	China: South China Sea	2008	*Seriola dumerili*	FJ237922	CFCS261-08
UMTF04035	Kota Kinabalu, Sabah (KKJ)	2010	*Seriolina nigrofasciata*	JX261025	DBMF-M787
UMTF04036	Kota Kinabalu, Sabah (KKJ)	2010	*Seriolina nigrofasciata*	JX260996	DBMF-M788
UMTF04037	Kota Kinabalu, Sabah (KKJ)	2010	*Seriolina nigrofasciata*	JX261240	DBMF-M789
UMTF04038	Kota Kinabalu, Sabah (KKJ)	2010	*Seriolina nigrofasciata*	JX261538	DBMF-M790
UMTF03813	Kudat, Sabah (KDT)	2010	*Seriolina nigrofasciata*	JX261648	DBMF-M567
UMTF03814	Kudat, Sabah (KDT)	2010	*Seriolina nigrofasciata*	JX261335	DBMF-M568
AHM17-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Seriolina nigrofasciata*	HQ560985	DBMF-M52
UMTF03879	Sandakan, Sabah (SDK)	2010	*Seriolina nigrofasciata*	JX261162	DBMF-M631
BP14-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Seriolina nigrofasciata*	HQ560968	DBMF-M33
NPPF1099	Iran: Bushehr, Nayband National Park Coast	2009	*Seriolina nigrofasciata*	HQ149949	NPPF1099
NPPF1085	Iran: Bushehr, Nayband National Park Coast	2009	*Seriolina nigrofasciata*	HQ149950	NPPF1085
WL-M74	India	N/A	*Seriolina nigrofasciata*	EU014234	N/A
WL-M75	India	N/A	*Seriolina nigrofasciata*	EU014235	N/A
WL-M76	India	N/A	*Seriolina nigrofasciata*	EU014236	N/A
UMTF03357	Mersing, Peninsular Malaysia (MG)	2010	*Trachinotus baillonii*	JX261097	DBMF-M109
UMTF03356	Mersing, Peninsular Malaysia (MG)	2010	*Trachinotus baillonii*	JX261081	DBMF-M108
UMTF03355	Mersing, Peninsular Malaysia (MG)	2010	*Trachinotus baillonii*	JX261175	DBMF-M107
UMTF03354	Mersing, Peninsular Malaysia (MG)	2010	*Trachinotus baillonii*	JX261383	DBMF-M106
BIOUG<CAN>:BW-A1406	Australia: Queensland	1999	*Trachinotus baillonii*	EF609480	FOAC407-05
MBIO1276.4	French Polynesia: Society Islands, Moorea	2006	*Trachinotus baillonii*	JQ432196	MBFA763-07.COI-5P
MBIO1437.4	French Polynesia: Society Islands, Moorea	2006	*Trachinotus baillonii*	JQ432197	MBFA842-07.COI-5P
UMTF04071	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Uraspis uraspis*	JX261636	DBMF-M823

UMTF03832	Sandakan, Sabah (SDK)	2010	*Uraspis uraspis*	JX261300	DBMF-M584
BP05-01	Bagan Panchor, Peninsular Malaysia (BP)	2009	*Uraspis uraspis*	JX261251	DBMF-M24
AHM16-01	Hutan Melintang, Peninsular Malaysia (AHM)	2009	*Uraspis uraspis*	HQ560984	DBMF-M51
UMTF04070	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Uraspis uraspis*	JX261028	DBMF-M822
UMTF03833	Sandakan, Sabah (SDK)	2010	*Uraspis uraspis*	JX261040	DBMF-M585
UMTF04069	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Uraspis uraspis*	JX261408	DBMF-M821
UMTF03803	Kudat, Sabah (KDT)	2010	*Uraspis uraspis*	JX261207	DBMF-M555
UMTF03816	Kudat, Sabah (KDT)	2010	*Uraspis uraspis*	JX261072	DBMF-M559
UMTF03989	Kota Kinabalu, Sabah (KKJ)	2010	*Uraspis uraspis*	JX261088	DBMF-M741
UMTF03988	Kota Kinabalu, Sabah (KKJ)	2010	*Uraspis uraspis*	JX261042	DBMF-M740
UMTF03987	Kota Kinabalu, Sabah (KKJ)	2010	*Uraspis uraspis*	JX261151	DBMF-M739
UMTF03986	Kota Kinabalu, Sabah (KKJ)	2010	*Uraspis uraspis*	JX261256	DBMF-M738
UMTF04072	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Uraspis uraspis*	JX261168	DBMF-M824
UMTF04073	Kuala Perlis, Peninsular Malaysia (KP)	2010	*Uraspis uraspis*	JX261577	DBMF-M825
UMTF03834	Sandakan, Sabah (SDK)	2010	*Uraspis uraspis*	JX261212	DBMF-M586
UMTF03985	Kota Kinabalu, Sabah (KKJ)	2010	*Uraspis uraspis*	JX261495	DBMF-M737
UMTF03815	Kudat, Sabah (KDT)	2010	*Uraspis uraspis*	JX261541	DBMF-M558
UMTF03805	Kudat, Sabah (KDT)	2010	*Uraspis uraspis*	JX261497	DBMF-M557
UMTF03835	Sandakan, Sabah (SDK)	2010	*Uraspis uraspis*	JX261317	DBMF-M587
UMTF03836	Sandakan, Sabah (SDK)	2010	*Uraspis uraspis*	JX261132	DBMF-M588
UMTF03804	Kudat, Sabah (KDT)	2010	*Uraspis uraspis*	JX261485	DBMF-M556
NPPF1125	Iran: Bushehr, Nayband National Park Coast	2009	*Uraspis uraspis*	HQ149964	NPPF1125

Appendix 2

Biological characteristics attributed to Carangidae taxa examined.

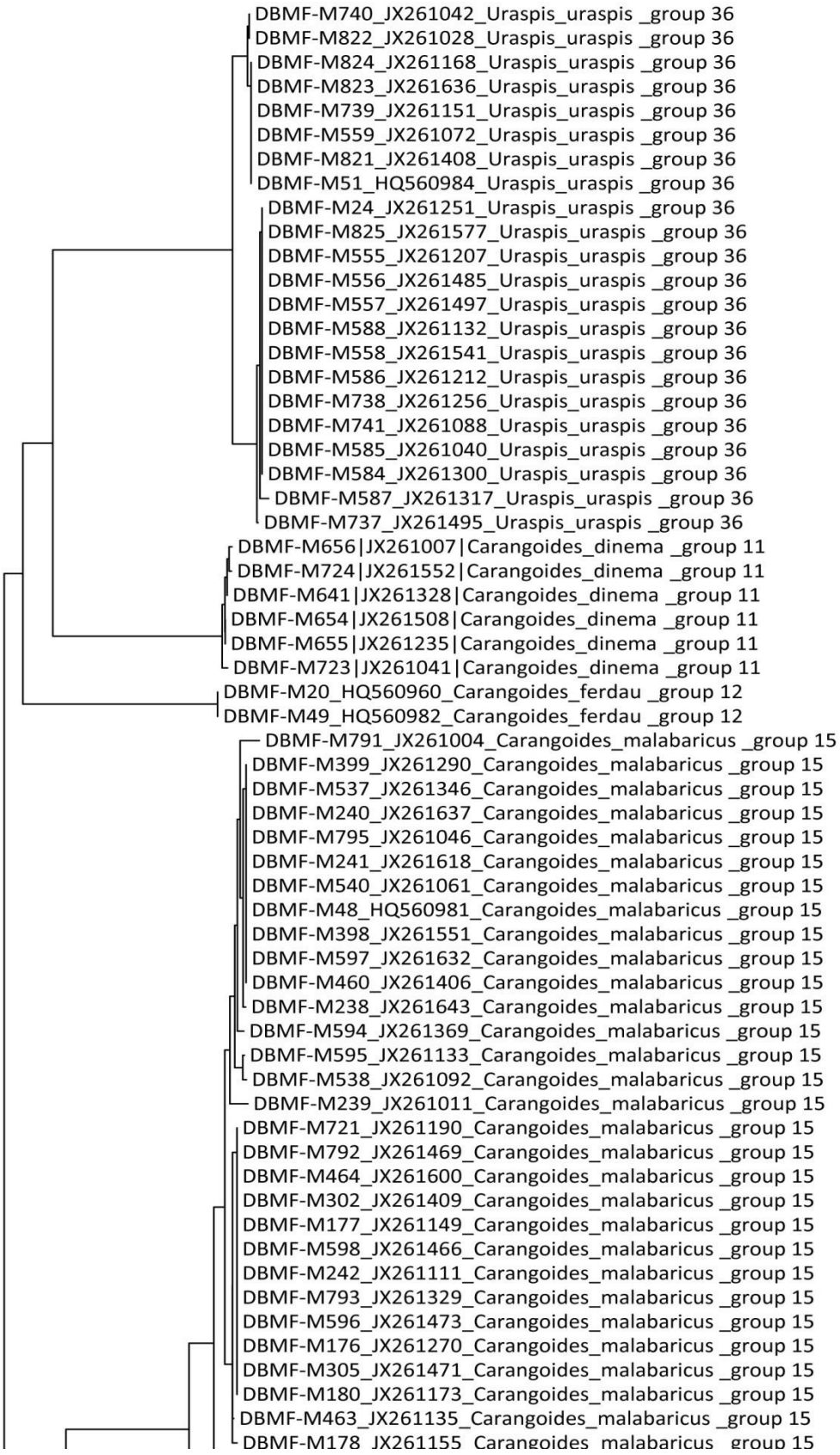
Species	Biological Characteristics		
	Body shape	Maximum body size*	Habitat use
Alectis ciliaris	Compressed	Large	Semi demersal/pelagic
Alectis indicus	Compressed	Large	Semi demersal/pelagic
Alepes djedaba	Compressed	Small	Pelagic
Alepes kleinii	Compressed	Small	Pelagic
Alepes melanoptera	Compressed	Small	Pelagic
Alepes vari	Moderately compressed	Small	Pelagic
Atropus atropus	Compressed	Small	Semi demersal/pelagic
Atule mate	Moderately compressed	Small	Pelagic
Carangoides bajad	Moderately compressed	Small	Semi demersal/pelagic
Carangoides chrysophrys	Compressed	Medium	Demersal
Carangoides dinema	Moderately compressed	Medium	Semi demersal/pelagic
Carangoides ferdau	Compressed	Medium	Semi demersal/pelagic
Carangoides fulvoguttatus	Round	Medium	Semi demersal/pelagic
Carangoides gymnostethus	Moderately compressed	Medium	Semi demersal/pelagic
Carangoides hedlandensis	Compressed	Small	Demersal
Carangoides malabaricus	Compressed	Small	Demersal
Caranx ignobilis	Moderately compressed	Large	Pelagic
Caranx sexfasciatus	Compressed	Medium	Demersal
Caranx tille	Compressed	Medium	Semi demersal/pelagic
Decapterus kurroides	Round	Small	Pelagic
Decapterus macrosoma	Round	Small	Pelagic
Decapterus maruadsi	Round	Small	Pelagic
Elagatis bipinnulata	Round	Large	Pelagic
Gnathanodon speciosus	Compressed	Medium	Demersal
Megalaspis cordyla	Round	Medium	Pelagic

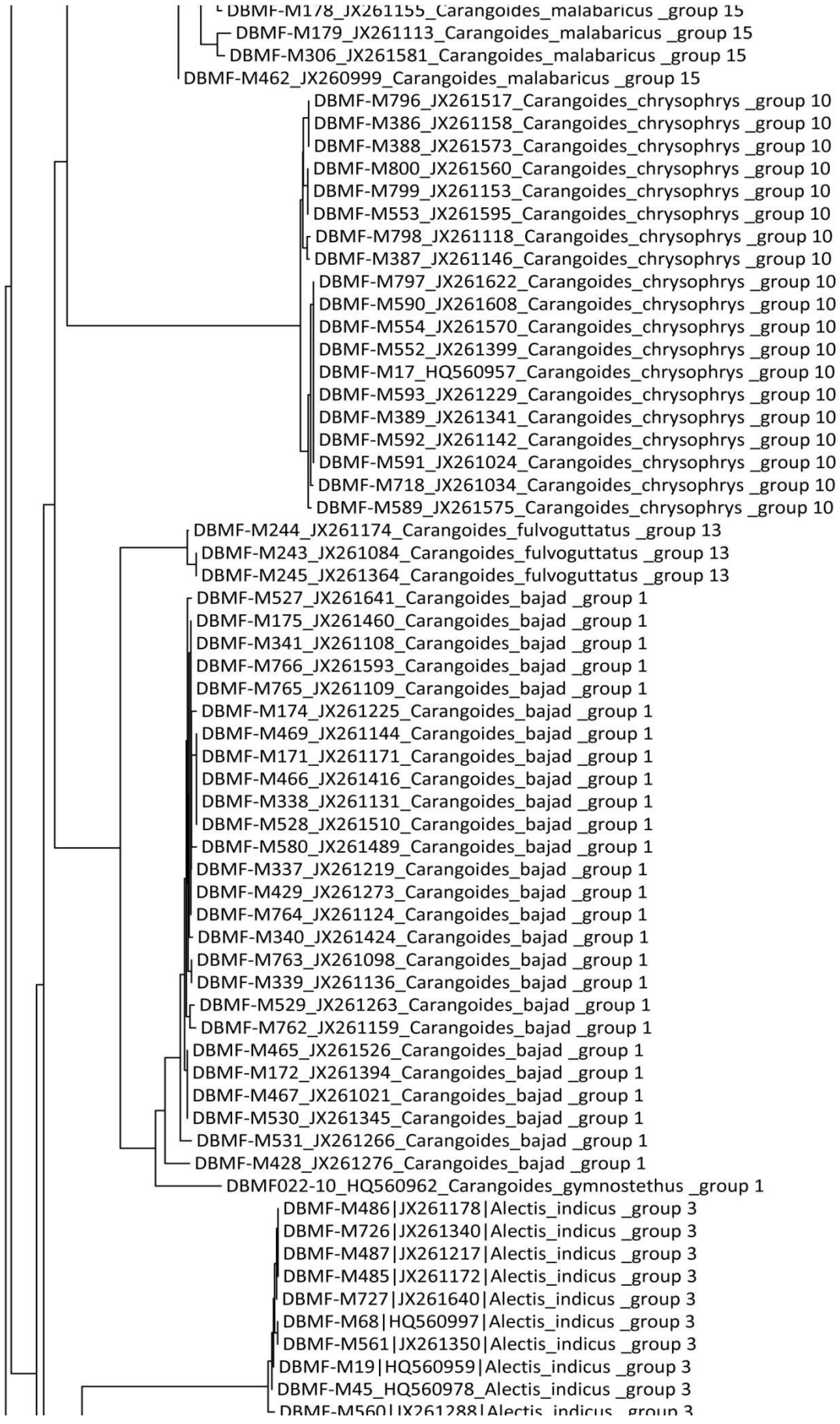
Parastromateus niger	Compressed	Medium	Pelagic
Scomberoides commersonnianus	Compressed	Medium	Pelagic
Scomberoides tala	Compressed	Medium	Pelagic
Scomberoides tol	Compressed	Small	Pelagic
Selar boops	Moderately compressed	Small	Pelagic
Selar crumenophthalmus	Moderately compressed	Small	Pelagic
Selaroides leptolepis	Moderately compressed	Small	Demersal
Seriola dumerili	Round	Large	Pelagic
Seriolina nigrofasciata	Round	Medium	Demersal
Trachinotus baillonii	Compressed	Small	Pelagic
Uraspis uraspis	Compressed	Small	Demersal

*Small = 0-63cm; Medium = 64-127cm; Large = 128-190 cm

Appendix 3

Tree corresponding to partition detected by ABGD method.




