

Bangor University

MASTERS BY RESEARCH

Where did all these woodlice come from?

Armadillidium Brandt, 1833 (Isopoda: Oniscidea) diversifica@on in the Mediterranean

Hughes, Thomas

Award date:
2023

Awarding institution:
Bangor University

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 09. Apr. 2024

**Where did all these woodlice come from?
Armadillidium Brandt, 1833 (Isopoda: Oniscidea)
diversification in the Mediterranean**

Thomas D. Hughes (Student no. 500379287) bsuaf1@bangor.ac.uk

Supervisor: Alexander Papadopoulos

Bangor University School of Natural Sciences

February 2023

Thesis Declaration I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy

CONTENTS

ABSTRACT	1
1 — INTRODUCTION	2
1.1 — Geological History of the Mediterranean	3
1.2 — The Genus <i>Armadillidium</i>	5
1.3 — Project Aims	11
2 — METHODOLOGY	11
2.1 — Sample collection and genome extractions	11
2.2 — Processing, Alignments and Analysis	12
2.3 — Morphology, dissection, imaging and illustration preparation	12
3 — RESULTS	12
4 — DISCUSSION	15
4.1 — The Armadillidae	15
4.2 — Mitochondrial Tree and the <i>Armadillidium</i> Species-groups	17
4.3 — Nuclear tree and the <i>maculatum</i> -group	19
4.4 — <i>maculatum</i> -group Morphology	20
4.5—Evolutionary History of the <i>maculatum</i> -group	22
4.6 — <i>Armadillidium</i> diversification in the Mediterranean	26
5 — CONCLUSION	30
6 — REFERENCES	31

ABSTRACT

Current assessments show the highest diversity of terrestrial Isopods is within Mediterranean Biomes and insular tropical regions. Despite our growing understand of terrestrial Isopod biogeography, there has been little research examining the speciation mechanisms behind the high diversity within these particular regions. We chose the genus *Armadillidium* within the Mediterranean Basin as a model system, focusing on the French fauna, particularly those represented in the occidental-subgroup of the *maculatum* species group. We sequenced the mitochondrial CO1 gene and nuclear CDS, and collated morphological and biogeographic data to examine the family placement of the *Armadillidium*, the relationship of the species-groups and investigated the evolutionary history of the *maculatum*-group in France. Our results support other findings that *Armadillidium* is polyphyletic. Species with duplocarinate-type cephalons belong to undiagnosed genera with evolutionary origins within the Iberian peninsula. The *maculatum*-group is also polyphyletic with only 4 members of the subgroup having a shared origin, likely during the Miocene. We tentatively tied the diversification of the subgroup to three major geological events: the formation of the Molasse basin, development of rias after the Massinian Salinity Crisis and climatic oscillations of the Pliocene. Two modes of allopatry were identified relating to these geological events, in addition to parapatry linked with petrological change. Our findings suggest the geological heterogeneity and dynamic history of the continent is responsible for diversification within the occidental-subgroup. However, other possible contributors to diversification have been identified, which likely correlate with the adaptive characteristics of terrestrial Isopods.

1 — INTRODUCTION

Species richness varies across taxonomic groups, geographic regions and habitat types, resulting in an uneven distribution of biodiversity over the Earth's surface. The intertwining relationship between speciation and extinction results in spatio-temporal variation of biodiversity. The factors contributing to species diversification can be broken down into abiotic (such as orogenic processes and climate change) and biotic (such as sexual selection, predation and hybridisation). Studies have attempted to identify which factor(s) have influenced diversification within specific clades, which in almost all cases is the result of a synergistic relationship of both biotic and abiotic selective forces (Bouchenak-Khelladi et al., 2015; Donoghue & Aanderson, 2015; Condamine et al., 2018), in addition to demographic processes and genetic drift (for example; Willi et al., 2020; Jones et al., 2021). Furthermore, it has been shown that diversification is often associated with particular events or evolutionary innovations that have large-scale selective implications and can result in widespread diversification within specific clades, e.g. adaptive radiation of Eutherian mammals after the Cretaceous mass extinction (Halliday & Goswami 2016). Patterns of explosive diversification often correlated with the exploitation of novel or the subdivision of previously neglected ecological niches (Gavrilovs & Losos, 2009).

There are just over 3,700 valid species of the Isopod suborder Oniscidea (woodlice), the only suborder of Crustacea to be comprised of almost entirely terrestrial living species. In contrast, only a few other Crustacean groups have developed terrestriality and of their total diversity only a fraction have developed this lifestyle. For instance, only 3% of amphipods and 1.7% of decapods are terrestrial (Broly et al., 2013 and references therein). The success of the Oniscidea has allowed them to occupy almost all terrestrial environments across the world with the exception of polar regions and high altitudes exceeding 4,800masl (Beron, 1997). As such, a great degree of morphological and physiological adaptations have arisen to overcome the difficulties of colonising terrestrial environments. The most significant of these adaptations are a closed brood pouch (marsupium), a highly evolved gill structure for atmospheric respiration (pleopodal lungs), a water-resistant cuticle, a water conducting system, and a reduction in overall body size (Hornung, 2011). Although the adaptive successes of the Oniscideans are well known, very little is known about their biogeography and the factors that have driven their diversification on land.

Schmalfuss (2004) assembled the first world list of terrestrial isopods which provided an initial framework from which distribution patterns could be analysed on a global scale. Due to the limited distributional data present for most species, the analysis was comprised of low-resolution information typically at the country level. Despite this, Sfenthourakis et al. (2007) (using the data of Schmalfuss, 2004), revealed a consistent trend that the highest diversity was present in Mediterranean-like biomes and in some insular regions of the tropics. This is additionally supported by latitudinal gradients of species richness which peaks at both mid-latitudes (Sfenthourakis et al., 2007; Sfenthourakis & Hornung, 2018). In Europe, hotspots of species diversity with high levels of endemism are found in the circum-Mediterranean region (Sfenthourakis et al., 2007). There is also a distinct latitudinal gradient of woodlouse species richness, which declines as distance increases north of the Mediterranean. This trend is taxonomically consistent across multiple speciose families (e.g., Armadillidiidae and Philosciidae), with a clear decrease in species richness towards northerly latitudes in Europe (Hornung & Sólmos, 2007). Therefore, the majority of Europe's woodlouse species richness, and a large proportion of the world's species diversity, are concentrated within the Mediterranean basin biodiversity hotspot.

At present there are thirty-six recognised biodiversity hotspots, of which five are categorised as Mediterranean-type climate regions and are represented by the presence of evergreen sclerophyll scrubland (Myers et al., 2000; Medail & Myers, 2004). These Mediterranean-type climate regions include;

the Mediterranean Basin, California (USA), central Chile, the Cape Region (South Africa) and southwestern Australia. The Mediterranean Basin, in particular, is the largest Mediterranean-type climate region in the world and the second largest known biodiversity hotspot. It encompasses an area of 2 million square kilometres, spanning from the Macaronesian archipelago in the west to the Levant in the east and represents the contact zone of Europe, Africa and Asia (CEPF, 2010). Despite representing only 1.6% of the Earth's surface, it houses 20% of the world's vascular plant species (Cowling et al., 2005). The high level of endemism within this region has been attributed to three main factors (Micó et al., 2009): a complex geological history which has led to speciation via allopatry and geographic vicariance (Krijgsman, 2002), a refugial site for species during the Quaternary glaciations (Hewitt, 2004), and finally, the influence of humans modifying and fragmenting species distributions over time (Galante, 2005), leading to rapid levels of diversification (Valente et al., 2010).

1.1 — Geological History of the Mediterranean

The Mediterranean region has experienced a complicated geological and climatic history, brought about by a suite of geodynamic, eustatic and orbital processes. The region originated in the early-middle Jurassic, when Pangea was undergoing its peak fragmentation causing the region to develop into a complex of ribbon continents and slender basins. These later formed into two independent oceanic basin systems of the ancient Tethys realm (van Hindbergen et al., 2020 and references therein). In the northwest was the Alpine Tethys Ocean (formed during the middle-late Jurassic, and correlated with the opening of the central Atlantic) and in the southwest was the Neotethys Ocean (formed during the Triassic-Jurassic between the two subcontinents Laurasia and Gondwana) (van Hindbergen et al., 2020 and references therein). From the Cretaceous to the early Neogene, the African-Arabian continent drifted northwardly into the Eurasian continent, causing the Paleotethys (and the Alpine Tethys Ocean) to close, resulting in the formation of the Proto-Mediterranean sea system (Dercourt et al., 1986; Krijgsman, 2002; Rosenbaum et al., 2002). Simultaneously, the broad line of contact between these continents forced upwards a long chain of collisional mountains in a tectonic episode known as the Alpine orogeny, stretching from the Pyrenees (Spain) in the west to the Zagros mountains (Iran) in the east (Khadiji, 2010). The formation of these mountain chains isolated an area of shallow sea off of the Tethys ocean (and Proto-Mediterranean) known as the Paratethys during the Eocene-Oligocene (Rögl, 1996). Throughout its history, the Paratethys reconnected intermittently with the Tethys or its descendants, the Mediterranean Sea and the Indian Ocean, due to a combination of eustatic and tectonic changes (Rögl, 1999).

Nearing the final stages of this tectonic episode at the border between the Oligocene and Miocene, the weight of the Alpine orogenic wedge resulted in flexure of the European plate, causing an extensive (1,200km long) foreland basin to develop north of the Alps. The Alpine foredeep, also referred to as the Molasse Basin, stretched from the French to the Austrian Alps (Schlunegger & Kisslin, 2015). This basin became incorporated within the western Paratethys, and later allowed a seaway to connect with the Mediterranean Sea through eastern France during the Burdigalian (Miocene). This seaway formed due to a decline in the thrust rate of the Alpine orogeny, eustatic sea level rise and reduced sedimentation into the central and western regions of the Molasse (Kuhlmann, 2000; Schlunegger et al., 2001; Zweigel et al., 1998). The eastern part of the Molasse had already experienced marine conditions since the Oligocene (Kuhlemann & Kempf, 2002) but it wasn't until the early Miocene c.20Ma that shallow seas formed across the entire foredeep, eventually causing the seaway to connect with the Paratethys (Bieg, et al., 2008). This connection was short-lived as the foredeep closed around the Langhian (Miocene), although the Mediterranean Sea was still intermittently connecting with the central Paratethys through the Adriatic from the early-mid Miocene (Meulenkamp & Sissingh, 2003; Kovac et al., 2017). The presence of the Paratethys in Europe during the Oligocene-Miocene strongly influenced the climatic conditions of the continent during

that time. The epicontinental sea provided oceanic conditions to the surrounding landmass, bringing a large amounts of water vapour into the atmosphere, in addition to its high water heat capacity decreased the seasonal thermal gradient (Ramstein et al., 1997; Fluteau et al., 1999). The gradual disappearance of the Paratethys is correlated with a shift to more continental conditions, particularly colder winters (Ramstein et al., 1997).

The disconnection of the Mediterranean Sea from the Indian Ocean c.11Ma. meant the Mediterranean could only undertake hydrological exchange with the Atlantic Ocean through two gateways: the Betic strait in southern Spain, and the south-Riffian gateway in northern Morocco, which progressively narrowed due to the convergence of the African and Arabian plates (Flecker et al., 2015). It is believed that this plate convergence in addition to changes in the Earth's precession resulted in the closure of the Atlantic passage and caused almost complete desiccation of the Mediterranean Sea (Krijgsman et al., 1999 and references therein). This period is known as the Messinian Salinity Crisis (MSC) c. 5.96-5.33Ma. The MSC played a significant role in the biogeography of the flora and fauna of the region. The presence of prehistoric dwarf mammals, such as elephants and hippopotamuses on small Mediterranean islands such as Cyprus, Malta and Sicily, provide evidence that a migration of terrestrial animals occurred across the dehydrated basin (Marra, 2005; Zazzo et al., 2015). These land bridges across the Mediterranean also aided the migration and speciation of invertebrate fauna such as *Troglophilus* (cave crickets) in the Aegean region (Kaya et al., 2013), but also acted as a barrier to migration in *Phlebotomus* (sandflies) (Trájer et al., 2021).

Tectonic subsidence eventually led to the Mediterranean finally reconnecting to the Atlantic Ocean through the Strait of Gibraltar, causing the basin to refill 5.33Ma, an episode known as the Zanclean flood (Blanc, 2002). The refilling of the Mediterranean basin has been strongly correlated with vicariance of some terrestrial invertebrate groups between Europe and north Africa such as Pachydeminae (leaf chafers) (Sanmartín, 2003) and *Euscorpius* (wood scorpions) (Graham et al., 2012).

During the MSC, deep canyons developed along the lower sections of rivers that discharged into the Mediterranean basin (Clauzon et al., 2008 and references therein). Regression of marine conditions within the basin submerged the fluvial canyons, resulting in the formation of rias along the majority of the major rivers that connected into the Mediterranean, particularly along southern France, Spain and northern Morocco (Tassy et al., 2014). It is likely that the rias acted as physical barriers to migration at this time. Vandel (1962) observed that some woodlouse species such as *Cylisticus esterelanus*, *Helleria brevicornis* and *Trichoniscus biformatus*, which are characteristic of the soil fauna west of the Var river (France) are absent east of it. This implied that the Var and its ancient ria may have played a role in the biogeography of these species. More recently, the differentiation of the wall lizards *Podarcis bocagei* and *P. hispanica* (now *P. guadarramae*) on the mainland and the Ria de Arosa Archipelago (Spain) were the result of vicariance and dispersal, with genetic drift presumed to act as the main force leading to divergence of island populations (Arntzen & Sousa, 2007).

The warm and humid conditions experienced during the Oligocene-Miocene persisted into the early Pliocene but began to drastically deteriorate during the middle-late Pliocene (3.1-2.4Ma) which lead into the glacial-interglacial climatic oscillation of the Pleistocene-Holocene; a period better known as the Ice Age. It is characterised by extended, cold and dry glacials which were interspersed by short, warm and wet interglacials (Salzmann et al., 2011). The rapid change in climatic conditions during this time dramatically influenced species distributions (Hewitt, 2000). During glacial episodes northern latitudes and high altitudes became almost uninhabitable to most life whilst lower latitudes and altitudes remained habitable (Stewart et al., 2010 and references therein). Therefore, species present in the uninhabitable regions either adapted to the prevailing conditions, migrated along corridors of suitable habitat into lower latitudes or became extinct (Stewart et al., 2010). Species that existed at lower latitudes, particularly around the northern

Mediterranean, endured the cold periods within glacial refugia (specific regions that were minimally impacted by the glacial periods (Taberlet et al., 1998; Hewitt, 1999; Stewart et al., 2010)). When an interglacial reappeared, some species were once again released from the bounds of the refugia and spread rapidly to take advantage of the empty habitats freed from the retreating glaciers (Kyrkjeeide et al., 2014; Kühne et al., 2017). Evidence of these constrictions and expansions are evidenced by the variance of genetic diversity within species across the continent. Populations associated with glacial refugia typically experience an extended period of demographic stability, compared to populations established as a result of post-glacial expansion. As such, refugial populations can be diagnosed by a higher level of genetic diversity than extra-refugial populations that became established after the end of the last glacial episode (Wielstra et al., 2013; Esquer-Garrigos et al., 2019).

The extensive and ‘reticulated’ physical history of the Mediterranean has clearly helped generate the significant richness of species diversity within the region. However, spatio-temporal and climatic changes alone cannot explain this entirely. Buira et al. (2020) revealed that edaphic stresses such as pH has help facilitate diversification in Mediterranean angiosperms, particularly within the Eudicotidae. Whilst Rundel et al. (2018) showed recurrent fires during summer droughts within the world's Mediterranean hotspots played a decisive role in plant diversification. Furthermore, biological agents have also been identified as major factors in driving speciation, such as divergent sexual selection of Mediterranean barn swallow (*Hirundo rustica*) population causing phenotypic differentiation (Wilkins et al., 2016). Despite the successes of the aforementioned phylogenetic and comparative research in the identification of these factors and specific date ranges for divergence within clades, the results of some studies however, have shown that unusual distributions can often be the product of human-mediated dispersal; such as the results seen in the woodlouse *Helleria brevicornis* (Gentile et al., 2010), and the slug *Geomalacus maculosus* (Reich et al., 2015).

1.2 — The Genus *Armadillidium*

The genus *Armadillidium* Brandt Oniscidea: Armadillidiidae) is speciose (c.189 species; Schmalfuss, 2003) and has its highest degree of diversity and endemism along the Northern Mediterranean Sea between Italy (>60 species) and Greece (>55 species) with a declining richness across the Mediterranean from east to west. This trend has led many to believe that the diversification centre for the *Armadillidium* is located around the northeastern Mediterranean (Schmalfuss, 2000), although this view has been debated due to the presence of “primitive” species groups in Iberia and north west Africa (Vandel, 1962). The high degree of localised endemic species confined within the region confirms *Armadillidium* as a *sensu latu* Mediterranean taxon with very limited mobility and dispersal rates (Schmalfuss, 2000; Sfenthourakis & Hornung, 2018). *Armadillidium* are the archetypal conglobating (ball rolling) woodlice, a behaviour shared by many other terrestrial isopod genera to conserve water and/or act as protection from predators (Smigel & Gibbs, 2008). Furthermore, *Armadillidium* have highly evolved invaginated pleopodal lungs (called pseudo-tracheae) (Wright & Ting, 2006). As Pleopodal lungs are derivations of gills of marine ancestors, their function and evolutionary development is the result of phylogenetic and adaptative progression along humidity gradients (Hornung, 2011). Oniscidean species with simple plate-like, weakly folded respiratory surfaces are believed to characterise the first stage of the pleopodal lung developmental line. This is replaced by a more heavily folded surface with partial invagination of the respiratory field. In the final evolutionary stage, the pleopodal lungs are fully invaginated with a water-repellant surface, so gaseous exchange is only permissible through small respiratory spiracles (Hornung, 2011). Therefore, the invagination of the pleopodal lungs in *Armadillidium* is likely partly responsible for their success in the xeric environs of the Mediterranean biome. Therefore, *Armadillidium* presents an opportunity to understand oniscidean diversification and adaptation within the Mediterranean Basin.

