WYDZIAŁ BIOLOGII
i OCHRONY ŚRODOWISKA
Uniwersytet Łódzki

Full-time PhD studies of
Ecology and Environmental Protection

Kamil Hupało

Diversity and origin of freshwater amphipods of Mediterranean islands

Różnorodność i pochodzenie słodkowodnych obunogów wysp Morza Śródziemnego

PhD Thesis

performed in Department of Invertebrate Zoology and Hydrobiology in Institute of Ecology and Environmental Protection

Supervisor:

- Prof. dr hab. Michał Grabowski

Co-supervisor:

- Dr Tomasz Mamos

Łódź, 2019

For my Mom and my Wife – for your everlasting support on this great journey

Table of contents

Acknowledgements

Firstly, I would like to express my greatest gratitude to my Supervisor and my Mentor, Prof. Michał Grabowski for his continuous support throughout my Ph.D studies, for his great advices, time, patience, dedication, motivation and immense knowledge. His constant guidance helped me in so many ways during this great journey, at the very beginning of my scientific career. I also want to thank my Co-supervisor, dr Tomasz Mamos for his support and share of knowledge that made my work constantly better. I could not have imagined having a better advisors for my Ph.D studies and I am utterly grateful for their support.

I would like to thank all the great scientists I was priviliged to meet on the way, which inspired me to make my work better. Especially I would like to thank Fabio Stoch and Ioannis Karaouzas for providing the study material and for many fruitful discussions that enhanced my thesis even further. I would also like to thank Tomasz Rewicz, Jean-François Flot and Anna Wysocka for their insightful comments, which helped me to widen my research from various perspectives. My sincere thanks also goes to fellow amphipodologists and carcinologists that I was priviliged to meet and that have inspired me with their comments and discussions that have broaden my scientific perspectives in numerous ways.

I also thank the members of Department of Invertebrate Zoology and Hydrobiology of University of Lodz, who have provided me with support, guidance and immense hospitality, that made me feel welcomed and at home in the University.

I would like to thank my Friends for their support and for always being there for me.

Last but not the least, I would like to thank my Mum and my Wife – the two most important persons in my life for their invaluable support through my ups and downs and their unconditional love that kept me going and motivated me to bring the best out of me. Words cannot really express how grateful and lucky I am to have you in my life.

Summary

The Mediterranean islands are considered to be natural laboratories of evolution and places with extremely high level of endemism. Even though the fresh waters are among most diverse and also the most endangered ecosystems, still little is known about its biodiversity in the Mediterranean islands, as most of the studies on insular fauna focus mainly on terrestrial and marine biota. One of the most abundant organisms, being often keystone species in the freshwater macroinvertebrate communities are gammarid amphipods. In my PhD thesis, I investigated the diversity and origin of the freshwater gammarids of the Mediterranean islands. In the first part of my PhD, the available resources on the diversity of the freshwater malacostracan crustaceans from the Mediterranean islands were investigated and gathered together. Chapter I, is arguably the first such an extensive study on the freshwater fauna of the Mediterranean Islands, with valuable insight on its biogeographical affiliations. The findings indicate that amphipods are the most speciose group, being the most species-rich order on each of studied islands and archipelagos, with also one of the highest rate of endemism. In the main core of the PhD thesis, the diversity and origin of the freshwater gammarids from Aegean islands including Crete as well as Sicily were studied by gathering both morphological and molecular characteristics and by reconstructing the time-calibrated phylogenies using multimarket dataset. Chapter II provides the first evidence of the presence of freshwater populations of *Gammarus* on six Aegean islands with at least three endemic species, most probably new to science. The molecular methods were implemented to analyse their evolutionary history combining the information from the adjacent regions, including populations of recently described *Gammarus plaitisi* from Crete. Moreover, the divergence of these new species is strongly connected with the geological history of the Aegean region and its islands. In Chapter III, the substantial level of intraspecific diversity was detected within each of the Sicilian gammarid morphospecies. Moreover, these results support the different timescales and separate colonisation events of the gammarid fauna of Sicily.