DBMF-M178_JX261155_Carangoides_malabaricus _group 15
DBMF-M179_JX261113_Carangoides_malabaricus _group 15
DBMF-M306_JX261581_Carangoides_malabaricus _group 15
DBMF-M462_JX260999_Carangoides_malabaricus _group 15
DBMF-M796_JX261517_Carangoides_chrysophrys _group 10
DBMF-M386_JX261158_Carangoides_chrysophrys _group 10
DBMF-M388_JX261573_Carangoides_chrysophrys _group 10
DBMF-M800_JX261560_Carangoides_chrysophrys _group 10
DBMF-M799_JX261153_Carangoides_chrysophrys _group 10
DBMF-M553_JX261595_Carangoides_chrysophrys _group 10
DBMF-M798_JX261118_Carangoides_chrysophrys _group 10
DBMF-M387_JX261146_Carangoides_chrysophrys _group 10
DBMF-M797_JX261622_Carangoides_chrysophrys _group 10
DBMF-M590_JX261608_Carangoides_chrysophrys _group 10
DBMF-M554_JX261570_Carangoides_chrysophrys _group 10
DBMF-M552_JX261399_Carangoides_chrysophrys _group 10
DBMF-M17_HQ560957_Carangoides_chrysophrys _group 10
DBMF-M593_JX261229_Carangoides_chrysophrys _group 10
DBMF-M389_JX261341_Carangoides_chrysophrys _group 10
DBMF-M592_JX261142_Carangoides_chrysophrys _group 10
DBMF-M591_JX261024_Carangoides_chrysophrys _group 10
DBMF-M718_JX261034_Carangoides_chrysophrys _group 10
DBMF-M589_JX261575_Carangoides_chrysophrys _group 10
DBMF-M244_JX261174_Carangoides_fulvoguttatus _group 13
DBMF-M243_JX261084_Carangoides_fulvoguttatus _group 13
DBMF-M245_JX261364_Carangoides_fulvoguttatus _group 13
DBMF-M527_JX261641_Carangoides_bajad _group 1
DBMF-M175_JX261460_Carangoides_bajad _group 1
DBMF-M341_JX261108_Carangoides_bajad _group 1
DBMF-M766_JX261593_Carangoides_bajad _group 1
DBMF-M765_JX261109_Carangoides_bajad _group 1
DBMF-M174_JX261225_Carangoides_bajad _group 1
DBMF-M469_JX261144_Carangoides_bajad _group 1
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DBMF-M528_JX261510_Carangoides_bajad _group 1
DBMF-M580_JX261489_Carangoides_bajad _group 1
DBMF-M337_JX261219_Carangoides_bajad _group 1
DBMF-M429_JX261273_Carangoides_bajad _group 1
DBMF-M764_JX261124_Carangoides_bajad _group 1
DBMF-M340_JX261424_Carangoides_bajad _group 1
DBMF-M763_JX261098_Carangoides_bajad _group 1
DBMF-M339_JX261136_Carangoides_bajad _group 1
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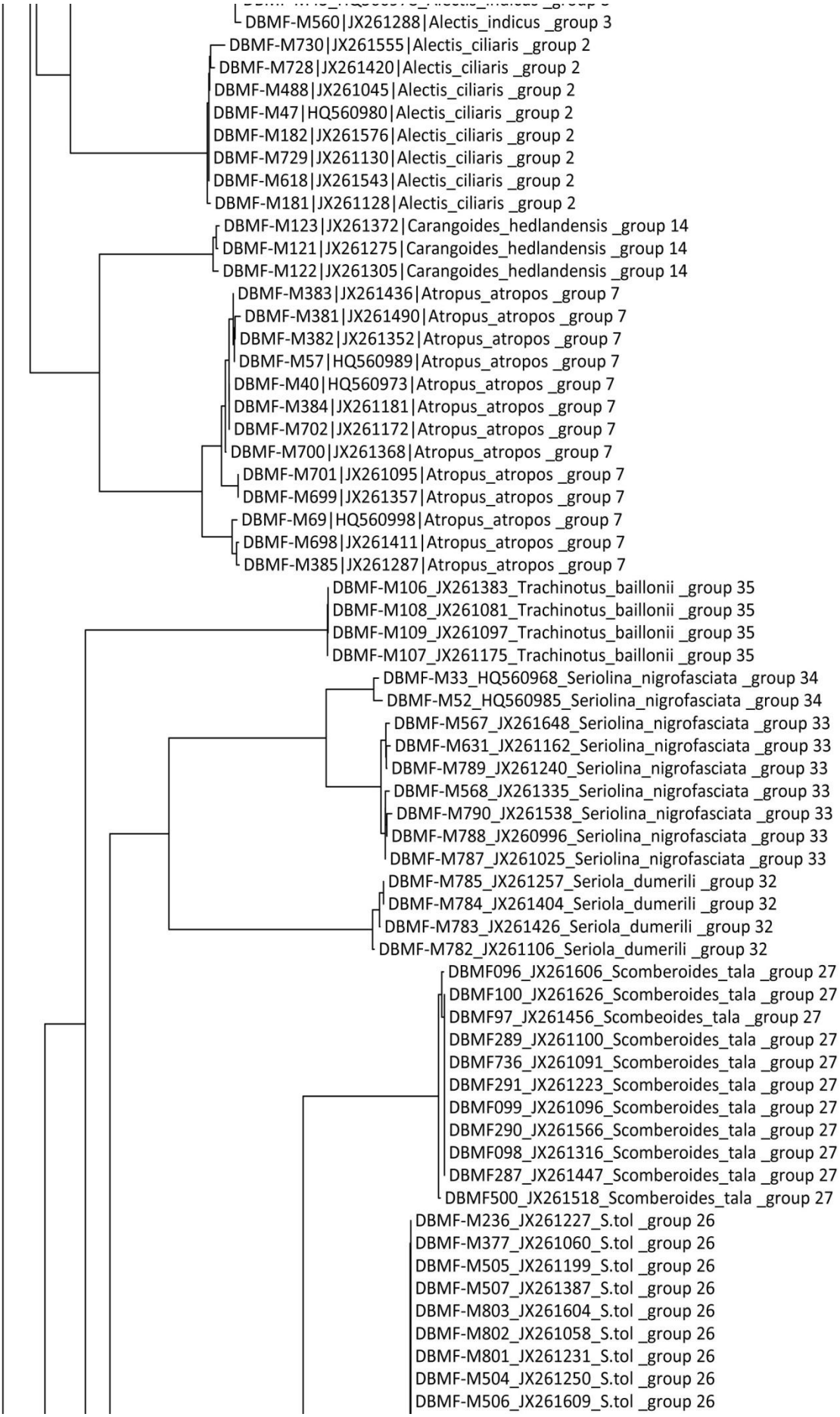
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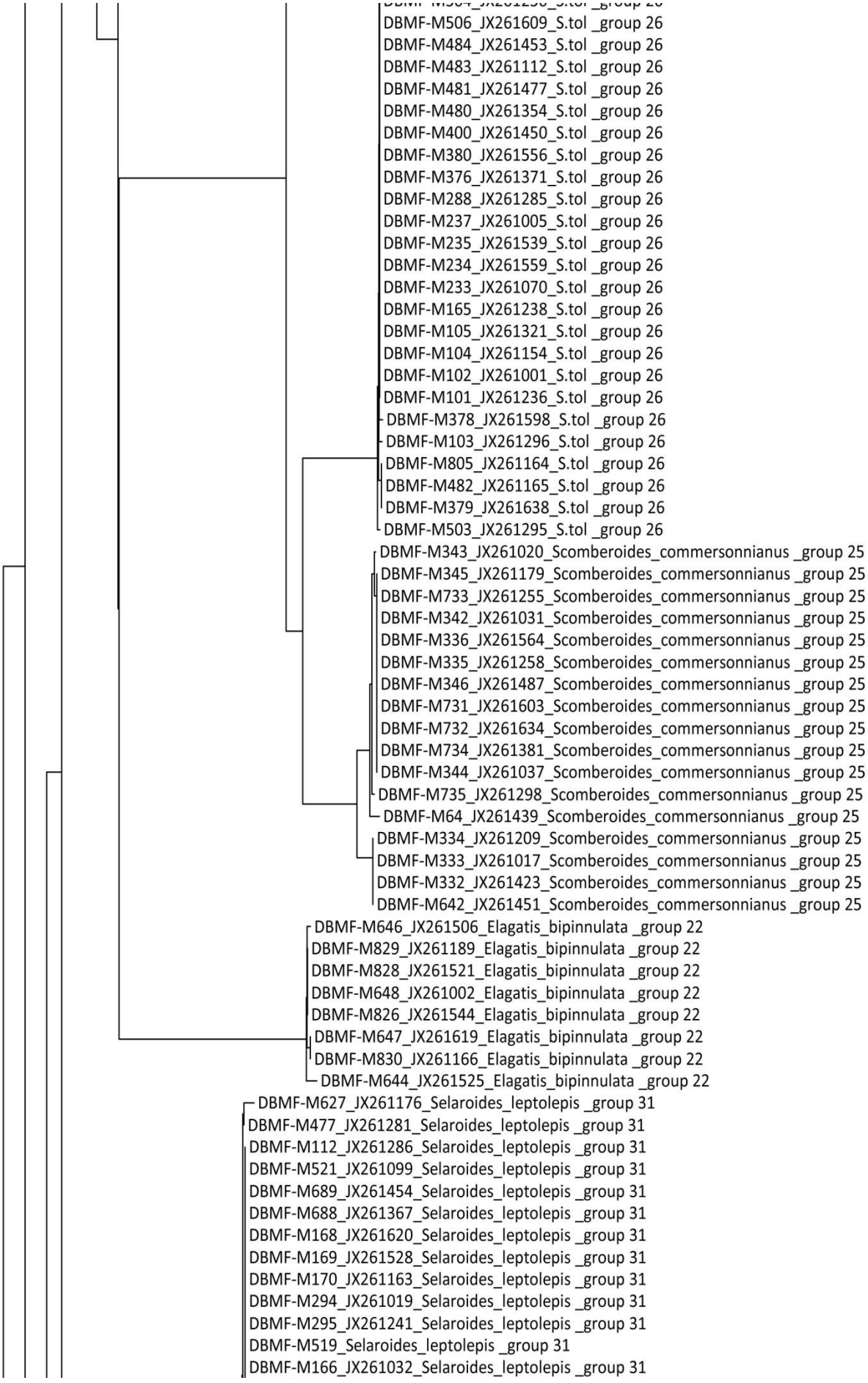
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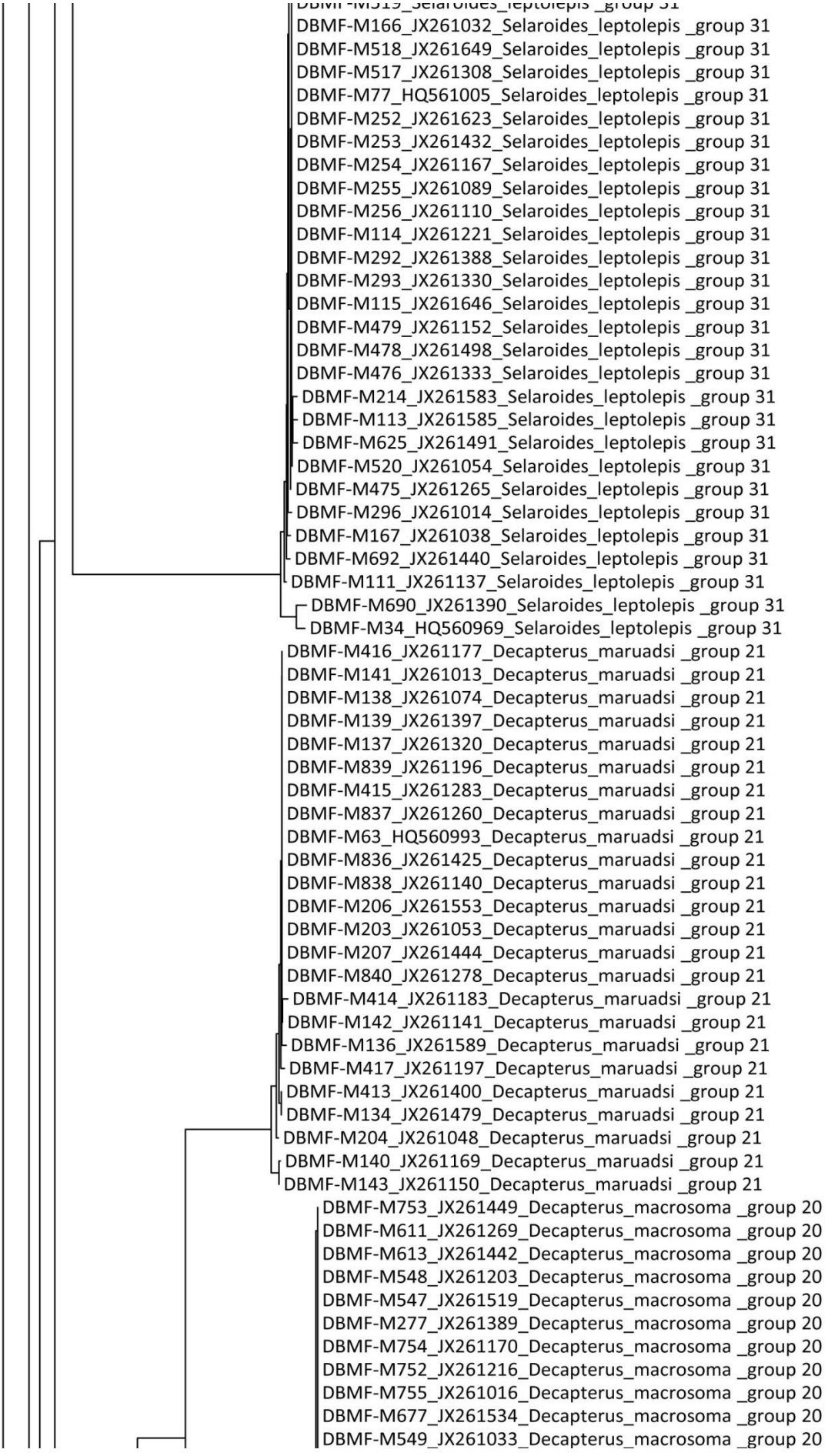
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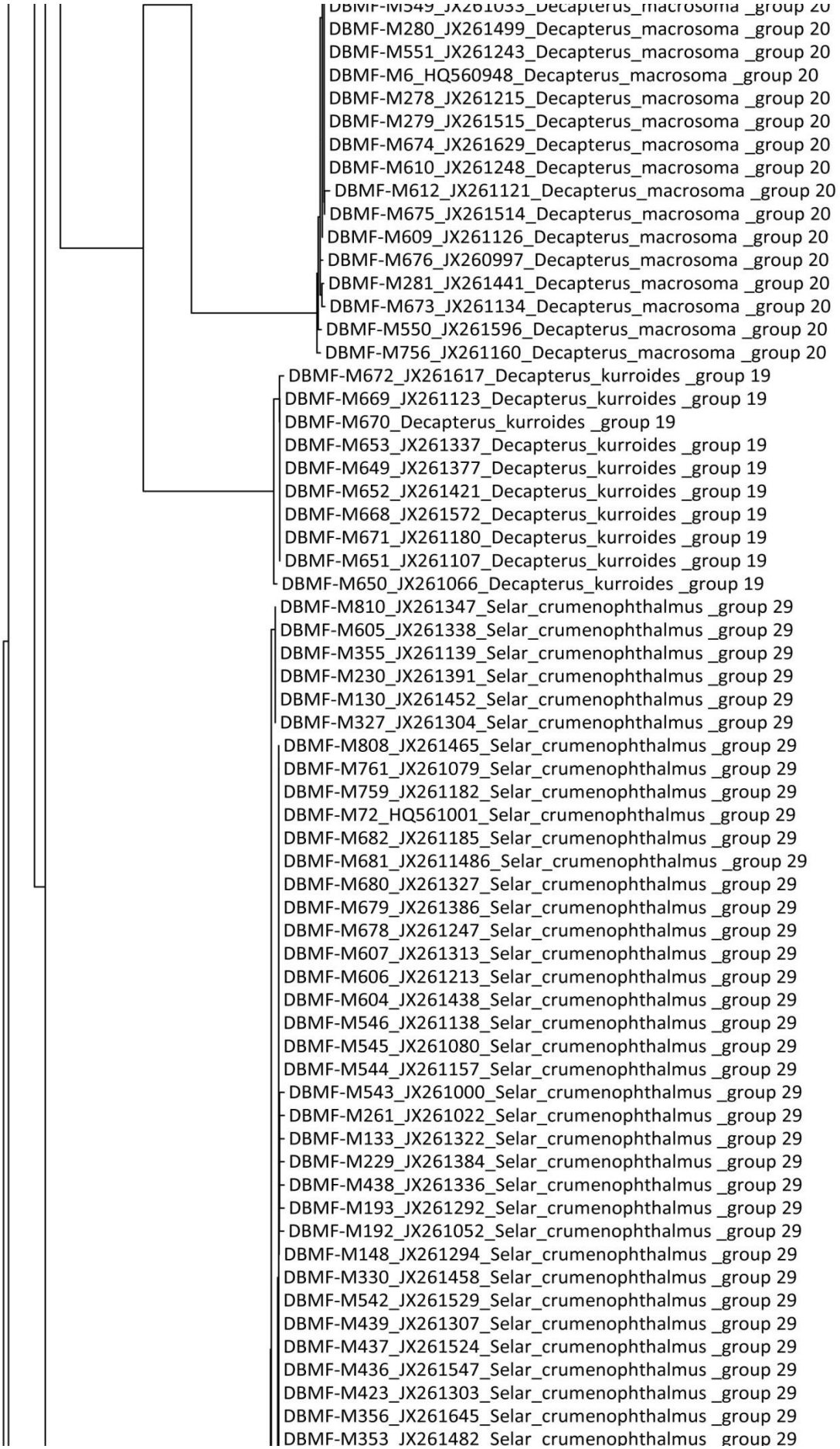
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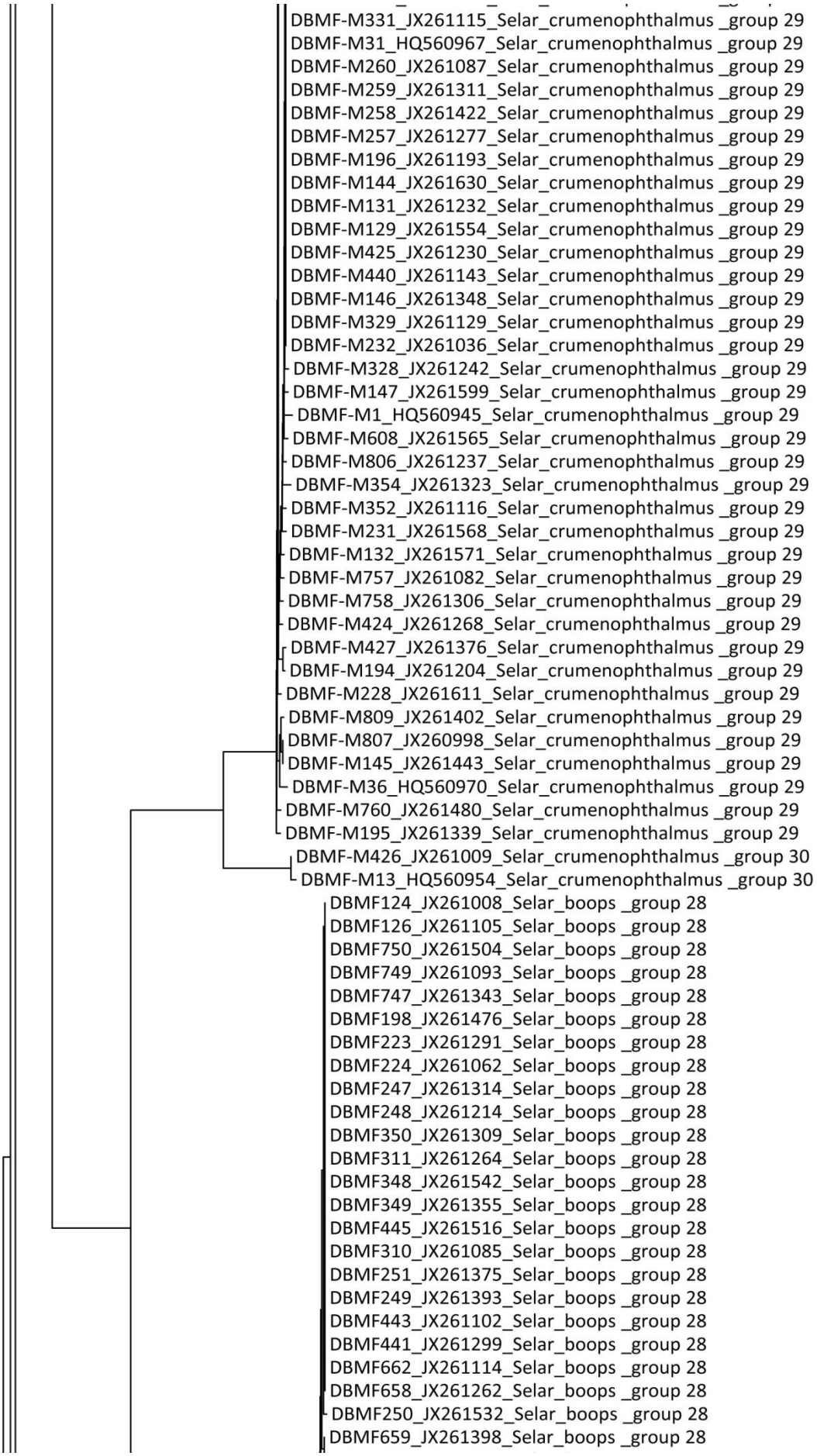
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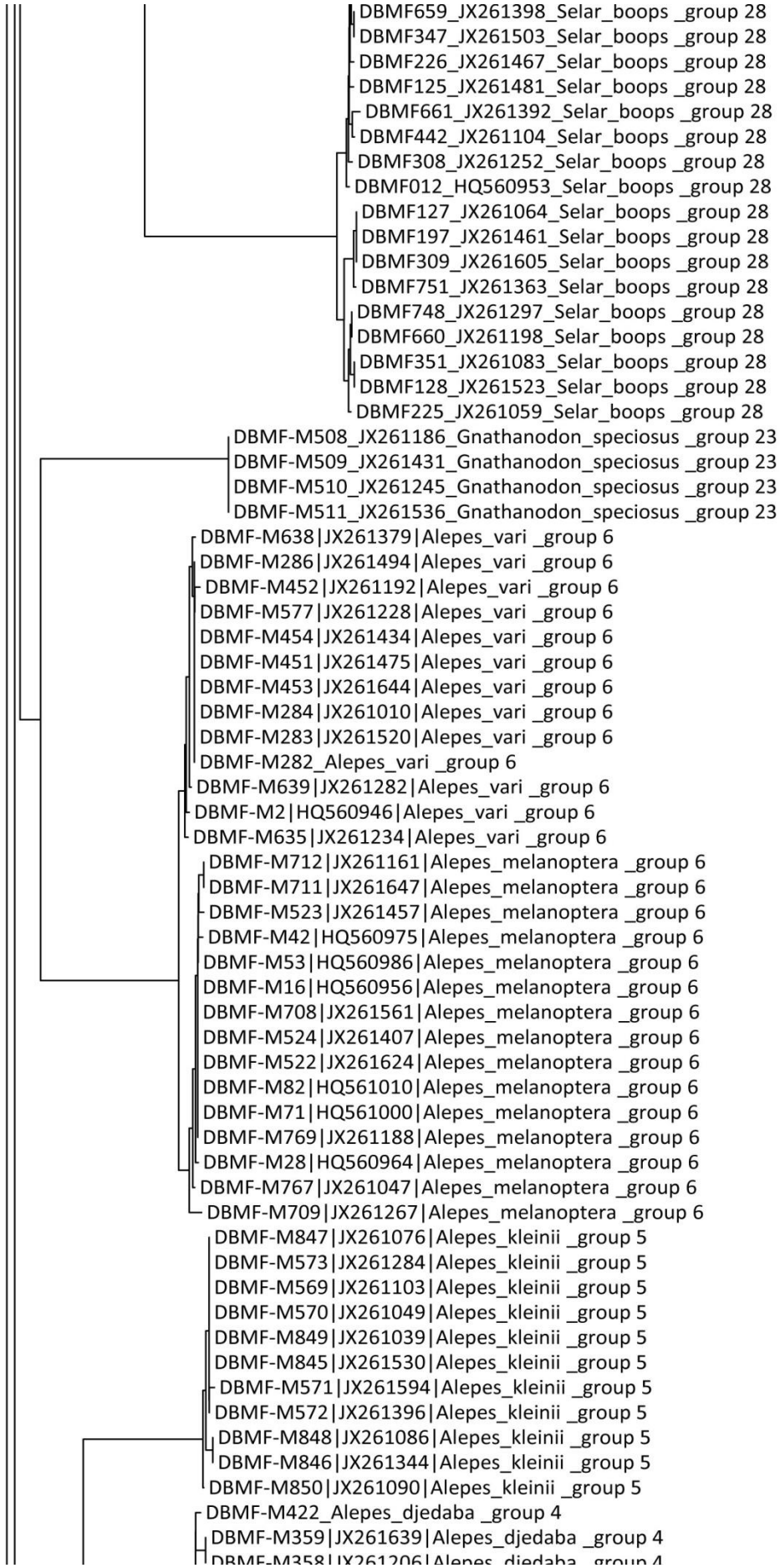
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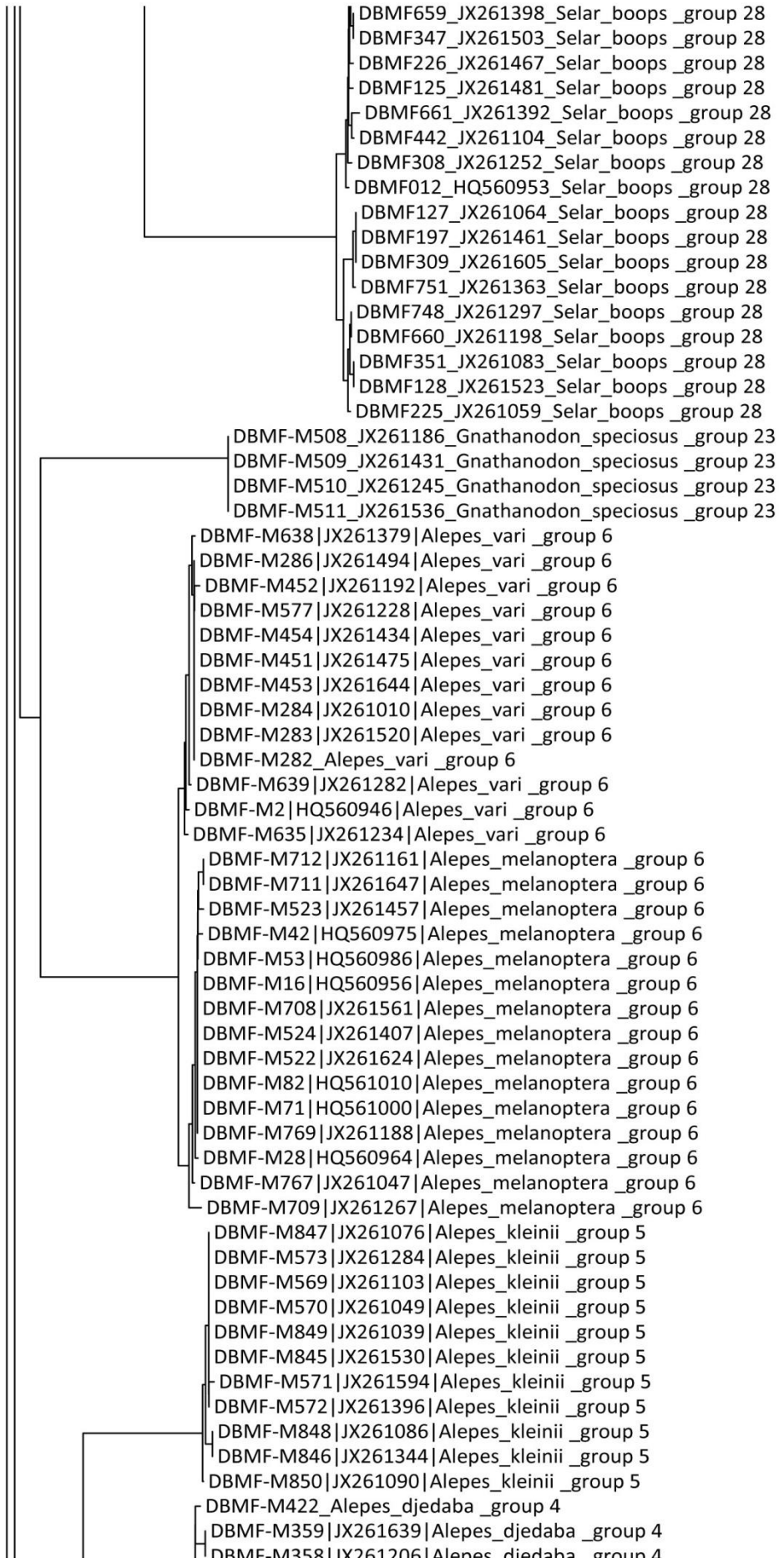
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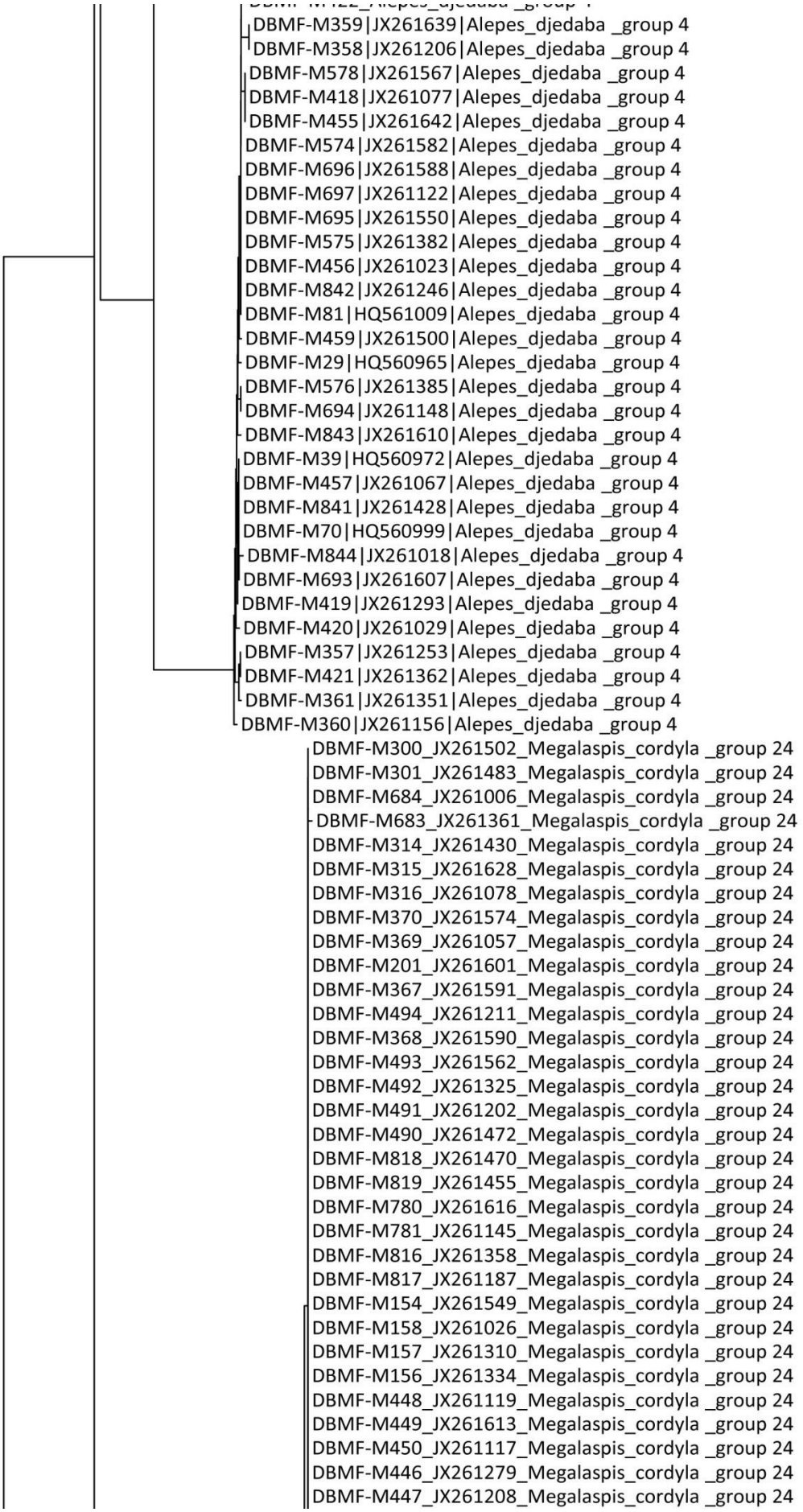
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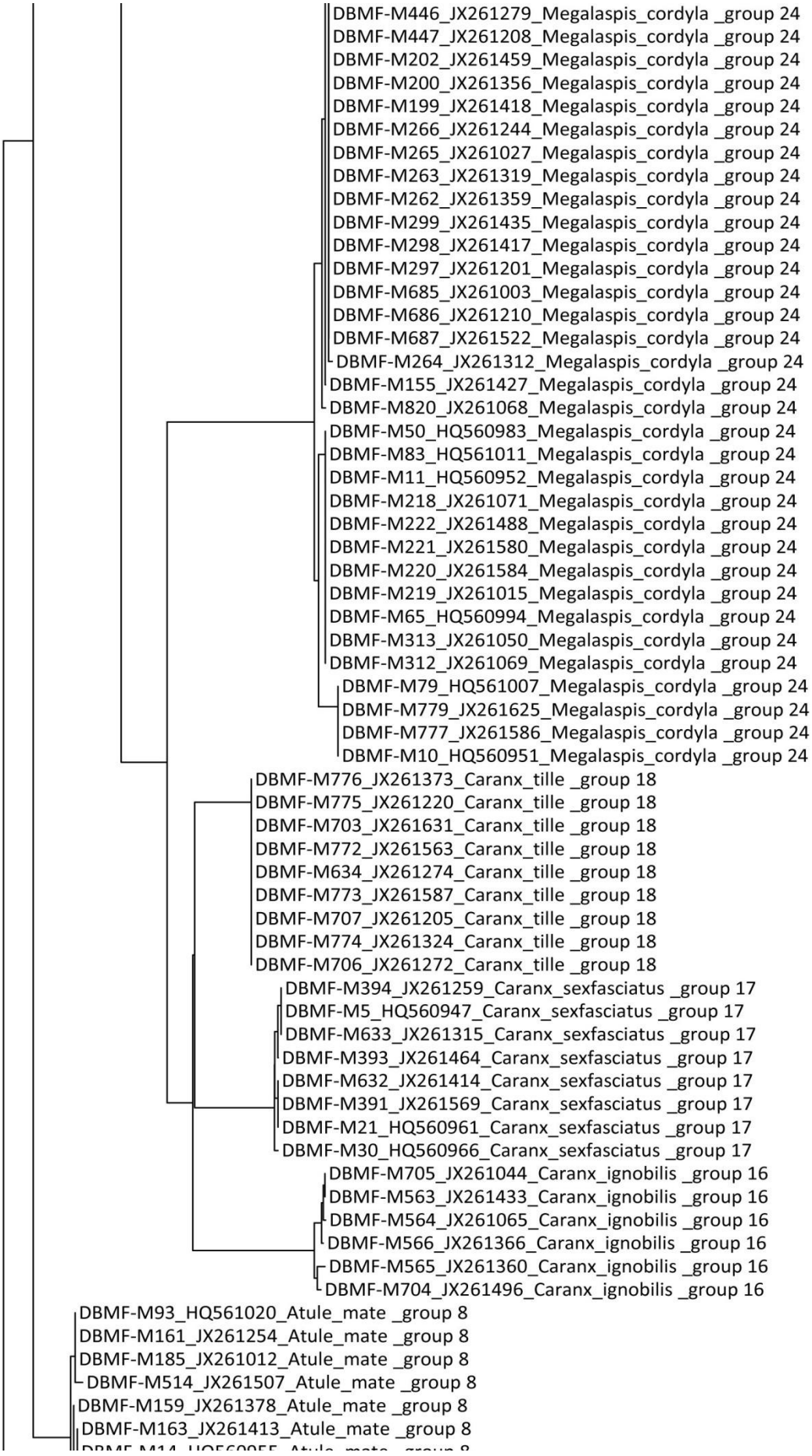
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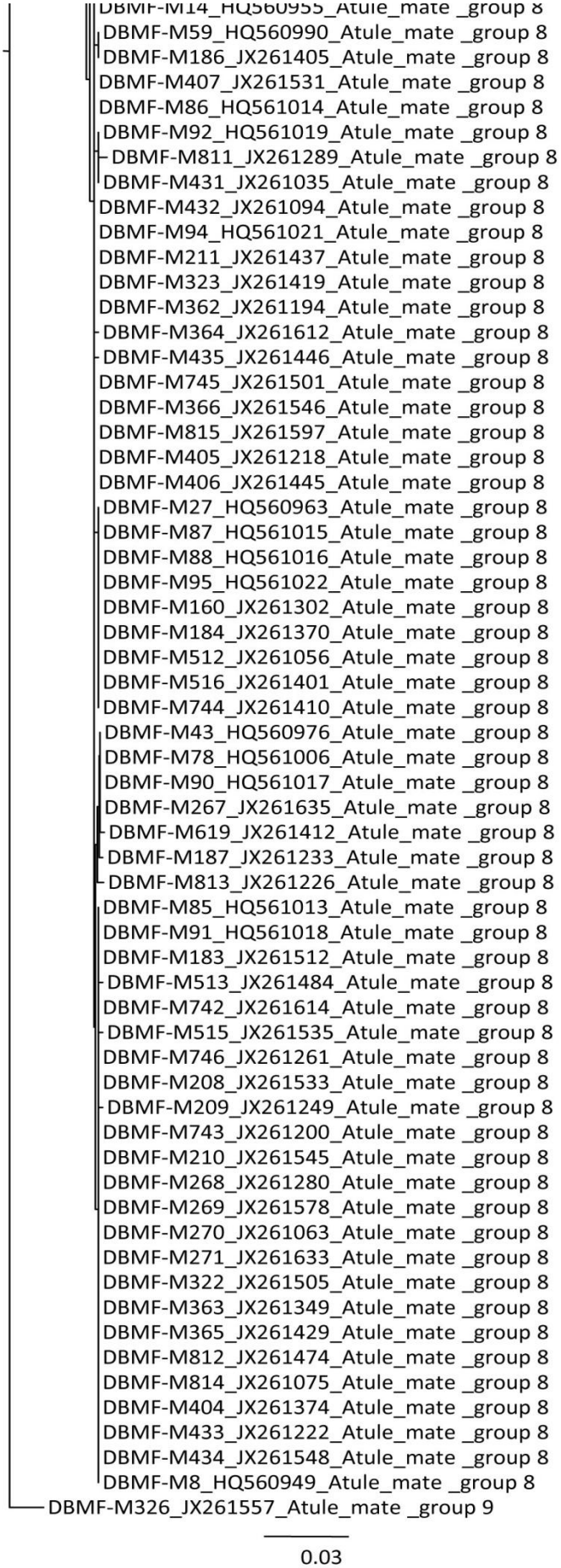
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DBMF-M744_JX261410_Atule_mate _group 8
DBMF-M43_HQ560976_Atule_mate _group 8
DBMF-M78_HQ561006_Atule_mate _group 8
DBMF-M90_HQ561017_Atule_mate _group 8
DBMF-M267_JX261635_Atule_mate _group 8
DBMF-M619_JX261412_Atule_mate _group 8
DBMF-M187_JX261233_Atule_mate _group 8
DBMF-M813_JX261226_Atule_mate _group 8
DBMF-M85_HQ561013_Atule_mate _group 8
DBMF-M91_HQ561018_Atule_mate _group 8
DBMF-M183_JX261512_Atule_mate _group 8
DBMF-M513_JX261484_Atule_mate _group 8
DBMF-M742_JX261614_Atule_mate _group 8
DBMF-M515_JX261535_Atule_mate _group 8
DBMF-M746_JX261261_Atule_mate _group 8
DBMF-M208_JX261533_Atule_mate _group 8
DBMF-M209_JX261249_Atule_mate _group 8
DBMF-M743_JX261200_Atule_mate _group 8
DBMF-M210_JX261545_Atule_mate _group 8
DBMF-M268_JX261280_Atule_mate _group 8
DBMF-M269_JX261578_Atule_mate _group 8
DBMF-M270_JX261063_Atule_mate _group 8
DBMF-M271_JX261633_Atule_mate _group 8
DBMF-M322_JX261505_Atule_mate _group 8
DBMF-M363_JX261349_Atule_mate _group 8
DBMF-M365_JX261429_Atule_mate _group 8
DBMF-M812_JX261474_Atule_mate _group 8
DBMF-M814_JX261075_Atule_mate _group 8
DBMF-M404_JX261374_Atule_mate _group 8
DBMF-M433_JX261222_Atule_mate _group 8
DBMF-M434_JX261548_Atule_mate _group 8
DBMF-M8_HQ560949_Atule_mate _group 8
DBMF-M326_JX261557_Atule_mate _group 9
0.03

Appendix 4

Kimura 2-parameter pairwise distances for each Indo-Malay Carangidae species.

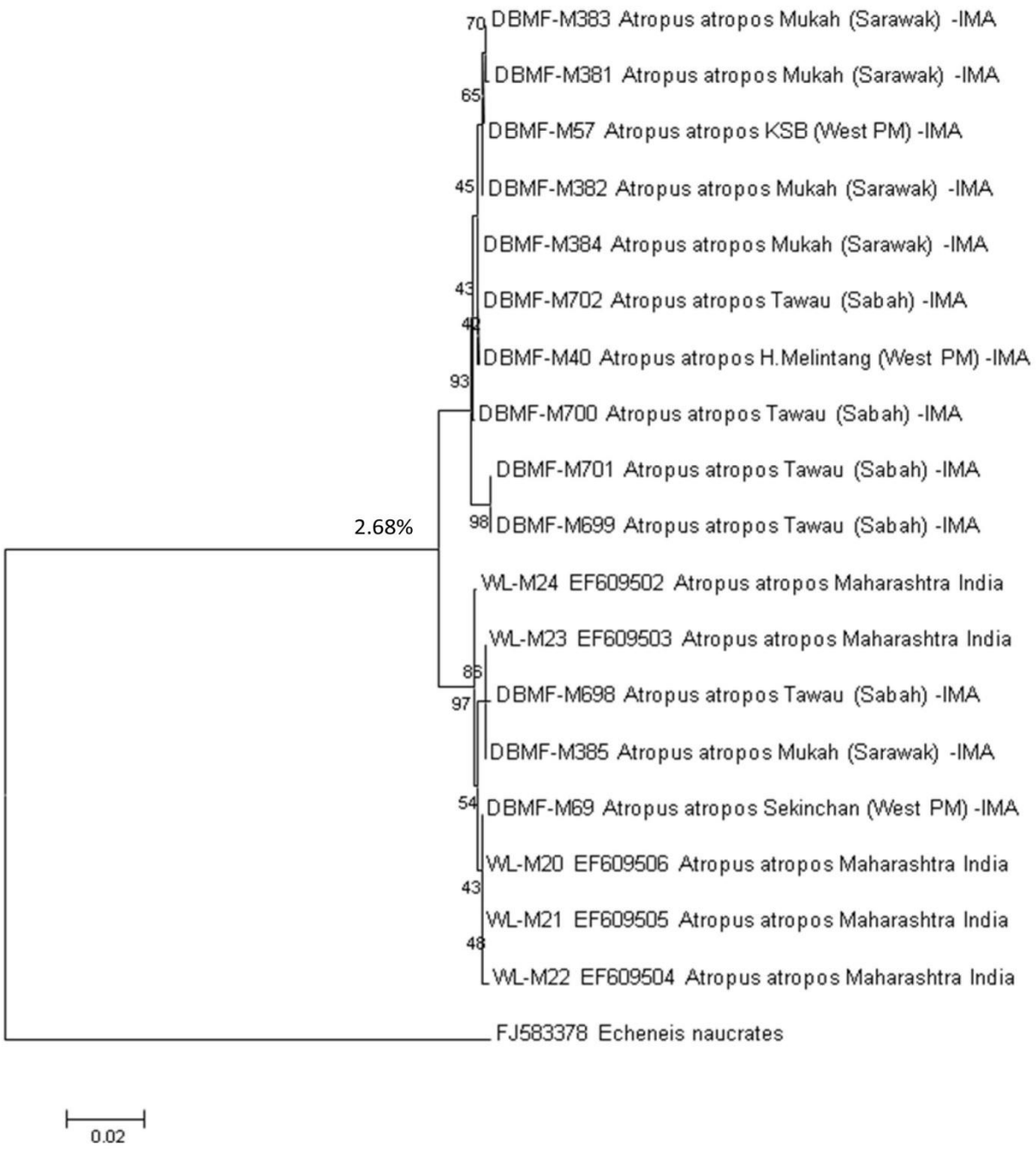
Species	**Common name**	**n**	**min**	**mean**	**max**	**SE**
Alectis ciliaris	African pompano	8	0	0.16	0.63	0.04
Alectis indicus	Indian threadfish	10	0	0.17	0.62	0.02
Alepes djedaba	Shrimp scad	31	0	0.25	0.62	0.01
Alepes kleinii	Razorbelly scad	11	0	0.16	0.46	0.02
Alepes melanoptera	Blackfin scad	15	0	0.40	1.65	0.03
Alepes vari	Herring scad	13	0	0.16	0.64	0.02
Atropus atropus	Cleftbelly trevally	13	0	1.13	2.68	0.11
Atule mate	Yellowtail scad	67	0	0.34	4.82	0.02
Carangoides bajad	Orangespotted trevally	26	0	0.39	1.93	0.02
Carangoides chrysophrys	Longnose trevally	19	0	0.33	0.81	0.02
Carangoides dinema	Shadow trevally	6	0	0.03	0.16	0.02
Carangoides ferdau	Blue trevally	2				
Carangoides fulvoguttatus	Yellowspotted trevally	3	0	0.21	0.31	0.09
Carangoides gymnostethus	Bludger	1				
Carangoides hedlandensis	Bumpnose trevally	3	0.16	0.31	0.47	0.07
Carangoides malabaricus	Malabar trevally	33	0	0.54	2.05	0.16
Caranx ignobilis	Tille trevally	6	0	0.506	1.09	0.09
Caranx sexfasciatus	Redtail scad	8	0	0.16	0.31	0.02
Caranx tille	Shortfin scad	9	0	0.07	0.31	0.02
Decapterus kurroides	Round scad/ Japanese scad	10	0	0.09	0.47	0.02
Decapterus macrosoma	Rainbow runner	26	0	0.08	0.48	0.01

Decapterus maruadsi	Golden trevally	24	0	0.15	0.66	0.01
Elagatis bipinnulata	Torpedo scad	8	0	0.22	0.63	0.04
Gnathanodon speciosus	Black pomfret	4	0	0	0	0
Megalaspis cordyla	Talang queenfish	63	0	0.53	2.06	0.01
Parastromateus niger	Barred queenfish	51	0	0.3	1.09	0.01
Scomberoides commersonnianus	Needlescaled queenfish	17	0	0.56	1.78	0.05
Scomberoides tala	Oxeye scad	11	0	0.08	0.34	0.01
Scomberoides tol	Bigeye scad	32	0	0.09	0.46	0.01
Selar boops	Yellowstripe scad	40	0	0.37	1.27	0.01
Selar crumenophthalmus	Greater amberjack	75	0	0.39	4.66	0.02
Selaroides leptolepis	Blackbanded trevally	39	0	0.18	1.62	0.01
Seriola dumerili	Small spotted dart	4	0	0.31	0.47	0.06
Seriolina nigrofasciata	Snubnose pompano	9	0	1.79	4.317	0.30
Trachinotus baillonii	Whitemouth jack	4	0	0	0	0
Uraspis uraspis	Whitemouth jack	22	0	0.67	1.72	0.04

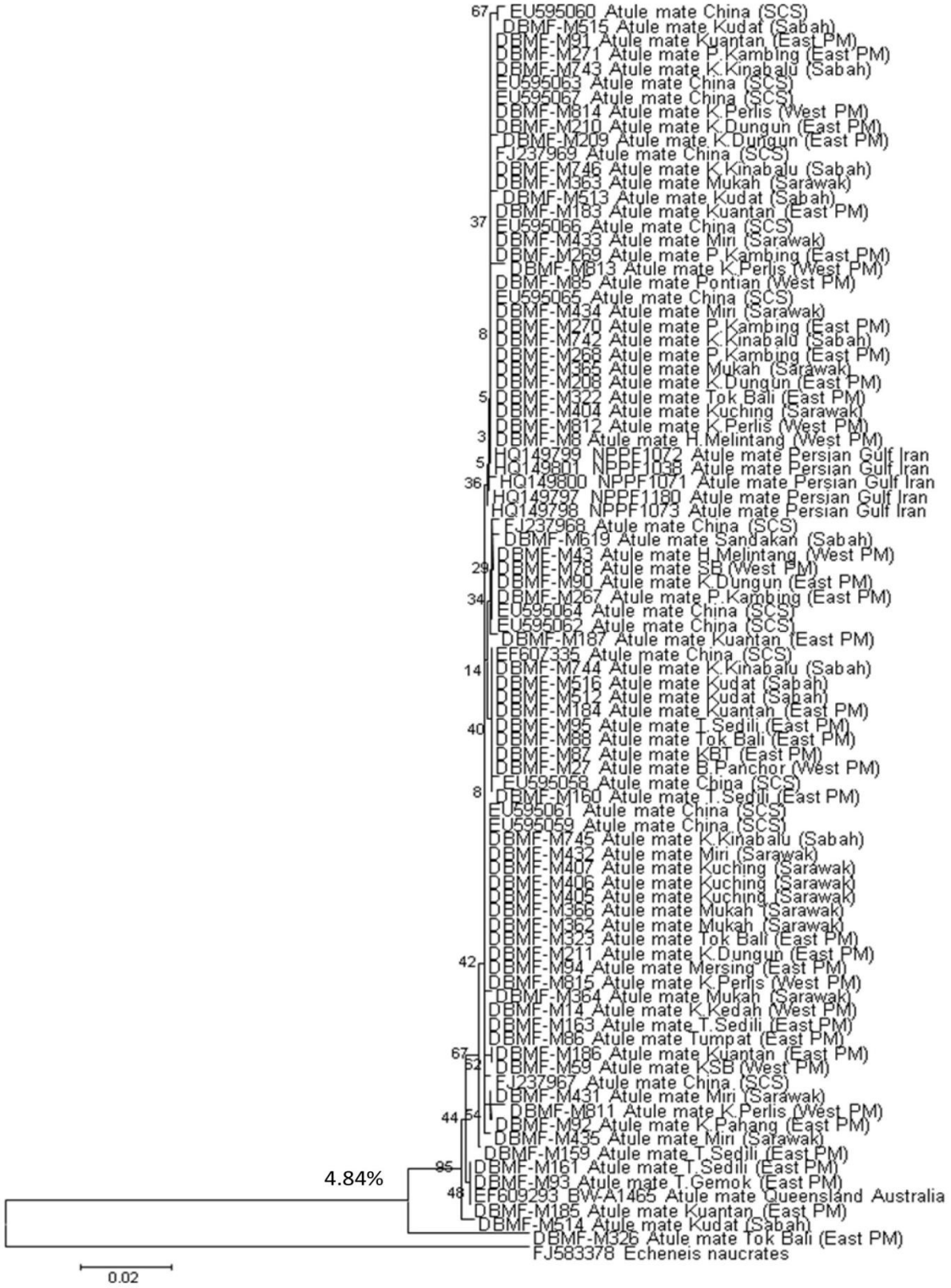
Appendix 5

Taxon ID tree of 23 widespread Carangidae species generated by MEGA5 including conspecifics from other geographical regions. (Kimura 2-parameter, pairwise deletion). Percentage showed maximum *COI* divergence between clades.