During the early to mid-twentieth century Verhoeff and Strouhal independently produced classification systems to manage the numerous *Armadillidium* species being described. Verhoeff developed a series of subgenera whilst Strouhal used sixteen non-taxonomic “groups” (Schmölzer, 1954 and references therein). However, due to the inability to provide any taxonomic merit to the approach of Verhoeff (as stated also by Schmölzer, 1954), the use of the subgenera fell out of favour and Strouhal’s more reasonable approach superseded it, and is still maintained to this day (see Schmalfuss, 2008 and Garcia, 2020 as examples). Despite this, some of Verhoeff’s subgenera survive, with the monotypic subgenus *Pleuarmadillidium* which contains only the species *Armadillidium omblae* Verhoeff, 1910, and the subgenus *Armadillidium* which contains all other *Armadillidium* species.

The incomplete cataloging of woodlouse diversity in the Mediterranean is likely the result of three main factors. Firstly, there is a deficit of skilled individuals who can undertake species identification within these hyper-diverse regions, secondly, there is large degree of synonymy, particularly within the *Armadillidium*, and finally, there is a lack of updated and refined resources, such as figured dichotomous keys, species photographs in life and well delineated species diagnosis. Furthermore, many species and their distributional data are often poorly described and restricted to their original descriptions, which can be difficult to access due to the prevalence of small obscure journals during the 20th century. Despite these factors, the woodlouse fauna of France is particularly well-resolved compared to other Mediterranean countries. This is primarily due to the prodigious contribution by the woodlouse taxonomist and evolutionary biologist Albert Vandel, who amassed an incomparable resource to the country’s woodlice during the 20th century, which contains detailed maps of occurrence points and distributional notes for the known fauna at the time (Vandel, 1960a; 1962). In addition to this, the national species recording database, Inventor National du Patrimoine Naturel (INPN) stores extensive coordinate data (typically at a resolution of 10km²) for the majority of the countries flora and fauna. With this information it is possible to elucidate interesting distribution and species relationship patterns within the French Mediterranean, and target specific groups with qualities suitable for phylogeographic research.

Séchet and Noël (2015) documented 299 terminal woodlouse taxa (218 species) in France, of which twenty-one are within the genus *Armadillidium*. With the exclusion of *A. kossuthi*, which was a single introduction from Naples (Italy), *A. rojanum* which is reportedly synonymous with *A. allassiense*, the addition of *A. cf. arcangelii* (Noël et al., 2022), and the unpublished new species *A. ventosum* (Hughes & Northfield) brings the total known French *Armadillidium* to twenty-one species. Vandel (1962) previously documented eighteen *Armadillidium* within France and segregated them into ten species groups, six of which are those originally proposed by Strouhal and Verhoeff (*depressum*, *granulatum*, *maculatum*, *nasatum*, *pictum* and *vulgare*), with the *pictum*-group being synonymous with the *pulchellum*-group of Strouhal (1927). The remaining four groups (*opacum*, *pruvoti*, *serratum* and *sordidum*) were added (Vandel, 1962). The *pruvoti*-group was a derivation of the *Alloschizidium* by Vandel, which previously was treated as a subgenus of *Armadillidium* (Verhoeff, 1918) before being raised to genus level (Verhoeff, 1933). The reason Vandel placed *Alloschizidium* back into *Armadillidium* was because he divided the family Armadillidiidae into two subfamilies; the Eluminae, which lacked a postscutellar line (comprising eleven genera) and the monogeneric Armadillidiinae, which bear a postscutellar line (containing only the *Armadillidium*) (Vandel, 1944; 1954; 1962). As *Alloschizidium* bore a postscutellar line (a feature only present within the Armadillidiinae) and because the schisma on the posterior-lateral corner of pereonite one was likely a derived trait, Vandel reinstated *Alloschizidium* back into the *Armadillidium* as the *pruvoti*-group. However, a recent reevaluation of *Alloschizidium* found that it did deserve its own distinct generic status (Taiti & Ferrara, 1996). Therefore, the French *Armadillidium* are allocated between nine of Strouhal’s species-groups, not ten as proposed by Vandel.

Of the twenty-one species of *Armadillidium* present within France (as indicated by Séchet and Noël, 2015), eight are distributed beyond the limits of the Mediterranean Biome whilst thirteen are confined within it. Of the Mediterranean species, *A. assimile* and *A. granulatum* are the most widely distributed, with *A. assimile* being found between Spain and Italy and *A. granulatum* across the majority of the countries surrounding the Mediterranean. The remaining eleven species can be described as localised endemics, with small geographic ranges. *A. lanzai*, *A. littorale* and *A. torchiai* are endemic to the island of Corsica. *A. serratum* is endemic to the Pyrénées, *A. esterelanus*, *A. quinquepustulatum*, *A. simoni* and *A. ventosum* are endemic to the Provence region. *A. allassiense* and *A. maculatum* are endemic to the Provence region and the neighbouring parts of northwestern Italy, and *A. sordidum* is distributed between Corsica, Provence and northwest Italy. Of the thirteen regions of France, Provence contains the highest number of *Armadillidium* species (sixteen) and the greatest number of endemics (seven). Of these endemics, *A. allassiense* and *A. sordidum* belong to the *sordidum*-group whilst *A. esterelanus*, *A. maculatum*, *A. quinquepustulatum*, *A. simoni* and *A. ventosum* belong to the *maculatum*-group (Vandel, 1962). *A. lanzai* and *A. assimile* also belong to the *maculatum*-group, bringing its total to seven species. Therefore, the *maculatum*-group is the most speciose group in France, representing 33% of its entire *Armadillidium* fauna and 54% of its Mediterranean species. The *maculatum*-group, particularly within the Provence region, are chosen here as a model system to explore the processes of localised diversification within the Mediterranean biome.

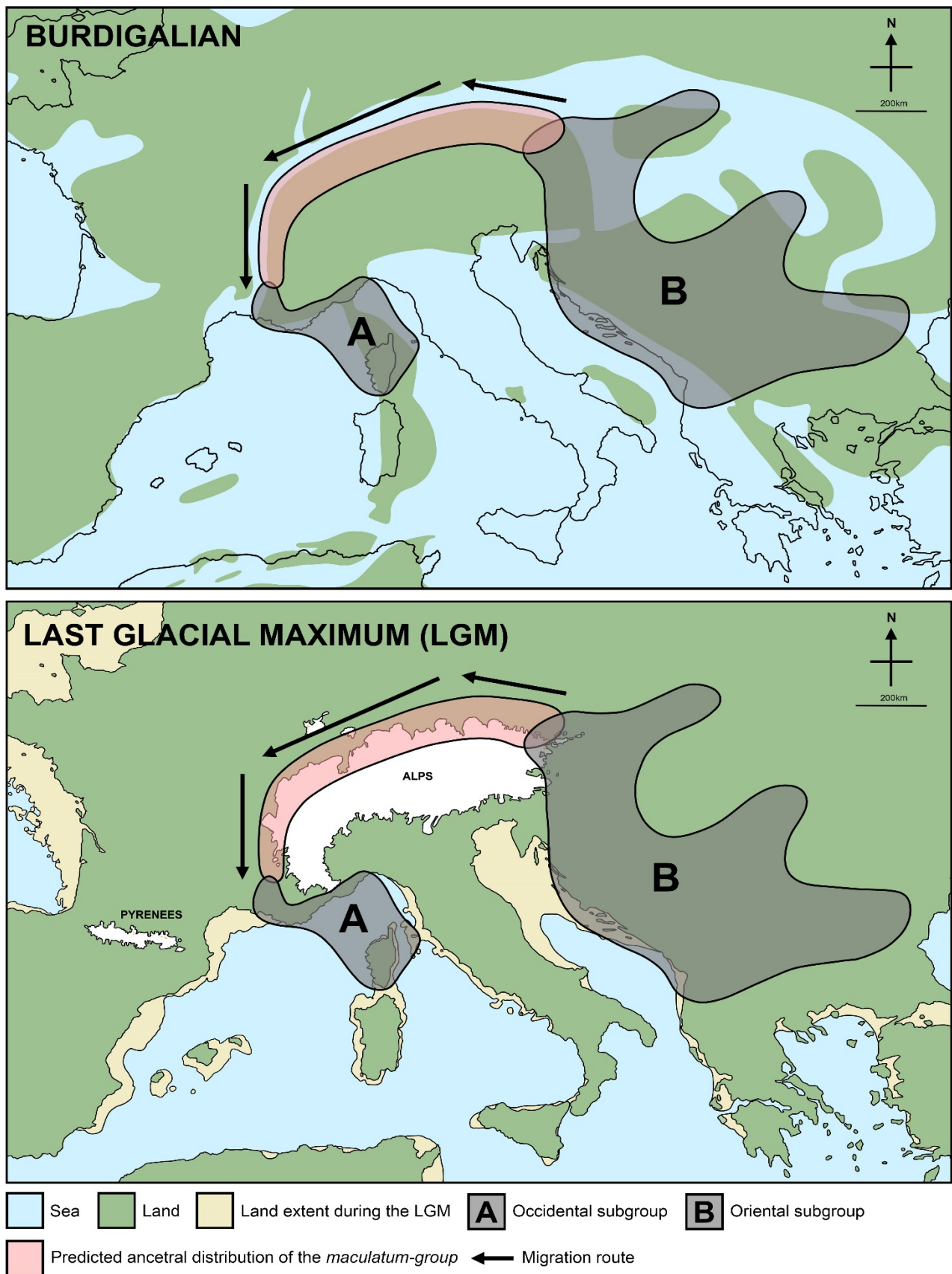


Figure 1: Distribution of the two *maculatum*-subgroups (A and B) with the predicted ancestral migration route and location of intermediate species in red (subgroup ranges modified from Vandel, 1962; Burdigalian map modified from Sissingh, 2006; LGM map modified from Jaunsproge, 2013).

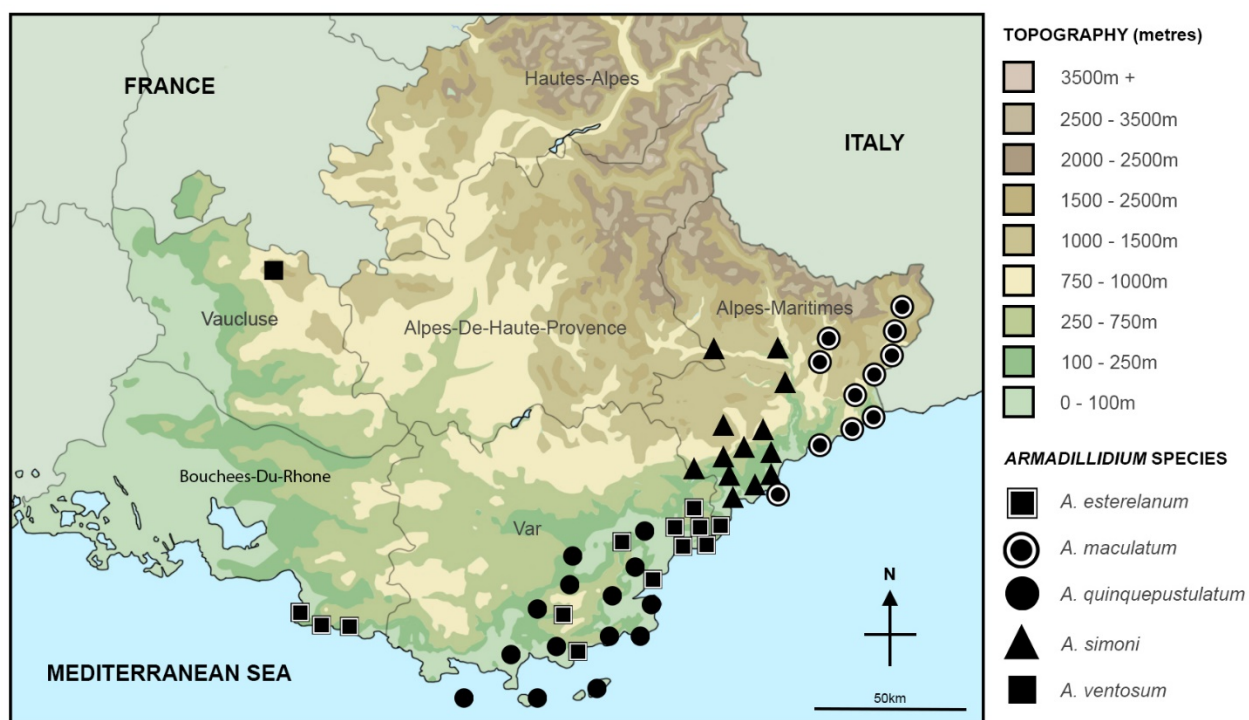


Figure 2: Distribution of the five Provençal endemic *maculatum*-group species showing the influence of the regional topography (note that the majority of species are below 250masl). (Data points after Vandel, 1962 and from 2019/2020 fieldwork by the author; topographic map modified from Gaba, 2009).

Verhoeff (1910) produced the first publication describing the *Armadillidium* species known from the Provence region and allocated them into distinct morphological groups. Verhoeff's *maculatum*-group was instated to accomodate *A. esterelanus*, *A. pujetanum* (a junior synonym of *A. simoni*), *A. maculatum* and *A. quinquepustulatum*. He observed that this group was restricted between the western Alps to the north and the Mediterranean to the south, along a 10km strip of low-lying land where a stable, humid climate is strongly influenced by the sea (Fig. 2). It was also apparent that the species were allocated by their geological domain, with the western species *A. esterelanus* and *A. quinquepustulatum* living on an igneous complex whereas *A. maculatum* and *A. simoni* in the east were isolated to Mesozoic limestone. Following this, an updated diagnosis for the *maculatum*-group was provided by Vandel (1960a, 1962). The group has since been expanded and now includes species present in western Europe. Vandel (1960a, 1962), provides two subgroups to denote these regional clusters:

Oriental subgroup: *A. klugii*, *A. versicolor*, *A. narentanum*, *A. albomarginatum*, *A. flavoscutatum*, *A. cetinjense* (now a junior synonym of *A. klugii*) and *A. bulgaricum* (likely a junior synonym of *A. klugii*).

Occidental subgroup: *A. assimile*, *A. esterelanus*, *A. maculatum*, *A. quinquepustulatum* and *A. simoni*.

Since then, several more species have been (in some cases tentatively) added to the *maculatum*-group (see Gruia et al., 1994; Taiti & Ferrara, 1996). In addition, Schmalfuss (2013) suggests the *klugii*-group for *A. klugii*, *A. flavoscutatum* and *A. wernerii* whilst also making three species synonymous with *A. klugii*. This group almost certainly constitutes a part of the oriental subgroup of the *maculatum*-group, but has likely been allocated its own grouping due to its localised nature along the Adriatic. The distribution of the *maculatum*-group according to Vandel (1962), can be further divided into three geographical units. The

Occidental cluster, present in southern France, northwestern Italy and Corsica, the Adriatic cluster, found on the Adriatic coast, into the northern Balkans from eastern Bulgaria to northern Croatia and lastly the Balkan cluster, comprised solely of the species *A. versicolor* which is distributed from southern Austria, Czech Republic and Slovakia into Romania and Bulgaria (Vandel, 1962).

The presence of a major distributional hiatus (c. 600km) across the Alpine chain between the Oriental and Occidental subgroup has been attributed to the extinction of intermediate species during the last glacial cycle (Vandel, 1960a, 1962). The presence of *A. versicolor* at Lake Traunsee (Austria) in the eastern Alps represent a possible vestigial population of this migration. In addition, the later discovery of *A. ventosum* at Mont Ventoux (France) in the western Alps may also provide another extreme alpine limit for the species group, providing further support of this hypothesis.

The high diversity and broader distributional range of the *maculatum*-group in eastern Europe in addition to reflecting the ancient radiation centre for the *Armadillidium* genus, strongly suggest that the *maculatum*-group originated here too. Therefore, based on this view, the Occidental subgroup should have arisen from a historical migration of species from the Oriental subgroup across the Alps (Vandel, 1960a, 1962). Vandel (1960a, 1962) noted that the unusual distribution of the *maculatum*-group across Europe mirrored the construction of the European landmass during the Miocene, and hypothesised that the migration could have occurred during this time. As aforementioned, the Miocene was characterised by oceanic conditions such as the high water heat capacity of the intercontinental sea at this time lowered the seasonal thermal gradient causing a subtropical climate to be present over much of the European continent (Ramstein et al., 1997; Fluteau et al., 1999; Jiménez-Moreno et al., 2008; Henrot et al., 2017). As the *maculatum*-group is comprised of predominantly thermophilic species adapted to arid Mediterranean scrubland, the conditions of the Miocene would have been conducive for long-distance migration along corridors of suitable habitat. With the majority of the *maculatum*-group living in close proximity to the coast (or in areas that historically had coastlines) where there is a greater stability of climatic conditions. It is possible that coastal sprawl was the major mode for migration and diversification within the species group (Fig. 1: Burdigalian). As no representatives of the *maculatum*-group are present across northern Italy, where the southern foothills of the Alps was once a coastline, it is more feasible that the migration would have occurred to the north of the Alps instead. Therefore, the presence of the Alpine foredeep during the Burdigalian provides a potential geomorphological structure that could facilitate this long distance migration, which would also be supported by the distributional data on *A. ventosum* and *A. versicolor* (Fig. 1: Burdigalian) (Kuhlmann, 2000; Schlunegger et al., 2001; Zweigel et al., 1998).