Altogether, these results indicate connectivity of the evolutionary history of the insular freshwater gammarids with the geological history of the islands as well as the entire Mediterranean region. Moreover, the level of overlooked diversity detected supports the importance of using molecular tools in the biodiversity assessments. The results of this thesis also highlight the need for future studies on the insular freshwater Mediterranean biota and

deliver a valuable insight for better understanding of the mechanisms of the diversification of the freshwater organisms.

Introduction

Due to its complex geological history and unique combination of geological and climatic factors, the Mediterranean region is recognized as one of the one of the 25 most important biodiversity and endemism hotspots worldwide (Myers et al. 2000, Woodward 2009, Blondel et al. 2010). Although the Mediterranean represents only 1.6% of the Earth's surface, it hosts nearly 10% of the global flora, 3% of all the discovered vertebrates and over 7% of world marine biodiversity, with a high level of endemism (Medail & Quezel 1997, Myers et al. 2000, Coll et al. 2010). Even if the freshwater fauna of the Mediterranean Basin is still largely understudied, it is estimated that the region is housing around 35% of all the Palearctic species, which corresponds to more than 6% of the world freshwater species. At least 43% of the freshwater Mediterranean fauna is considered to be endemic to the area with the majority inhabiting the Mediterranean islands (Figueroa et al. 2013). Even though, they cover about 100.000 km^2 corresponding to 5% of the entire area of the Region, they are considered to be the natural laboratories of evolution with high level of endemism, mainly due to their isolation as well as climatic, topographic, and geological heterogeneity (Schüle 1993, Hopkins 2002, Vogiatzakis et al. 2008). There are more than 5,000 islands in the Mediterranean Basin and they are either of oceanic or continental origin with some of them being the wedges of the oceanic plates, formed by volcanic activity, created by fragmentation of tectonic plates or by the sea level changes (Whittaker & Fernández-Palacios, 2007). Despite now they are situated in the same basin, they emerged at different time and during separate geological events which in some cases combined with its temporal connectivity the mainland, largely affect the composition of insular floral and faunal communities.

Amphipods are one of the most diverse crustacean orders with more than 10.000 species described (Horton et al. 2017). They are mostly marine organisms, which were able to colonize terrestrial as well as freshwater habitats, with nearly 2000 species currently known from continental waters, with Gammaridae (= gammarids) family being the most diverse epigean family among the freshwater amphipods (Vader 2005, Väinölä et al. 2007). Due to the emergence of DNA-based methods of biodiversity assessment, a plethora of cryptic species has been identified in the recent years (for summary see Fišer et al. 2018). A similar trend has also been observed in the gammarids, where extraordinarily high rate of cryptic diversity was discovered within several morphospecies (e.g. Weiss et al. 2014, Wysocka et al.

2014, Mamos et al. 2014, 2016; Copilaş-Ciocianu and Petrusek 2015; Katouzian et al. 2016, Grabowski et al. 2017a,b). Gammarids are also known to be the keystone species in macroinvertebrate communities, playing a crucial role in the trophic chains in the aquatic environment (MacNeil et al., 1999, Casagranda et al. 2006). Due to their widespread distribution, high abundance, significance in the food web and sensitivity to a wide range of pollutants, members of this group are commonly used as bioindicators and assay organisms in ecotoxicological studies (e.g. Costa et al. 2005, Neuparth et al. 2005, Gerhardt et al. 2011).