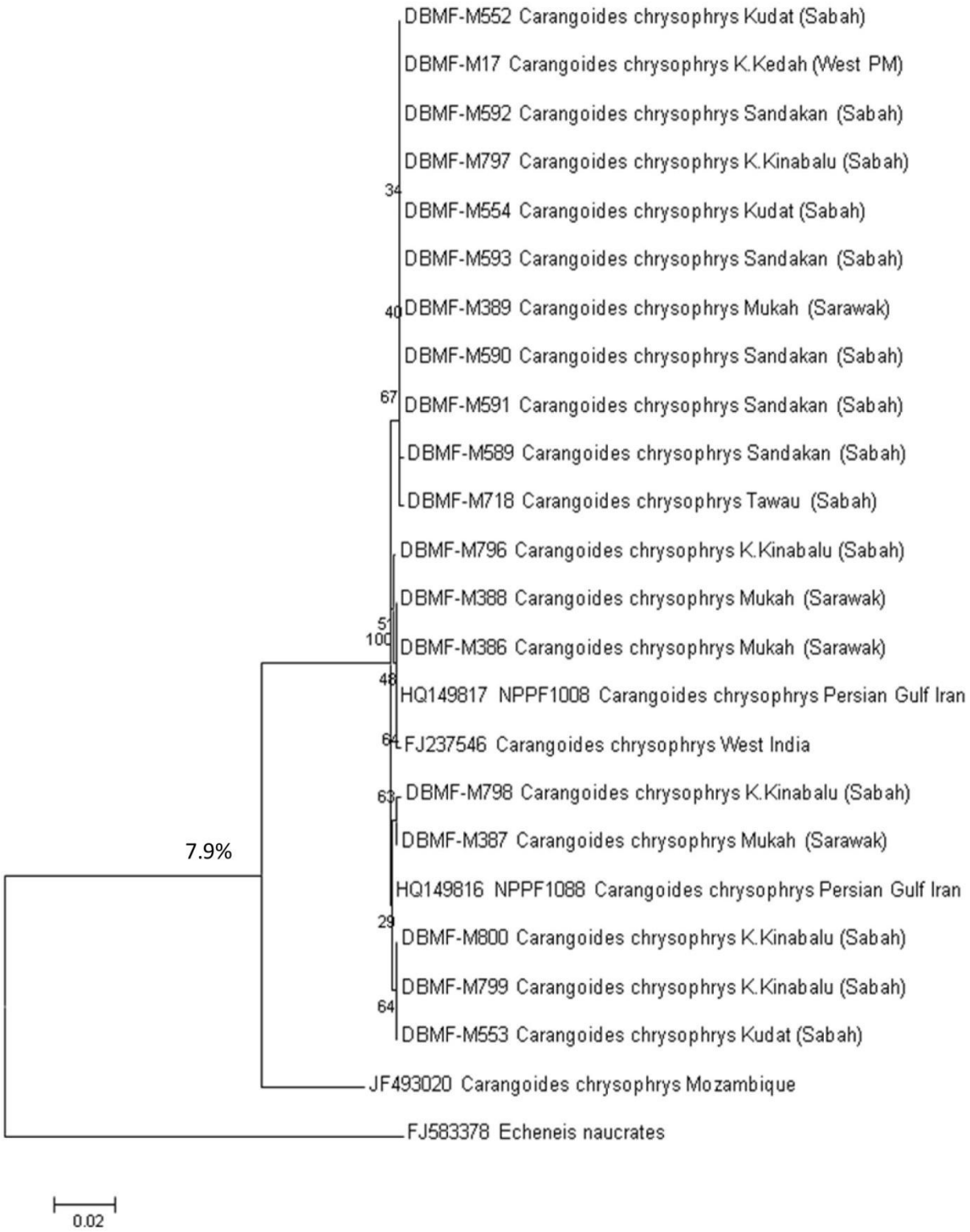
5.1 *Atropus atropos*



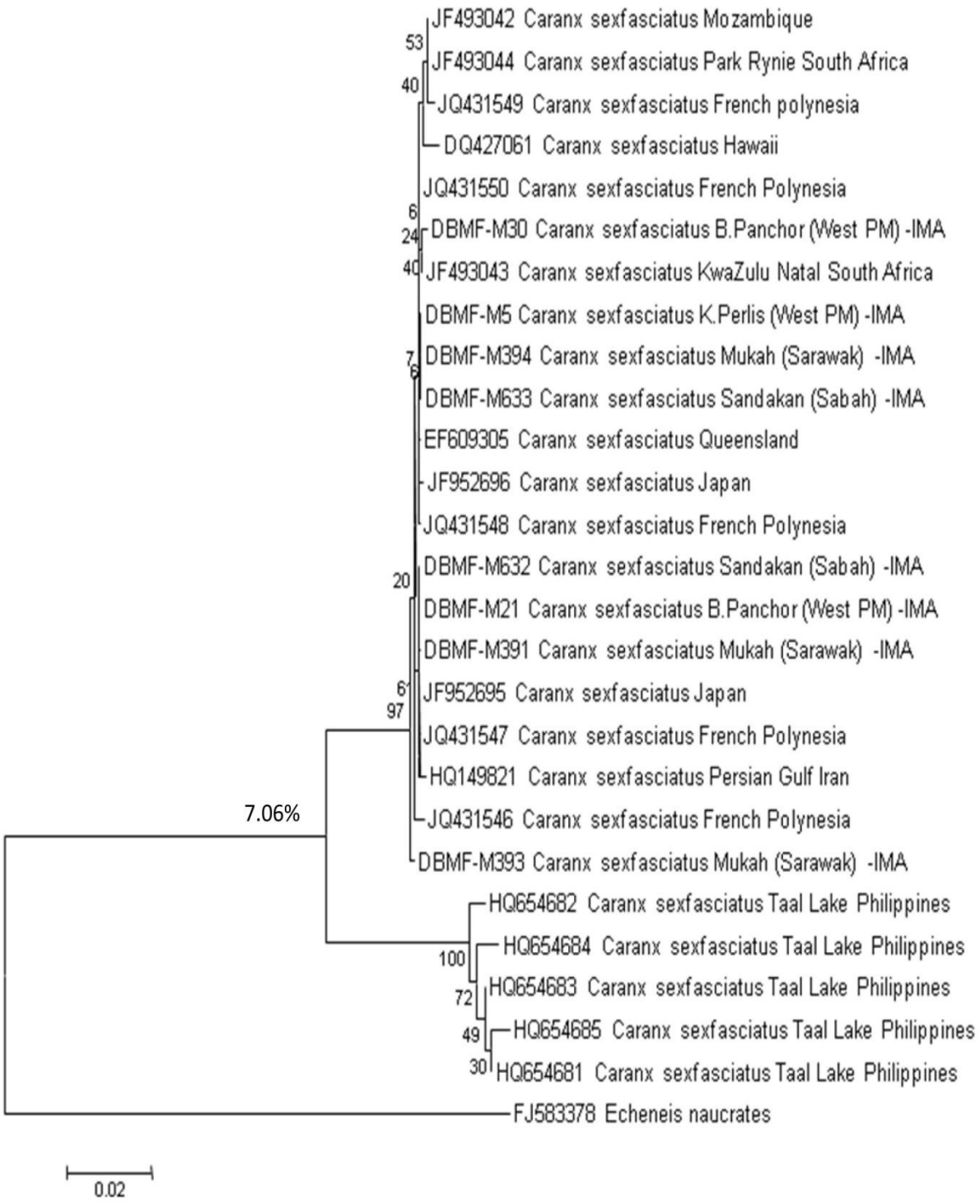
5.2 *Atule mate*



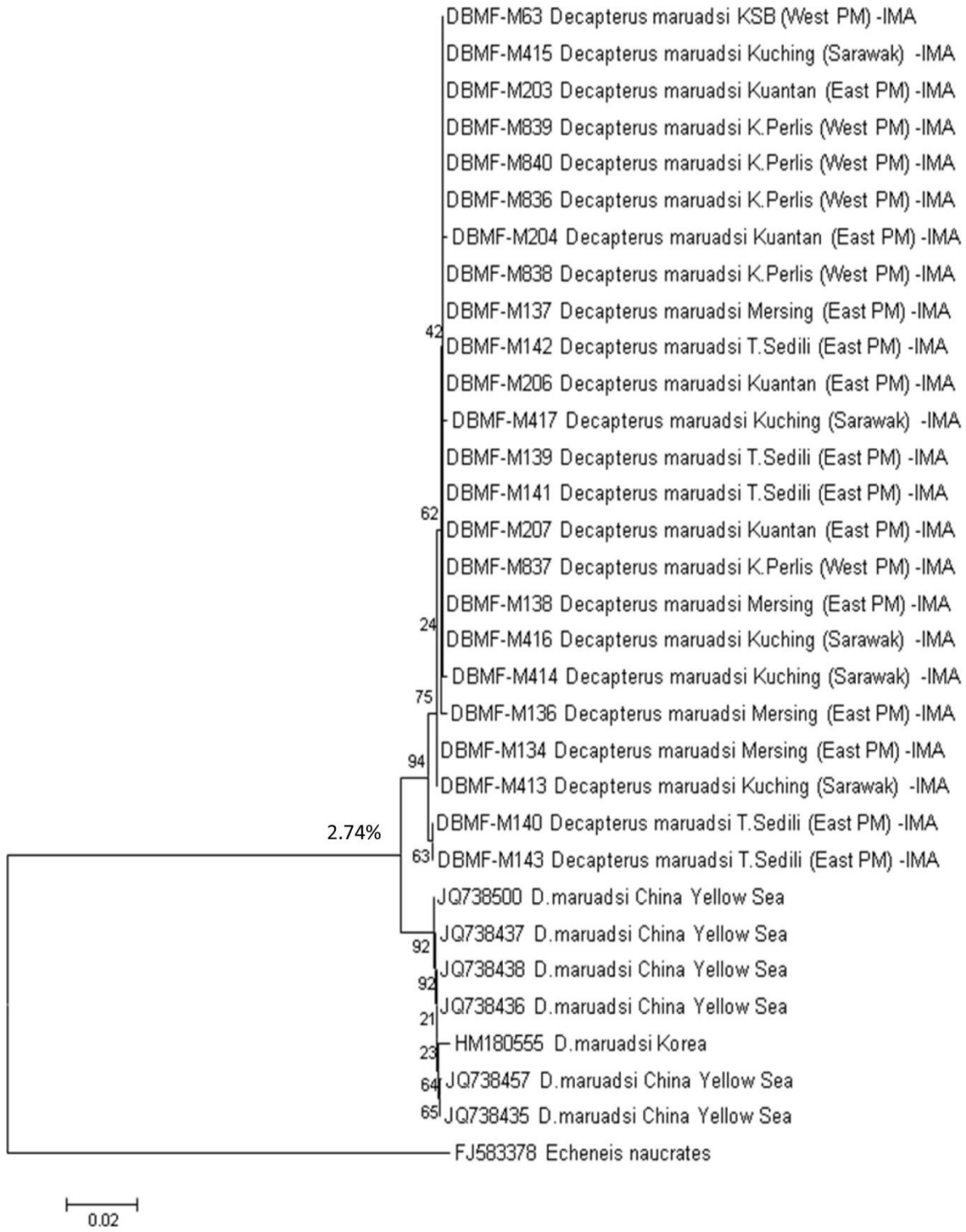
5.3 *Carangoides chrysophrys*



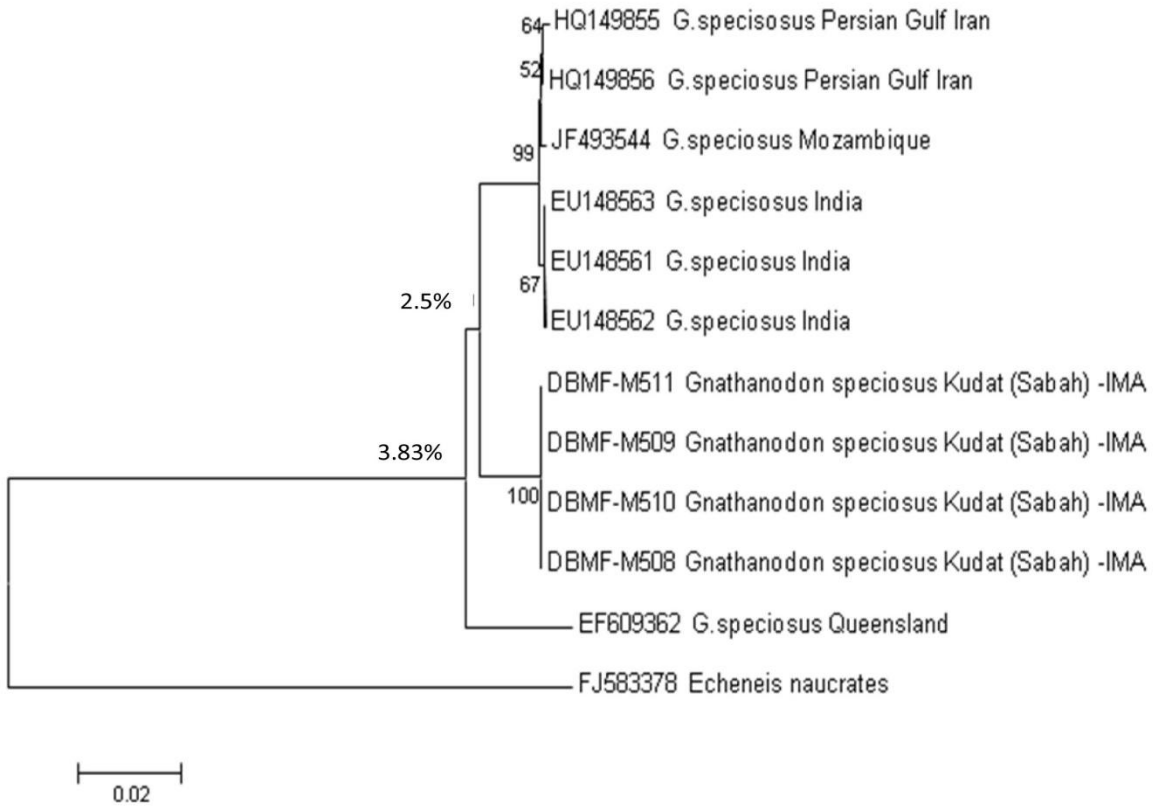
5.4 *Caranx sexfasciatus*



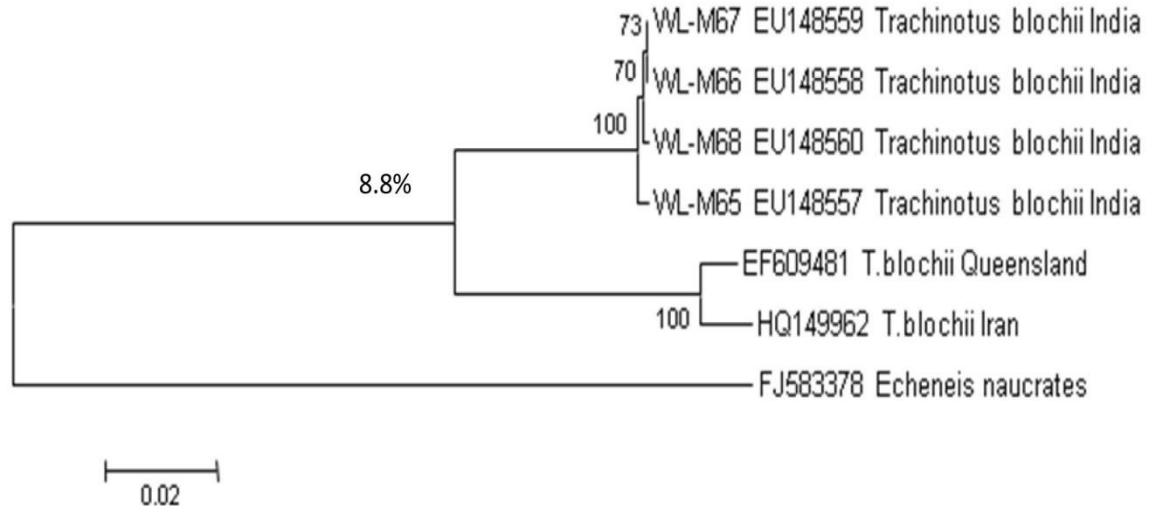
5.5 *Decapterus maruadsi*



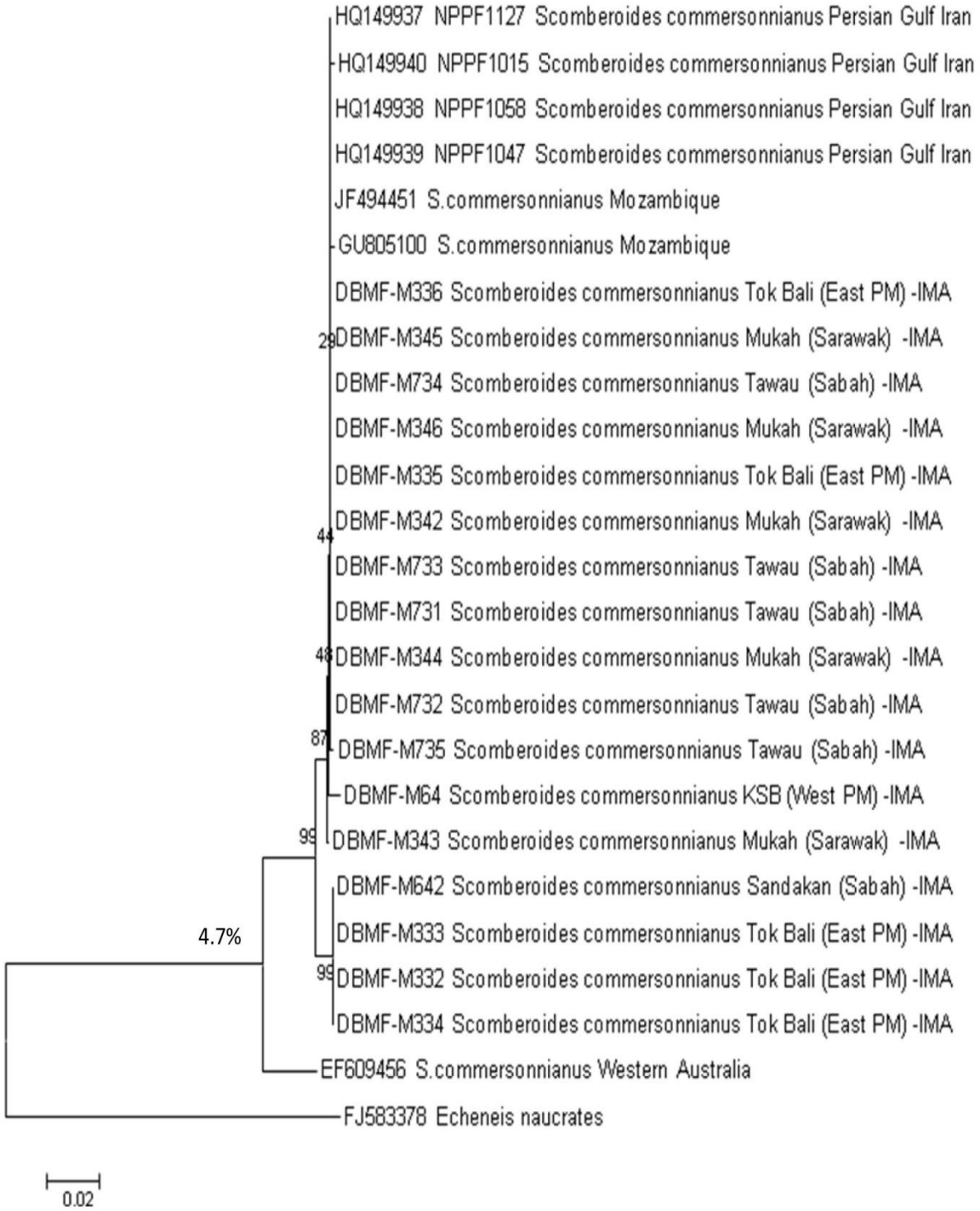
5.6 *Gnathanodon speciosus*



5.7 *Trachinotus blochii*

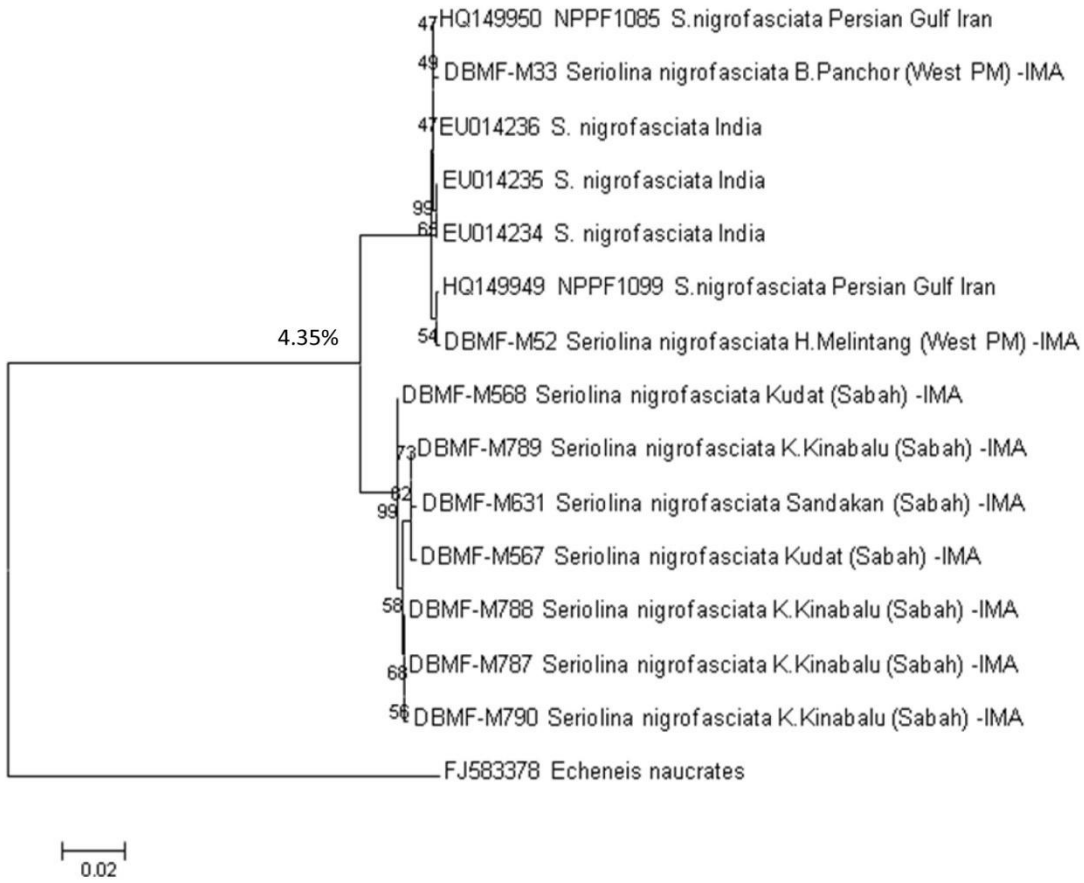


5.8 *Scomberoides commersonnianus*

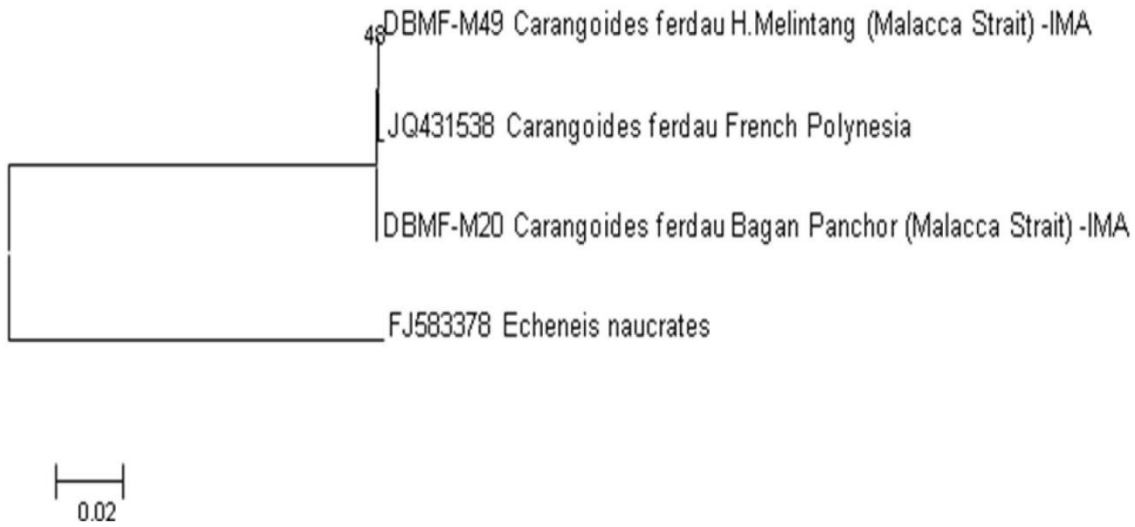


5.9 *Selar crumenophthalmus*

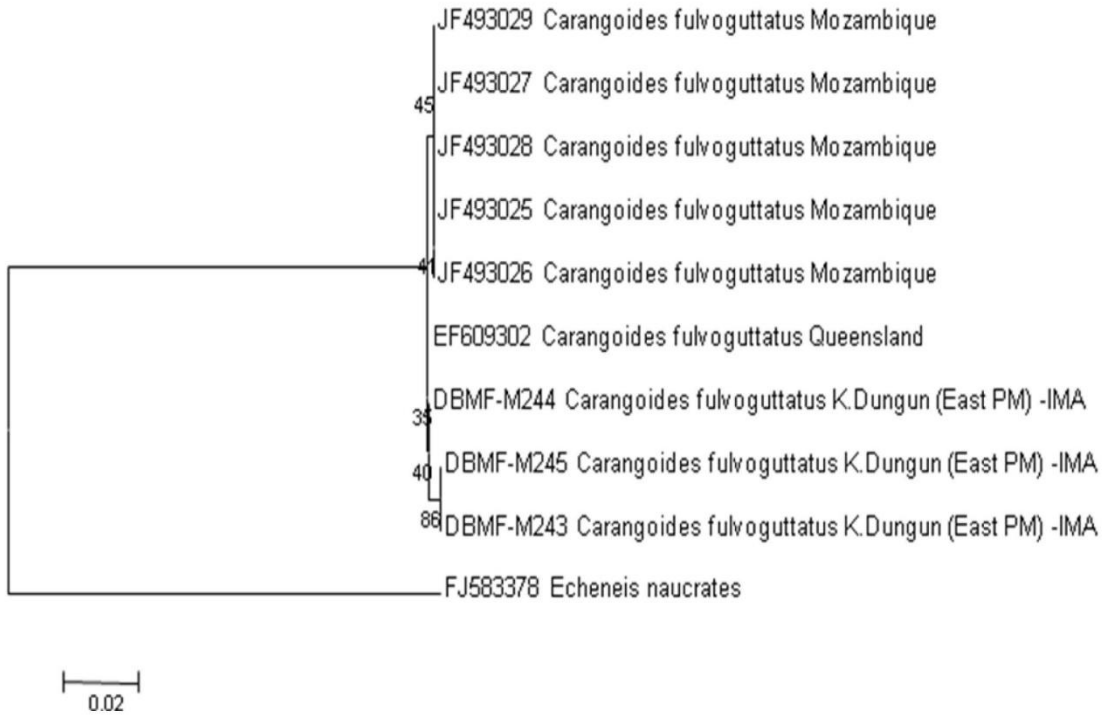
5.10 *Seriolina nigrofasciata*



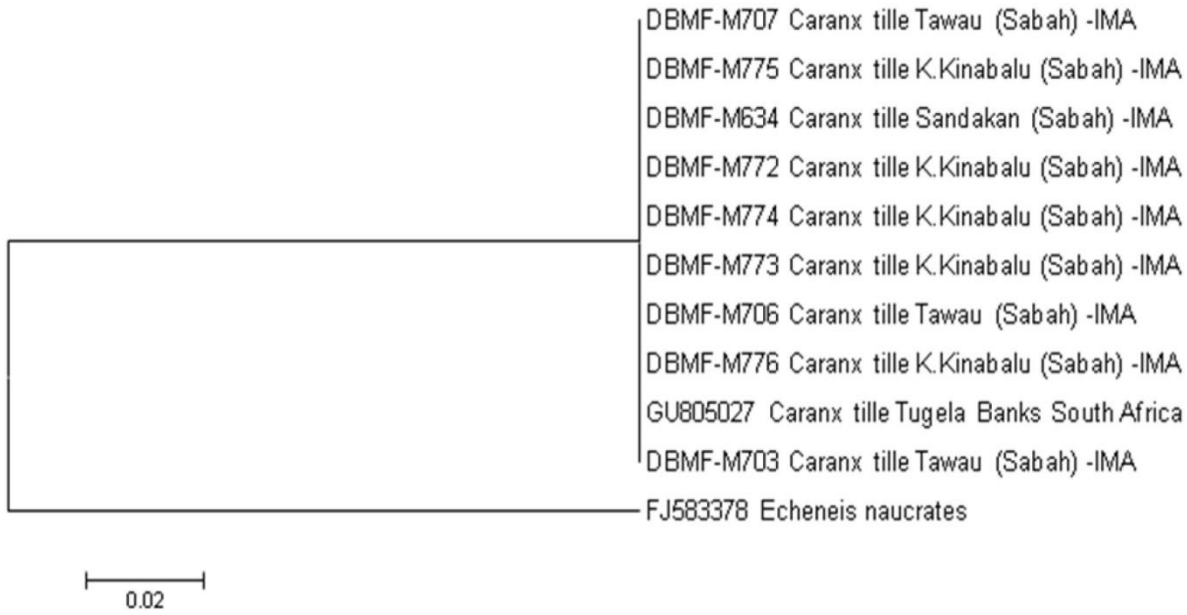
5.11 *Carangoides ferdau*