This hypothesis would suggest that intermediate populations between the two subgroups had to exist around the Alps but have since disappeared. As such, the Quaternary glaciation provides a suitable explanation for the disappearance of these woodlice (Vandel, 1960a, 1962) (Fig. 1: Last Glacial Maximum). Therefore, it is possibly to hypothesis that the origin and diversification of the French *maculatum*-group was the result of widespread migration into the region during suitable climatic conditions followed by strong selective stresses caused by the MSC, refilling of the Mediterranean basin and Quaternary glaciation. However, the peculiarity of the four coastal Provençal endemics to specific geological domains also suggests that habitat structure or edaphic effects may also play a particular role in their diversification.

1.3 — Project Aims

The radiation of the *maculatum*-group in Provence provides a unique opportunity to assess the mechanisms driving woodlouse diversification within the Mediterranean biome on a localised scale. Morphological information and genetic data has been acquired for all of the species groups present in France, with the exception of the *serratum*-group. Furthermore, all of the *maculatum*-group species of the Occidental subgroup are included, with the exception of *A. lanzai* from Corsica. Therefore, thirteen species representing 62% of the total French *Armadillidium* fauna are included within this study.

The project is broken down into four key aims. First, to understand some of the broad relationships within the Armadillidae family and the possibly polyphyletic nature of the *Armadillidium*. Second, to interpret the value of the *Armadillidium* species groups within a phylogenetic context, whilst comparing these results against our traditional knowledge on their morphology and biogeography. Third, to uncover the evolutionary history of the occidental-subgroup of the *maculatum*-group, and explore Vandel's migration theory through the incorporation of the widespread eastern European species, *A. versicolor*. Finally, with the combination of our results on the *maculatum*-group and those of other studies to explore possible causes for the high diversity of woodlice in the European Mediterranean.

2 — METHODOLOGY

2.1 — Sample collection and genome extractions

Live specimens of *A. esterelanum*, *A. maculatum*, *A. opacum*, *A. quinquepustulatum* and *A. ventosum*, were collected by the author during fieldwork undertaken in the eastern regions of France in 2018/19. 3 distinct populations of *A. simoni* were collected by Max Callens and live specimens were sent to the author. A dataset of partial mitochondrial cytochrome c oxidase subunit 1 (CO1) representing 20 localities of 7 *Armadillidium* species were provided by Franck Noël via Boldsystems, whilst a further 9 CO1 sequences of 6 *Armadillidium* species were sourced from NCBI Genbank. Live specimens from captive populations of *A. versicolor* from 2 distinct localities and *A. granulatum* were provided by Mark Titterton and specimens of *A. depressum* were collected by the author in north Wales in 2021. In total, 41 localities of 16 *Armadillidium* species were made available for the analysis. The closely related species *Eluma caelata* was also chosen as an out-group and sourced from NCBI Genbank. Unless made available via online repositories, the remaining material was subject to total genomic DNA extraction.

Live woodlouse specimens were immersed in a -80 freezer and DNA extraction commenced within half an hour of them being deceased. The potential risk of DNA contamination due to the high prevalence of feminising Wolbachia and the large diversity of enteric fauna found in woodlice led to the decision to select only male specimens (where possible) for extraction and to starve the woodlice for several days before freezing to ensure the gut was free of soil particles. 1 individual was contributed from each population sampled, totalling 11 samples. Extractions were undertaken using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers supplementary protocol for insects. Control of extraction quality and quantity was performed on a NanoDrop, and successful DNA eluates were sent for paired-end, Illumina platform sequencing (average read length 151) and for library construction using a TruSeq Nano DNA Kit at Macrogen (Amsterdam, Netherlands).

2.2 — Processing, Alignments and Analysis

The raw genomic data was then cleaned using Cutadapt v.4.1 (Martin, 2011) and Trimmomatic v.0.40 (Bolger et al., 2014) to crop and filter reads and remove unwanted adapters. FLASH (Fast Length Adjustment of SHort reads) (Magoč and Salzberg, 2011), was then utilised to merge the paired-end reads. The quality screened data was then mapped using BWA-SW (Burrows-Wheeler Aligner) to CD's (coding regions) of the reference Isopod genome of *Armadillidium vulgare* (assembly no. MT5521281.1) (Li & Durbin, 2010). SpeedSeq v.0.1.0 (Chiang et al., 2015), was then employed to create genotype calls across the mapped genomes with a 20% maximum filter for missing data points. The CO1 regions also mapped to the reference *A. vulgare* genome.

A DNA maximum likelihood tree was then constructed using SNPhylo (Lee et al., 2014), using all usable CD's, and supported by 1,000 bootstraps. For the CO1 regions, sequences were manually trimmed then aligned using Muscle (Edgar, 2004) in Mega 11 (Tamura et al., 2021). Following the default settings, a maximum likelihood tree was made using non-parametric bootstrap supports obtained through 200 tree replicates. In both trees Kimura 2-parameter (K2P) nucleotide substitution model were used. Trees were later manipulated in FigTree v1.4.4 (Figtree, 2022), rooted to the most likely *Armadillidium* outgroup, *A. opacum*, and edited in Photoshop (Adobe, San Jose, California, USA).

2.3 — Biogeographic data, morphology, dissection, imaging and illustration preparation

Dissection of key *Armadillidium* species was undertaken using an Aspen GTS6 stereo microscope and Aspen GTC10 Trinocular Biological Microscope. The first pleopod of male specimens were prepared as permanent mounts using Euparal and photographed using a Canon EOS 60D Mounted on the Aspen GTC10. Photo-stacked images (c.25 photographs) were made using Zerene Systems (Richland, Washington, USA), and post edited in Lightroom and Photoshop (Adobe, San Jose, California, USA). Habitus images of live specimens were taken on a Fujifilm XT-2. Examination of important characters, including; cephalic structure, male pleopod exopodite 1, male pereopod 7 and the posterior-lateral corner of pereonite 1 were made to compare species morphologies with the genetic data. Where specimens were not available illustrations present within the current literature was used (particularly Vandell, 1962). Biogeographic information on the species was also extracted from the literature with the addition of co-ordinate data collected by the author.

3 — RESULTS

Amplification and sequencing of all our *Armadillidium* samples were successful, with the final alignments covering 422,049 bp of coding sequence for the nuclear tree and 634 base pairs for the CO1 tree.

The Nuclear and CO1 maximum likelihood trees producing similar clade topologies for the extent of *maculatum*-group. The bootstrap values for the Nuclear tree were all above 92%, with the exclusion of 52% for *A. simoni*, but this would have no effect on the overall tree topology. The tree includes all sampled species with the exclusion of the outgroup, *A. opacum* under the *maculatum*-group clade with *A. quinquepustulatum* representing the most basal species of the group. This would involve the subsuming *A. granulatum* (*granulatum*-group) and *A. depressum* (*depressum*-group) into a much broader *maculatum*-group. The CO1 tree has less well supported bootstrap values, but two major clades are visible with a 79% support value delineating the duplocarinate-*Eluma* cephalic type clade and an *Armadillidium*-type clade. The CO1 tree when encompassing all of the known *maculatum*-group species present within the tree

extends into a large clade within the *Armadillidium*-type clade that is supported by an 88% bootstrap value. This clade also includes *A. granulatum* (*granulatum*-group) and *A. depressum* (*depressum*-group) embedded within the *maculatum*-group. *A. quinquepustulatum* also represents the most basal species within the clade. Outside of this, the *sordidum*-group and *nasatum*-group are the two most closely related species groups to the *maculatum*-group clade, followed by the *opacum*- then the *vulgare*-group. The *pictum*-group sits within the other duplocarinate-*Eluma* clade outside of all the other *Armadillidium* species groups and is rooted with *E. caelata*, indicating a non monophyletic *Armadillidium*. Between both trees the relationship between *A. esterelanus* and *A. simoni* and *A. ventosum* is well supported as a cladistic unit with *A. ventosum* representing the basal species in the nuclear tree and the *A. esterelanus* x *simoni* hybrid (*A. simoni* "Peymeinade") representing the basal species for the CO1 tree. Below this the placement of species within the *maculatum*-group clade becomes less structured. In the Nuclear tree the following cladistic unit is formed with a basal *A. maculatum*, whereas in the CO1 tree this is followed by *A. granulatum*. In the Nuclear tree *A. granulatum* and *A. versicolor* produce a clade whereas in the CO1 tree *A. maculatum* and *A. versicolor* form a clade, as does *A. quinquepustulatum* and *A. depressum*. Furthermore, in the CO1 tree, *A. assimile* forms its own clade which is well defined by its Corsican and mainland populations despite it being recovered with a weak node support of 46.5%. Of the occidental-subgroup members of the *maculatum*-group, the most distant within the trees is *A. assimile* followed by *A. quinquepustulatum*, with the core Provençal endemic species forming a unique clade, which includes *A. esterelanus*, *A. simoni* and *A. ventosum*. From biogeographic data *A. maculatum* should also be closely related to this core clade, but this is only evidenced within the Nuclear tree.

Like the genetic data, the morphological examinations and literary analysis also supports a non-monophyletic *Armadillidium*. The clear morphological divide in cephalic structure between the *pictum*- and *serratum*-group and the remaining *Armadillidium* groups in France is supported by their geographic distribution. No hyper-endemic species with *Armadillidium*-type cephalic structures are found west of the Rhone whereas no hyper-endemic species with duplocarinate-type cephalic structures are found east of the Rhone. Only cosmopolitan and expansive Mediterranean *Armadillidium* species (*A. assimile*, *A. granulatum* and *A. depressum* to name a few) overlap the ranges of the two cephalon types in France. This pattern is also mirrored across genera and geographic areas. The *maculatum*-group clade as defined by the trees, contains species with both granulated and smooth teguments, pseudospheric and mesospheric conglobation types and broad scutellums with either rounded or trapezoidal tips. Furthermore, *A. depressum* and *A. granulatum* which are present in the clade, have their closest morphological relatives in the Italian Riviera and the southern Aegean respectively. Of the French endemic *maculatum*-group species, all share a smooth tegument and triangular telson with the exception of *A. quinquepustulatum* which has a granulated tegument and a trapezoidal telson. There is also shared structural similarities in the male pleopod exopodite 1 within these core species, with the exception of *A. quinquepustulatum*. The medial edge is regularly sinuous and ends with a posterior point that is either well rounded or notched. Whereas in *A. quinquepustulatum* it has as almost straight margin the the medial edge with a regularly curved posterior point.

SPECIES GROUPS & CEPHALON TYPE

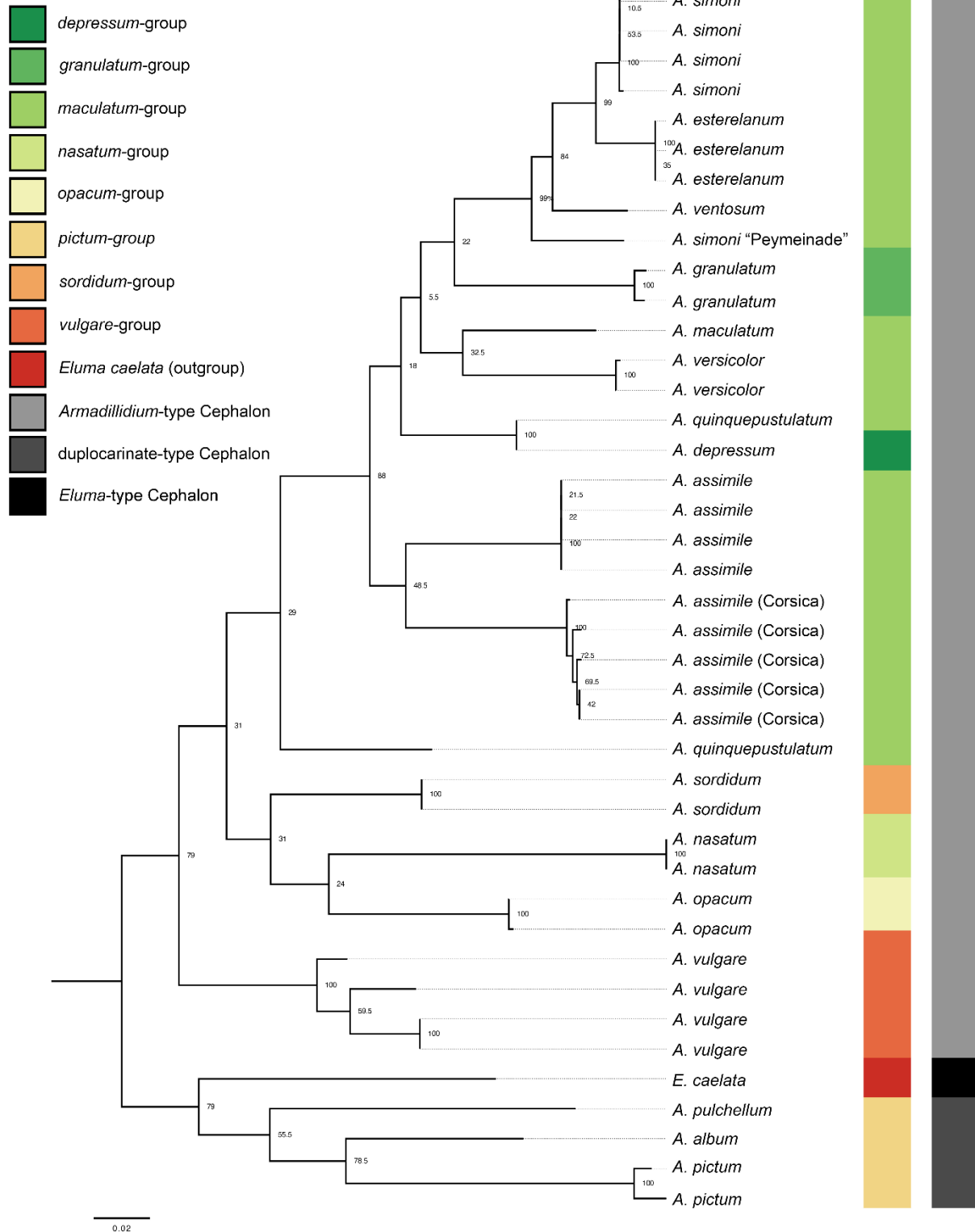


Figure 3: Mitochondrial CO1 maximum likelihood tree of 13 *Armadillidium* species present within France, in addition to *Eluma Caelata* included as an outgroup (200 BS supports).

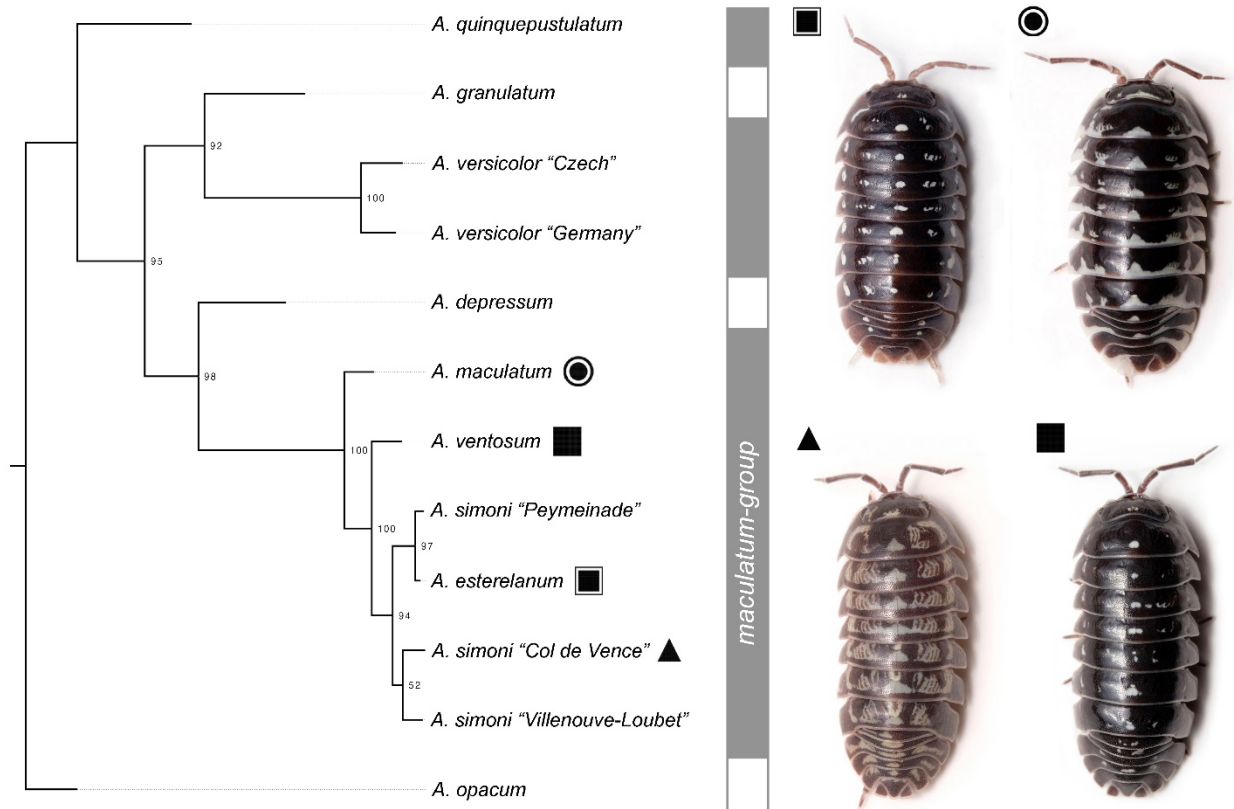


Figure 4: Nuclear maximum likelihood tree using CDs, used on 12 *Armadillidium* samples (1,000 BS supports).