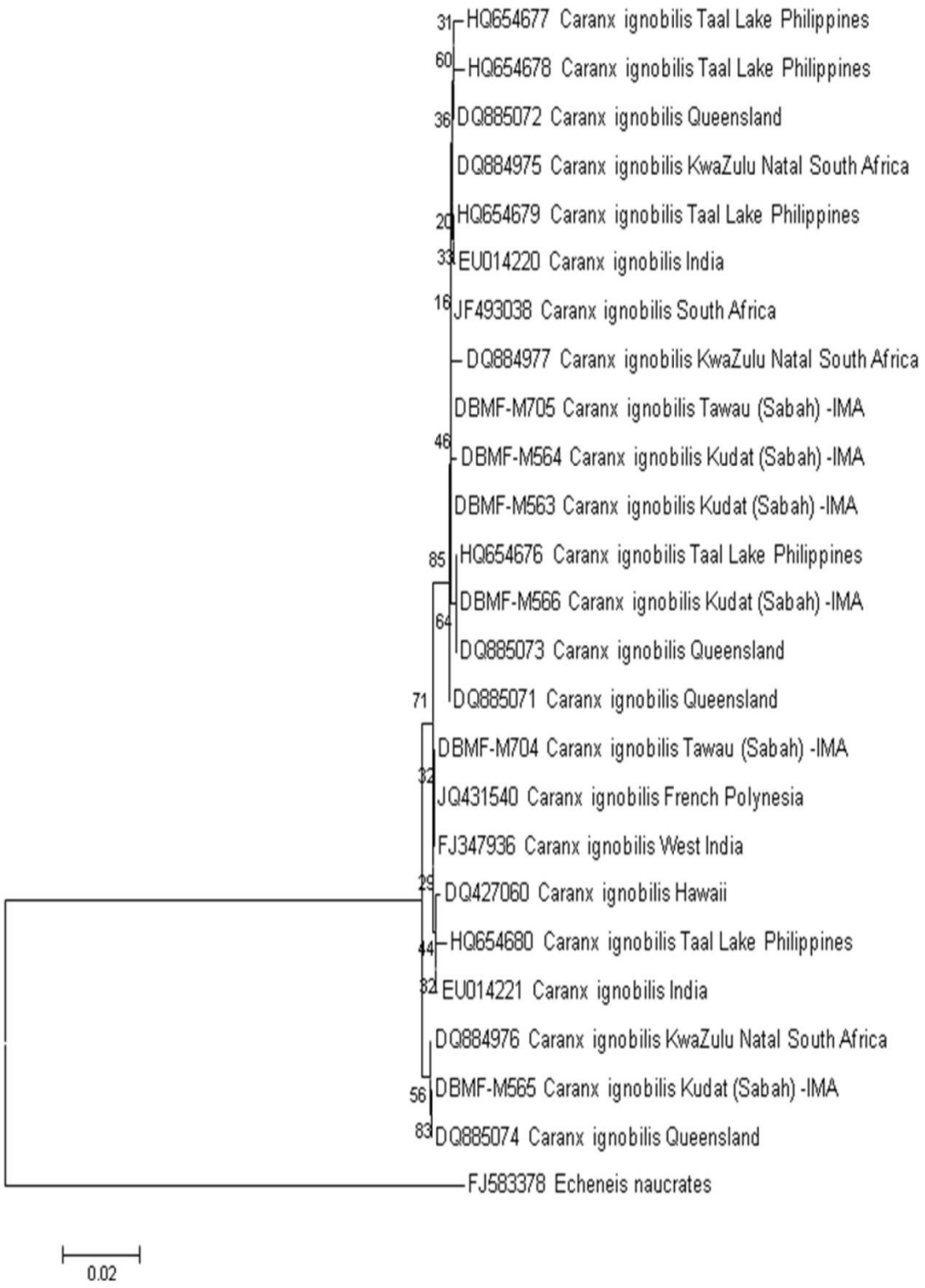
5.12 *Carangoides fulvoguttatus*



5.13 *Caranx tille*

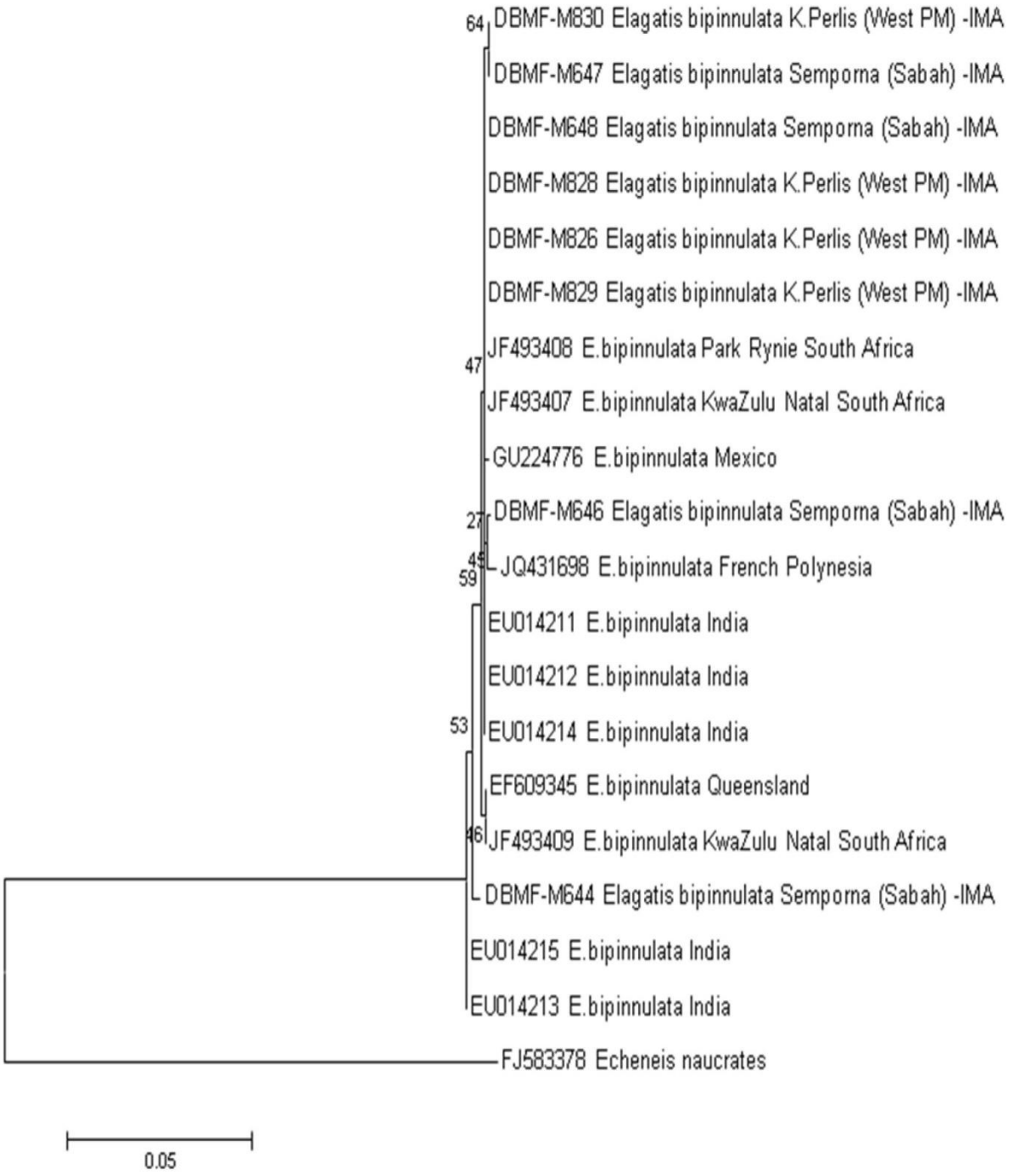


5.14 *Caranx ignobilis*



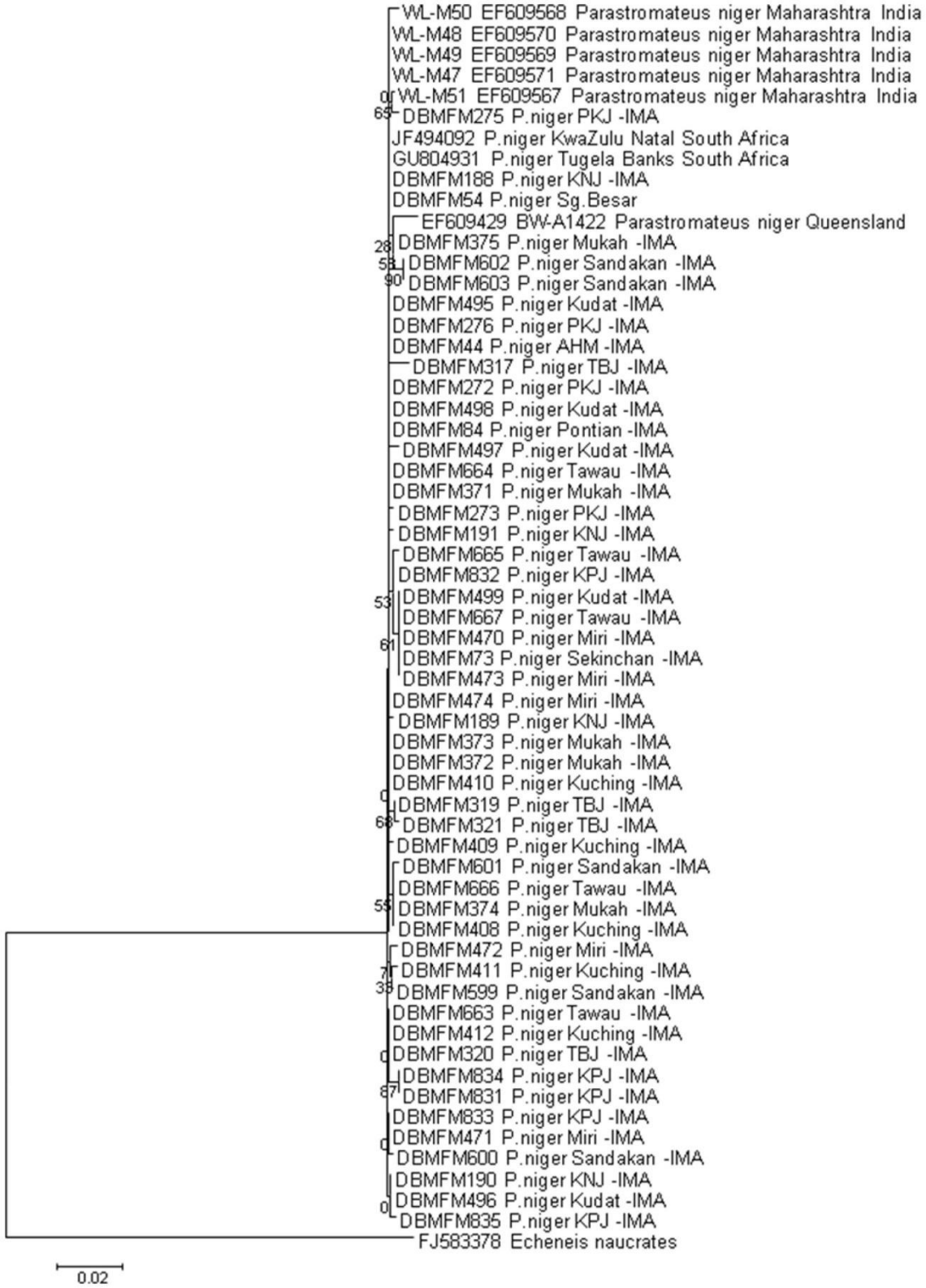
5.15 *Decapterus macrosoma*

5.16 *Elagatis bipinnulata*

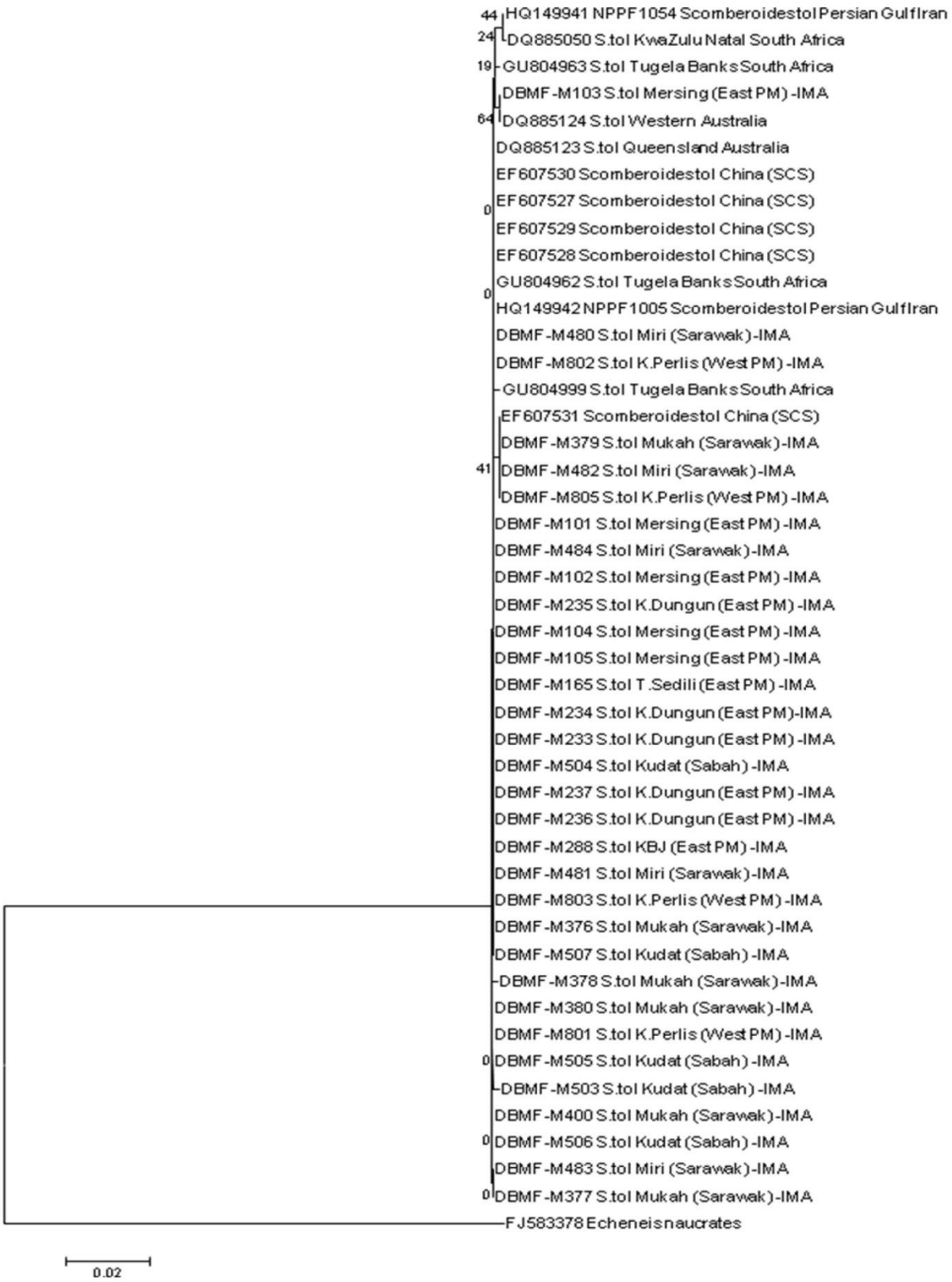


5.17 *Megalaspis cordyla*

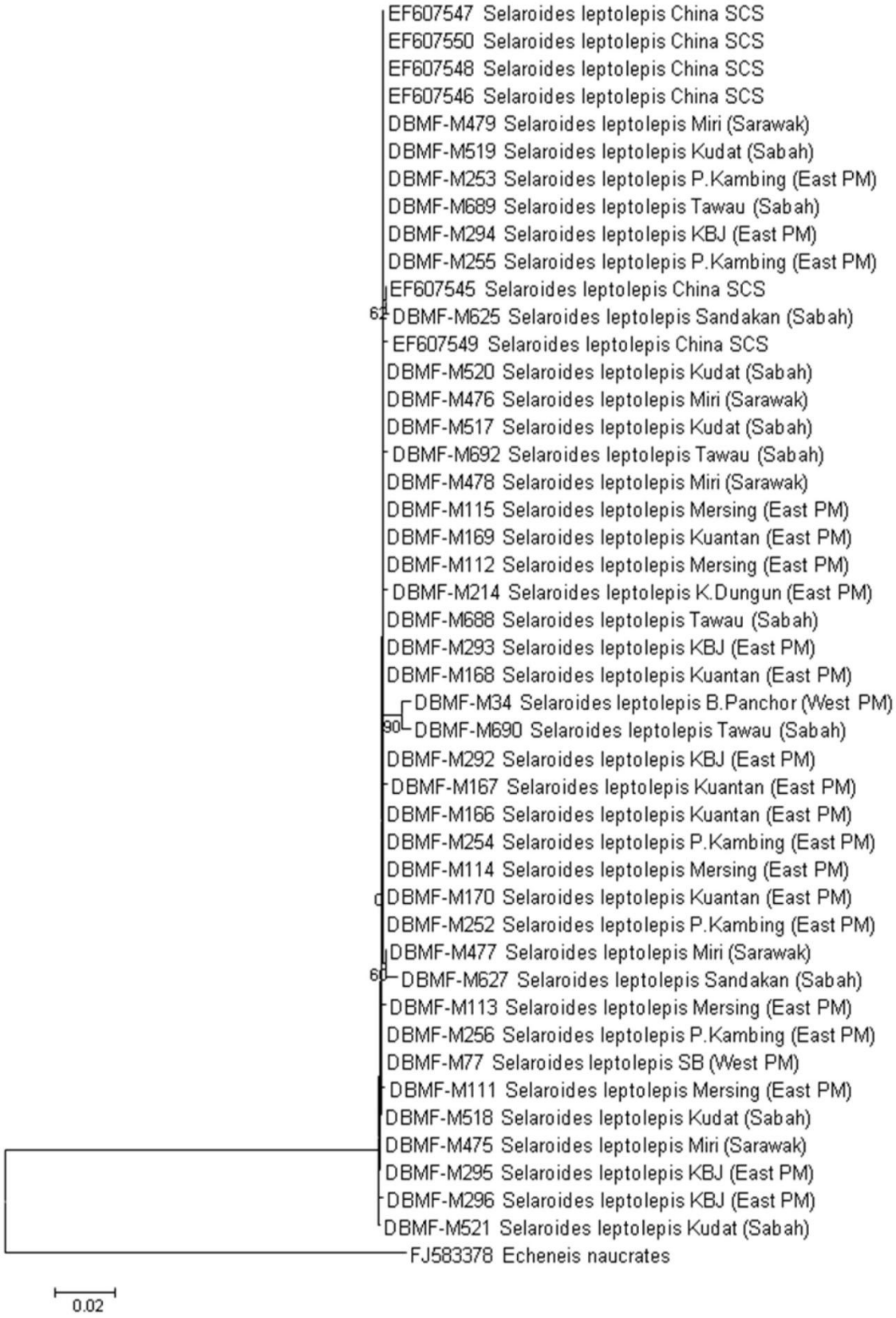
5.18 *Parastromateus niger*



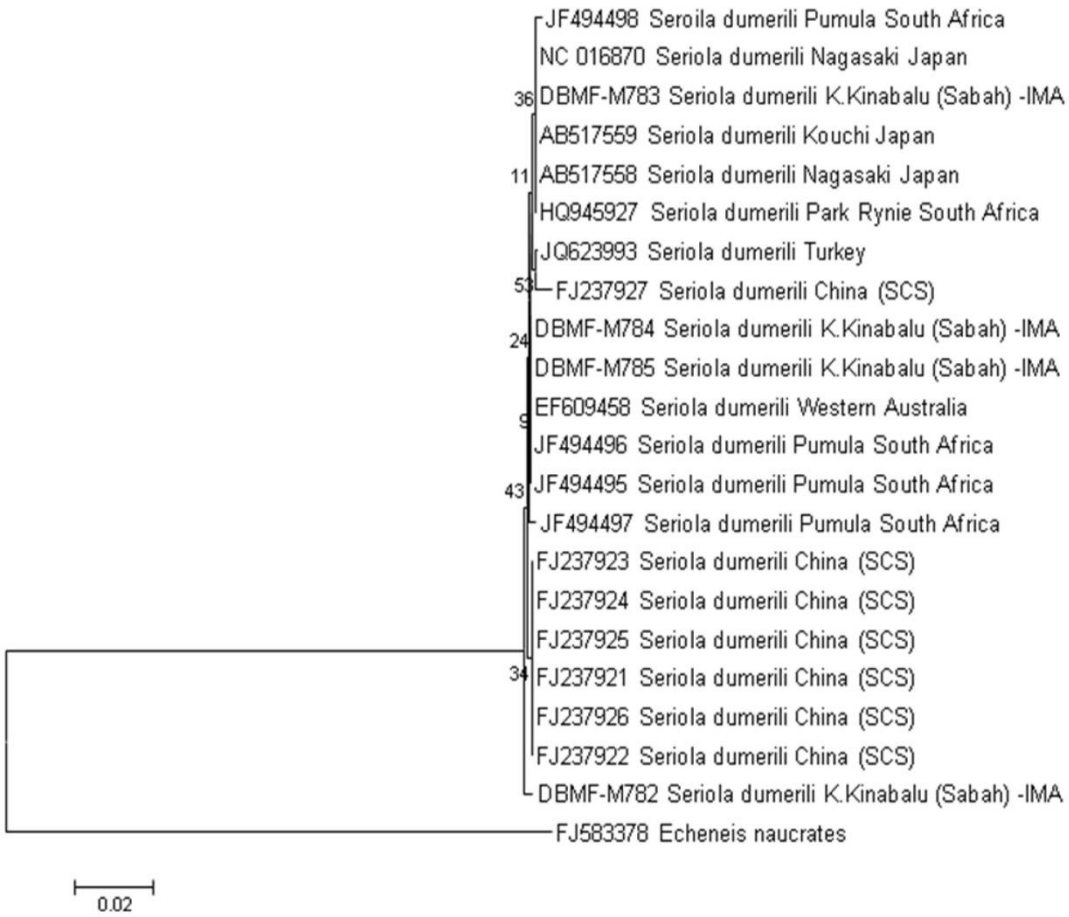
5.19 *Scomberoides tol*



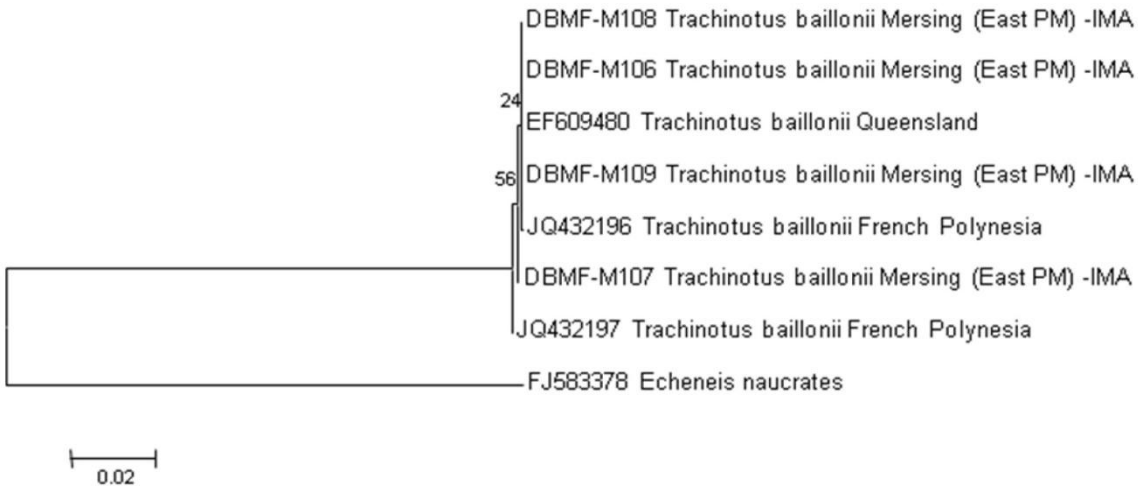
5.20 *Selaroides leptolepis*



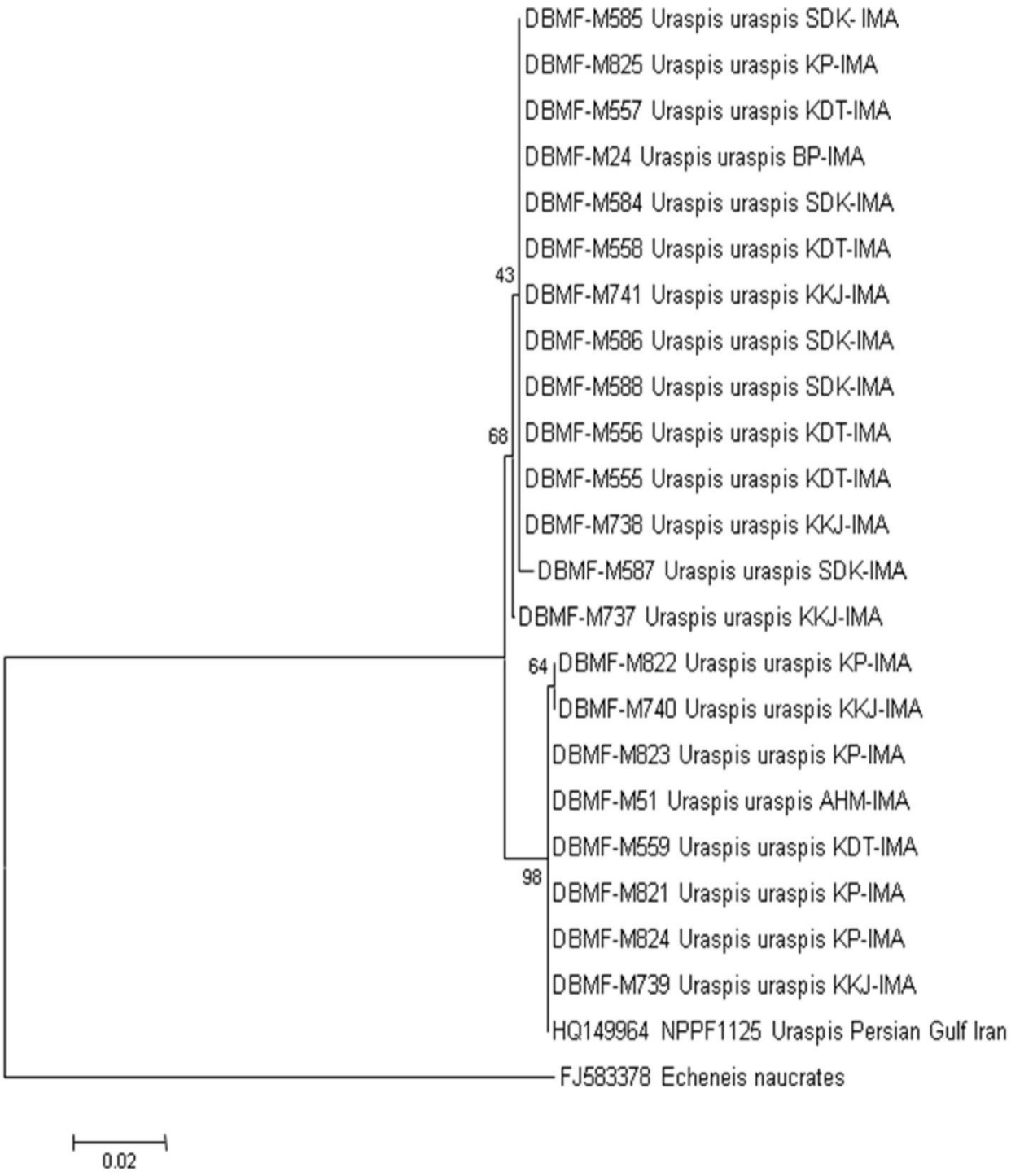
5.21 *Seriola dumerili*



5.22 *Trachinotus baillonii*



5.23 *Uraspis uraspis*



Appendix 6

Statistical test of *COI* divergence rates correspond with biological characteristics.