4 — DISCUSSION

4.1 — The Armadillidae

The family Armadillidae which is comprised of 18 genera (including the *Armadillidium*) has been shown, morphologically, to represent a clearly monophyletic entity (Schmidt, 2003, 2008; Schmalfuss, 2013b). Although this family is well resolved, the accepted morphological boundaries at generic level are not as well supported by clear derived character states, with many originally being instated as subgenera (Recuero et al., 2008 and references therein). As such, the monophyly of many genera have been extremely unsettled.

Two of the main characters used in Armadillidae taxonomy include the type of cephalic structure and whether a schisma is present on the pereon. The cephalic structure can be divided into three main groups which can be recognised by the composition of two line-like features that either connect to the margins of the scutellum, known as the frontal line (*linea frontalis*), or sits above it, known as the postscutellar line (*linea postscutellaris*). The first, *Eluma*-type group has only a *linea frontalis* which is usually continuous, dividing the vertex from the front of the cephalon. This type of cephalic structure is well represented in typically species-poor genera; *Ballodillium*, *Cristarmadillidium*, *Eleoniscus*, *Eluma*, *Estenarmadillidium*, *Iberiarmadillidium*, *Paraschizidium*, *Paxodillidium*, *Platanosphaera*, *Schizidium*, *Trichodillidium*, *Troglarmadillidium*, *Trogleluma* and *Typhlarmadillidium*. The second, duplocarinate-type group possess a *linea frontalis* and a variably defined *linea postscutellaris*. This configuration is present in a few species within the *Armadillidium*, the *Alloschizidium*, and tentatively the *Cyphodillidium* (schmölzer, 1965). The

final, *Armadillidium*-type group, has a heavily reduced, almost absent linea frontalis but a strongly defined linea postscutellaris. It is found in most species within the *Armadillidium* and *Echinarmadillidium*.

It was assumed that a subfamily classification could be constructed to accommodate the different cephalic structures, with the Elumiinae (lacking a well-defined linea postscutellaris) and the Armadillidiinae (with a linea postscutellaris) (Vandel 1944, 1962). Even though the structure of the cephalon has significant taxonomic value some authors noticed that the present layout was taxonomically insufficient. As mentioned in the introduction, the placement of *Alloschizidium pruvoti* within the Armadillidiinae (a monogeneric subfamily) was dependent on its ovate conglobation shape and the presence of a schisma on the first pereonite being derived traits (Vandel, 1962). Another example is the circumstance with *Echinarmadillidium* which bears an *Armadillidium*-type cephalic structure but also possesses a schisma on pereonites I and II (Schmalfuss & Sfenthourakis, 1995). Therefore, based on the current taxonomic model of the Armadillidiidae all genera bearing a duplocarinate- and *Armadillidium*-type cephalic structure would in theory, exist within the Armadillidiinae and be synonymous with the genus *Armadillidium*. It is therefore unwise to progress with the sub-familial taxonomic structure as the Armadillidiinae is clearly not monogenetic. In addition, some genera such as the *Schizidium* contains species with both duplocarinate and *Eluma*-type cephalic structures which breaks the boundary between the two Armadillidiidae subfamilies (Recuero et al., 2022). It is then not surprising that the current definition in place for the most diverse genus within the family, the *Armadillidium*, is very likely to be polyphyletic (Schmalfuss, 2013b).

Only recently have the two main characters of the Armadillidiidae been investigated with the use of molecular techniques (Recuero et al., 2022). Using mitochondrial DNA (Cox1) and nuclear DNA (18s, 28s and H3) it was shown that the *Eluma*-type cephalic structure is most probably the ancestral state of the family, with the *Armadillidium*- and duplocarinate-type cephalic structures representing derived states which evolved along independent lineages. The presence of a schisma also appears to be a convergent character, arising independently within unrelated clades. It is likely the schisma is an example of parallel evolution, where selective pressures have driven the formation of a more efficient conglobation strategy (Schmalfuss, 2008b).

The Multilocus Bayesian phylogenetic reconstruction of several groups of the Armadillidiidae by Recuero et al. (2022), revealed that duplocarinate-type *Armadillidium* (comprising the *pictum*— and *serratum*-group) evolved along an independent lineage from the *Armadillidium*-type (all other species groups). The results of our mitochondrial (CO1) maximum-likelihood tree supports an identical topology when *Eluma caelata* was included as an outgroup; where *A. album*, *A. pictum* and *A. pulchellum* occupy a separate clade to the remaining *Armadillidium* species (Fig. 3). The fact that different gene regions have produced identical phylogenetic outputs provide increasing supports of the polyphyletic nature of the *Armadillidium*.

Interestingly, the *Armadillidium* groups containing duplocarinate-type cephalic structures, totalling thirteen species, have a west European to northwestern African distribution from Morocco, Spain, Portugal (including the Azores) in the west, through France (including Corsica), United Kingdom, Belgium and the Netherlands in the east, with the highest species diversity being found in the Iberian Peninsula. However, there are also some sporadic records of *A. album* from Greece (Schmalfuss, 2013b). As the *Armadillidium* species diversity is greatest in eastern Europe than western Europe, with the likely centre of origin being located around the northeastern Mediterranean (Schmalfuss, 2000), It is increasing more likely that these groups (as defined by their cephalic structure) represent two distinct evolutionary origins that have converged on similar adaptive characteristics.

The only other genus with a duplocarinate-type cephalic structure is the *Alloschizidium*. This genus is comprised of fourteen species which are distributed in southern France and Corsica, Sardinia and northwest

Italy. Recuero et al. (2022) consider *Echinarmadillidium*, with a Greek distribution as a duplocarinate-type but it is certainly an *Armadillidium*-type (Schmalfuss & Sfenthourakis, 1995). This confusion likely arose due to the mis-interpretation of which lines constitute the linea frontalis, linea postscutellaris and the linea antennalis. Schmölzer (1965) also suggests that the monotypic *Cyphodillidium* along the Adriatic could belong to the duplocarinate-type, but the presumed presence of a linea postscutellaris may be a misinterpretation due to the overall spiny tegument making this character appear present when it is not. It is likely this genus has a *Eluma*-type cephalic structure, and as such, is closely related to the genus *Paxodillidium* (Schmalfuss & Sfenthourakis, 1995). Therefore, the duplocarinate-type cephalic structure may be a character trait limited to western Europe with its highest diversity being found within the Iberian peninsula and the islands of Corsica and Sardinia. This biogeographic pattern can be easily attributed to regional plate tectonics. Corsica and Sardinia were originally attached to the Iberian peninsula but broke off on a single microplate and underwent anticlockwise rotation c. 29Ma (Boccaletti et al., 1990). It was during this period of rotation that land bridges with southeastern France (c. 20-15Ma) and northwestern Italy (c. 8Ma) formed. As such, the ancestor to all the duplocarinate-type genera likely originated on the Iberian peninsula. The presence of duplocarinate-type *Armadillidium* as far east as the Netherlands can be attributed to range expansion of some species out of Iberia since the last glacial maximum. The only exception to the above is *A. album* which is a littoral expansive duplocarinate-type whose broad distribution across Europe is likely the result of tidally moved beach debris.

Therefore, the distributional pattern of the duplocarinate-type in western Europe and the *Armadillidium*-type in eastern Europe supports the results of Recuero et al. (2022) that the *Eluma*-type is very likely to be the ancestral character state. This is because genera with the *Eluma*-type cephalic structure can be found across the entirety of Europe and overlap the distributions of both the *Armadillidium*- and duplocarinate-types. Therefore, with the combined evidence of two separate genomic studies, distinct morphological disparity and a clearly geographically separate distribution, the duplocarinate-type *Armadillidium* species (comprising the *pictum*- and *serratum*-groups) almost certainly represent a new genus within the Armadillidiidae. Despite the mounting evidence, further taxonomic work will be required to allocate the correct placement of this new genus in context with the other genera. Therefore, of the nine subgroups of the *Armadillidium* in France, the two duplocarinate-types can be disregarded.

4.2 — Mitochondrial tree and *Armadillidium* species-groups

Our gene tree reflects the evolutionary history of the singular mitochondrial gene (CO1) and doesn't infer true species relationships as the nuclear (species) tree provides. Nonetheless, it can still be useful to observe potential cladistic patterns across the *Armadillidium* species-groups, and see if there is congruence with the species tree; particularly within the *maculatum*-group itself. The gene tree (Fig. 3), does reveal that the aforementioned, duplocarinate- and *Eluma*-type cephalic structures represent a distinct clade to all other *Armadillidium* species groups with an *Armadillidium*-type cephalic structure, comprising the; *depressum*-, *granulatum*-, *maculatum*-, *nasatum*-, *sordidum*- and *vulgare*-group. Despite the relatively high bootstrap values of these two major clades; the *Armadillidium*- and duplocarinate clades, both with 79% support values, the bootstrap values do dwindle significantly at some nodes making their interpretations impracticable. Furthermore, the two *A. quinquepustulatum* samples have appeared in completely separate locations within the *maculatum*-group clade, suggesting their discrete placements maybe the results of polymorphisms or hybridisations of the species CO1 gene.

According to the results, *A. vulgare* (*vulgare*-group) represents the most basal species group with an *Armadillidium*-type cephalic structure in France. This group is identified by a smooth (rarely weakly granulated) tegument, a eusperhic conglobation type and with a scutellum that weakly protrudes above the

vertex (Vandel, 1962). In fact, the group includes all *Armadillidium* species with a euspheric conglobation type that isn't within the *pictum*-group. The presence of euspheric conglobation within the *pictum*-group, the basal *vulgare*-group and within the *Eluma*, may indicate that this conglobation type is an ancestral character state within the Armadillidiidae and that any modification of a perfect sphere is a derived characteristic. Although speculative, a larger generic dataset incorporating a character state tree would be required to determine the true value of this feature in Armadillidiidae evolution.

The tree does not support a monophyletic *maculatum*-group due to the placement of *A. depressum* and *A. granulatum* (*depressum*- and *granulatum* group). The *depressum*-group is defined by the relatively granulated tegument, a pseudospheric conglobation type and a very broad scutellum. Vandel (1962) suggests that the group has morphological affinities with the *nasatum*-group and likely originated within the Italian riviera. The *granulatum*-group on the other hand, is characterised by a strongly granulated tegument, a mesospheric conglobation type and a very broad scutellum. Vandel (1962), suggests the group has morphological affinities with the *depressum*-group and likely originated within the southern Aegean where the groups is most diverse. Therefore, the disparate centres of endemism between these two groups may suggest their placements, especially with in the *maculatum*-group could be erroneous, or the defined boundaries of the group are not resolved. Furthermore, *A. assimile*, which is well represented as a distinct clade basely within the *maculatum*-group, has clear definition between its French mainland and Corsican populations. *A. assimile*, was included in the *maculatum*-group due to it fitting the major morphological criterions of the group, but differed from all the other French species by its colouration, being comprised of mottled brown markings, and having a distributional pattern that overlaps with all the other occidental-subgroup species (bar *A. ventosum*). This distribution and the results of the tree suggest it is either the product of a very basal speciation event within the occidental-subgroup, or is in a closely related but evolutionary distinct sister-group to the *maculatum*-group. There is also a possibility that some regional records of *A. assimile* could be a misidentification of another, very recent expansive species; *A. cf. arcangelii* (Gregory 2014; Garcia & Cabanillas, 2021; Noël et al., 2022).

The remaining clade comprises the *nasatum*-, *opacum*- and *sordidum*-group. Both the *opacum*- and *sordidum*-groups share a mesospheric conglobation, a smooth tegument and a short scutellum, but are deemed separate group by the structural differences in the male pereopod VII and the linea frontalis on the cephalon (Vandel, 1962). In contrast, the *nasatum*-group is characterised by its pseudospheric conglobation, heavily granulated tegument and presence of rectangular, forwardly projecting scutellum, in addition to several other minor characteristics (Vandel, 1962). It is likely the *opacum*- and *sordidum*-groups share a common origin and that diversification within them occurred with the emergence of the Italic peninsula where the majority of the extant species reside. The few exceptions being *A. opacum* which has a boreo-Alpine distribution across central and north eastern Europe, and *A. alassienne* and *A. sordidum* which are also present in southern France. Furthermore, the *nasatum*-group has an Italo-Dalmatian distribution, occurring on both sides of the Adriatic (Vandel, 1962). An identical distribution pattern had also been observed in the monophyletic *Niphargus* subterranean amphipods (Delić et al., 2020). Their dispersal and isolation being the product of regression-transgression cycles of Miocene/Pleistocene Mediterranean Sea. As such, if the *nasatum*-group arose on Italy and then migrated across the Adriatic we could presume a possible evolutionary origin linked to the *opacum*- and *sordidum*-group whose distributions are solely attributed to the Italic peninsular and adjacent continental countries. If the migration occurred in the opposite direction, it is likely that their common ancestor should be much more ancient and sit beyond the limits of their clade within the CO1 tree. Despite these speculations, the morphological disparity of the *nasatum*-group and the weak support values for the clade raises considerable doubts on its placement within our phylogeny.

4.3 — Nuclear tree and the *maculatum*-group

The nuclear tree (Fig. 4) provides a more robust topology than the mitochondrial tree. All species used within the tree fell under a single clade with the exception of *A. opacum*, which was chosen as an outgroup. *A. quinquepustulatum* was the most basal species within the main clade, indicating the *maculatum*-group, as with the CO1 tree is not monophyletic due to the clade also including *A. depressum* (*depressum*-group) and *A. granulatum* (*granulatum*-group).

Of the originally defined occidental-subgroup, *A. quinquepustulatum* was the most aberrant species of the group, bearing a granulated tegument and a trapezoidal telson, a combination of features not seen in any other species within the group. Furthermore, the similar morphologies of *A. versicolor* (a member of the to the oriental-subgroup) with the occidental-subgroup suggested a possibly linked evolutionary origin. However, *A. versicolor* sits in a separate clade with *A. granulatum* away from the occidental-subgroup species. The four endemic species in Provence all have a close evolutionary origin, sitting in a clade supported by a 100% bootstrap support, with the nearest related species being *A. depressum*. This relationship seems to be supported biogeographically, where the centre of endemism for the *depressum*-group is found in the Italian Riviera, adjacent to the region where the occidental-subgroup is located. However, the morphological differences highlighted in the mitochondrial section (4.2), for *A. granulatum* and *A. depressum*, do not correspond with the morphological characteristics used to define the *maculatum*-group. Therefore, the criterion used in species-group definitions are not functional from a phylogenetic standpoint. The only well supported subset of the *maculatum*-group as defined by our trees is the Provençal endemic cluster (*A. esterelanum*, *A. maculatum*, *A. simoni*, *A. ventosum*), who's morphological and biogeographic relationships appear congruent. As such, the monophyly of the occidental-subgroup could be retained for this tree if everything below *A. maculatum* was excluded, with the shared morphological characters of *A. versicolor* and *A. quinquepustulatum* being down to convergence. This would also resolve the morphological disparity seen with the inclusion of the *depressum*- and *granulatum*-group in addition to their contrasting centres of diversification across Europe. Therefore, It is most plausible to assume that the Provençal endemic occidental-group species arose through the migration of an ancestral *depressum*-group species out of northwestern Italy.

It is uncertain whether the remaining *maculatum*-group species, *A. lanzai* (which was not included in our study) belong within the group. But we can suggest from the extremely close morphology of *A. lanzai* to *A. quinquepustulatum* (Taiti & Ferrara, 1996), in addition to their shared geological preferences and paleogeographic affiliations, likely suggests it also does not belong within the occidental-subgroup.

As such, the morphological criterion of the *maculatum*-group clearly incorporates species with different evolutionary histories but have converged on similar morphological structure. As such the exclusion of *A. versicolor* would also indicate all members of the oriental-subgroup must have an unrelated origin to the remaining species found in the occidental-subgroup. Therefore, our nuclear data has revealed that morphology alone cannot support phylogenetic relatedness, as many characteristics used in *Armadillidium* systematics appear to be homoplastic.

4.4 — *maculatum*-group morphology

As the morphological and genetic evidence clearly reveals that *A. quinquepustulatum* does not belong to the *maculatum*-group, it can be excluded from further examination here. This leaves certainty four species that share a common evolutionary origin within the region; *A. esterelanum*, *A. maculatum*, *A. simoni* and *A. ventosum*. The topology of the *maculatum*-group clade in the nuclear tree places *A. maculatum* as the basal species. This position goes against the original assumptions that members of the *maculatum*-group with banded colouration represented the most recently derived state, with spots being intermediate and normal (mottled/striated) colours being primitive (Vandel, 1962).