Divergences by size

ONEWAY

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Large	5	0.2560	0.15372	0.06875	0.0651	0.4469
Medium	9	0.3989	0.62311	0.20770	-0.0801	0.8779
Small	22	0.3495	0.40930	0.08726	0.1681	0.5310
Total	36	0.3489	0.44027	0.07338	0.1999	0.4979

	Minimum	Maximum
Large	0.10	0.51
Medium	0.00	1.98
Small	0.00	1.79
Total	0.00	1.98

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
1.132	2	33	0.335

ANOVA

	Sum of Squares	df	Mean Square	F	Sig
Between groups	0.066	2	0.033	0.161	0.852
Within groups	6.719	33	0.204		
Total	6.784	35			

Divergences by shape

ONEWAY

	N	Mean	Std. Deviation	Std. Error
Round	8	0.3975	0.57091	0.20185
Compressed	20	0.2540	0.28445	0.06360
Moderately compressed	8	0.5375	0.59545	0.21052
Total	36	0.3489	0.44027	0.07338

	95% Confidence Interval for Mean		Minimum	Maximum
	Lower bound	Upper bound		
Round	-0.0798	0.8748	0.08	1.79
Compressed	0.1209	0.3871	0.00	1.13
Moderately compressed	0.0397	1.0353	0.14	1.98
Total	0.1999	0.4979	0.00	1.98

Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
0.933	2	33	0.404

ANOVA

	Sum of Squares	df	Mean Square	F	Sig
Between groups	0.484	2	0.242	1.266	0.295
Within groups	6.301	33	0.191		
Total	6.784	35			

Divergences by habitat use

Group Statistics

habitat	N	Mean	Std. Deviation	Std. Error
Pelagic	19	0.2479	0.16085	0.03690
Demersal	8	0.4650	0.58118	0.20548

Independent Samples Test

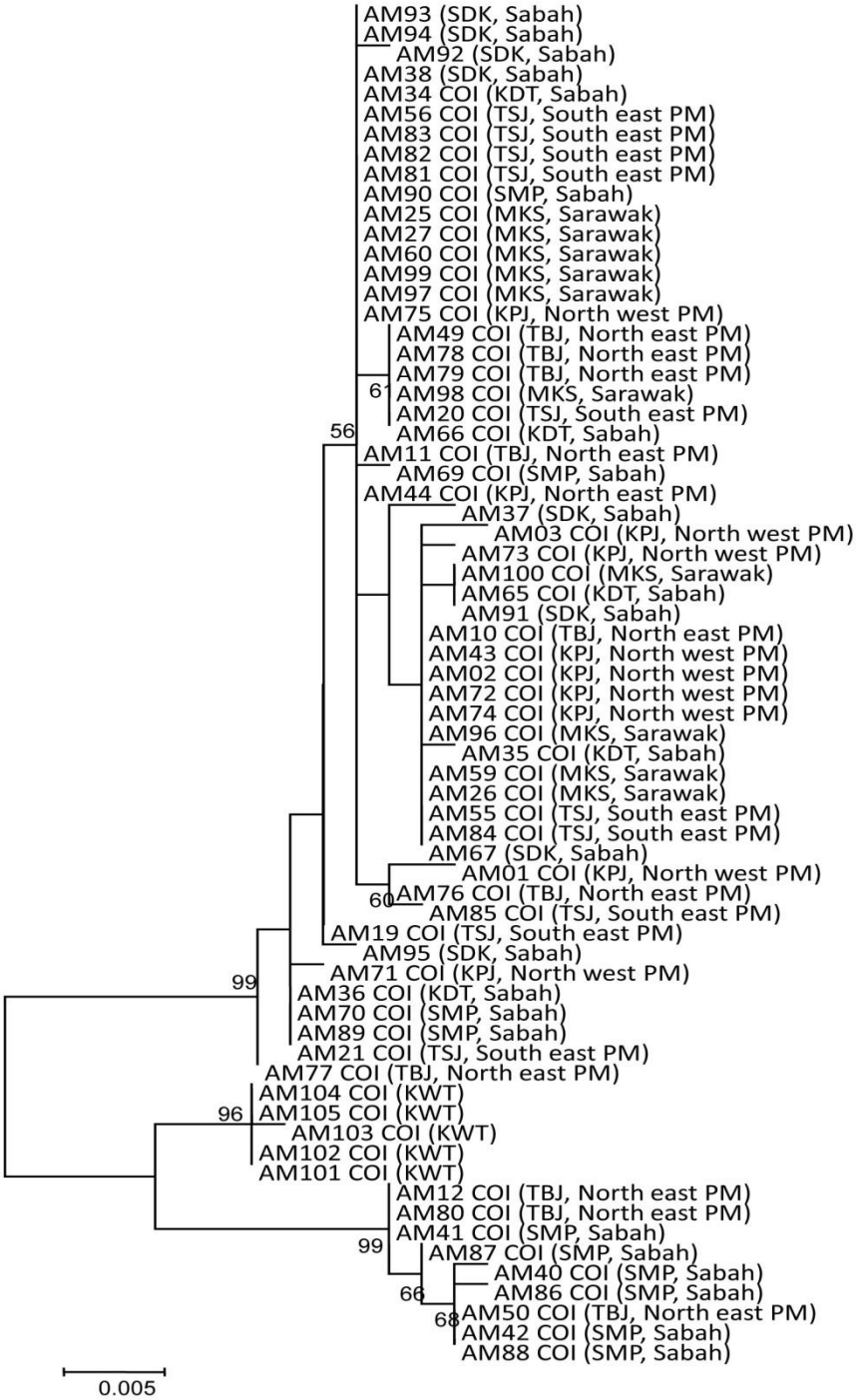
	Levene;s Test for Equality of Variances		t-test for Equality of Means			
	F	Sig.	t	df	Sig. (2-tailed)	Mean difference
Equal variances assumed	6.703	0.016	-1.531	25	0.138	-0.21711
Equal variances not assumed			-1.040	7.456	0.331	-0.21711

	t-test for Equality of Means		
	Std. Error Difference	95% Confidence Interval of the Difference	
		Lower	Upper
Equal variances assumed	0.14181	-0.50916	0.07495
Equal variances not assumed	0.20877	-0.70471	0.27050

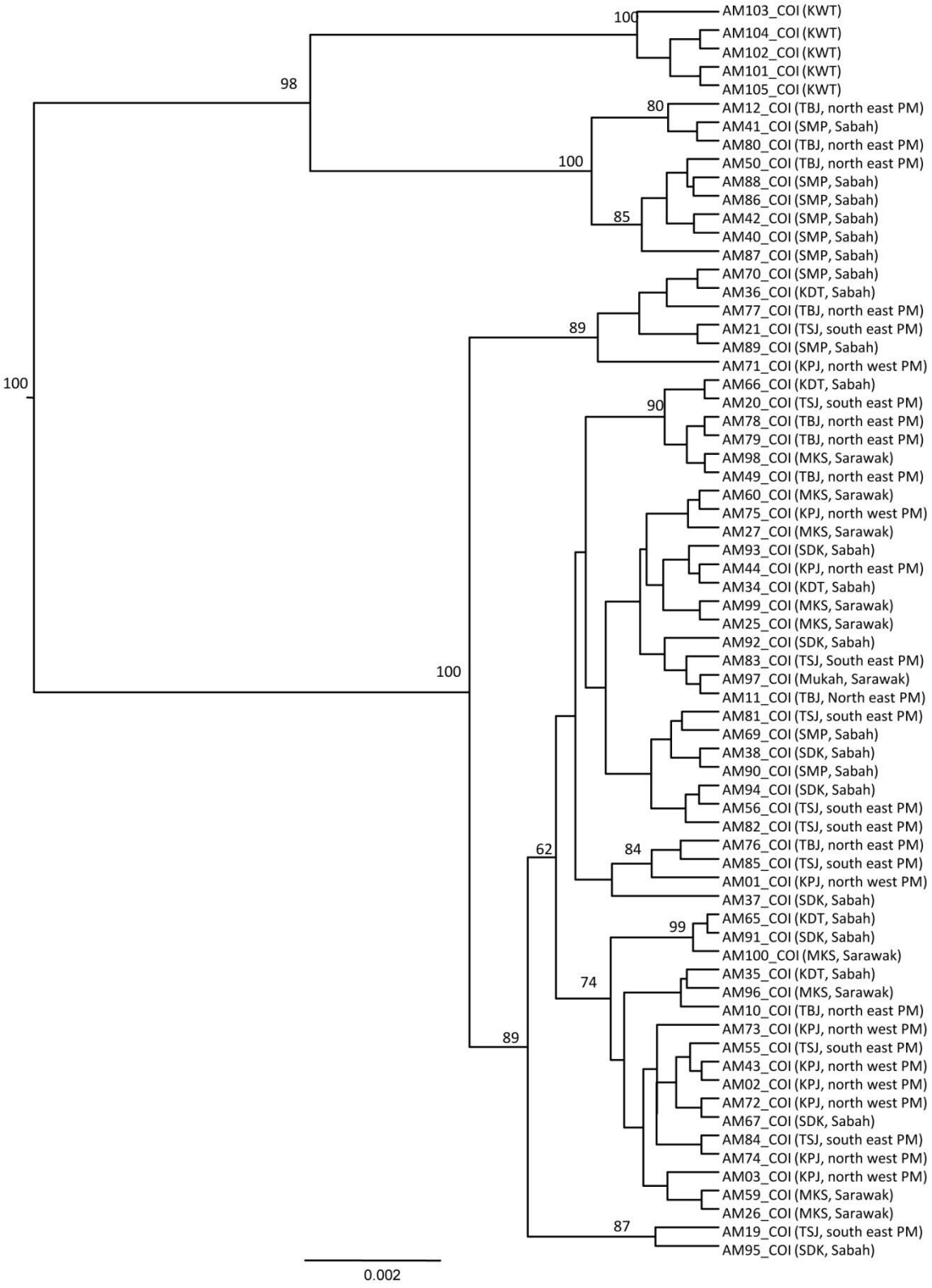
Appendix 7

Maximum-likelihood and Bayesian trees of *Atule mate* by genes.

7.1 a) Maximum-likelihood and b) Bayesian trees of 68 *COI* sequences.

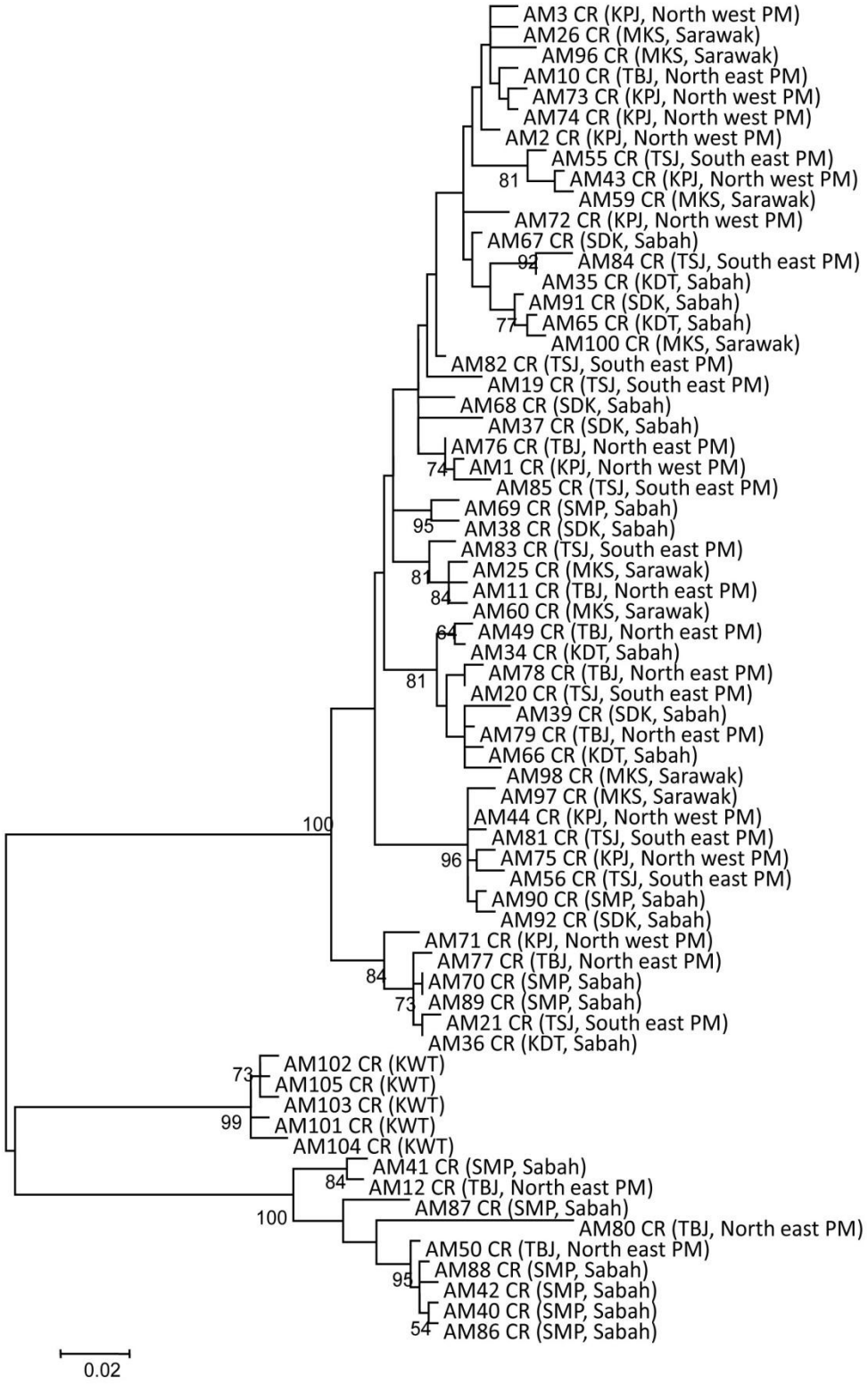


a) Maximum-likelihood tree

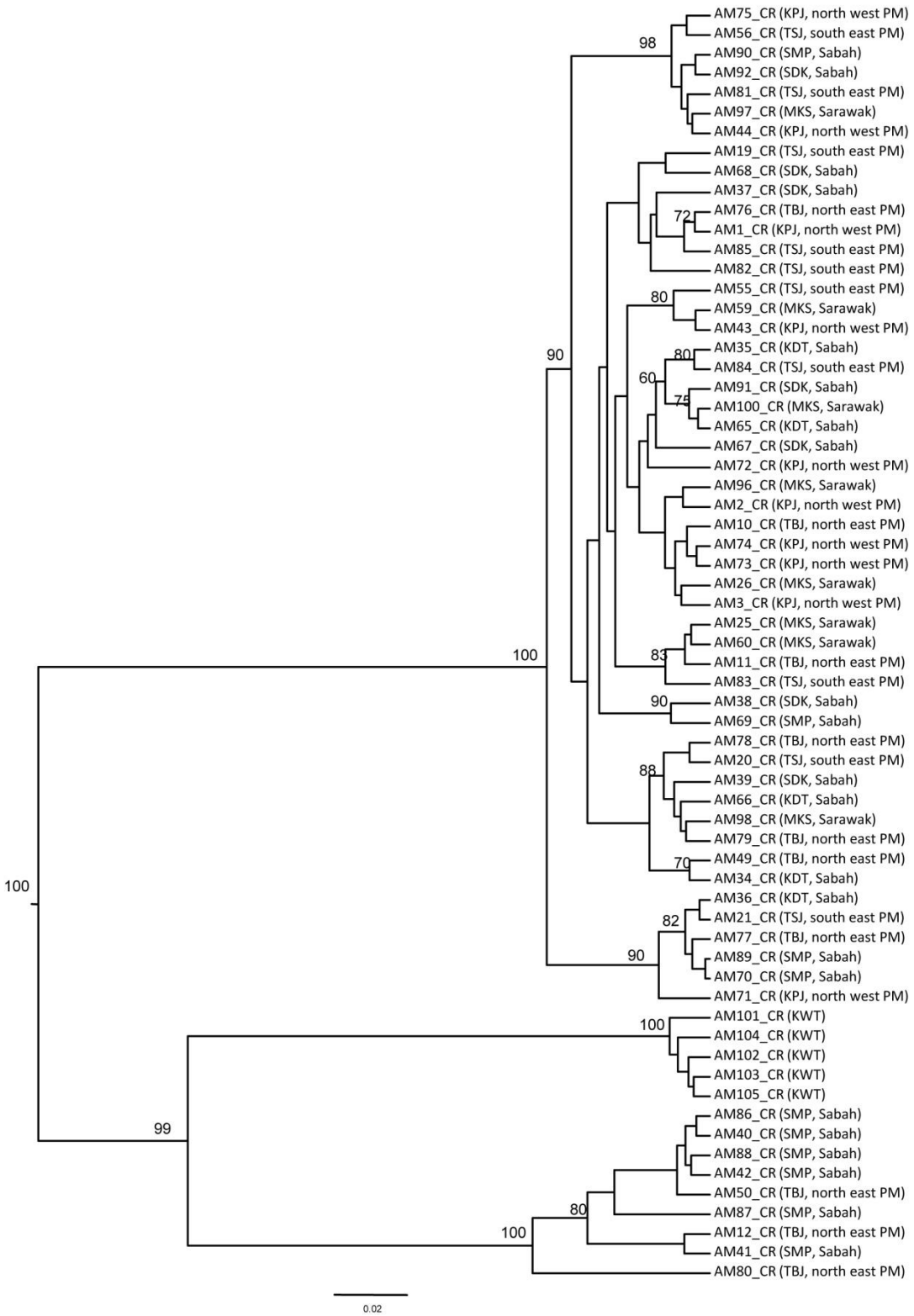


b) Bayesian tree

7.2 a) Maximum-likelihood and b) Bayesian trees of 65 control region sequences.

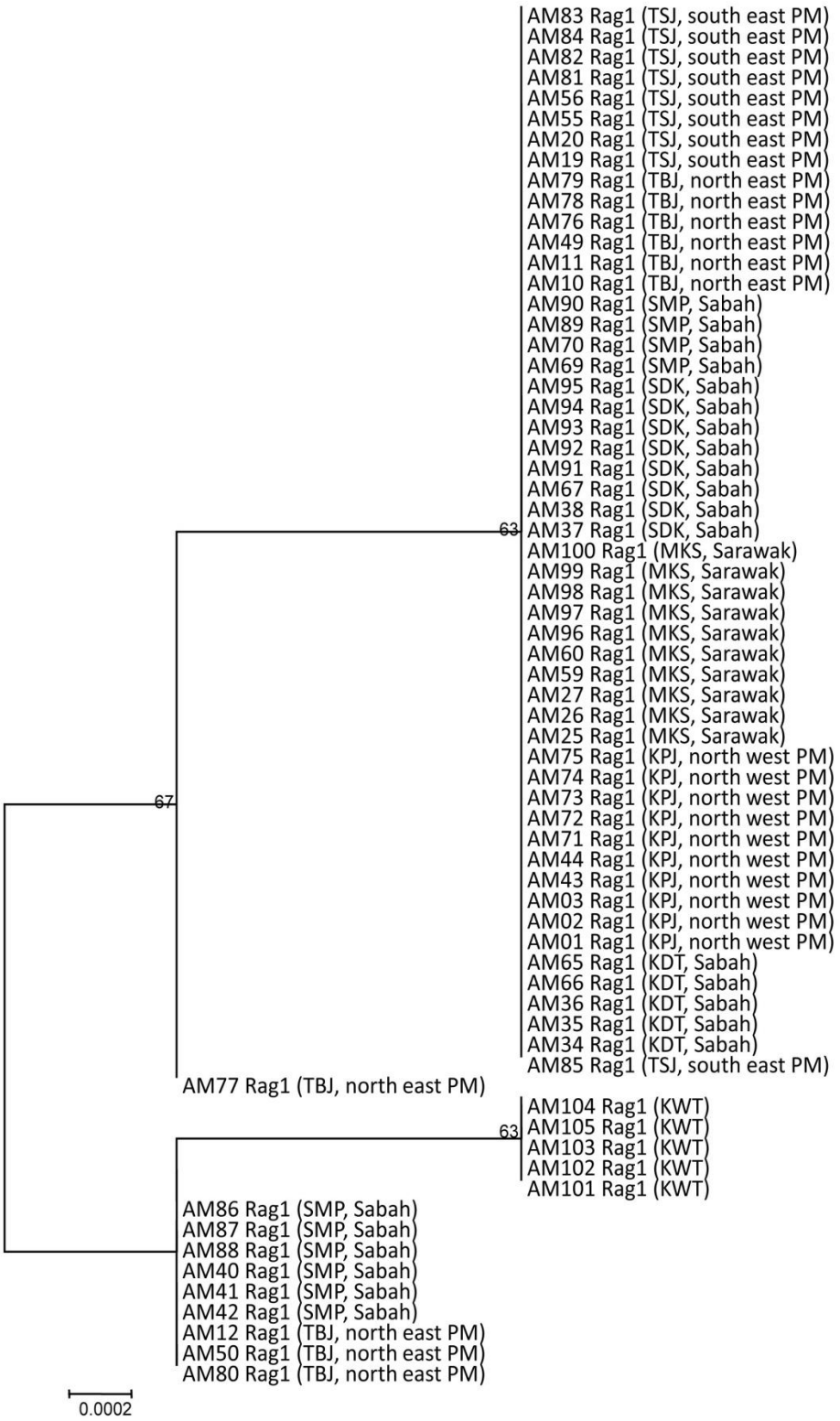


a) Maximum-likelihood tree

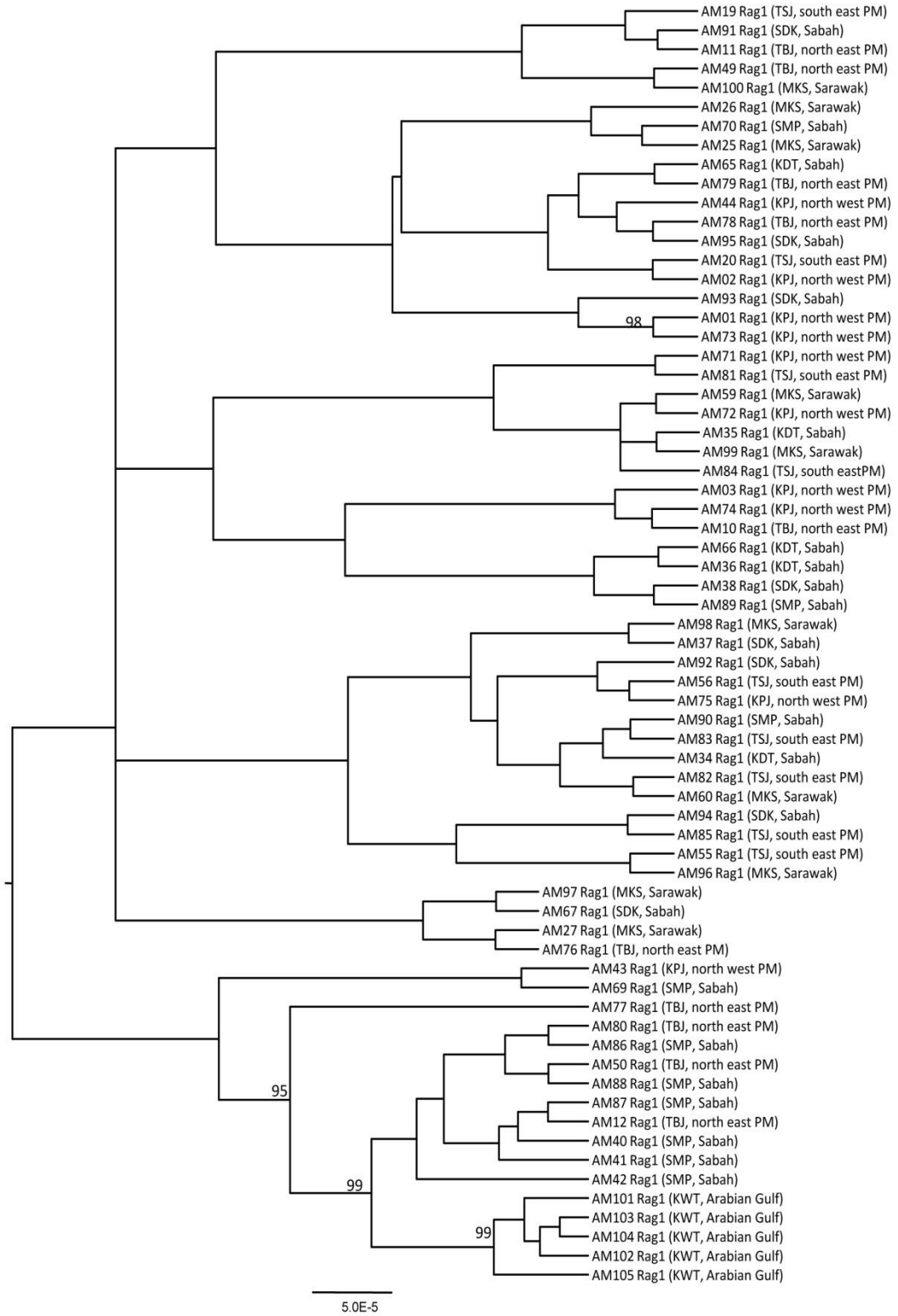


b) Bayesian tree

7.3 a) Maximum-likelihood and b) Bayesian trees of 67 Rag1 sequences.



a) Maximum-likelihood tree

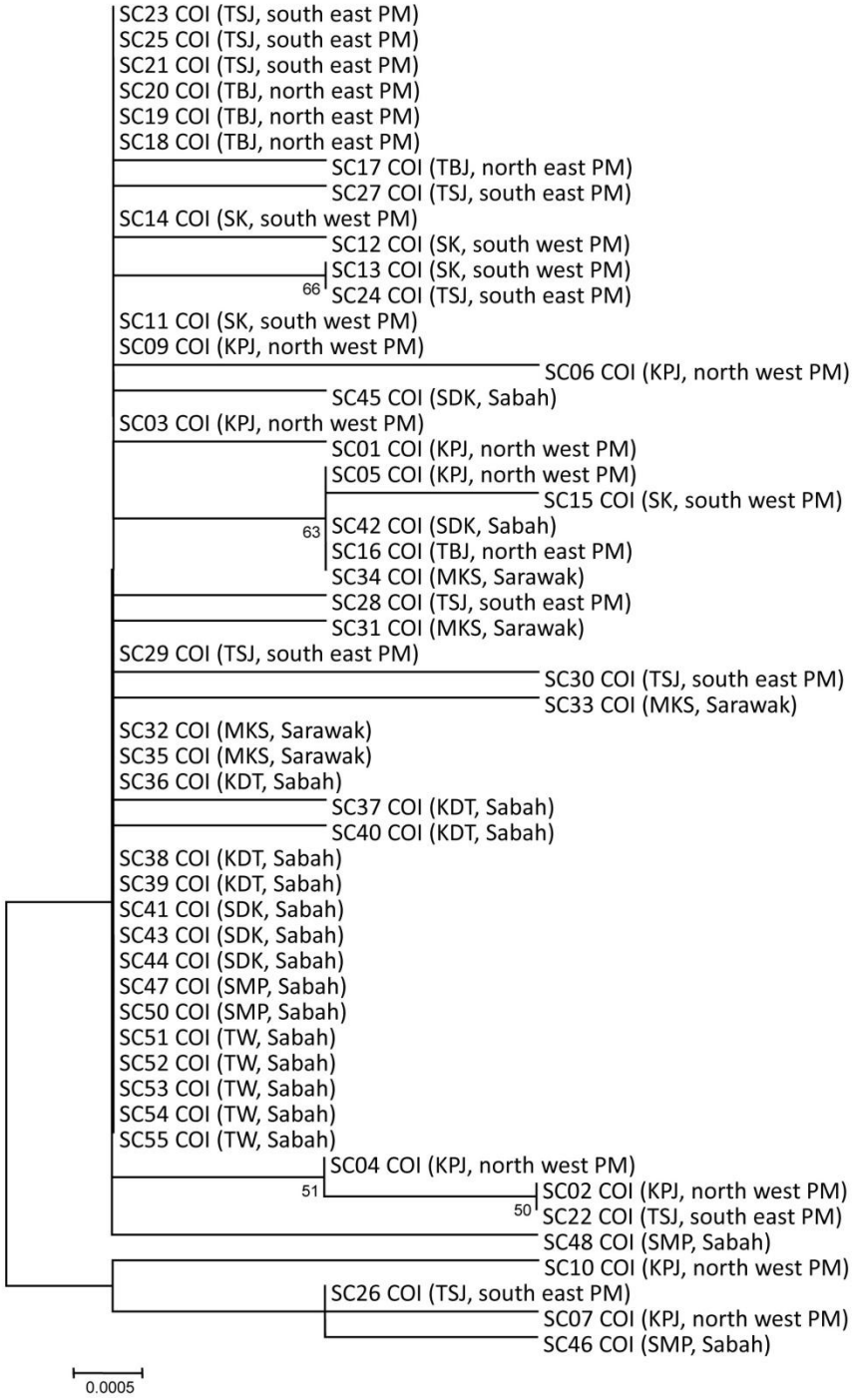


b) Bayesian tree

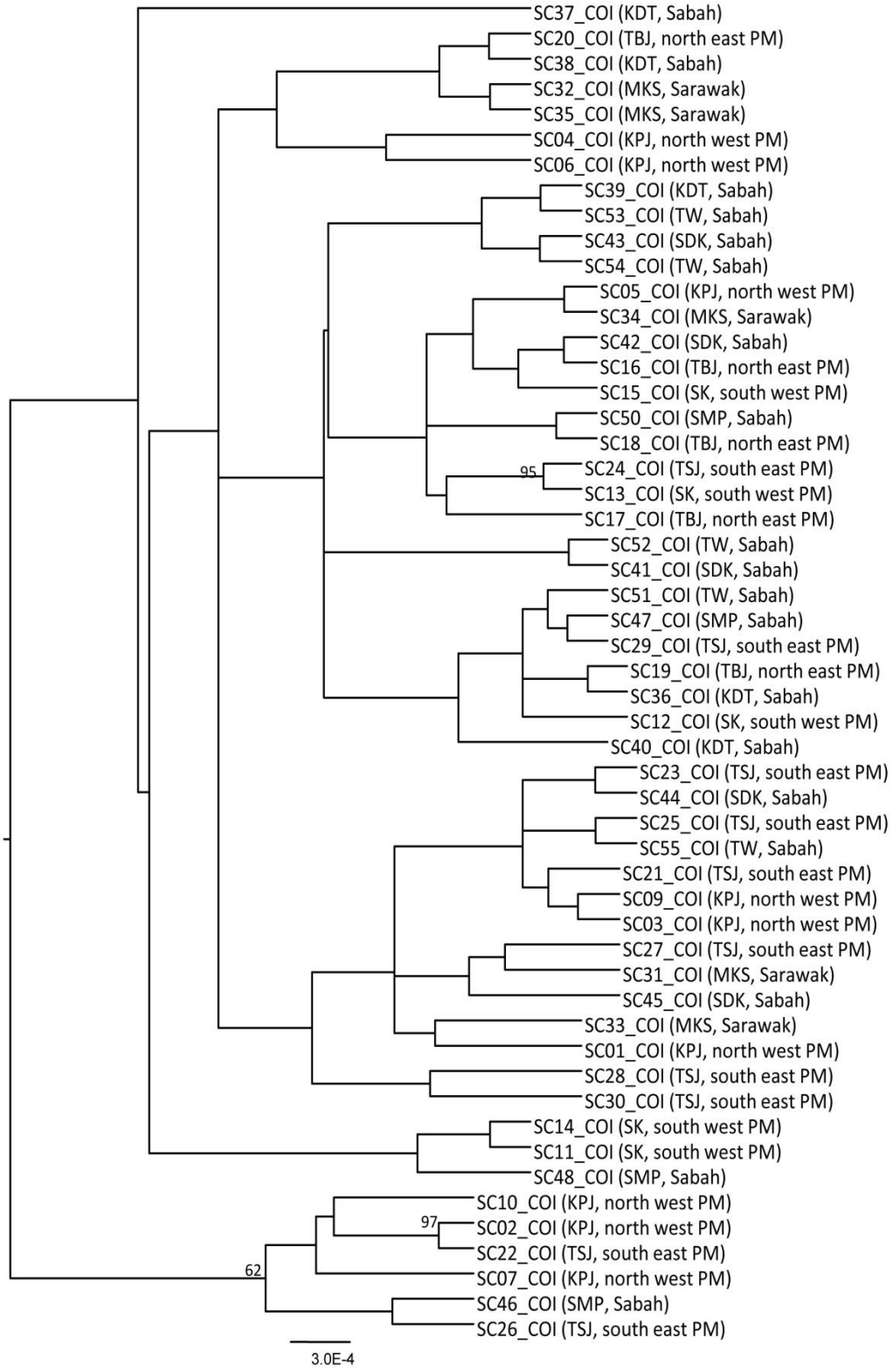
Appendix 8

Maximum-likelihood and Bayesian trees of *Selar crumenophthalmus* by genes.

8.1 a) Maximum-likelihood and b) Bayesian trees of 53 *COI* sequences.