A. simoni was originally believed to represent the most ancestral *maculatum*-group species in Provence due to its striated body markings, with *A. esterelanus* being a spotted intermediate and *A. maculatum* being the most derived with complete bands. This assumption was also supported by male sexual characters, where an indentation on the internal edge of the exopodite of the first pleopod, which is present in *A. esterelanus* and *A. simoni*, was absent in *A. maculatum* (Fig. 5B). Vandel (1962), also mentions the subtle differences of the male pereopod VII, particularly the variable number of prominent spines on the ischium, where *A. esterelanus* and *A. simoni* have between 7-9 spines whilst *A. maculatum* has 12-13. However, after examining several dissected pereopods, the number of spines appears variable within each species, so we are excluding this here as a possible identifying character. In addition, the clubbed shape and hair fields of the ischium are also not immediately different between the species. Morphometrics may reveal ratio differences in the overall structure and shape of the concavity of the clubbed end, but this is beyond the limits of this current study.

As our results indicate an alternative evolutionary scenario it is worth describing, what we believe to be the three most important morphological character (colouration, angle of the postero-lateral corner of pereonite I and the shape of the male exopodite I) relate to one another.

Firstly, the colouration and body markings do not have much phylogenetic significance as there is a high degree of variability between and within populations of the same species. Despite this, the four species can be tentatively separated from one another when observing the size and shape of the body markings. These species all bear a dark brown to black base colouration with stripes or spots which can either be white or yellow. In *A. maculatum* the dorsal markings range from five large spots through a series of intermediates where they form completely intact bands in the subspecies *A. maculatum zonatum*. In the spotted form, the two peripheral spots and the central, often triangular spot on the vertex are well defined, and the inner pair is weakly striated. An additional pair of spots can sometimes be present on the extreme margins of the epimeron. The markings are usually white, rarely yellow (Fig. 4 bordered circle). In *A. ventosum* only white markings are present, which usually comprise five relatively reduced spots per pereonite, although additional and missing spots are commonly observed, the central spot is always the most well defined (Fig. 4 square). In *A. simoni* the markings are extremely variable, with roughly five spot region on each pereonite that can either be white or yellow. The central spot is often well defined and typically triangular in shape. The peripheral spots are elongated and thickset and the inner pair is heavily striated and broad so that it merges with both the central and marginal spots (Fig. 4 triangle). Finally, *A. esterelanus* is the most regularly marked species of the group, almost always bearing five clearly defined white or yellow spots per pereonite. The central spot is rarely larger than the peripheral pair and the inner pair are usually weakly striated (Fig. 4 bordered square). Secondly, the postero-lateral corner of pereonites I can be either angular or regularly sinuous. An angular corner is present only in *A. esterelanus* and *A. ventosum*, with it appearing more exaggerated in *A. ventosum* where the top portion of the angle forms a slight concavity. In contrast, this corner is regularly sinuous in *A. maculatum* and *A. simoni*. From this we can determine that an angular posterior-lateral corner of pereonite I is likely a derived trait as it is not found within the basal *A. maculatum*. The reappearance of a sinuous corner in *A. simoni* may suggest that this character was present in the ancestral species C (Fig. 6B), with it only persisting in *A. simoni*. Therefore, an angular corner may be homoplastic in this clade where it has arisen twice during the evolution of the group.

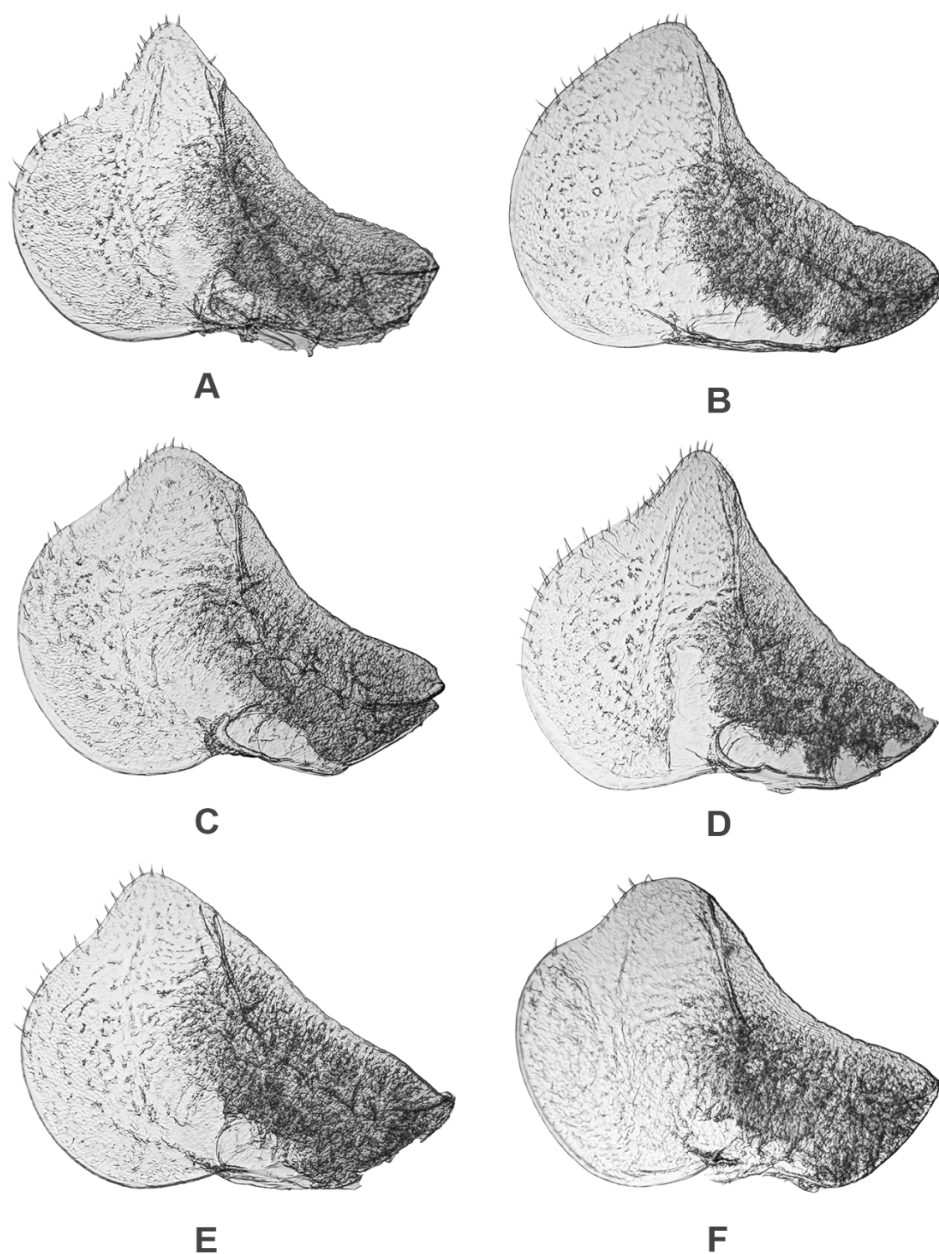


Figure 5: Pleopod I Exopodites — **A)** *A. esterlanum* (Les Adrets-de-l'Estérel), **B)** *A. maculatum* (Vallon de Roya) **C)** *A. Simoni* (Col de Vence), **D)** *A. cf. simoni x esterlanum* (Peymeinade), **E)** *A. simoni* (Villeneuve-Loubet), **F)** *A. ventosum* (Mont Ventoux).

Finally, the lack of an indentation on the internal edge of the male 1st exopodite appears to be an ancestral character state within these four species. The evolutionary progression in the shape of the exopodite when regarding the tree topology starts with *A. maculatum* where it is regularly rounded so that the posterior point is incorporated with it and does not appear prominent (Fig. 5B). Next is *A. ventosum* where an indentation has developed on the internal edge. The posterior point is still ill-defined and appearing somewhat truncated (Fig. 5F). Finally, the posterior point becomes progressively pointed in *A. simoni* (Fig. 5C & 5E) where it reaches its final extent in *A. esterlanum* (Fig. 5A).

4.5—Evolutionary history of the *maculatum*-group

The biogeographical history of the *maculatum*-group had been briefly discussed by Verhoeff (1910) and a full evolutionary narrative was proposed for the origin of the occidental-subgroup in France by Vandel (1960a, 1962). Our results indicate a different diversification scenario within southeastern France than that of Vandel's, but we cannot exclude the possibility of his Alpine migration hypothesis during the Miocene (Fig. 1). Although our results indicate *A. versicolor* is not associated with the ancestral progenitor of occidental-subgroup of the *maculatum*-group, it doesn't detract from the possibility that a similar migration route was utilised by a different group of *Armadillidium*.

A. maculatum is the most easterly representative of the occidental-subgroup, extending its range into northwestern Italy where the nominate species is replaced by the subspecies *A. maculatum zonatum* in the vicinity of Alassio (Verhoeff, 1910). This is surprising when the routes of migration are considered around the forming Alpine chain. As coastal sprawl appears to be the most common process in which *Armadillidium* species appear to spread along the extremities of their ranges in France (Vandel 1962), two options become apparent during the Miocene. Coastal sprawl can occur in the northern Alps along the shoreline of the Alpine molasse, or to the south through northern Italy (Fig. 1, northern route arrowed). Vandel (1962) suggests the absence of the *maculatum*-group throughout Italy was the result of it being completely submerged during the Miocene. If the migration had occurred along the southern Alps we should have expected a higher diversity of this group in Italy. The ancestral Apennines started to appear out of the Mediterranean Sea during the mid Miocene (c.17-16 Ma) in what is now the Tyrrhenian Sea, and was connected to south eastern France. The Italic peninsula didn't start taking on a recognisable form until the end of the Miocene (Schmidt et al., 2021 and references therein). Under the Alpine migration hypothesis, a most likely route of migration based on the morphological and biogeographic information would suggest a route following the northern Alps (Fig. 1). However, our nuclear tree indicates the occidental subgroup has closer genetic affinity to the *depressum*-group than to *A. versicolor*. As the *depressum*-group is believed to have arisen in the Italian riviera and has its greatest species diversity there, it is then unsurprising to find that the most basal occidental subgroup species, *A. maculatum* is the most easterly species and the only one to occur into northern Italy. Therefore, our original assumption that the migration occurred along the northern Alps and the Molasse basin is incorrect, and the occidental-subgroup likely arrived into southeastern France from the southeast via Italy.

Using the topology of the nuclear tree we can tentatively derive the following biogeographic scenario for the four Provençal *maculatum*-group species. Although our phylogeny did not include a molecular clock, we still examined the geomorphological history of the region to deduce the series of events that may have contributed to the speciation and present distribution patterns of these *Armadillidium*. It is likely that the founding species for the occidental-subgroup arrived into Provence during the mid-Miocene, c.20.4-16Ma, probably around the Burdigalian when the climatic conditions were more suitable for these thermophilic woodlice (Jiménez-Moreno et al., 2008; Henrot et al., 2017).

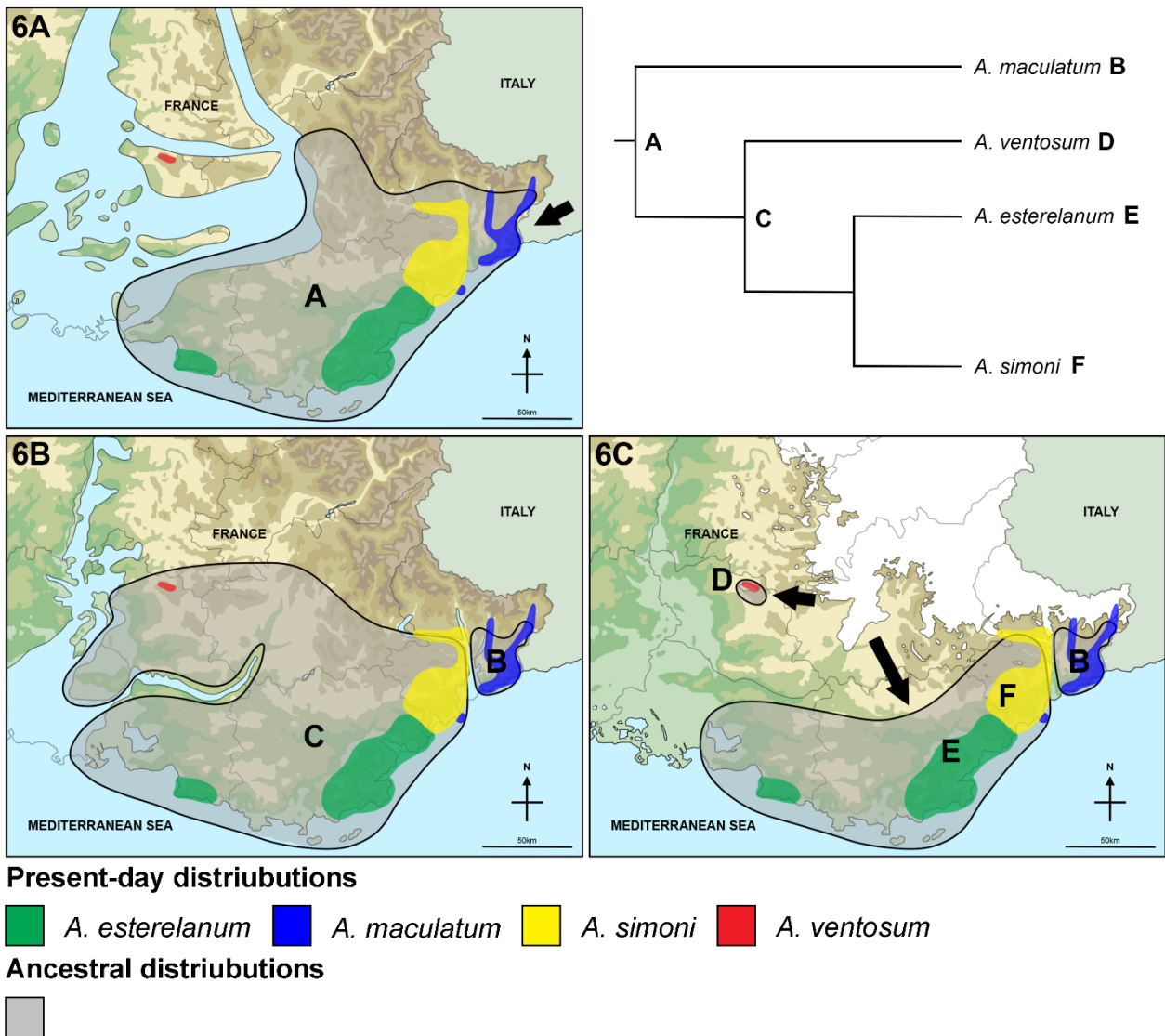


Figure 6: Prediction models outlining the distribution of the ancestral species and modern representatives of the four modern *maculatum*-group species (Figs. 6A-C), in relation to the nuclear phylogenetic tree results (top right). 6A) Extent of the Alpine Molasse during the Burdigalian (after Sissingh, 2001), 6B) Extent of the Pliocene Rias (after Mocochain et al., 2011; Tassy et al., 2014), 6C) Extent of glacial ice over during the LGM (after Seguito et al., 2018).

The distribution of this common ancestor was likely very broad and covered much of the low-lying areas of Provence, being constricted from further expansion by the Alps to the northeast, the Mediterranean Sea to the south and the Rhone valley seaway (Alpine Molasse) to the west (Fig. 6A). This distribution likely persisted throughout the Miocene. During the mid-Miocene a final warm episode occurred, known as the Miocene Climatic Optimum, before the region slowly began to cool (Flower & Kennett, 1994; Zachos, 2001). By the late Miocene the climate trended towards a decrease in precipitation, conversion to more continental conditions and the development of widespread open habitats; particularly sclerophyllous vegetation and C4-grasses (Bernor et al., 1996; Bruch et al., 2011). The slow climatic and vegetative changes experienced during the Miocene, in our opinion, do not sufficiently explain how *A. maculatum* could differentiate from the ancestral species. However, the strong present-day distributional relationship of *A. maculatum* within the valleys of the Var and Roya, suggests its origins are likely linked to the history of these waterways. Firstly, Vandel (1962) highlighted the Var as biogeographically significant for the terrestrial isopod diversity of the region, with common species west of the Var, such as *Cylisticus*

esterelanus. *Helleria brevicornis* and *Trichoniscus biformatus* being completely absent to the east of it. This clearly indicates that the Var is a major barrier to terrestrial isopod dispersal. Secondly, the bayesian phylogenetic analysis of Becking et al. (2017) during their study on the diversity and evolution of sex determination in terrestrial isopods, revealed the divergence time of *A. maculatum* and *A. simoni* to c.4ma. This divergence time coincides approximately with the re-filling of the Mediterranean Sea after the MSC (c.5.33mya), where the valleys of the Var developed into a ria and were completely submerged underwater (Blanc, 2002). The extent of this ria has been illustrated by Mocochain et al. (2011) and Tassy et al. (2014), showing that it reached far up the valleys of the Tinée, Var and Vésubie to the foothills of the western Alps (Fig. 6B). The physical barrier of the ria and the mountains to the north would have caused, almost, if not complete separation of the eastern Var population of the ancestral species from those to the west. It is likely from the current evidence that *A. maculatum* appeared through an allopatric mode of speciation, with its distinct morphology being the result of its extended period of isolation from the other occidental-subgroup species of the *maculatum*-group.