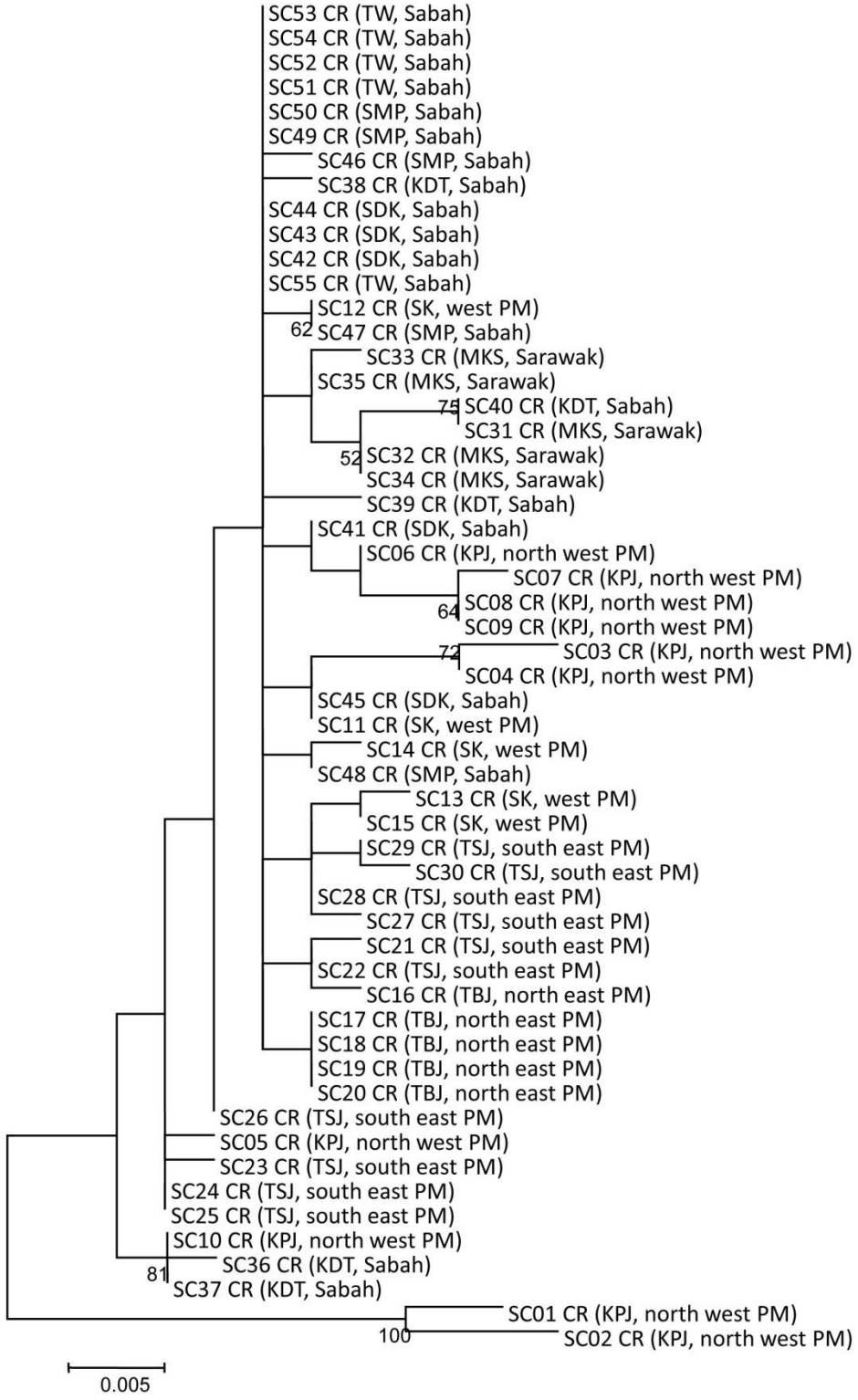


a) Maximum-likelihood tree

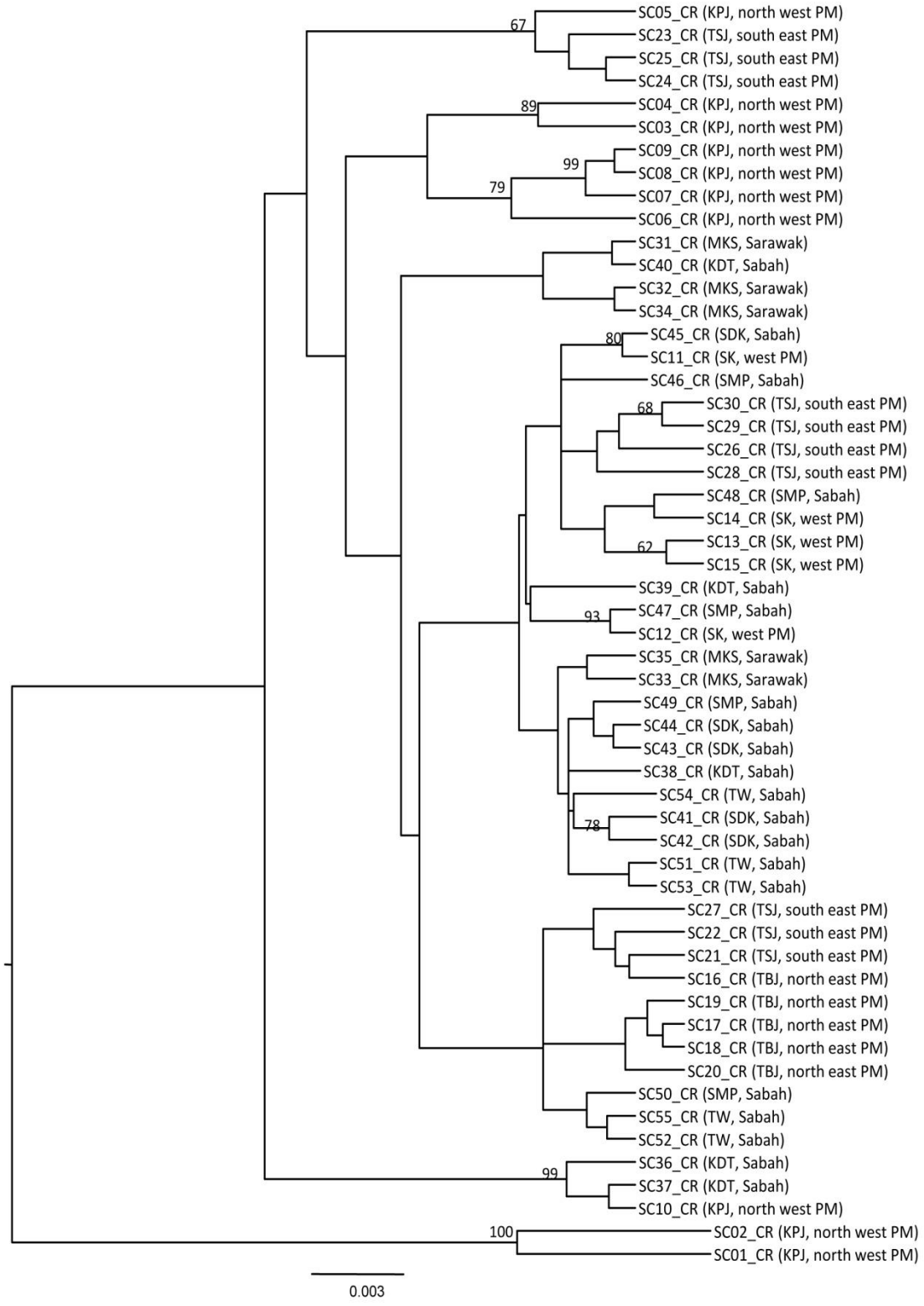


b) Bayesian tree

8.2 a) Maximum-likelihood and b) Bayesian trees of 55 control region sequences.



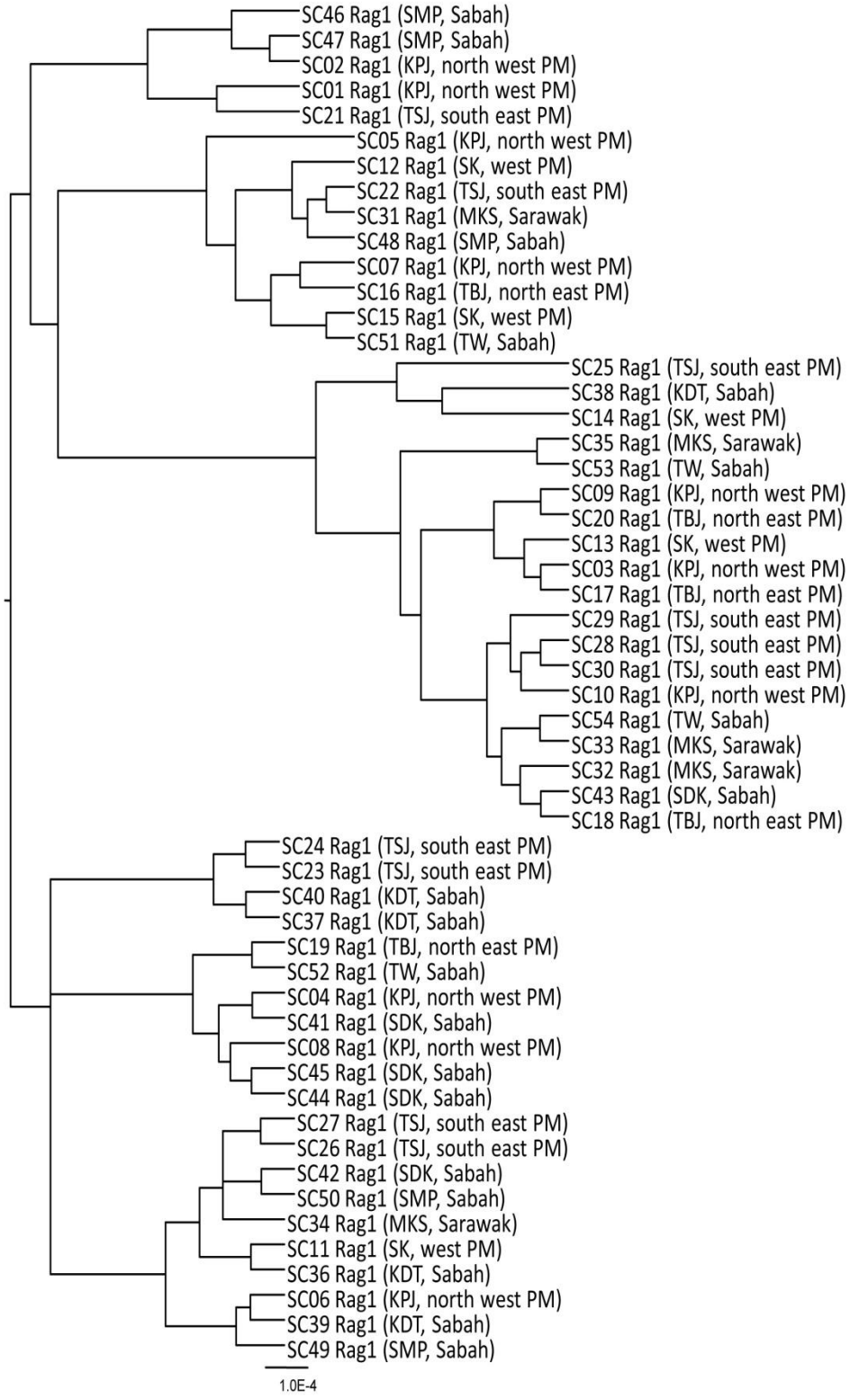
a) Maximum-likelihood tree



b) Bayesian tree

8.3 a) Maximum-likelihood and b) Bayesian trees of 54 Rag1 sequences.

a) Maximum-likelihood tree

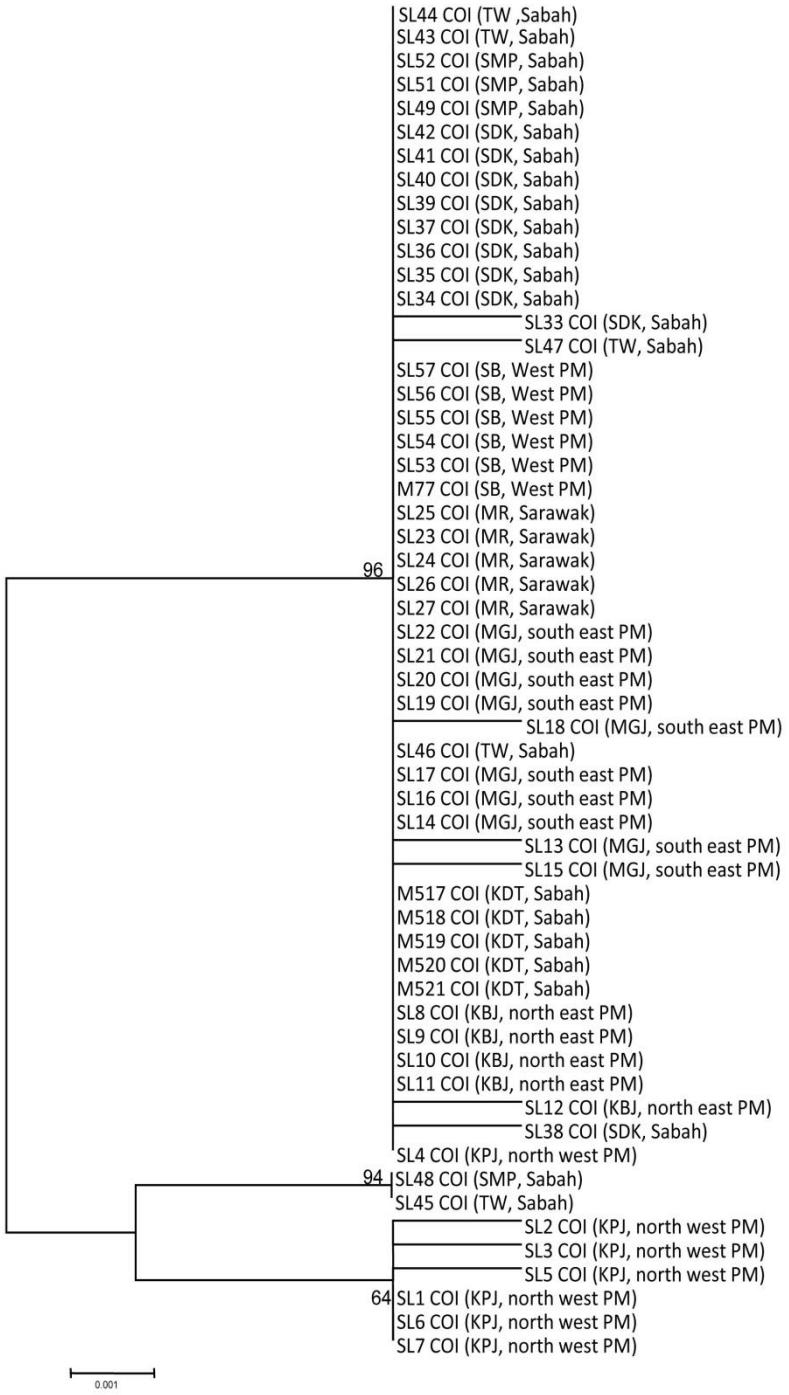


b) Bayesian tree

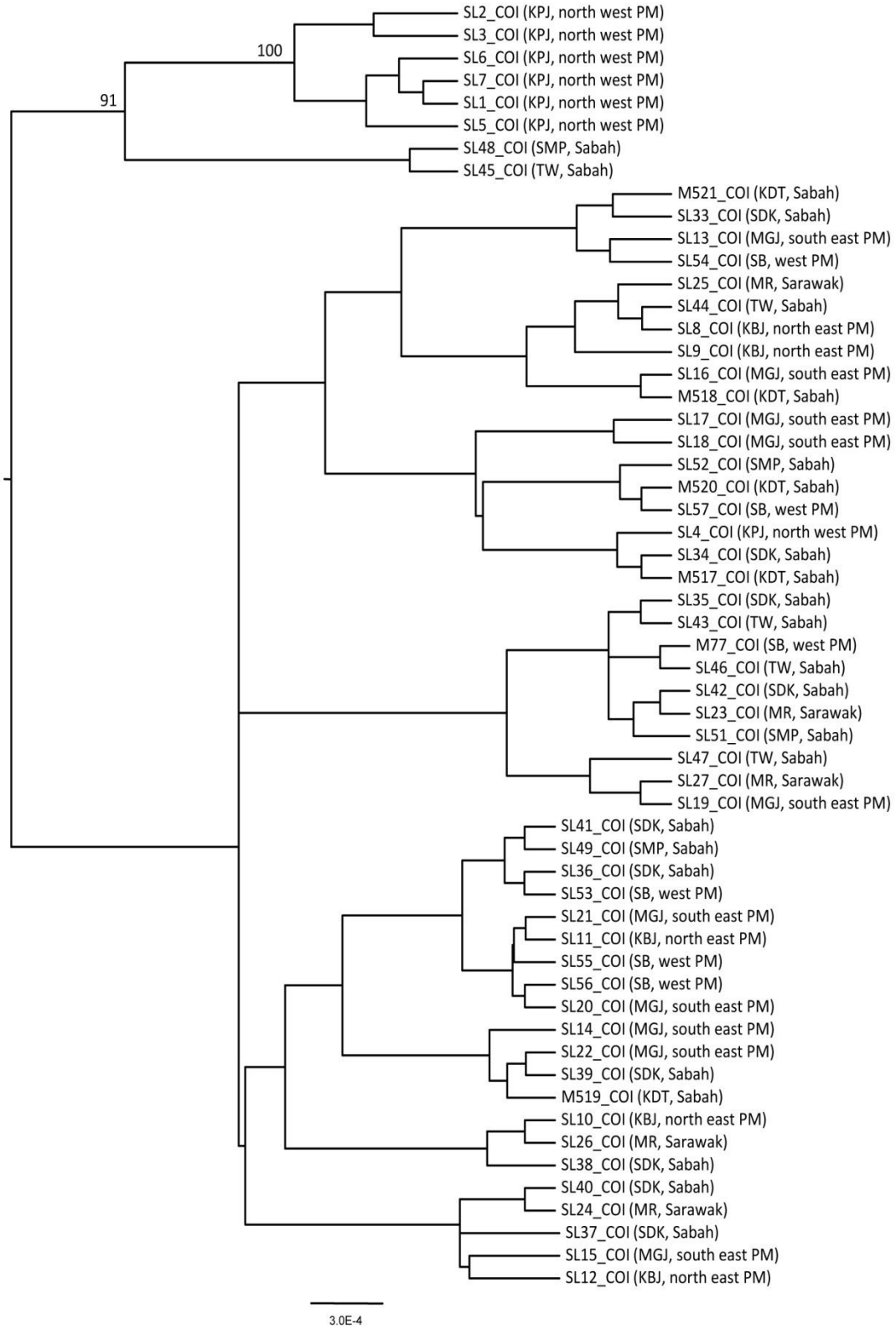
Appendix 9

Maximum-likelihood and Bayesian trees of *Selaroides leptolepis* by genes.

9.1 a) Maximum-likelihood and b) Bayesian trees of 57 *COI* sequences.

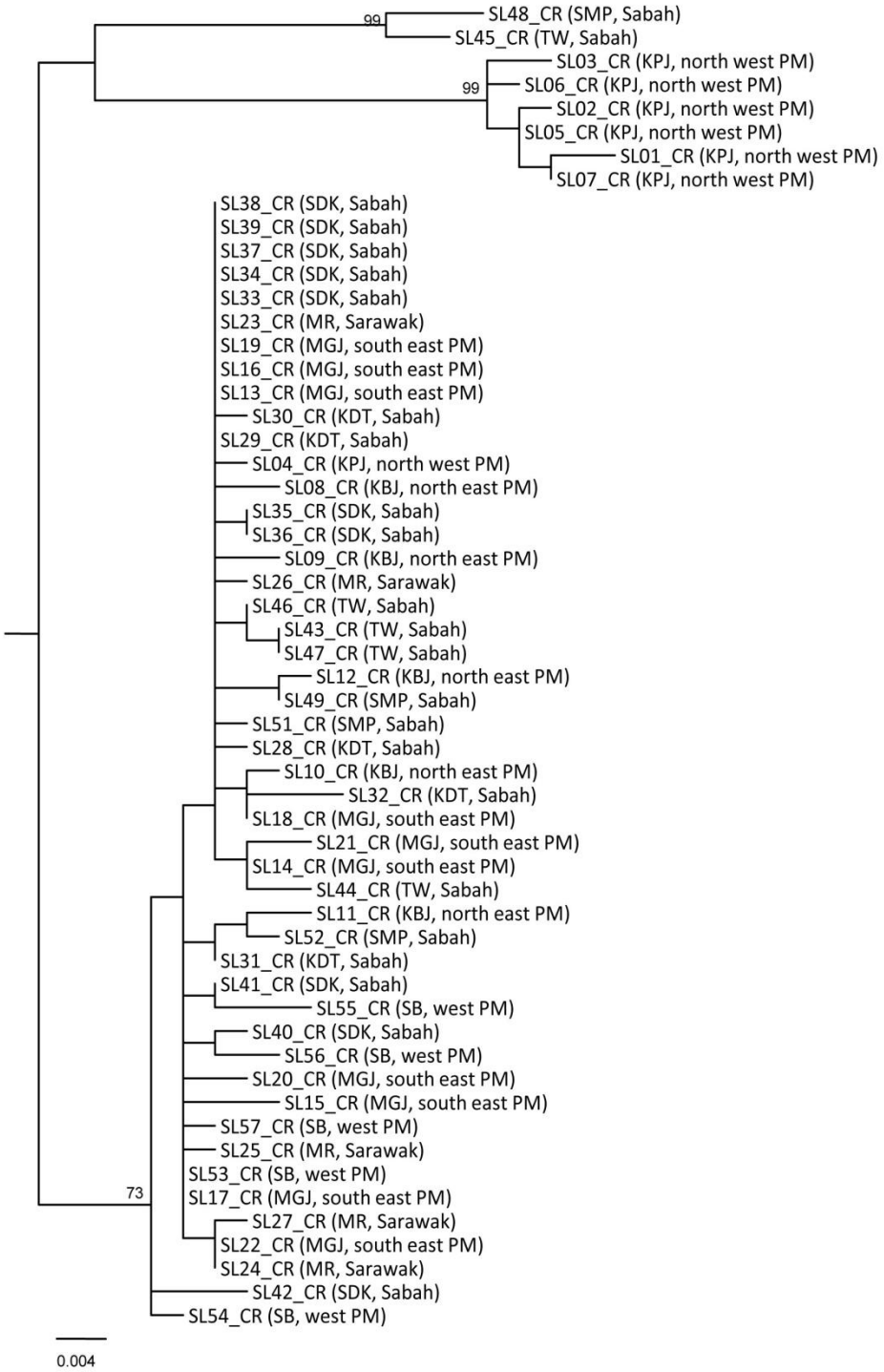


a) Maximum-likelihood

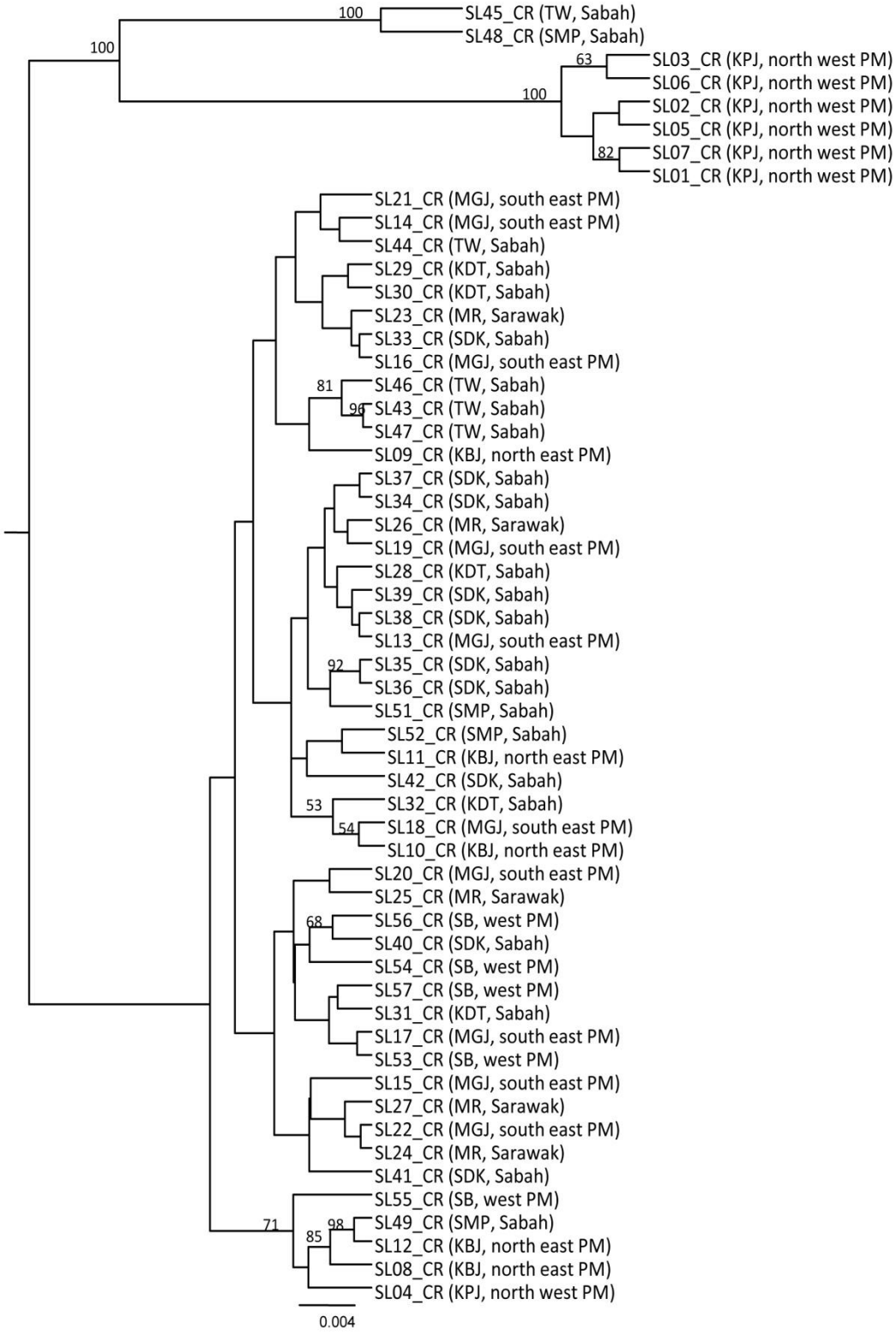


b) Bayesian tree

9.2 a) Maximum-likelihood and b) Bayesian trees of 56 control region sequences.

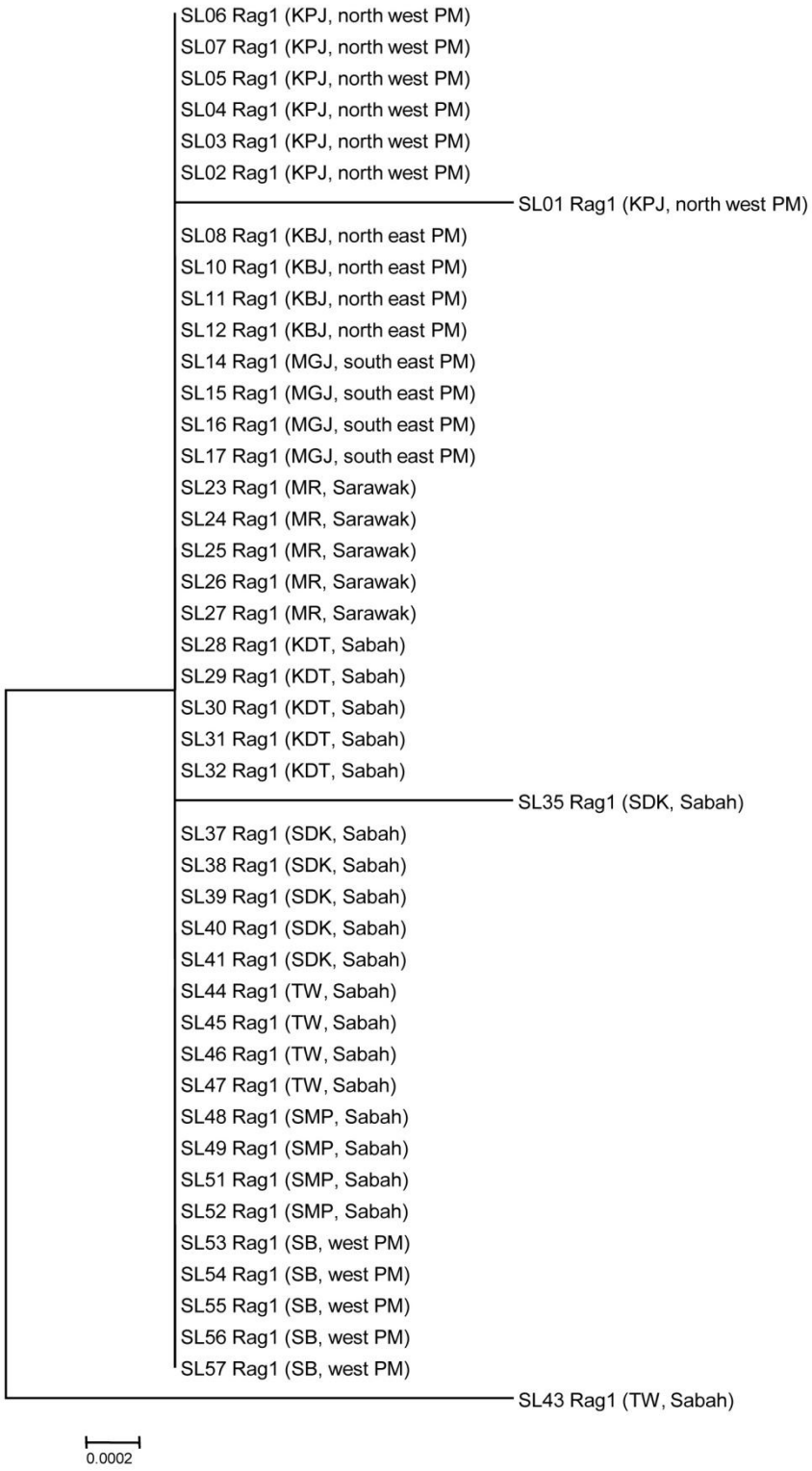


a) Maximum-likelihood tree

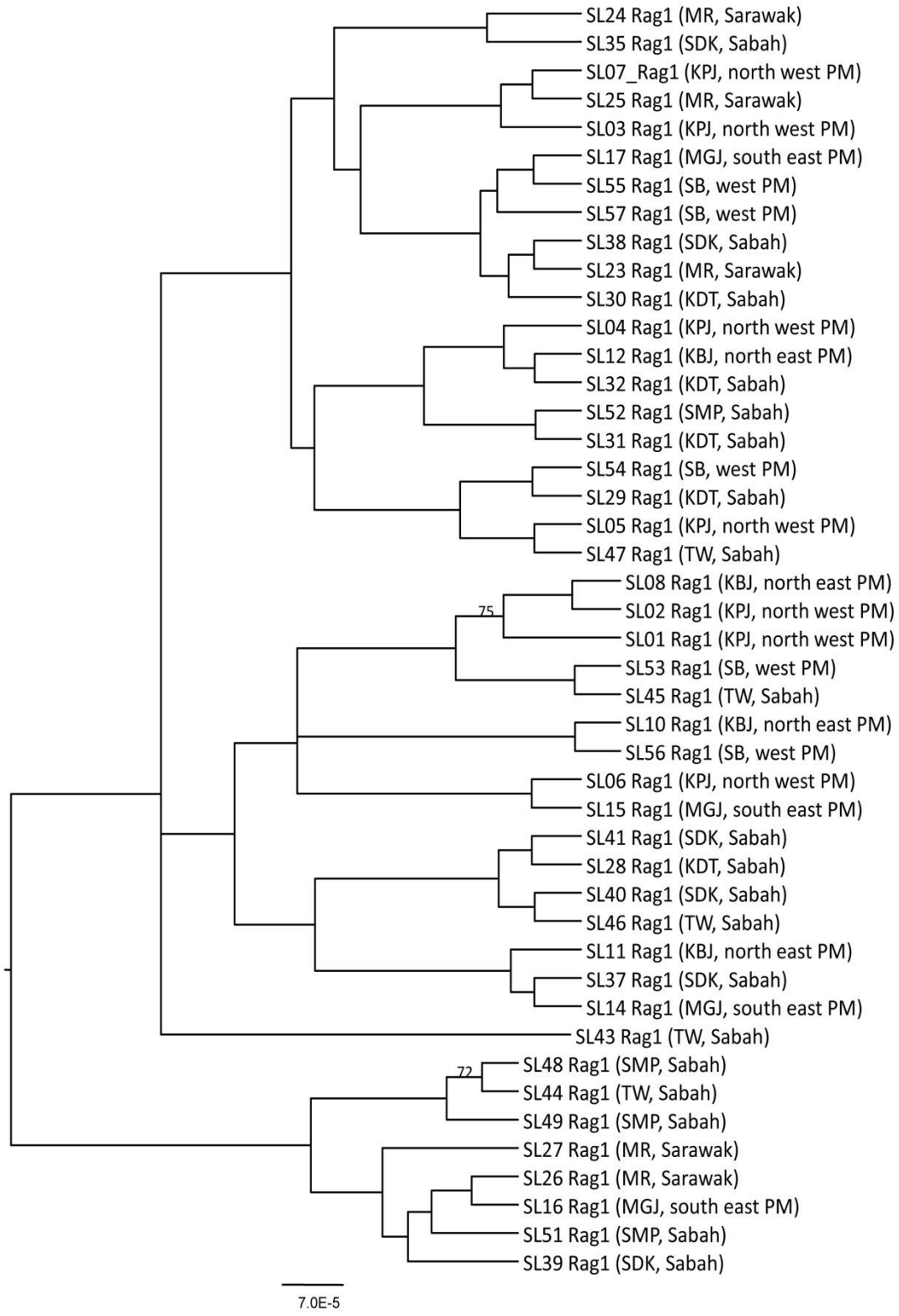


b) Bayesian tree

9.3 a) Maximum-likelihood and b) Bayesian trees of 45 Rag1 sequences.



a) Maximum-likelihood



b) Bayesian tree

Appendix 10

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PLOS ONE

DNA Barcoding Reveals Cryptic Diversity within Commercially Exploited Indo-Malay Carangidae (Teleosteii: Perciformes)

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Abstract

Background: DNA barcodes, typically focusing on the *cytochrome oxidase I gene (COI)* in many animals, have been used widely as a species-identification tool. The ability of DNA barcoding to distinguish species from a range of taxa and to reveal cryptic species has been well documented. Despite the wealth of DNA barcode data for fish from many temperate regions, there are relatively few available from the Southeast Asian region. Here, we target the marine fish Family Carangidae, one of the most commercially-important families from the Indo-Malay Archipelago (IMA), to produce an initial reference DNA barcode library.