Deterioration of the stable, warm and humid conditions during the middle-late Pliocene (3.1-2.4Ma) caused a marked change in climatic conditions and habitat structure of the region resulting in dynamic shifts in species distributions (Coope, 1994; Hewitt, 2000). This likely led to a major constriction in the ranges of the *maculatum*-group species in Provence at that time. A southerly shift in range towards the Mediterranean coast, reflecting the current distributions of *A. esterelanus*, *A. maculatum* and *A. simoni* (Fig. 2), can be attributed to this area being more climatically stable due to the warm, humid influences of the Mediterranean Sea (Verhoeff, 1910). In addition to acting as a major glacial refugia (Schmitt & Varga, 2012; Chiochio et al., 2019). However, presuming a population of the *maculatum*-group was located north of the Durance, it must have been almost completely restricted in this area since the formation of a ria along its course (Mocochain et al., 2011; Tassy et al., 2014) (Fig. 6B). Once the Ice Age had begun, intermediate *maculatum*-group populations that extended into the western Alpine chain likely expired under the cold and dry glacial conditions (Fig. 1 Last Glacial Maximum) (Vandel, 1962; Salzmann et al., 2011 and references therein). The same likely occurred around, and to the north of the Durance, where glaciers extended along its upper reaches and on several of the peaks of the pre-Alps (Fig. 6C). Therefore, the origin of *A. ventosum* can be tentatively attributed to the ria and glacial activity of the Durance separating it from the species of the southern Provence refugia. Additionally, the presence of *A. ventosum* at the summit of Mont Ventoux is unusual considering the thermophilic nature of these *Armadillidium*, but several lines of evidence provide explanation for its existence at this atypic location. Firstly, it has been well documented that Mont Ventoux had acted as a glacial refugia during the LGM (Schmitt & Varga, 2012; Crégut-Bonnouire et al., 2018) with other endemic invertebrates being present at the same location and within the same habitat, such as the short-horned grasshopper *Podisma amedeignatoae* (Fontana & Pozzebon, 2007). Secondly, the geomorphological arrangement of the mountain, with an elongated form, stretching from east to west, results in the southern slopes being exposed to continuous harsh sun, whilst the northern slopes receives comparatively little.

The gradually sloping southern slopes also receives mild sea winds giving it a curiously Mediterranean climate despite its high altitude. The fact only these particular regions of the Vaucluse mountain chain have Mediterranean like conditions in juxtaposition to the surrounding landscape can explain how a thermophilic, typically Mediterranean species could survive in such a location. In addition, there are some potentially subtle morphological and behavioural adaptations that we have observed in *A. ventosum* that may explain its survival here. First, the ground colour is consistently very black, noticeably darker than any other species of the occidental-subgroup. It is likely this provides a thermoregulatory advantage at high altitude and corresponds to the thermal melanism hypothesis (Clusella-Trullas et al., 2007); an adaptation feature seen in other highland species in Europe (Köhler & Samietz, 2017; Martínez-Freiría et al., 2020). *A. ventosum* is also more diurnal in nature than the other occidental-subgroup species, which is likely a

strategy to avoid being active during the significantly cooler nocturnal temperatures. In addition, *A. ventosum* is extremely abundant at the summit. This phenomenon has been attributed to ecological release within other mountain-top terrestrial isopods due to the potential absence of predatory species at high altitude (Sfenthourakis & Hornung, 2018). This may also support why *A. ventosum* has adopted a diurnal active period over the other occidental-subgroup species, which likely face much greater diurnal predatory risks.

The final stage of diversification of the occidental-subgroup occurred after the formation of *A. ventosum*, and likely corresponds to a time period during the Ice age when *A. esterelanus* and *A. simoni* were isolated in a Provençal glacial refugia (Fig. 6C). Unlike *A. maculatum* and *A. ventosum* there is no clear evidence of vicariance between these two species and their adjacent distributions appear to be solely controlled by the different geological domains they are found on (Verhoeff, 1910; Vandel, 1962). *A. esterelanus* has been recorded almost exclusively from areas of crystallophyllian rock (Porphyries and micaschists) and very rarely from areas of calcareous soil. It is very abundant in the Estérel massif becoming less so into the Maures massif. There are some disjunct populations on calcareous rock east of Marseilles in the Calanques massif (Vandel, 1962; Pavon, 2021), and some atypical specimens that likely correspond to *A. esterelanus* from the Hérault and Gard departments (Franck Noël pers. comm.). This atypic population has also been found in association with *A. cf. arcangelii* (a known introduced species from Italy), probably indicates these populations arose through anthropogenic dispersal. Additionally, the populations recorded around the Calanques massif are suggested to have appeared through coastal sprawl (Vandel, 1962). It is relatively clear that *A. esterelanus* as a species originated within the Esterel massif due to its ubiquitousness there, but its presence on calcareous rock is hard to explain, especially with the clear hiatus between the populations. Unfortunately, we were unable to include any specimens from these calcareous populations within the genetic analysis, but it is likely they will not deviate significantly from *A. esterelanus* due to their almost identical somatic and sexual characteristics. In contrast, *A. simoni* is restricted entirely to Mesozoic limestone, with a range that extends from the boundary of the crystallophyllian rock of the Maures and Tanneron Massif to the west and the valley of the Var to the east. *A. simoni* is also relatively common within its range despite having a localised distribution, occupying an area of c.30km wide, although there are some atypical specimens that likely correspond with *A. simoni* from Verdon (Franck Noël pers. comm.). Vandel (1962), noted that individuals encountered in the Tanneron massif, the contact point between the igneous and calcareous geologies, displayed intermediate characteristics between *A. esterelanus* and *A. simoni* indicating a possible hybrid zone. The specimens he examined from this region displayed an *A. esterelanus* type male pleopod I exopodite, however, the somatic characteristics deviated between the two species. With body colouration varying from spotted to striated and the posterior-lateral corner of Pereonite I was either angular or sinuous dependent on the individual. It is worth mentioning that the character of the Pereonite I has been used as an important characteristic for differentiating these two species, where it is always angular in pure *A. esterelanus* populations and sinuous in pure *A. simoni* populations. We were fortunate to examine specimens initially labelled as *A. simoni* “Peymeinade” which were collected northeast of the Tanneron massif and include it within the genetic analysis. It is clear from the nuclear tree that the Peymeinade population corresponds more closely to *A. esterelanus* than *A. simoni* and the male pleopod I exopodite also matches the *A. esterelanus* form, with a clearly defined medial indentation (Fig. 5C). Furthermore, the colouration is relatively intermediate between the two species with striations that are defined into spot-like regions. The posterior-lateral corner of Pereonite I is most often sinuous like *A. simoni* in the individuals we examined. As such the *Armadillidium* from Peymeinade clearly represents an intermediate population and match the observations made by Vandel (1962). Therefore, it appears that these two species have recently separated from one another, probably sometime during the LGM, via parapatric speciation in correspondence with the underlying geology and subsequent changes in the flora community and microhabitat structure. It is likely that the common ancestor of these two species corresponds to a more *A. esterelanus* type morphology. This is because we

see more similarities to *A. ventosum* with *A. esterelanus* than we do with *A. simoni*, particularly with the composition of the body marking and the angular shape of the posterior-lateral corner of Pereonite I. The broader distributional extent on multiple geologies of *A. esterelanus* indicates it may be less adaptively constrained than *A. simoni*, suggesting *A. esterelanus* or its ancestor may have had a broader distributional range along the Tyrrhenian coast. In addition, the growing number of atypic populations being discovered, and with the recent discovery of *A. ventosum* too, it is beginning to suggest the region is likely much more rich with *maculatum*-group species comprised an extremely close species complex.

4.6 — *Armadillidium* diversification in the Mediterranean

Our analysis of the *maculatum*-group has revealed two broad processes that are likely significant when regarding larger scale diversification patterns within terrestrial Isopods.

The first is the synergy of climate, eustatism and geomorphology facilitating allopatry. The combination of these factors are well known in the diversification of other taxa within the Mediterranean (for example; Sanmartín, 2003; Marra, 2005; Kaya et al., 2013; Zazzo et al., 2015; Trájer et al., 2021), with only a few studies on terrestrial Isopods (for example; Poulakakis & Sfenthourakis, 2008; Gentile et al., 2010; Dimitriou et al., 2022). Our results indicate that the occidental-subgroup of the *maculatum*-group were affected by the following events; subtropical Miocene climate, formation of the Alps and the Alpine Foredeep, the Zanclean flood and the climatic oscillation of the Ice Age. But how does these relate on a much broader scale? The highest number of *Armadillidium* species can be found within Greece (+55 species) and Italy (+60 species), so comparison with these two regions would provide weight to the value of allopatry on larger scale diversification.

Schmalzfuss (2013b), gives a brief presumption on the diversification of the Greek *Armadillidium*. He suggest that differentiation likely occurred during periods of rapid climatic change (particularly during the Ice Age) where the combination of heterogeneity and the slow dispersal ability drove diversification. Furthermore, he mentions within the Peloponnese, a few ancient species existed within the region, but later migratory influxes during wetter periods of the Pleistocene brought species from the north, which differentiated as a result of isolation on the many mountain systems caused by a drier and warmer climate (Schmalzfuss, 2006a). Sfenthourakis & Hornung (2018), later expanded on this, suggesting that the combination of habitat heterogeneity alongside the sporadic, semi-isolated arrangement of favourable humid habitats in the arid Mediterranean region facilitated vicariance. Species could freely move during wetter geological periods but became isolated or even extinct when conditions dried again. This fluctuation of conditions likely enhanced allopatric differentiation. This process has been proposed for causing the genetic disparity between populations of Greek *Ligidium* (Klossa-Kilia et al., 2006), and the possible localised extinction of *L. cycladicum* on the island of Kythnos (Sfenthourakis & Hornung, 2018). Despite this, the hypothesis can greatly explain the diversification seen in mesic species, but it is becomes difficult to see how this process could contribute to the diversification of more xeric Isopods, such as those belonging to the *maculatum*-group of the *Armadillidium*. It is also, likely that insular speciation played a significant role within Greece, as a high number of its fauna are island endemics (Alexiou & Sfenthourakis, 2013). The same is likely true with Italy which was once a complex archipelago during the Pliocene (5.3-2.6 Ma), which was formed during the Zanclean refilling of the Mediterranean Sea after the MSC (Schmitt et al., 2021). The low dispersibility of terrestrial isopods and the combination of climate and insular formation within Greece and Italy could explains the high *Armadillidium* diversity within these particular regions.

The second is the significance of lithology and topographic heterogeneity developed during the formation of the Mediterranean region facilitating parapatry. The diversity of geology is known to be a factor that has

driven endemic diversity of vascular plants within the region (Molina-Venegas et al., 2013), particularly in regards to edaphic composition (Buira et al., 2020). The variability of plant diversity across different geologies has resulted in subtle variations in habitat and microhabitat structure over relatively short geographical regions. It is through these habitat composition changes that could be driving hyper-endemism in terrestrial Isopods through tenuous variation in selective pressures. For example, the habitat heterogeneity from coastal Mediterranean scrub, through wet Laurel forests to high altitude heather shrubland on Madeira, resulted in the specialisation of its endemic *Porcellio* to specific biotopes, ranging from waterfall hepatic mats, coastal scree to vertical rock faces (Vandel, 1960b). Therefore, the lack of any vicariant events show that adaptive shift of a generalised ancestral form(s) is likely the progenitor of the island's *Porcellio* diversity. Adaptive shift within the monophyletic genus *Hawaiioscia* has also been suggested as the likely cause of their diversification despite having an allopatric modern day distribution (Rivera et al., 2002). Therefore, as evidenced with *A. esterelanus* and *A. simoni*, parapatry could potentially be a common mode of speciation within the *Armadillidium*, particularly in habitats with contiguous petrologies or elevational changes.

Over 330 species of terrestrial Isopods are cave adapted (Troglobionts) (Derbák et al., 2018), and reflect the same global diversification patterns, with the highest number of species present in circum-Mediterranean countries (Manicasterri & Argano, 1989). The distribution of cave systems are strongly dependent on the lithology (eg. karst and pyroducts), suggesting that these patterns may actually be reflecting the global distribution of cave systems. The cave fauna of the Mediterranean is particularly well documented compared to other parts of the world, so this pattern may also be a product of recording bias. Despite this possibility, a large proportion of Mediterranean endemics are found in cave systems, which are almost entirely the product of karst and carbonate outcrops occurring across much of the Mediterranean region. It is believed at least 15% of the surface of the Mediterranean is occupied by this geology type (Nikolaidis et al. 2014; and Bakalowicz 2015). As such, adaptive shift to a subterranean lifestyle cannot be ignored when discussing the causes of large-scale diversification patterns. Although subterranean species are common in other families such as the Trichoniscidae (Vandel, 1960a), it is not particularly common within the *Armadillidium*, with only a handful of examples (Cruz, 1990; Gruia et al., 1994; Caruso & Bouchon, 2011, to name a few), however this lifestyle is curiously more prevalent in other genera of the Armadillidiidae, for example *Alloschizidium* (Taiti & Montesanto, 2018) *Cristarmadillidium* (Cifuentes, 2021) and *Trogletuma* (Reboleira et al., 2015).

In addition to our findings, there are other processes that could be contributing to the diversification of *Armadillidium* in Europe.

Sexual selection is a particularly important one to consider, despite being generally overlooked process within terrestrial Isopods. Lefebvre et al. (2019), revealed that body mass was a significant component of mating success in *Armadillidium vulgare*, where males were typically more successfully in mating with smaller females (20% lighter than males). Younger, smaller females (1-2 years old) were more responsive to pre-copulation stimulation from males than older and larger females (3+ years old). Although female fecundity increased with size, larger females almost never mate, suggesting spermathecae is stored from previous matings when they were smaller. These older females contribute up to half of the offspring into the next generation. The authors concluded that sexual selection by males for smaller females was significant in the success of copulation attempts, and that females were more responsive to larger males, suggesting that older males could infer a greater benefit to the offspring (longevity, better competitiveness). Despite these results, they failed to consider the much larger implication that reproductive selection and size partitioning may have on the diversification within the *Armadillidium*.

Copulation by larger/older males with smaller females ensures greater transfer of desirable male traits, and the genetic diversity of offspring produced by mating with younger females will always be greater than those produced by the small number of previously mated large females. Larger females are likely to possess traits which were adaptively superior to the current environment than smaller females that had died at a younger age. Therefore, the contribution of half the offspring by a small proportion of adaptively superior females skews the next generation to favour their genetic composition. As such, the offspring of young females will always provide the genetic diversity, whilst the larger females contribute an equal amount of presently superior genetic traits. Such a mechanism may contribute significantly to how a parapatric mode of speciation may occur within the *Armadillidium* over a comparatively short time period. Using our example of *A. esterelanus* and *A. simoni*, migration of an ancestral species across the two distinct geological substrates from acidic, igneous to alkaline, calcareous would confer different environmental pressures. *Armadillidium* arriving on these two new geologies will have to adapt to the different rock types. The high genetically diverse offspring produced by the young female will provide the genetic basis in which a greater amount of selection can occur. The females that mature beyond their third year will be the ones equipped with the most adaptively advantageous traits and will contribute a large proportion of the next generation. As the igneous selective pressures are different to those of experienced on the calcareous rocks, the more adaptively superior traits of the larger females will be slightly different dependant on the geology they are on. Therefore, over time, a population will adaptively shift to the conditions much faster due to the significant contribution of size orientated sexual selection and increasing fecundity with age. This pattern may bear true for a large proportion of *Armadillidium* speciation, both allopatrically and sympatrically, but similar reproductive cycle studies have not been made in other terrestrial isopods so whether this processes is mirrored across the entire suborder is yet to be determined.

Finally, predatory selective pressures could act as a substantial proponent in behavioural and morphological adaptive diversification of terrestrial Isopods. Isopods have developed a variety of anti-predatory mechanisms, including conglobation, running, clinging, tonic immobility, jumping, aggregation and stridulation (Tuf & Ďurajková, 2022 and references therein). In addition, some genera have developed elaborate tegumental projections such as those seen in *Calmanesia* and *Pseudolaureola*. An acidic proteinaceous secretion produced from the uropods also seems to be a widespread strategy for deterring predators such as ants, which readily get caught up in the stringy secretions (Deslippe et al., 1996). *Porcellio magnificus* has also been shown to mimic the posture of venomous *Buthus* scorpions when disturbed (Tuf & Ďurajková, 2022). Within the *Armadillidium*, however, conglobation (Tuf & Ďurajková, 2022) and uropodal secretions (Deslippe et al., 1996) appear to be the universal mechanisms for limiting predation risks.

Some *Armadillidium* species have developed a secondary anti-predatory mechanisms involving dishonest colour signalling (Herbert, 1965; Tuf & Ďurajková, 2022). Several general colour schemes in Mediterranean *Armadillidium* have been observed by the author: generalist species with cryptic uniform colour patterns (such as *A. badium*, *A. frontirostre* and *A. nasatum*), typically woodland species with cryptic but varied mottled colour patterns (such as *A. opacum*, *A. pictum* and *A. sordidum*), and aposematic scrub-dwelling species typically exhibiting brown or black ground colouration, with yellow, red or white spots and stripes (for example, *A. frontitriangulum*, *A. gestroi* and *A. wernerii*).