Methodology/Principal Findings: Here, a 652 bp region of *COI* was sequenced for 723 individuals from 36 putative species of Family Carangidae distributed within IMA waters. Within the newly-generated dataset, three described species exhibited conspecific divergences up to ten times greater (4.32–4.82%) than mean estimates (0.24–0.39%), indicating a discrepancy with assigned morphological taxonomic identification, and the existence of cryptic species. Variability of the mitochondrial DNA *COI* region was compared within and among species to evaluate the *COI* region's suitability for species identification. The trend in range of mean K2P distances observed was generally in accordance with expectations based on taxonomic hierarchy: 0% to 4.82% between individuals within species, 0% to 16.4% between species within genera, and 8.64% to 25.39% between genera within families. The average Kimura 2-parameter (K2P) distance between individuals, between species within genera, and between genera within family were 0.37%, 10.53% and 16.56%, respectively. All described species formed monophyletic clusters in the Neighbour-joining phylogenetic tree, although three species representing complexes of six potential cryptic species were detected in Indo-Malay Carangidae; *Atule mate, Selar crumenophthalmus* and *Seriolina nigrofasciata*.

Conclusion/Significance: This study confirms that *COI* is an effective tool for species identification of Carangidae from the IMA. There were moderate levels of cryptic diversity among putative species within the central IMA. However, to explain the hypothesis of species richness in the IMA, it is necessary to sample the whole family across their broad geographic range. Such insights are helpful not only to document mechanisms driving diversification and recruitment in Carangidae, but also to provide a scientific framework for management strategies and conservation of commercially-important fisheries resources.

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Introduction

Spectacular biodiversity exists in tropical marine ecosystems. One mega-diverse tropical region, where the ranges of many tropical marine species overlap, is the centre of maximum marine biodiversity of the Indo-Malay Archipelago (IMA) [1]. Various hypotheses giving rise to this extraordinary species richness have been proposed [2], though two in particular have been widely addressed [3–5]: the Centre-of-Overlap and the Centre-of-Origin hypotheses, both of which postulate contrasting patterns of species ranges and distribution of species richness. The former proposes geographic isolation and allopatric speciation with midpoint ranges of species distributions falling on each side of the IMA, with overlap across the IMA. Large scale genetic structure is expected to result from geographic isolation, and cryptic species may be expected to exhibit allopatric distribution ranges, potentially overlapping in the IMA. The Centre-of-Origin hypothesis proposes speciation centred in the IMA, with midpoint ranges of species distributions occurring within the IMA. Large scale genetic structure is expected to be shallow as a consequence of high connectivity and larval dispersal across the IMA. Since the IMA encompassess the centre of the distributional range of the

target taxa studied here, the Carangidae, we test whether there is any evidence of highly divergent cryptic lineages in sympatry, as predicted by the Centre-of-Origin hypothesis.

Given that only a small fraction of all global species have been formally described, between 1.5–1.8 million out of an estimated 10 million [6], efforts to catalogue and understand drivers of biodiversity need to be prioritised. Research on cryptic species has increased recently with studies [5,7–8] indicating the frequent occurrence of cryptic species occurring within and outside the IMA. One of the problems associated with identifying cryptic species is that many taxonomic protocols rely on phenotypic characters, and require lengthy and detailed inspection of the specimens [9]. Such traditional methods of identifying, naming and classifying organisms are largely based on visible morphology. Misidentification of economically important species in cryptic species-complexes can result in inaccurate data collection potentially leading to the overexploitation of stocks [10]. Therefore, in addition to disclosing potential drivers of diversification, accurate identification at the species-level is vital to ensure the successful management of commercially important fish stocks in IMA waters, and here, a DNA barcoding database can play an important role.

The introduction of the DNA barcoding approach, which utilises a short, standardised gene region [11] to identify species [12–17] has been shown to be useful in solving taxonomic ambiguities. Hebert *et al.* [11] proposed that within species, DNA sequences would be more similar than that among different species, and that this 'barcoding gap' could be used to delimit species. To date, the *Cytochrome Oxidase subunit I (COI)* mitochondrial protein-coding gene has been accepted widely as a practical, standardized species-level barcode for the majority of the animal kingdom [18]. The main goal of DNA barcoding is to facilitate rapid identification of potentially unidentified taxa in global biodiversity assessment and conservation, including cryptic and microscopic taxa, and organisms with morphologically ambiguous characters [11]. DNA barcoding has also focused on the development of a global barcoding database [19] as a species identification tool for large taxonomic assemblages of animals, representing a quick and easy method for non-specialists to identify disparate specimens. The identification process through DNA barcoding is relatively straight-forward, and depends upon the quantifiable matching of *COI* sequences from unknown specimens with previously documented and archived voucher specimens. Where marked discordance is found in the *COI* sequences of test and reference specimens, additional taxonomic and related studies are undertaken to assess likelihood of discovering novel taxa [20].

To date, many barcoding projects involving various organisms from different geographic regions can be accessed from the public barcode library, the Barcode of Life Data Systems (www.barcodinglife.com) [19]. Despite the wealth of DNA barcode information for fish from many temperate regions [21–24], there are relatively few data available from Southeast Asian waters, an area exceptionally rich in biodiversity. DNA barcoding should prove useful for rapid biodiversity assessment [25] in this region, where significant levels of biodiversity loss are escalating [1]. Our study provides the first barcode records for 723 specimens representing 36 putative species from Carangidae sampled from waters of the IMA. Variability of *COI* was compared both within and among species to evaluate its suitability for species identification. Samples for assaying the *COI* barcodes were analysed and compared with field-based morphological species identifications and additional molecular data from other geographical regions were obtained from GenBank and the BOLD System. Such analyses may identify hidden diversity in Carangidae, where such diversity exists.

The family Carangidae encompasses fishes whose body size ranges from small (TL = 16 cm) to large (TL = 250 cm) and body shapes vary from elongate and fusiform to deep and strongly compressed [26]. This diverse family of marine fishes are known variously by common names such as jacks, trevallies, amberjacks, pompanos, scads, kingfish, pilotfish, queenfishes and rainbow runner [27]. Carangids represent an important food source and play a significant role in the commercial fisheries industry in Southeast Asia [27]. All members, small or large are considered as edible protein and can be caught in large numbers every year (ca. 1,556,578 tonnes in 2010) [28]. Despite their high economic value and ecological importance, the taxonomy of Carangids remains poorly understood [29]. FishBase citations include many synonyms, which indicate taxonomic ambiguities in Carangids [30] due to morphological and meristic similarities across species, as well as plasticity in body shape, size and colour patterns [7,31]. In addition, Carangids typically display significant changes in morphology and pigmentation during growth [32], and such changes have likely lead to misidentification of specimens, and contributed to general taxonomic confusion. An interesting example of change with growth occurs in juveniles of African pompano (*Alectis ciliaris*), which are easily recognized by the presence of long filaments trailing from five to six dorsal and anal fins. As fish grow larger, these filaments shorten and eventually disappear [33]. The exact biological mechanism behind such developmental changeis unclear, as is the function of the filaments. Carangid eggs and newly hatched larvae are also difficult to distinguish from the eggs and larvae of many other families of marine fishes [34], making it difficult to map spawning grounds and identify ichthyoplankton [35]. Pigmentation changes during development in Carangid larvae and its diagnostic value is thereby of limited value for species identification [36]. Unambiguous delineation of such apparent phenotypic plasticity is required not only for taxonomy and systematics, but also is of critical importance for fisheries management, trade and conservation purposes. *Cytochrome oxidase subunit 1 (COI)* has been shown to accurately discriminate between closely related species of various animal groups [13,15–17,37], and is applied here to examine the integrity of species delineation in Carangids.

Materials and Methods

Sampling

We collected 845 Carangidae specimens from four geographic regions within the IMA: South China Sea, Straits of Malacca, Sulu Sea and Celebes Sea. The samples were collected from several fish landing sites during two field trips; from October to November 2009, and from June to July 2010 (Figure 1). Specimens encompassed 39 putative species and 18 genera from the Family Carangidae. Sample collections included tissue sampling for genetic analysis, as well as collection of whole specimens (adult fish and larvae) for storage as barcode voucher specimens. All samples were preserved in 99% ethanol. Digital photographs of all fishes were taken immediately and voucher specimens were tagged according to museum ID number and archived in the South China Sea Museum, Universiti Malaysia Terengganu (www.umt.edu.my). All details regarding collection dates, collection sites with geographical coordinates, taxonomy and vouchers can be found in the Barcode of Life Data System website (BOLD, www.barcodinglife.com) [19] under project 'DNA Barcoding of Malaysian Fish' (DBMF). At least five individuals of each species were collected from each sampling site depending on their

abundance. Few specimens were collected in some low abundance species (<5), while those that were abundant enabled the collection of more individuals (up to 75), with 29/36 species having sample sizes of >5 individuals. All fishes were identified based on morphology, with the help of expert local taxonomists in most cases, FAO-Fisheries Identification Sheets [38] and identification books published by the Department of Fisheries Malaysia [39–40].

Fin clips were removed from the right pectoral fin of each fish and preserved in 99% ethanol. Fish specimens were then placed in ice, frozen on site and transported to South China Sea Museum, University Malaysia Terengganu. Fin clips were sent to the Canadian Centre for DNA Barcoding (CCDB), University of Guelph Ontario, Canada for further processing. Total genomic DNA was extracted from fin clips of 39 putative species and PCR amplifications performed using the procedure of [41]. Following the CBOL standard practice, *COI* genes were sequenced in both directions. All *COI* sequences and trace files have been deposited in the Barcode of Life Data System (www.barcodinglife.com) under a project named 'DNA Barcoding of Malaysian Marine Fish' (DBMF). Sequences have also been deposited in GenBank (Table S1, Supporting information).

Data validation

For this study, we collected 845 individuals of Carangidae. However, a total of 110 individuals generated sequences of insufficient quality to be uploaded into the BOLD system, and were therefore not considered further. After exclusion of these 110 individuals, our *COI* data base encompasses a total of 735 sequences. Incorrect taxonomic classification may affect divergence assessment of our data set. Therefore, all 735 sequences were aligned and a Neighbour Joining tree produced using the BOLD platform. A small percentage (1.63%) of samples which did not cluster with their own taxa had their photographs reviewed and this revealed potential misidentification. The remaining three species (*Carangoides oblongus*, *Carangoides orthogrammus*, *Trachinotus blochii*) with one specimen each, failed to PCR amplify, leaving a total of 36 species in the data set. Subsequently, we analysed 723 sequences from 36 species and 18 genera from Family Carangidae.

COI divergence assessment

The diversity assessment for Carangidae were analysed from the data set with 723 sequences, 18 genera and 36 putative species. The Kimura 2-parameter (K2P) distance measure has become the most widely used in barcoding studies [42] and was employed here. Genetic distances between specimens were calculated for each intraspecies, intragenus and intrafamily with the 'Distance Summary' command implemented by BOLD. K2P was also used for Neighbour-joining (NJ) analysis (Figure S1, Supporting Information), using the BOLD Management and Analysis System. All sequences were aligned using the MUSCLE algorithm in the software programme MEGA5 [43], and the amino acid translation was examined to ensure that no gaps or stop codons were present in the alignment. NJ analyses were conducted using 1000 bootstrap replicates. Nucleotide divergences of *COI* variation across 36 species of Carangidae were analysed. Genetic distances among specimens were calculated for each intraspecies and intragenus pairwise comparison with the 'Distance Summary'

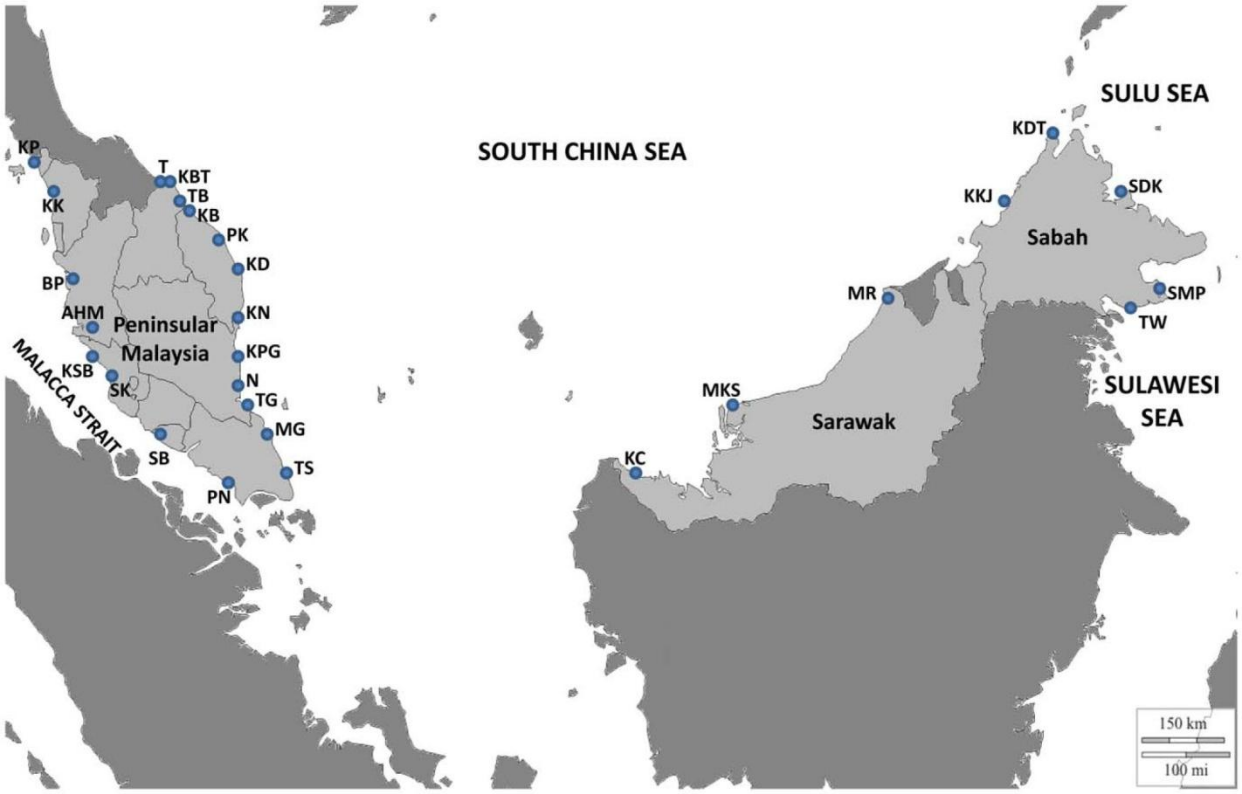

Figure 1. Distribution of locations for the 845 specimens sampled along the coast of Malaysia. See Table S1 for detailed sampling information.
doi:10.1371/journal.pone.0049623.g001

Table 1. Kimura 2-parameter (K2P) distances between Indo-Malay Carangidae.

Comparison within	*n*	Number of comparisons	Min (%)	Mean (%)	Max (%)	SE (%)
Species	36	13445	0	0.37	4.82	0.006
Genus	18	10680	0	10.53	16.4	0.028
Family	1	240503	8.64	16.56	25.39	0.006

doi:10.1371/journal.pone.0049623.t001

analysis in BOLD. Other analytical tools in BOLD such as Nearest Neighbour, Identify Unknown and BOLD Identification System were also applied to the data. The Maximum Likelihood (ML) approach was also conducted by determining the highest likelihood tree bootstrapped 1000 times using RAxML 7.2.8 [44] (Figure S2, Supporting Information). Bayesian phylogenetic analyses was conducted in Mr Bayes v3.2.1 [45], though outputs showed no convergence after 10 million generations. We thus discarded these analyses and present here only NJ and ML analyses. We also employed the recently described bioinformatics tool, Automatic Barcode Gap Discovery (ABGD) [46] for species delimitation analysis. ABGD automatically detects the breaks in the distribution of genetic pairwise distances, referred to as the 'barcode gap' and uses it to partition the data. The method proposes a standard definition of the barcode gap and can be used even when the two distributions overlap to partition the data set into candidate species. The same species therefore should be grouped in the same partition.

Additional *COI* sequences from GenBank and BOLD Systems were added to compare *COI* sequences of 23 selected species from this study with conspecifics from West (South Africa, Mozambique, Iran, India and Turkey) and East (Australia, Philippines, China, Japan, Hawaii, French Polynesia and Mexico) of the IMA. All species and GenBank accession numbers are listed in Table S1.

Results

General findings

COI barcodes were recovered for a total of 36 species and 18 genera from the Family Carangidae, for the first time from the IMA. The number of sequences per species varied between 1 (*Carangoides gymnosthetus*) for species that were rare, to 75 (*Selar crumenophtalmus*) for species that were abundant in Malaysian waters. Thus a total of 723 *COI* barcodes with an average length of 652 bp were obtained for this commercially important fish family. No insertions/deletions, heterozygous sites or stop codons were observed, supporting the view that all of the amplified sequences constitute functional mitochondrial *COI* sequences.

COI divergence assessment

COI nucleotide divergences were calculated for the dataset of 723 sequences of 36 species and 18 genera. Sample sizes and mean divergences at various taxonomic levels are given in Table 1. As expected, genetic divergence increased progressively with higher taxonomic level: 0% to 4.82% between individuals within species, 0% to 16.4% between species within genera, and 8.64% to 25.39% between genera within family, which support a marked change in genetic divergence at the species boundary (Figure 2).

The average within species K2P distance is 0.37% with far less, 0.00% for *Carangoides ferdau*, *Gnathanodon speciosus* and *Trachinotus*

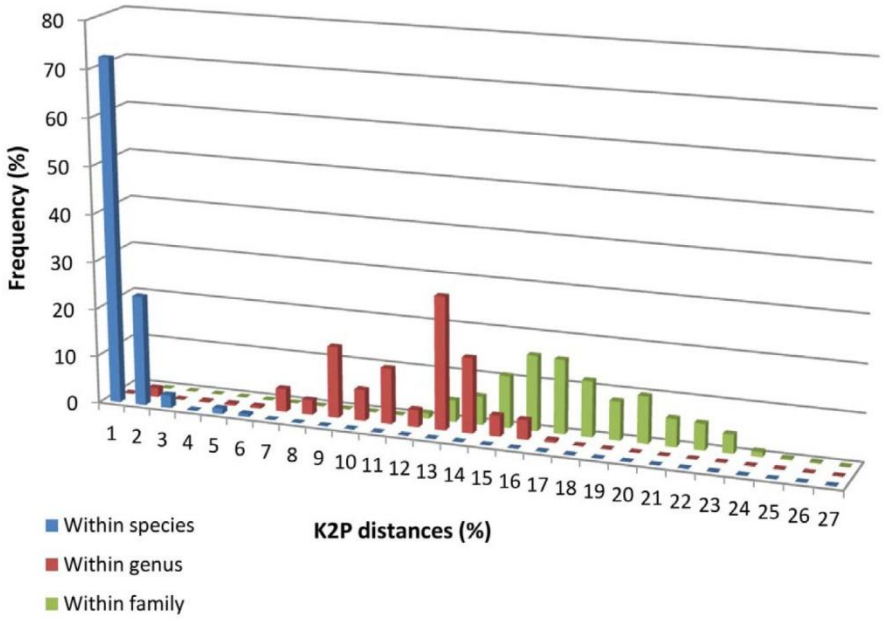

Figure 2. Frequency distributions of *COI* K2P distances (%) intraspecies, intragenus and intrafamily. 36 species, 18 genera and 1 family.
doi:10.1371/journal.pone.0049623.g002

Table 2. Intraspecific nucleotide K2P distances for 36 species of Indo-Malay Carangidae.

Species	No. of sequences (n)	Mean K2P distance (%)
Alectis ciliaris (Bloch, 1787)	8	0.16
Alectis indicus (Rüppell, 1830)	10	0.17
Alepes djedaba (Forsskål, 1775)	31	0.25
Alepes kleinii (Bloch, 1793)	11	0.16
Alepes melanoptera (Swainson, 1839)	15	0.40
Alepes vari (Cuvier, 1833)	13	0.16
Atropus atropos (Bloch & Schneider, 1801)	13	1.13
Atule mate (Cuvier, 1833)	67	0.34
Carangoides bajad (Forsskål, 1775)	26	0.39
Carangoides chrysophrys (Cuvier, 1833)	19	0.33
Carangoides dinema (Bleeker, 1851)	6	0.03
Carangoides ferdau (Forsskål, 1775)	2	0.00
Carangoides fulvoguttatus (Forsskål, 1775)	3	0.21
Carangoides gymnostethus (Cuvier, 1833)*	1	N/A
Carangoides hedlandensis (Whitley, 1934)	3	0.31
Carangoides malabaricus (Bloch & Schneider, 1801)	33	0.54
Caranx ignobilis (Forsskål, 1775)	6	0.51
Caranx sexfasciatus Quoy & Gaimard, 1825	8	0.16
Caranx tille Cuvier, 1833	9	0.07
Decapterus kurroides Bleeker, 1855	10	0.09
Decapterus macrosoma Bleeker, 1851	26	0.08
Decapterus maruadsi (Temminck & Schlegel, 1843)	24	0.15
Elagatis bipinnulata (Quoy & Gaimard, 1825)	8	0.22
Gnathanodon speciosus (Forsskål, 1775)	4	0.00
Megalaspis cordyla (Linnaeus, 1758)	63	0.53
Parastromateus niger (Bloch, 1795)	51	0.30
Scomberoides commersonnianus Lacepède, 1801	17	0.56
Scomberoides tala (Cuvier, 1832)	11	0.08
Scomberoides tol (Cuvier, 1832)	32	0.09
Selar boops (Cuvier, 1833)	40	0.37
Selar crumenophthalmus (Bloch, 1793)	75	0.39
Selaroides leptolepis (Cuvier, 1833)	39	0.18
Seriola dumerili (Risso, 1810)	4	0.31
Seriolina nigrofasciata (Rüppell, 1829)	9	1.79
Trachinotus baillonii (Lacepède, 1801)	4	0.00
Uraspis uraspis (Günther, 1860)	22	0.67

*only 1 sequence available.
doi:10.1371/journal.pone.0049623.t002

baillonii. The latter estimates were largely due to the low number of specimens collected, and all specimens were from the same landing site (n = 1–4). *Atropus atropos* (1.13%) and *Seriolina nigrofasciata* (1.79%) displayed slightly higher divergence rates than average (Table 2). The average congeneric distance was 10.53%, which was higher than the conspecific distance. The congeneric distances were lowest among queen fishes, *Scomberoides* (7.52% - 3 species), followed by *Caranx* (7.53% - 3 species); *Alepes* (8.84% - 4 species); *Decapterus* (8.89% - 3 species); *Alectis* (11.37% - 2 species); *Carangoides* (11.66% - 7 species) and the highest variation observed in the genus *Selar* (12.25% - 2 species) (Table 3).

Mean intraspecific K2P divergence of Indo-Malay Carangidae was 0.37% (range 0–4.82%), while mean congeneric species K2P divergence was 10.53% (range 0–16.4%) (Table 1). In the NJ analyses, the majority of recognised species formed monophyletic clusters (Figure 3). Such patterns illustrate the utility of *COI* sequences to provide species-level resolution. All assemblages of conspecific individuals had bootstrap support of 98–100%. However, in ML analyses (Figure S2, Supporting Information), four species which have been identified as different species formed two monophyletic clusters; *Alepes vari* grouped together with *Alepes melanoptera*, while *Carangoides bajad* grouped in the same cluster as *Carangoides gymnosthetus*. These results were also supported by the ABGD analysis (Figure S3, Supporting Information).

Table 3. Congeneric nucleotide K2P distances for seven genera in Indo-Malay Carangidae

Genus	No. of sequences (n)	Mean K2P distance (%)
Alectis	18	11.37
Alepes	70	8.84
Carangoides	93	11.66
Caranx	23	7.53
Decapterus	60	8.89
Scomberoides	60	7.52
Selar	115	12.25

doi:10.1371/journal.pone.0049623.t003

Cryptic diversity in the Indo-Malay Archipelago

In three species, we detected deep divergences among individuals that had been assigned to a single taxon. Closer observation of the data associated with *Atule mate, Selar crumenopthalmus* and *Seriolina nigrofasciata* showed maximum intraspecific divergences of 4.82%, 4.66% and 4.32% (Table S2, Supporting information) respectively, revealing that the specimens of each in fact formed two clusters in both NJ and ML analyses with 99–100% bootstrap support (Figures 4–9). Divergent as they were, members of the two clusters nonetheless were more similar to each other than to members of any other species in our data set.

Atule mate

Phylogenetic analyses also revealed two clusters generated from 67 *Atule mate* samples (Figures 4 and 5). Mean K2P distance within species was 0.34% with a maximum of 4.82% nucleotide divergence. These clusters were separated by a mean *COI* nucleotide divergence of 4%. Cluster I, the major lineage containing most specimens from all sampling regions exhibited no obvious geographic structuring, and was strongly supported with a bootstrap value of 100% in the NJ tree. In contrast, Cluster II is a minor lineage, containing only a single specimen from Tok Bali, Kelantan, eastern Peninsular Malaysia (TB). Phylogenetic trees constructed from control region and Rag 1 (nuclear DNA) data were consistent with the pattern observed at *COI* (unpublished data).

Selar crumenophthalmus

Seventy five specimens of *Selar crumenophthalmus* also formed two clusters in the *COI* NJ and ML trees (Figures 6 and 7). Mean K2P distance within species was 0.39% with a maximum of 4.66% nucleotide divergence. Cluster I comprised the majority of the specimens with a high bootstrap value of 100%, while Cluster II comprised only two individuals from Kuala Kedah, western Peninsular Malaysia (KK) and Kuching, Sarawak (KC), also supported by a high bootstrap value of 100%. A mean pairwise distance of 4.5% separated these two clusters. No geographic pattern was apparent.

Seriolina nigrofasciata

Mean K2P distance within species of *Seriolina nigrofasciata* was 1.79% with a maximum nucleotide divergence of 4.32%. Nine specimens of this species formed two clusters with Cluster I comprising the specimens from Kota Kinabalu (KKJ) and Kudat (KDT), Sabah. Cluster II comprised only two individuals from Hutan Melintang (AHM) and Bagan Panchor (BP) from western Peninsular Malaysia, supported by a bootstrap value of 100% (Figures 8 and 9). A mean pairwise distance of 4.32% separated these two clusters.

COI sequences of 23 species examined here were compared with data available from conspecifics from other geographical regions (downloaded from BOLD and GenBank), and NJ trees were produced for each species (Figure S4, Supporting Information). From these 23 widespread species, 13 species exhibited shallow genetic structure with mixed *COI* lineages found on either side of the IMA. The other 10 species each formed two clusters with maximum nucleotide divergences ranging from 2.68–8.81%.

Discussion

According to the Fish Barcode of Life project database (www.fishbol.org), in 2009, 69% of species from Family Carangidae had been barcoded in Southeast Asia, but with some species represented by only a single sample. DNA barcodes had increased to 83% with 43 species having more than four barcodes in November 2011, including our data. We sequenced a total of 723 specimens from 18 genera and 36 species of Carangidae at the *COI* barcoding region. Thirty-three species could be accurately discriminated, illustrating the effectiveness of the *COI* gene for identifying commercial marine fish from Malaysian waters, and providing resolution at the species-level. However, the remaining three species showed deep divergences (4.32–4.82%) among individuals that had been assigned to a single taxon. Divergent as they were, members of the two clusters nonetheless were more similar to each other than members of any other species. These high sympatric divergences suggested that each may comprise cryptic species.

The average K2P distance of individuals within species was 0.37% compared with 10.53% for species within genera. Hence, congeneric species were approximately 28 times more divergent than conspecific individuals. The mean intraspecific K2P distance observed was similar to the intraspecific K2P distance reported for marine (0.24–0.39%) [23] and freshwater species (0.3–0.45%) [21]. The branch length among species tends to be much deeper than among conspecific individuals leading to a gap in the distribution of the pairwise distance among conspecific individuals and among species that has been referred to as the barcoding gap [47]. Mean divergence among species within families increased to 16.56%. These data show that increasing genetic divergence was observed with increasing taxonomic level, supporting a marked difference in genetic divergence at the species boundary. Such patterns in taxonomic distribution of nucleotide divergence supports observations obtained by Ward *et al.* [22] with genetic distances of 0.39% for conspecifics, 9.93% for congenerics and 15.46% for confamilial species of 754 *COI* sequences representing 207 species of Australian fish. Data obtained in our study were also consistent with those obtained by Asgharian *et al.* [48] for 187 individuals of Persian Gulf fish with values of 0.18%, 12% and 17.43% among conspecifics, congenerics and confamilial species respectively.