As we have found no reference to body colouration being linked to sexual selection in terrestrial Isopods, it is almost certain that the function of these colour patterns are tailored to predatory vision, either for camouflage, aposematism, or a combination of the two (Schmalfuss & Ferrara, 1982).

With regards to aposematism, only *A. klugii* has been identified within the literature as a putative Batesian mimic of the widespread Mediterranean black widow spider *Latrodectus tredecimguttatus* (Herbert, 1965), but nothing else has been discussed on the reason why so many others display aposematism within the

Mediterranean. The general lack of any major toxicity or distastefulness within terrestrial Isopods (Herbert, 1965), would indicate that aposematic *Armadillidium* have co-opted a Batesian mimicry strategy to reduce predation risk where they live in habitats with high numbers of predators. Hebert (1965), speculated that mimicking *Latrodectus* may be a strategy to avoid predation by Lacertid lizards, which are common predators of woodlice. This assumption seems feasible when considering the large distribution of both Lacertids and *Latrodectus* throughout the Mediterranean Basin, in addition to favouring habitat types often seen with the aposematic *Armadillidium*. This is further supported by the work of Bonacci et al. (2008), who showed that Lacertids are capable of recognising the difference between aposematic and non-aposematic prey items, in this case, four species of Carabids (ground beetles).

Despite these observations, woodlice tend to be in much greater abundance in a habitat than its aposematic model which would violate some of the understood principles of Batesian mimicry. That being, the mimic must be less numerous than the model to ensure that the predator doesn't receive high levels of positive encounters with a particular colouration (Finkbeiner et al., 2018). Therefore, there is a possibility that *Latrodectus* is not the sole model for this system and that multiple species have co-opted an aposematic colouration strategy against the same frequent predators. Other known toxic/ aposematic species that are frequent and widespread in Mediterranean scrub habitats include; the firebug (*Pyrrhocoris apterus*), starweb spider (*Uroctea durandi*), members of the Coccinellidae and some Lepidopteran caterpillars.

Despite this, it is impossible to infer at this stage how much impact this has on speciation, but it is clear with the high variability seen, especially within the *Armadillidium* were different visual strategies, from camouflaged to highly conspicuous, indicates that predatory interaction likely play a large part in Isopod diversification, even resulting in convergence of colouration in homogenous habitats. Unfortunately, many of the strategies and predator-prey relationships have not been studied in great detail, and thus, conclusions and theories regarding their implications on diversification and speciation in terrestrial isopods are lacking in clarity. However, since it is possible that these factors may play some part in the diversification of Mediterranean *Armadillidium*, they are worth highlighting as points of consideration.

5 — CONCLUSION

Using information on the taxonomic structure of the Armadillidiidae, the phylogenetic results from Recuero et al. on Iberian Armadillidiidae (2022) and the relationships of the *Armadillidium* species groups in France, we were able to identify key character traits and biogeographical patterns that are apparent within the Mediterranean region. The *Eluma*-type cephalic structure appears to represent an important ancestral character state within the Armadillidiidae, with it being found in numerous genera throughout the Mediterranean. The derived duplocarinate- and *Armadillidium*-type cephalic structures show a clear geographic divide with the former likely originating in the western Mediterranean and the latter to the east. As France borders the Iberian peninsula, it is situated at a biogeographic contact point between the duplocarinate- genera and the *Armadillidium*, therefore, creating an overlap of morphologically similar but phylogenetically distinct lineages. As such, of the original ten *Armadillidium* species groups in France (as proposed by Vandel, 1962) only seven should remain. The duplocarinate *pruvoti*-group had previously gained generic status as the *Alloschizidium*, but the *duplocarinate pictum*- and *serratum*-group should be instated under new genera.

The use of morphological species groups within the *Armadillidium* likely carries little phylogeographic and cladistic value unless used in reference to a close species complex with a clearly defined small biogeographic distribution. Our phylogenetic results revealed the occidental-subgroup of the *maculatum*-

group as a monophyletic unit only if *A. quinquepustulatum*, and tentatively *A. assimile* and *A. lanzai* are excluded. The evolutionary origin of the subgroup is not associated with the oriental *maculatum*-group arising from a migration across the northern Alps, but from an ancestor of the *depressum*-group out of northwestern Italy. Therefore, the *maculatum*-group as a whole, is not a monophyletic unit, indicating that the convergence of morphological characteristics can mislead species relationships. This is also apparent with *A. quinquepustulatum* which is genetically closer to species of the *granulatum*-group than the *maculatum*-group, but shares very little morphological similarity to members of that group. As such, it would be advisable in the future, that a more detailed study be undertaken, particularly ones containing a greater number of species within each of these groups to properly validate their phylogenetic significance and the relationship of the major morphological characters.

The particular model system (the *Occidental*-subgroup) we chose revealed a close evolutionary relationship of four out of the five endemic *maculatum*-group species in Provence. Of the four remaining species we tentatively revealed the evolutionary history of the group whilst identifying the key events that may have facilitated their speciation. The particular mechanisms we identified comprised two allopatric drivers; eusatism and climate change and one parapatric driver; habitat and petrological change resulting in ecological shift. To validate our interpretation of the occidental-subgroup, it would be recommended that a time calibrated phylogenetic tree be used to help narrow down potential date ranges in which speciation had occurred and correctly correlate them with particular events. Through our results and the examination of other studies, in addition to speculating other potential modes of speciation within the Mediterranean, it is clear that multiple abiotic and biotic factors could be working synergistically to drive diversification of terrestrial Isopods. However, the physiology of *Armadillidium* in particular, with their low dispersal rate, high adaptability and age related fecundity and sexual selection may be the inadvertent tools in the speciation tool box that has allowed them to diversify under the heterogeneous climate and geological landscape of the Mediterranean. So to answer the broader question “where did all these woodlice come from?”, the answer lies somewhere within the adaptive composition of woodlice and the extreme diversity of spatio-temporal conditions experienced within this Mediterranean basin.

6 — REFERENCES

- Arntzen, J. W & Sousa, P. S. (2007). Morphological and Genetic Differentiation of Lizards (*Podarcis bocagei* and *P. hispanica*) in the Ria de Arosa Archipelago (Galicia, Spain) resulting from Vicariance and Occasional Dispersal. *Biogeography, Time, and Place: Distribution, Barriers, and islands*. Dordrecht: Springer. pp. 365-401.
- Beron, P. (1997). On the high mountain Isopoda Oniscidea in the Old World. *Historia naturalist bulgarica*. 8. pp. 85-100.
- Becking, T., Isabelle, G., Raimond, M., Moumen, B., Chandler, C. H., Cordaux et al., Diversity and evolution of sex determination systems in terrestrial isopods. *Scientific Reports*. **7** (1084). DOI:10.1038/s41598-017-01195-4.
- Bieg, U., Suss, M. P & Kuhlemann, J. (2008). Simulation of tidal flow and circulation patterns in the Early Miocene (Upper Marine Molasse) of the Alpine foreland basin. *Special Publications of the International Association of Sedimentologists*. **40** pp. 145-169.
- Blanc, P. -L. (2002). The opening of the Plio-Quaternary Gibraltar Strait: assessing the size of cataclysm. *Geodinamica Acta*. **15** (5-6). pp. 303-317.

- Bolger, A. M., Lohse, M & Usadel, B (2014). Trimmomatic: A flexible trimmer for Illumina Sequence Data. *Bioinformatics*. **30** (15). pp. 2114-2120.
- Bouchenak-Khelladi, Y., Onstein, R. E., Xing, Y., Schwery, O & Linder, H. P. (2015). On the complexity of triggering evolutionary radiations. *New Phytologist*. **207**. pp. 313–326.
- Broly, P., Deville, P & Maillet, S. (2013). The origin of terrestrial isopods (Crustacea: Isopoda: Oniscidea). *Evolutionary Ecology*. **27** (3). pp. 461-476.
- Buira A., Fernández-Mazuecos, M., Aedo C & Molina-Venegas, R. (2020). The contribution of the edaphic factor as a driver of recent plant diversification in a Mediterranean biodiversity hotspot. *Journal of Ecology*. **109** (2). pp. 987-999.
- Caruso, D & Bouchon, D. (2011). *Armadillidium virgo* n. sp. from caves in southeastern Sicily: Is it a parthenogenetic species? (Crustacea, Isopoda, Oniscidea). *Italian Journal of Zoology*. **78** (1). pp. 96-100.
- CEPF. (2010). Critical Ecosystems Partnership Fund (CEPF): Ecosystem Profile, Mediterranean Basin Biodiversity Hotspot. https://www.cepf.net/sites/default/files/mediterranean_ep_final_2010.pdf [Accessed 15th February 2022].
- Chiang, C., Layer, R. M., Faust, G. F., Lindberg, M. R., Rose, D. B., Garrison, E. P et al. (2015). SpeedSeq: ultra-fast personal genome analysis and interpretation. *Nature Methods*. **12**. pp. 966-968.
- Chiocchio, A., Arntzen J. W., Martínez-Solano, I., de Vries, W., Bisconti, R., Pezzarossa, A et al. (2019). Reconstructing hotspots of genetic diversity from glacial refugia and subsequent dispersal in Italian common toads (*Bufo bufo*). *Scientific Reports*. **11**, 260. <https://doi.org/10.1038/s41598-020-79046-y>.
- Cifuentes, J. (2021). El género *Cristarmadillidium* Arcangeli, 1935, en la península ibérica, y descripción de una nueva especie *C. alticola* n. sp. de Sierra Nevada (Oniscidea, Armadillidiidae). *Boletín - Asociación Española de Entomología*. **45** (1-2). pp. 61-72.
- Clauzon, G., Suc, J-P., dumurdzanov, N., Melinte-Dobrinescu, M. C & Zagorchev, I. (2008). The Pliocene Gilbert-type fan delta of Dracevo (Skopje area, Republic of Macedonia): paleogeographic inference. Neotectonics of Southern Bulgaria and Northern Greece. *Geologica Macedonica*. **2**. pp. 21-28.
- Clusella-Trullas, S., van Wyk, J. H & Spotila, J. R. Thermal melanism in ectotherms. *Journal of Thermal Biology*. **32**. pp. 235-245.
- Condamine, F. L., Rolland, J., Höhna, S., Sperling, F. A & Sanmartín, I. (2018). Testing the role of the red queen and court jester as drivers of the macroevolution of Apollo butterflies. *Systematic Biology*. **67**. pp. 940–964.
- Cowling R.M., Ojeda, F., Lamont, B.B., Rundel, P. W & Lechmere-Oertel, R. (2005). Rainfall reliability, a neglected factor in explaining convergence and divergence of plant traits in fire-prone Mediterranean climate ecosystems. *Global Ecology and Biogeography*. **14**. pp. 509-519.

- Cruz, A. (1990). Descripción de dos especies del género *Armadillidium* Brandt, próximas pertenecientes al grupo *serratum*: *A. espanyoli* sp. n. y *A. pretusi* sp. n. (Isopoda, Oniscidea, Armadillidiidae). *Miscellanea Zoologica*. **14**. pp. 47-52.
- Derbák, D., Dányi, L & Hornung, E. (2018). Life history characteristics of a cave isopod (*Mesoniscus graniger* Friv.). *ZooKeys*. **801**. pp. 359-370.
- Donoghue, M. J & Sanderson, M. J. (2015). Confluence, synnovation, and depauperons in plant diversification. *New Phytologist*. **207**. pp. 260–274.
- Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*. **32** (5). pp. 1792-1797.
- Rambaut, A. (2010). FigTree v1.4.1. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. <http://tree.bio.ed.ac.uk/software/figtree/>.
- Esquer-Garrigos, Y. S., Streiff, R., Party, V., Nidelet, S., Navascués, N. de & Greenfield, M. D. (2019). Pleistocene origins of chorusing diversity in Mediterranean bush-cricket populations (*Ephippiger diurnus*). *Biological Journal of the Linnean Society*. **126** (3). pp. 598-613.
- Flecker, R., Krijnsman, W., Capella, W., de Castro Martins, C., Dmitrieva, E., Mayser et al. (2015). Evolution of the late Miocene Mediterranean-Atlantic gateways and their impacts on regional and global environmental change. *Earth Science Review*. **150**. pp. 365-392.
- Fluteau, F., Ramstein, G & Besse, J. (1999). Simulating the evolution of the Asian and African monsoons during the past 30 Myr using an atmospheric general circulation model. *Journal of Geophysical Research*. **104** (D10). pp. 11,995-12,018.
- Gaba, E. (2009). France map Lambert-93 topographic with regions-blank. https://upload.wikimedia.org/wikipedia/commons/2/23/Provence_topographic_map-fr.svg [Accessed 15th February 2022].
- Galante, E. (2005). *Diversité entomologique et activité agrosylvopastorale. Conservation de la biodiversité dans les paysages ruraux européens* (ed. By J.P. Lumaret, S. Jaulin, F. Soldati, G. Pinault and P. Dupont).. pp. 31-41. UVP/CIBIO/PNR de la Narbonnaise en Méditerranéas/OPIE-LR, Montpellier.
- Garcia, L. (2020). *Armadillidium nahumi* n. sp. (Crustacea: Oniscidea: Armadillidiidae), a new terrestrial isopod from catalan Pre-Pyrenees (Iberian Peninsula). *Bolleti de la Societ  d'Historia Natural de les Balears*. **63**. pp. 89-96.
- Garcia, L & Cabanillas, D. (2021). Los is podos terrestres (Crustacea, Isopoda, Oniscidea) del entorno de las Lagunas de Ambroz: una zona urbana en proceso de renaturalizaci n en el municipio de Madrid (Espa a). *Bolet n de la Asociaci n espa ola de Entomolog a*. **45**. (3-4). pp. 161-175.
- Gavrilets, S & Losos, J. B. (2009). Adaptive radiation: contrasting theory with data. *Science*. **323**. pp. 732–737.
- Gentile, G., Campanaro, A., Carosi, M., Sbordoni, V & Argano, R. (2010). Phylogeography of *Helleria brevicornis* (Crustacea, Oniscidea): old and recent differentiation of an ancient lineage. *Molecular Phylogeography and Evolution*. **54** (2). pp. 640-646.

- Graham, M. R., Stoev, P., Akkari, N., Blagoev, G & Fet, V. (2012). *Euscorpius sicanus* (Scorpiones: Euscorpiidae) from Tunisia: DNA barcoding confirms ancient disjunctions across the Mediterranean Sea. *Serket*. **13** (1/2). pp. 16-26.
- Gregory, S. (2014). Woodlice (Isopoda: Oniscidea) from the Eden Project, Cornwall, with descriptions of new and poorly known British species. *Bulletin of the British Myriapod and Isopod Group*. **27**. pp. 3-26.
- Gruia, M., Iavorschi, V & Sarbu, S. M. (1994). *Armadillidium tabacarui* (Isopoda: Oniscidea: Armadillidiidae), A new troglodytic species from a sulfurous cave in Romania. *Proceedings of the Biological Society of Washington*. **107** (4). pp. 699-706.
- Halliday, T. J. D & Goswami, A. (2016). Eutherian morphological disparity across the end-Cretaceous mass extinction. *Biological Journal of the Linnean Society*. **118**. pp. 152-168.
- Henrot, A. -J., Utescher, T., Erdei, B., Dury, M., Hamon, N., Ramstein, G. et al. (2017). Middle Miocene climate and vegetation models and their validation with proxy data. *Palaeogeography, Palaeoclimatology, Palaeoecology*. **467**. pp. 95-119.
- Hewitt, G. M. (1999). Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*. **68**. pp. 87–112.
- Hewitt, G. M. (2000). The genetic legacy of the Quaternary ice ages. *Nature*. **405**. pp. 907-913.
- Hewitt, G. M. (2004). Genetic consequences of climatic oscillation in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*. **359**. pp. 183-195.
- Hornung, E. (2011). Evolutionary adaptations on oniscidean isopods to terrestrial life: Structure, physiology and behaviour. *Terrestrial Arthropod Review*. **4**. pp. 95-130.
- Hornung, E., Tóthmérész, B., Magura, T & Vilisics, F. (2007) Changes of isopod assemblages along an urban-suburban-rural gradient in Hungary. *European Journal of Soil Biology*. **43** (3). pp. 158–165.
- Jaunsproge, M. (2013). Masters thesis: Cross-Media 3D Cartography of 'Europe at the Last Ice Age' Based on Initial Data Compilations. https://cartographymaster.eu/wp-content/theses/2013_Jaunsproge_Thesis.pdf [Accessed 15th February 2022].
- Jiménez-Moreno, G., Fauquette, S & Sus, J-P. (2008). Vegetation, climate and palaeoaltitude reconstructions of the Eastern Alps during the Miocene based on pollen records from Austria, Central Europe. *Journal of Biogeography*. **35** (9). pp. 1638-1649.
- Jones, M. R., Winkler, D. E & Massatti, R. (2021). The demographic and ecological factors shaping diversification among rare *Astragalus* species. *Diversity and Distribution: A Journal of conversation Biogeography*. **27** (8). pp. 1407-1421.
- Kaya, S., Boztepe, Z & Çiplak, B. (2013). Phylogeography of *Troglophilus* (Ophoptera: Troglophilinae) based on Anatolian members of the genus: radiation of an old lineage following the Messian. *Biological Journal of the Linnean Society*. **108**. pp. 335-348.