The NJ tree revealed that species identification and phylogenetic relationships based on morphological evidence and molecular methods are broadly consistent. However, the ML analyses suggested that four species might comprise only two taxonomic units, as these four species formed two reciprocally monophyletic clusters in the ML tree (*Alepes vari* and *Alepes melanoptera*; *Carangoides bajad* and *Carangoides gymnosthetus*). ABGD analysis supports such findings as the same pattern was evident. Further analyses should be undertaken by the inclusion of more genes and larger sample sizes to confirm the relationships across these four species. Phylogenetic relationships among species with NJ analysis were

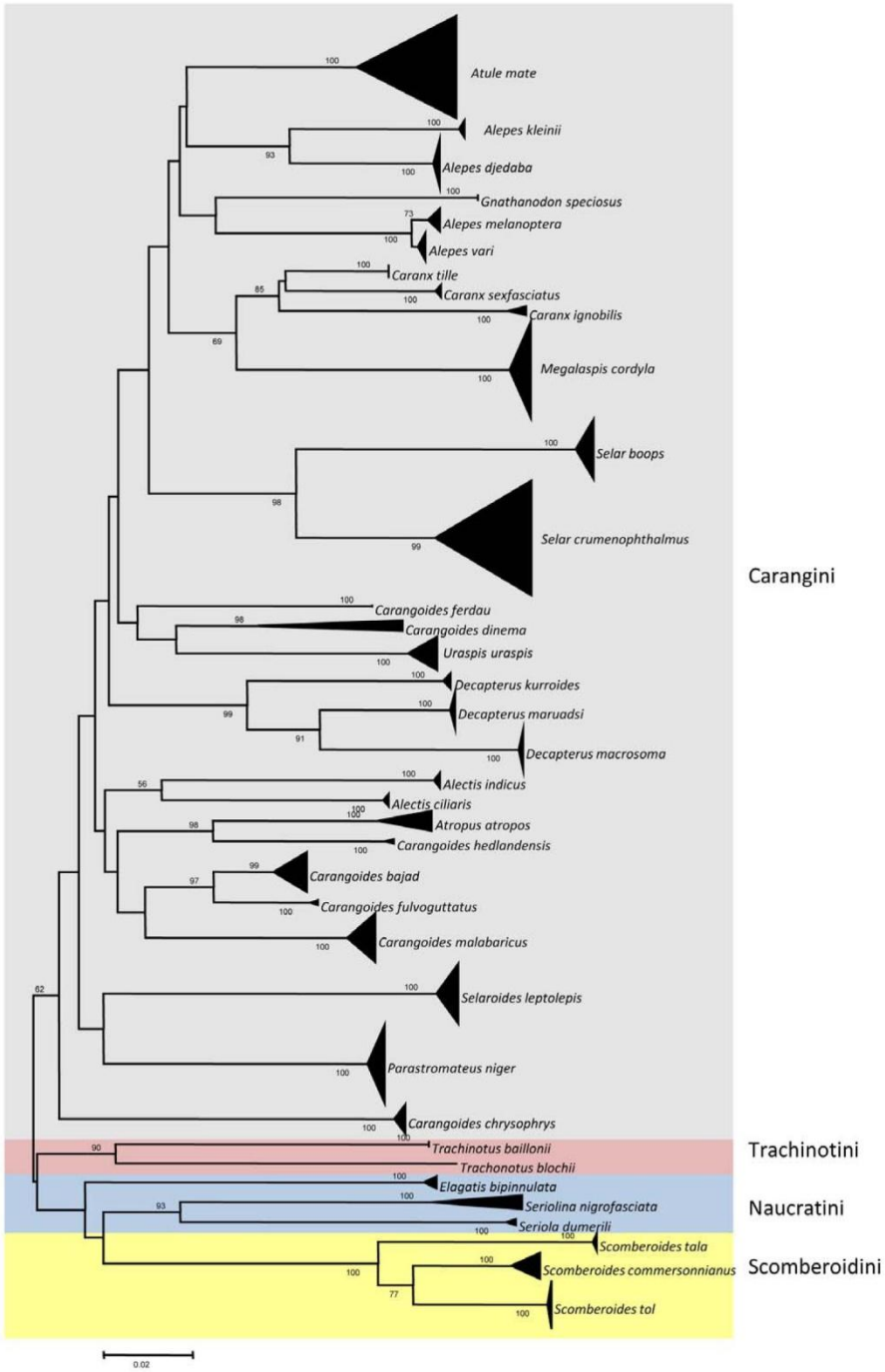

Figure 3. Neighbour-joining tree (K2P distance) of 36 Carangidae species. All species formed monophyletic clusters. Only bootstrap values greater than 50 are shown.
doi:10.1371/journal.pone.0049623.g003

clearly established, and individuals from the same species were grouped in the same taxonomic cluster with 98–100% bootstrap support. According to Smith-Vaniz [49], Carangidae can be categorized into four tribes based on morphological evidence; the Carangini, Trachinotini, Naucratini and Scomberoidini. All species of Carangidae in our study grouped according to Smith-Vaniz [49] (Figure 3), with the larger clade consisting of specimens known as jacks, trevallies, scads and black pomfret (tribe Carangini). The second clade comprised the other three tribes; Trachinotini, Naucratini and Scomberoidini, representing pompano, amberjacks and queen fishes. The emergence of these four tribes in NJ analyses clearly demonstrates that there is deep

Figure 4. Neighbour-joining tree (K2P distance) of 67 *COI* sequences of *Atule mate*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g004

phylogenetic signal in the relatively short *COI* sequence fragments, even though barcode analysis seeks only to delineate species boundaries. However, the phylogenetic relationships of these four tribes remain questionable [50–52], and our approach in isolation is not sufficient to explore such questions in depth. Additional gene regions, together with more comprehensive analytical methods including parsimony, ML and Bayesian approaches should be included to resolve such apparently deep phylogenetic relationships.

The main goals of DNA barcoding are to assign unknown specimens to a species category, and enhance the disclosure of new

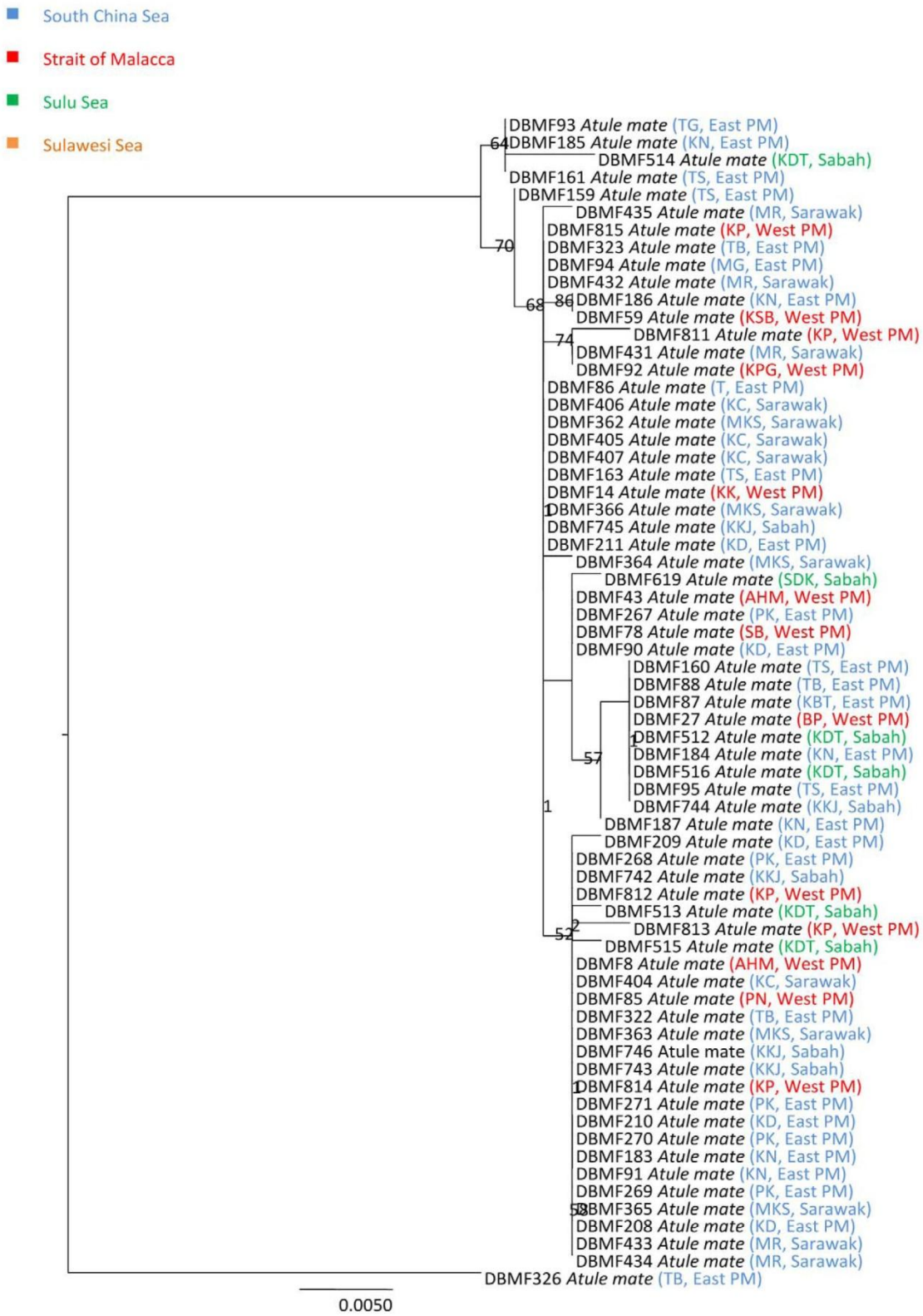

Figure 5. Maximum-likelihood tree of 67 *COI* sequences of *Atule mate*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g005

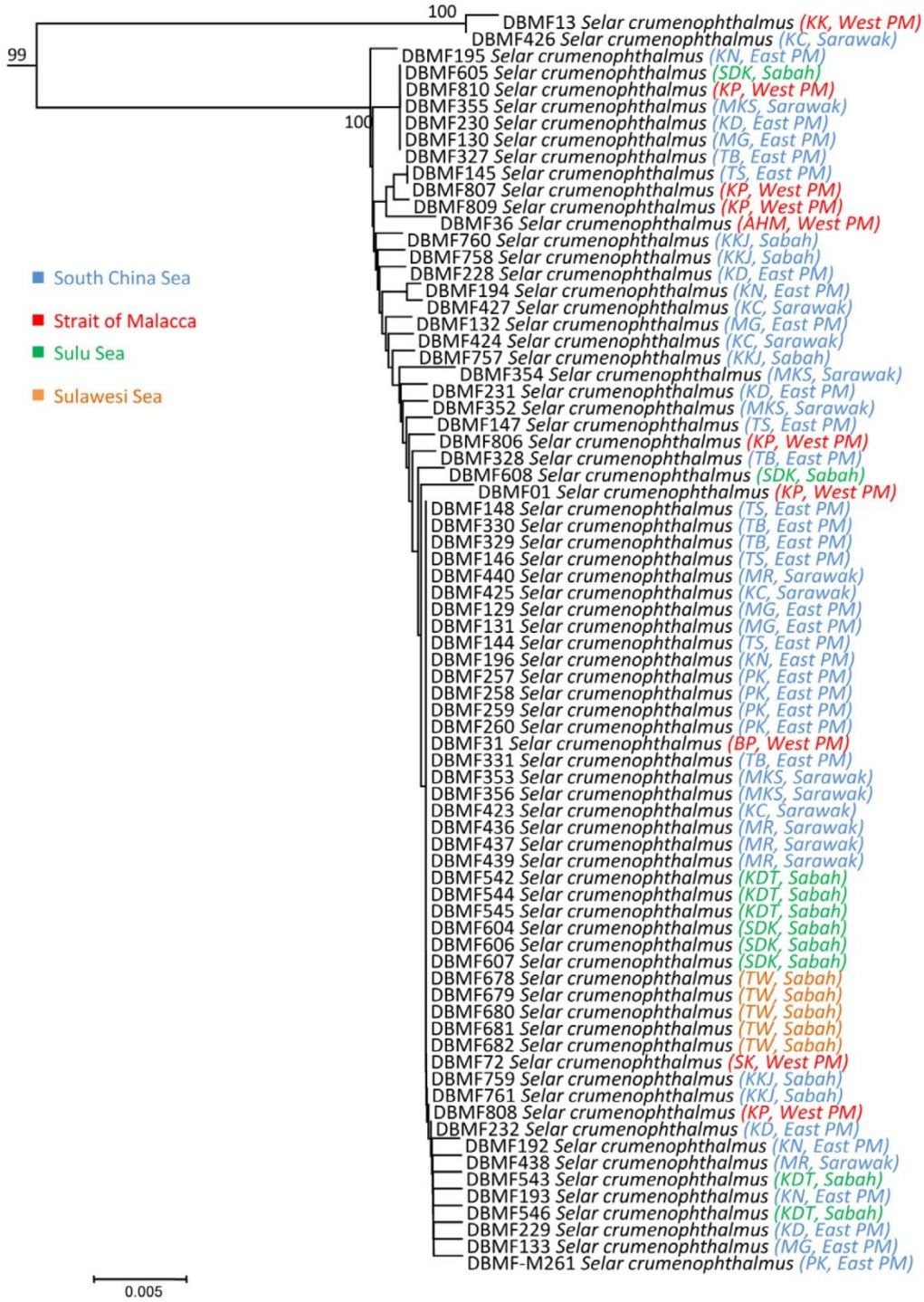

Figure 6. Neighbour-joining tree (K2P distance) of 75 *COI* sequences of *Selar crumenophthalmus*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g006

and cryptic species. DNA barcoding also facilitates identification, particularly in microscopic, diverse life history stages, and other organisms with complex or inaccessible morphology [11]. Furthermore, the approach is also able to discriminate species of highly similar morphology. The Carangids, which are morphologically very similar, such as the three species (*Caranx ignobilis*, *Caranx sexfasciatus* and *Caranx tille*), form a sister grouping (Figure 10). Because of such high similarity, they are sometimes

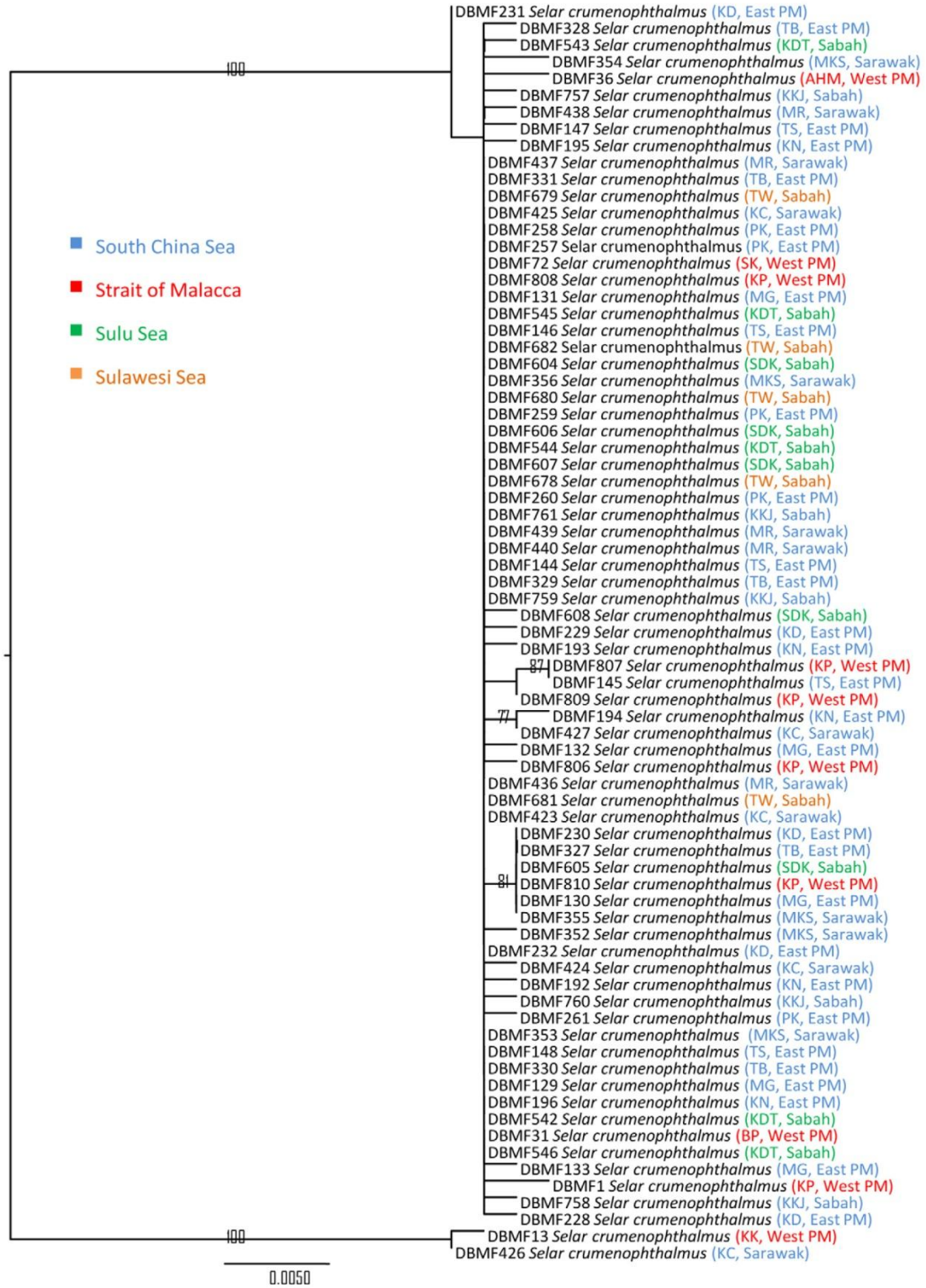

Figure 7. Maximum-likelihood tree of 75 *COI* sequences of *Selar crumenophthalmus*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g007

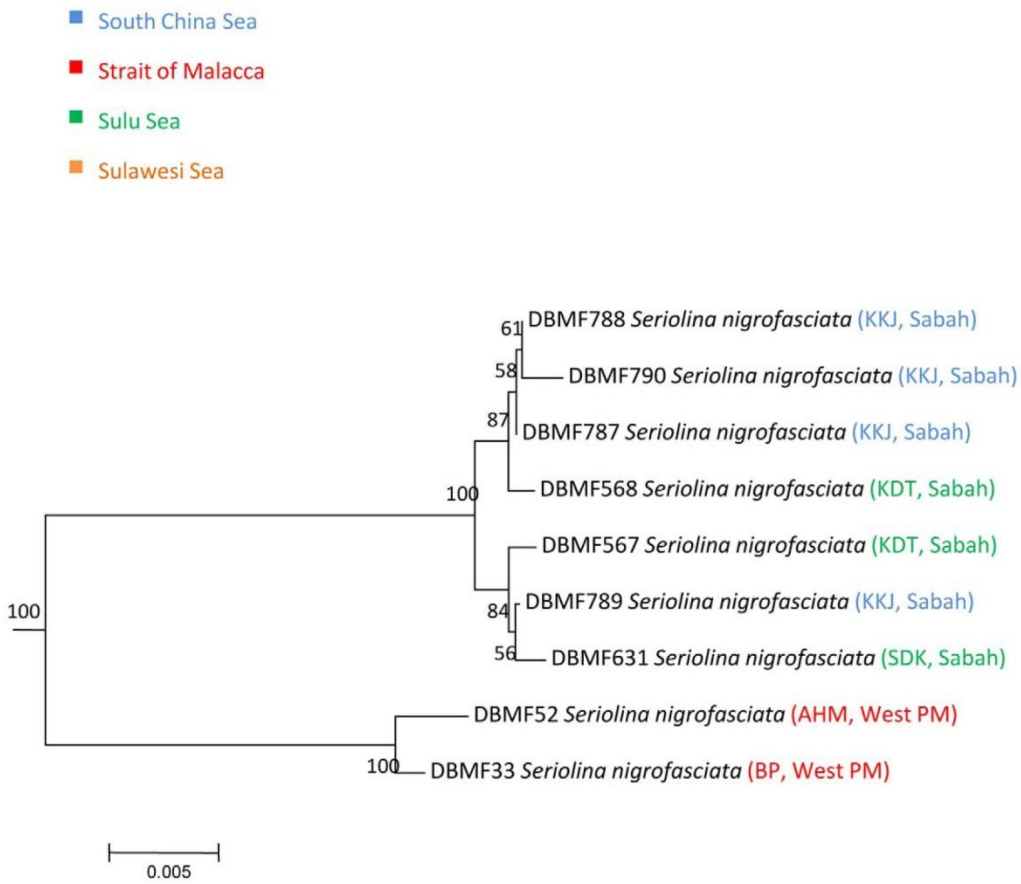

Figure 8. Neighbour-joining tree (K2P distance) of 9 *COI* sequences of *Seriolina nigrofasciata*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g008

misidentified. However, DNA barcoding discriminated these *Caranx* samples effectively on all occasions. Three distinct clusters were formed, separating the three species by an average interspecific distance of 7.53%, and average intraspecific distances of 0.51%, 0.16% and 0.07% for *Caranx ignobilis*, *Caranx sexfasciatus* and *Caranx tille*, respectively.

The Indo-Malay Archipelago has long been considered as the centre of maximum marine biodiversity [53–54]. A few studies based on the *COI* marker have discovered high cryptic diversity in coral reef fish around this region [5,22]. Several hypotheses have been proposed to explain the remarkably high diversity found in this region: 1) centre of origin [55], 2) centre of accumulation [56], and 3) centre of overlap [57]. Hypotheses 1 and 2 have recently been raised [5] to explain speciation and dispersal of marine species in the Indo-Malay Archipelago. It might either be the result of diversification within the region and subsequent species dispersed into peripheral areas (Centre of Origin), or the result of an overlap of the faunas from the Indian and Pacific Oceans (Centre of Overlap).

A few studies have identified high levels of cryptic species occurring within and outside the IMA [5,22,58], though here, we detected only a moderate frequency of potentially cryptic species within commercially exploited Indo-Malay Carangidae. Small sample size, bias in range of species collected, and restricted geographic ranges may have lead to fewer cryptic species being identified compared to previous studies. By increasing the geographic sampling range, more cyrptic diversity will likely be detected [5,22,58]. The majority of the species in Carangidae have a pelagic lifestyle. Interestingly, within marine ecosystems, most diversity is benthic, with such organisms including 98% of species diversity, while the remaining 2% are pelagic [59]. Three species representing complexes of six potential cryptic species were detected in Indo-Malay Carangidae; *Atule mate*, *Selar crumenophthalmus* and *Seriolina nigrofasciata*. All NJ and ML trees identified two separate lineages but only *Seriolina nigrofasciata* showed allopatric divergence, with the Sabah lineage separated from the West Peninsular Malaysia lineage by 4.32%. The other two showed sympatric divergences with both clusters consisting of geographically mixed *COI* lineages.

Comparison of COI sequences of 23 species from this study with conspecific sequences available from other geographical regions [48,60] revealed the existence of several more complexes of potentially cryptic species from outside the IMA. Using the ABGD analysis [46], 10 lineages were flagged as candidate cryptic species. Four recognised species (*Caranx sexfasciatus*, *Decapterus maruadsi*, *Gnathanodon speciosus* and *Seriolina nigrofasciata*) each comprised two lineages exhibiting allopatric divergences with a maximum nucleotide divergence of 7.1%, 2.7%, 3.8% and 4.35%, respectively (Figure S4, Supporting Information). As for *Seriolina nigrofasciata*, additional sequences from India and Iran clustered together, and samples from West Peninsular Malaysia were clearly separated from the western part of the IMA together with Sabah

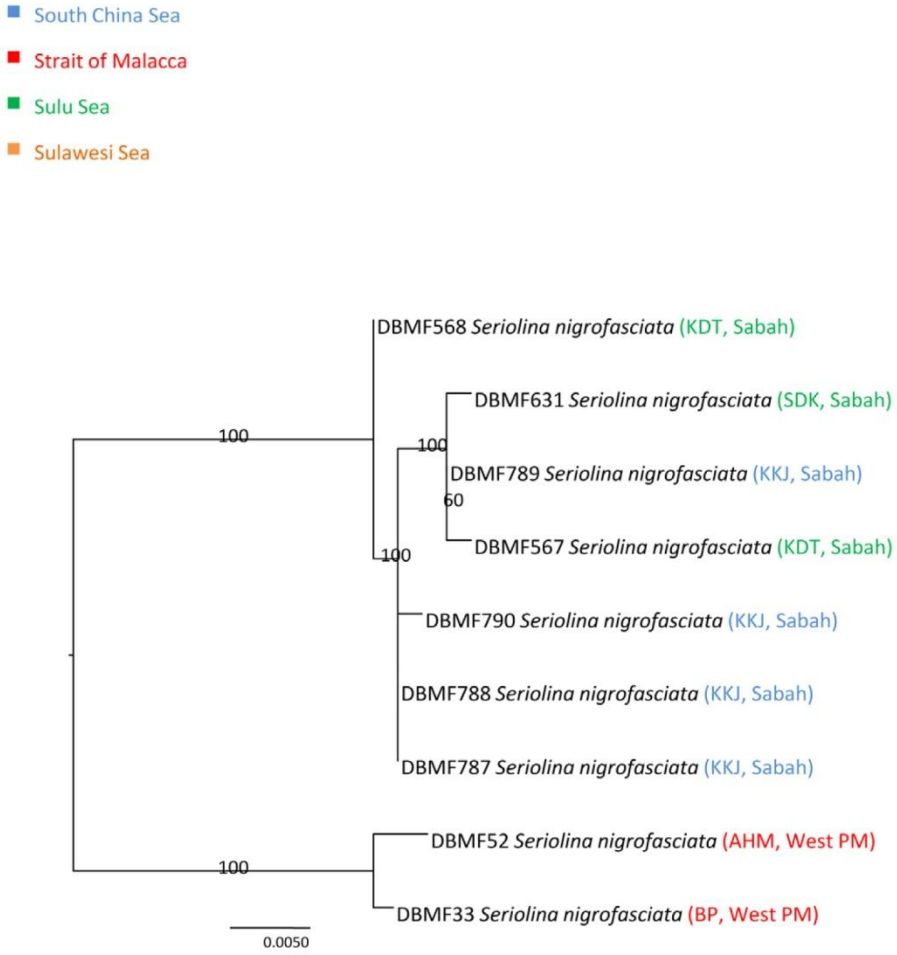

Figure 9. Maximum-likelihood tree of 9 *COI* sequences of *Seriolina nigrofasciata*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g009

(Borneo), representing an additional complex of two potential cryptic species. Such findings are consistent with large faunal discontinuities between Indian and Pacific Ocean ichthyofaunas as a consequence of geographic isolation on each side of IMA, as discussed by Springer and Williams [61]. However, our data is not sufficient to explain the hypothesis of species richness in the IMA. To explore hypotheses of species diversification it is necessary to sample the whole family across their broad geographic range.

Our study has examined only one family with different lifestyles, body shape and body size. We did not identify any significant association between genetic distances and these biological characteristics (pers. obs.). However, Zemlak *et al.* [58] used *COI* to examine patterns of divergences between fish species representing different lifestyles from opposite sides of the Indian Ocean. They detected deep divergences between certain inshore taxa, with the inshore taxa (mean *COI* divergence = 0.51%) exhibiting significantly higher levels of putative cryptic species than the offshore (mean *COI* divergence = 0.26%) fish. Such deep divergences were more representative of patterns in congeneric species than among populations of a single species, highlighting the possible genetic isolation of presumed cosmopolitan species. Out of the 35 species studied by Zemlak *et al.* [58], the one member of Carangidae sampled, the needlescaled queenfish (*Scomberoides tol*), appears to represent a broadly distributed sibling species pair whose distribution spans the Indian Ocean. Such findings reinforce the need in such *COI* barcoding studies to sample throughout the extremes of the geographic range to investigate the extent of hidden diversity in marine fauna.

Conclusion

In conclusion, the establishment of an Indo-Malay Carangidae *COI* barcoding library presented here contributes to the global DNA barcoding effort to document and catalogue the diversity of life, particularly with regard to conservation and management applications. We anticipate that the accumulation of biodiversity data will help drive and inform effective planning and monitoring of conservation and fisheries programmes in the Indo-Malay region. Intensification of industrial and commercial activities in Malaysian waters renders the biodiversity of the region highly vulnerable to threats and degradation. Therefore, such data are helpful not only to document mechanisms driving population structuring and recruitment in Carangidae, but also provide a scientific framework in support of effective management strate-

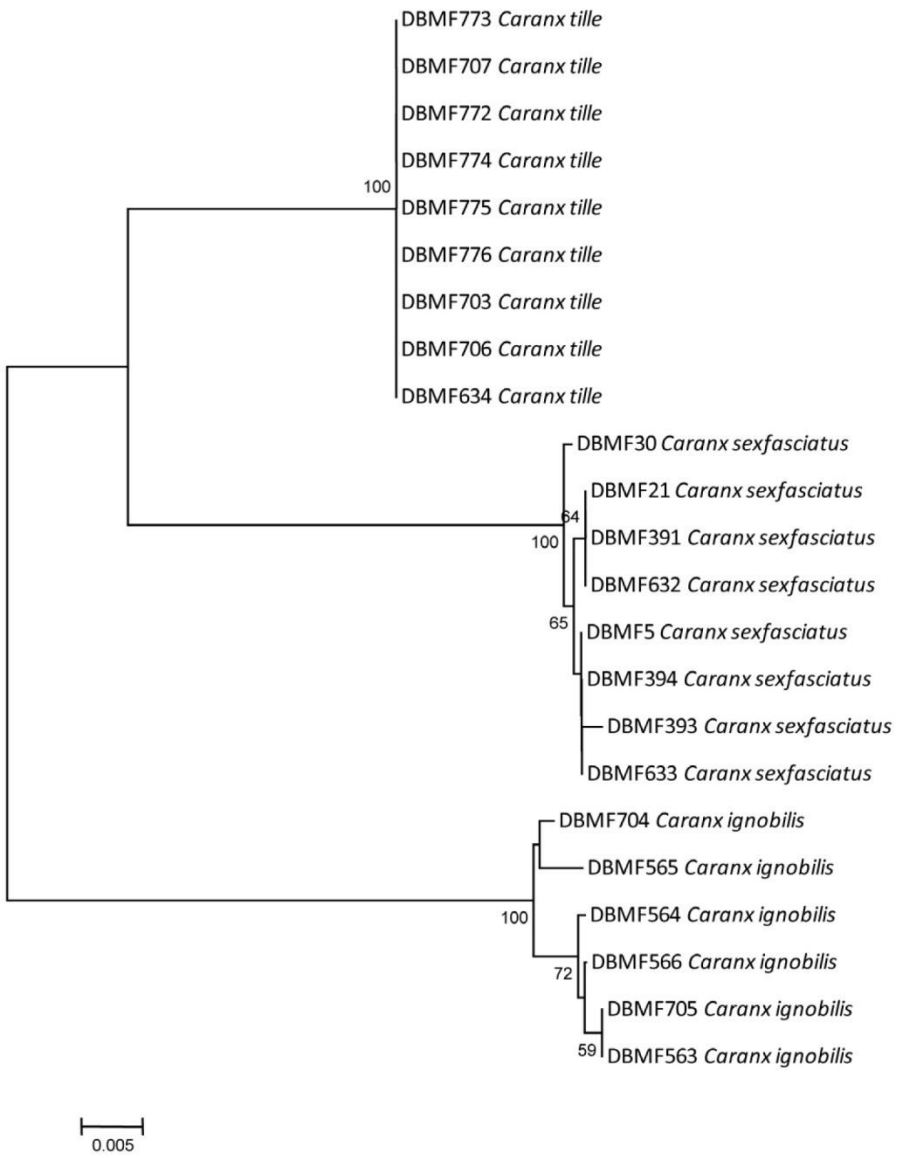

Figure 10. Neighbour-joining tree (K2P distance) of genus *Caranx*. Only bootstrap values greater than 50 are shown. Sample ID for the Barcode of Life Database (BOLD, www.barcodinglife.org) provided.
doi:10.1371/journal.pone.0049623.g010

gies and the conservation of commercially-important fisheries resources.

Supporting Information

Figure S1 Taxon ID Tree of Carangidae generated by BOLD. Neighbour-joining tree (Kimura 2-parameter, pairwise deletion). A total of 723 sequences from 36 species and 18 genera were analysed.
(PDF)

Figure S2 Phylogenetic tree from Maximum-likelihood analysis. Numbers above the branches represent bootstrap support based on 1000 replicates.
(PDF)

Figure S3 Tree corresponding to partition detected by ABGD method.
(PDF)

Figure S4 Taxon ID Tree of 23 widespread Carangidae species generated by MEGA5 including conspecifics from other geographical regions. Neighbour-joining tree (Kimura 2-parameter, pairwise deletion).
(PDF)

Table S1 Specimen data and GenBank accession numbers used in this study.
(DOC)

Table S2 K2P distances of Indo-Malay Carangidae.
(DOC)

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Author Contributions

Conceived and designed the experiments: TNAMJ MIT SAMN MdB GRC. Performed the experiments: TNAMJ. Analyzed the data: TNAMJ. Contributed reagents/materials/analysis tools: MIT SAMN MdB GRC. Wrote the paper: TNAMJ MIT SAMN MdB GRC.

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