- Khadiji, S. (2010). *Tectonic evolution and growth of the Zagros Mountain Belt (Fars, Iran): constraints from magnetostratigraphy, sedimentology and low- temperature thermochronometry*. Earth Sciences. Université Pierre et Marie Curie - Paris VI. English.
- Klossa-Kilia, E., Kiliadis, G., Tryfonopoulos, G., Koukou, K., Sfenthourakis, S & Parmakelis, A. (2006). Molecular phylogeny of the Greek populations of the genus *Ligidium* (Isopoda, Oniscidea) using three mtDNA gene segments. *Zoologica Scripta*. **35** (5). pp. 459-472.
- Köhler, G & Samietz, J. (2017). Morphological and colour morph cline along an altitudinal gradient in the meadow grasshopper *Pseudochorthippus parallelus*. *PLoS One*. **12** (12). e0189815.
- Kovac, M., Hudackova, N., Halasova, E., Kováčová, M., Holcová, K., Oszcypko-Clowes, M., et al. (2017). The Central paratethys palaeoceanography: A water circulation model based on microfossil proxies, climate, and changes of depositional environment. *Acta Geologica Slovaca*. **9** (2). pp. 75-114.
- Krijgsman, W. (2002). The mediterranean: Mare Nostrum of earth science. *Earth and Planetary Science Letter*. **205**. pp. 1-5.
- Krijgsman, W., Hilton, F. J., Raffi, I., Sierro, F. J & Wilson D. S. (1999). Chronology, causes and progression of the Messinian salinity crisis. *Nature*. **400**. pp. 652-655.
- Kuhlemann, J. (2000). Post-collisional sediment budget of circo-alpine basins (Central Europe). *Memorie di Scienze Geologiche Università di Padova*. **52**. pp. 1-91.
- Kuhlemann, J & Kempf, O. (2002). Post-Eocene evolution of the north Alpine Foreland Basin and its response Alpine tectonics. *Sedimentary Geology*. **152**. pp. 45-78.
- Kühne, G., Kosuch, J., Hochkirch, A & Schmitt, T. (2017). Extra-Mediterranean glacial refugia in a Mediterranean faunal elements: the phylogeography of the chalk-hill blue *Polyommatus coridon* (Lepidoptera, Lycaenidae). *Scientific Reports*. **7**. 43533.
- Kyrkjeeide, M. O., Stenøien, H. K., Flatberg, K. I & Hassel, K. (2014). Glacial refugia and post-glacial colonization patterns in European bryophytes. *Lindbergia*. **37** (2). pp. 47-59.
- Lee, T-H., Guo, H., wang, x., Kim, C & Paterson, A. H. (2014). SNPhylo: a pipeline to construct a phylogenetic tree from huge SNP data. *BMC Genomics*. **15** (1). pp. 162.
- Li, H & Durbin, R (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*. **26** (5). pp. 589-595.
- Manicasteri, C & Argano, R. (1989). An analytical synopsis of the troglitic terrestrial isopods. *Monitore zoologico italiano, Nuova Serie, Monografia*. **4**. pp. 63-73.
- Magoč, T & Salzberg, S. L. (2011). FLASH: fast length adjustment of Short reads to improve genome assemblies. *Bioinformatics*. **27** (21). pp. 2957-2963.
- Martin, M. (2011). Cutadapt Removes Adapter Sequences From High-Throughput sequencing Reads. *EMBnet.journal: Technical Notes*. **17** (1). pp. 10-12.

- Martínez-Freiría, F., Toyama, K. S., Freitas, I & Kaliontzopoulou. (2020). Thermal melanism explains macroevolutionary variation of dorsal pigmentation in European vipers. *Scientific Reports*. 10. (16122). <https://doi.org/10.1038/s41598-020-72871-1>.
- Médail, F & Myers, N. (2004). Mediterranean Basin. In: Mittermeier RA, Robles Gil P (eds) Hotspots revisited: Earth's biologically richest and most endangered terrestrial ecoregions. CEMEX (Monterrey), Conservation International, Washington and Agrupación Sierra Madre, pp 144–147.
- Meulenkamp, J. E & Sissingh, W. (2003). Tertiary palaeogeography and tectonostratigraphic evolution of the Northern and Southern Peri-Tethys platform and the intermediate domains of the African-Eurasian convergent plate boundary zone. *Palaeogeography, Palaeoclimatology, Palaeoecology*. **196** (1-2) pp. 209-228.
- Micó, E., Sanmartín, I., Galante, E & Hawkins, B. (2009). Mediterranean Diversification of the Grass-Feeding Anisopliina Beetles (Scarabaeidae, Rutelinae, Anomalini) as Inferred by Bootstrap-Averaged Dispersal-Vicariance Analysis. *Journal of Biogeography*. **36** (3). pp. 546-560.
- Mocochain, L., Audra, P & Bigot, J-Y. (2011). Base level rise and per ascensum model of speleogenesis (PAMS). Interpretation of deep phreatic karsts, vauclusian springs and chimney-shafts. *Bulletin de la Société Géologique de France*. **182** (2). pp. 87-93.
- Molina-Venegas, R., Aparicio, A., Pina F. J., Valdéa, B & Arroyo, J. (2013). Disentangling environmental correlates of vascular plant biodiversity in a Mediterranean hotspot. *Ecology and Evolution*. **3** (11). pp. 3879-3894.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A and Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*. **403**. pp. 853-858.
- Noël, F., Gregory, S. J & Agapakis, G. (2022). *Armadillidium arcangelii* Strouhal, 1929 (Isopoda: Oniscidea): a step towards the conquest of Europe? *Bulletin of the British Myriapod Isopod Group*. **34**. pp. 72-82.
- Pavon, D, Séchet, E & Noël, F. (2021). *Armadillidium esterelanus* Dollfus, 1887 et *Stenophiloscia glarearum* Verhoeff, 1908 dans le département des Bouches-du-Rhône (Crustacea, Isopoda, Armadillidiidae et Halophilosciidae). *Le Bulletin de la Société linnéenne de Provence*. **72**. pp. 117-122.
- Ramstein, G., Fluteau, F, Besse, J & Joussaume, S. (1997). Effect of orogeny, plate motion and land sea distribution on Eurasian climate change over the last 30 million years. *Nature*. **386**. pp. 788-795.
- Reich, I., Gormally, M., Allcock, A. L., McDonnell, R., Castillejo, J., Iglesias et al. (2015). Genetic study reveals close link between Irish and Northern Spanish specimens of the protected Lusitanian slug *Geomalacus maculosus*. *Biological Journal of the Linnean Society*. **116** (1). pp. 156-168.
- Rivera, M A. J., Howarth, F. G., Taiti, S & Roderick, G. K. (2002). Evolution in Hawaiian cave-adapted isopods (Oniscidea: Philosciidae): vicariant speciation or adaptive shifts? *Molecular Phylogenetics and Evolution*. **25** (1). pp. 1-9.
- Reboleira, A. S. P. S., Gonçalves, F., Oromí, P & Taiti, S. (2015). The cavernicolous Oniscidea (Crustacea: Isopoda) of Portugal. *European Journal of Taxonomy*. **161**. pp. 1-61.

- Rögl, F. (1996). Stratigraphic correlation of the Paratethys Oligocene and Miocene. *Mitteilungen der Gesellschaft der Geologie-und Bergbaustudenten in Österreich*. **41**. pp. 65-73.
- Rögl, F. (1999). Mediterranean and Paratethys. Facts and hypothesis of an Oligocene to Miocene palaeogeography (Short overview). *Geologica Carpathica*, **50** (4). pp. 339-349.
- Salzmann, U., Williams, M., Haywood, A. M., Johnson, A. L. A., Kender, S & Zalasiewicz, J. (2011). Climate and environment of a Pliocene warm world. *Palaeoecology*. **309** (1-2). pp. 1-8.
- Sanmartín, I. (2003). Dispersal vs. vicariance in the Mediterranean: historical biogeography of the Palearctic Pachydeminae (Coleoptera, Scarabaeoidea). *Journal of Biogeography*. **30**. pp. 1883-1897.
- Schlunegger, F., Melzer, J & Tucker G. (2001). Climate, exosource rock lithologies, crustal uplift and surface erosion: A theoretical analysis calibrated with data from the Alps/North Alpine Foreland Basin system. *International Journal of Earth Sciences*. **90**. pp. 484-499.
- Séchet, E & Noël, F. (2015). Catalogue commenté des Crustacés Isopodes terrestres de France métropolitaine (Crustacea, Isopoda, Oniscidea). *Mémoires de la Société Linnéenne de Bordeaux*. **16**. pp. 1-156.
- Schmalfuss H (2004) World catalog of terrestrial isopods (Isopoda: Oniscidea). https://www.zobodat.at/pdf/Stuttgarter-Beitraege-Naturkunde_654_A_0001-0341.pdf. [Accessed: 15th February 2022].
- Schmalfuss, H. (2008a). The terrestrial isopods (Isopoda: Oniscidea) of Greece. 25th contribution: The genus *Armadillidium* (Armadillidiidae) in the provinces Macedonia and Thrace. *Stuttgarter Beiträge zur Naturkunde A, Neue Serie*. **1**. pp. 153-201.
- Schmalfuss, H. (2008b). The terrestrial isopod genus *Schizidium* (Isopoda: Oniscidea): systematics, distribution, morphology. *Stuttgarter Beiträge zur Naturkunde A, Neue Serie*. **1**. pp. 143–151.
- Schmalfuss, H. (2013a). Revision of the *Armadillidium klugii*-group (Isopoda: Oniscidea). *Stuttgarter Beiträge zur Naturkunde A, Neue Serie*. **6**. pp. 1-12.
- Schmalfuss, H. (2013b). The species of the genus *Armadillidium* (Isopoda: Oniscidea) known from Greece. *Stuttgarter Beiträge zur Naturkunde A, Neue Serie*. **6**. pp. 13–20.
- Schmalfuss, H & Sfenthourakis S. (1995). The terrestrial isopods (Oniscidea) of Greece. 15th contribution: genera *Echinarmadillidium* and *Paxodillidium* (Armadillidiidae). *Stuttgarter Beiträge zur Naturkunde, Serie A*. **518**. pp. 1-521.
- Schmidt, C. (2003). Contribution to the phylogenetic system of the Crinocheta (Crustacea, Isopoda). Part 2. (Oniscoidea to Armadillidiidae). *Zoosystematics and Evolution*. **79**. pp. 3–179.
- Schmidt, C. (2008). Phylogeny of the terrestrial Isopoda (Oniscidea): a review. *Arthropod Systematics & Phylogeny*. **66**. pp. 191–226.
- Schmitt, T & Varga, Z. (2012). Extra-Mediterranean refugia: The rule and not the exception? *Frontier in Zoology*. **9**, 22. <https://doi.org/10.1186/1742-9994-9-22>.

- Schmitt, T., Fritz, U., Delfino, M., Ulrich, W & Habel, J. C. (2021). Biogeography of Italy revisited: genetic lineages confirm major phylogeographic patterns and a pre-Pleistocene origin of its biota. *Frontiers in Zoology*. **18** (34). pp. 1-13.
- Schmölzer, K. (1954). Beitrag Zur Kenntnis Der Gattung *Armadillidium* Latr. 1804 (Isopoda Terrestria). *Acta Zoologica Fennica*. **80**. pp. 1-63.
- Schmölzer K. 1965. Bestimmungsbücher zur Bodenfauna Europas. Lieferung 5. Ordnung Isopoda (Landasseln). Berlin: Akademie.
- Séchet, E & Noël, F. (2015). Catalogue commenté des Crustacés Isopodes terrestres de France métropolitaine (Crustacea, Isopoda, Oniscidea). *Mémoires de la Société Linnéenne de Bordeaux*. **16**. pp. 1-156.
- Sfenthourakis, S & Hornung, E. (2018). Isopod distribution and climate change. *ZooKeys*. **801**. pp. 25-61.
- Sfenthourakis, S., Kassara, C., Mylonas, M & Schmalfuss, H. (2007). *An analysis of the global distribution of known Oniscidea. Abstracts Volume of the 7th International Symposium on the Biology of Terrestrial Isopods*. Tunis, Tunisia.
- Sissingh, W. (2006). Syn-kinematic palaeogeographic evolution of the West European Platform: correlation with Alpine plate collision and foreland deformation. *Netherlands Journal of Geoscience*. **85** (2). pp. 131-180.
- Stewart, J. R., Lister, A. M., Barnes, I & Dalén, L. (2010). Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B*. **277**. pp. 661-671.
- Strouhal, H. (1927). Zur Kenntnis der Untergattung *Armadillidium* verh. (Isop. terr.). *Zoologischer Anzeiger*. **74**. pp. 5-34.
- Taberlet, P., Fumagalli, L., Wust-Sucy, A. G & Cosson, J. -F. (1998). Comparative phylogeography and postglacial colonisation routes in Europe. *Molecular Ecology*. **7**. pp. 453-464.
- Taiti, S & Ferrara, F. (1996). The terrestrial isopoda of Corsica (Crustacea, Oniscidea). *Bulletin du Muséum national d'Histoire naturelle*. 4th series. **18**, Section A (3-4). pp. 459-545.
- Taiti, S & Montesanto, G. (2018). New species of subterranean and endogeny terrestrial isopods (Crustacea, Oniscidea) from Tuscany (central Italy). *Zoosystema*. **40** (11). pp. 197-226.
- Tamura, K., Stecher, G & Kumar, S. (2021). Mega 11: Molecular Evolutionary Genetic Analysis. *Molecular Biology and Evolution*. **38** (7). pp. 3022-3027.
- Tassy, A., Fournier, F., Munch, P., Borgomano, J., et al. (2014). Discovery of Messinian canyons and new seismic stratigraphic model, offshore Provence (SE France): Implications for the hydrographic network reconstruction. *Marine and Petroleum Geology*. **57**. pp. 25-50.
- Trájer, A. J., Sebestyén, V & Padisák, J. (2021). The impacts of the Messinian Salinity Crisis on the biogeography of three Mediterranean sandfly (Diptera: Psychodidae) species. *Geobios*. **65**. pp. 51-66.

- Valente, L. M., Savolainen, V & Vargas, P. (2010). Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B*. **277** (1687). Pp. 1486-1496.
- Vandel, A. (1944). Isopodes terrestres récoltés par M. Remy au cours de son voyage en Corse. I. Description de *Paraschizidium remyi* n. sp., suivie de considérations sur l'histoire et la répartition de la famille des Armadillidiidae. *Archives de Zoologie Expérimentale et Générale*. **83**. pp. 83–98.
- Vandel, A. (1954). *Isopodes terrestres. Résultats biogéographiques*. In: Coiffait, H., Cassa-Gnau, P & Vandel, A., Contribution a la connaissance de la faune cavernicole et endogée du Liban (Mission H. Coiffait au Liban, 1951). Notes et mémoires sur le Moyen-Orient. **5**. pp. 166-169. Paris.
- Vandel, A (1954). Isopodes terrestres. Résultats biogéographiques. – In: COIFFAIT, H., CASSA- GNAU, P & VANDEL, A., Contribution a la connaissance de la faune cavernicole et endogée du Liban (Mission H. COIFFAIT au Liban, 1951). – Notes Mém. Moyen-Orient **5**: 166–169; Paris.
- Vandel, A. (1960a). Faune de France. Vol. 64: *Isopodes terrestres (Première partie)*. Paris. Ed. P. Lechevalier.
- Vandel, A. (1960b). Les isopodes terrestres de l'archipel Madérien. *Mémoires du Muséum national d'Histoire naturelle. Nouvelle Série. Série A. Zoologie*. **22**. pp. 1-155.
- Vandel, A. (1962). Faune de France. vol. 66: *Isopodes terrestres (Deuxième partie)*. Paris. Ed. P. Lechevalier.
- Verhoeff, K. (1910). Über Isopoden, 16. Aufsatz, *Armadillidium* und *Porcellio* an der Riviera. *Jahreshefte des Vereins für waterl. Naturkunde in Wuerttemberg*. **66**. pp. 115-143.
- Verhoeff, K. (1918). Ueber augenlose Armadillidien und kritische Prüfung der Familie Armadillidiidae. *Archiv für Naturgeschichte*. **83** (A). pp. 160-170.
- Wielstra, B., Crnobrnja-Isailović, J., Litvinchuk, S. N., Reijnen, B. T., Skidmore, A. K., Sotiropoulos, K., et al. (2013). Tracing glacial refugia of *Triturus* newts based on mitochondrial DNA phylogeography and species distribution modeling. *Frontiers in Zoology*. **10** (13). <https://doi.org/10.1186/1742-9994-10-13>.
- Wilkins, M. R., Karaardıç, H., Vortman, Y., Parchman, T. L., Albrecht, T., Pertrželková, L et al. (2016). Phenotypic differentiation is associated with divergent sexual selection among closely related barn swallow populations. *Journal of Evolutionary Biology*. **29** (12). pp. 2410-2421.
- Willi, Y., Fracassetti, M., Bachmann, O & Buskirk, J. V (2020). Demographic Processes Linked to Genetic Diversity and positive Selection across a Species' Range. *Plant Communications*. **1** (6). 100111.
- Zweigel, J., Aigner, T & Luterbacher, H. (1998). Eustatic versus tectonic controls on Alpine foreland basin fill: Sequence stratigraphy and subsidence analysis in the SE German Molasse. In: *Cenozoic Foreland Basins of Western Europe* (Eds. A. Mascle, C. Puigdefàbregas, H.P. Luterbacher and M. Fernàndez), *Geological Society London, Special Publications*. **134**. pp. 299-323.