So far, there has been around 120 freshwater gammarid species described from the Mediterranean region with only twenty, belonging to two genera: *Gammarus* Fabricius, 1775 and *Echinogammarus* Stebbing, 1899, being reported from the Mediterranean islands (Karaman & Pinkster 1977a,b, 1987, Pinkster 1993). However, given that no molecular studies prior to my PhD thesis has been conducted on the insular freshwater gammarids and taking into account the high level of cryptic diversity revealed recently from the mainland, one may conclude that this number is underestimated. Therefore, **the main aim of my PhD thesis** was to estimate the actual insular freshwater gammarid biodiversity of large Mediterranean islands, the Aegean islands, including Crete and Sicily, using molecular methods. Moreover, **as a secondary goal**, I investigated the biogeographical affiliations of the insular freshwater biota by providing first insights into the origin of the studied taxa, by reconstructing its multimarker time-calibrated phylogeny and interpret in the context of the geological history of the islands.

Freshwater gammarid fauna of the Mediterranean islands may be of continental origin, as a result of colonization through hydrological network of historical land connections, or it may be a result of the direct colonization from the sea. Considering the current knowledge upon Mediterranean Basin paleogeography as well as evolutionary history and ecology of gammarids, **it may be hypothesized** that:

- the insular species of the genus ***Gammarus* are probably the continental relicts** and/or they have colonized the islands through land connections in Miocene and/or in Pleistocene

- the insular species of ***Echinogammarus* have colonized the islands directly from marine waters** within a different timescale.

The results of my PhD thesis provide arguably the first insight into the actual insular freshwater biodiversity of gammarids and reveal their complex evolutionary history. These results contribute to general understanding of the history and evolution of insular freshwater fauna. They also stress the importance of geological events that played a critical role in shaping the freshwater biodiversity in the Mediterranean islands. These results provide the useful framework for future studies on the biodiversity and origin of the fauna of the Mediterranean insular fresh waters with the ultimate goal being understanding the mechanisms underlying the evolution of the insular freshwater biota.

Freshwater biodiversity of the Mediterranean islands

So far, the studies upon the biodiversity and origin of the fauna of the Mediterranean islands focused mainly on terrestrial or marine biota. There are several complex studies providing a thorough analysis of the fauna from selected islands or archipelagos, especially for herpetofauna (e.g. Lymberakis & Poulakakis 2010, Pinya & Carretero 2011, Stöck et al. 2016) and marine biota (e.g. Bianchi 2007, Mejri et al. 2011, Coll et al. 2012). The same holds true for crustaceans with majority of the studies from Mediterranean islands focusing on terrestrial or marine taxa (e.g. Pipitone & Arculeo 2003, Gentile & Argano 2005, Pavesi et al. 2013). Among very few studies targeting the freshwater crustacean diversity on Mediterranean islands, notable are studies on the inland assemblages of non-malacostracan crustaceans from Crete (Marrone et al. 2019) as well as on the evolutionary history of freshwater crabs on the Aegean islands (Jesse et al. 2011). Except for that, the knowledge about the diversity as well as the biogeographical affiliations and therefore, the origin of the insular freshwater crustaceans is scarce, with no molecular evidence confirming the hypotheses formed before the availability of molecular tools.

The freshwater ecosystems, being among the most species-rich ecosystems in the world, are also the most endangered ones (Darwall et al. 2009). Moreover, the extinction rate for freshwater biota is estimated to be even five times higher than for the terrestrial ones (Dudgeon et al. 2006), mainly due to the current rapid degradation caused by human activity (Darwall et al. 2009). Human pressure on the freshwater resources is especially high in the Mediterranean Basin, which combined with the consequences of the increasing tourist pressure, it is one of the most anthropogenically altered regions in the world with its islands being among the most severely threatened (Myers et al. 2000, Hopkins 2002, Cuttelod et al. 2008). Thus, gathering data on the diversity and distribution of the freshwater species in the area is of paramount importance to identify and prioritise the local biodiversity hotspots for planning conservation strategies, which could at least slow down the extinction process.

In the first part of my PhD, the available resources on the diversity of the freshwater malacostracan crustaceans from the Mediterranean islands were gathered and analysed together. **Chapter I** is arguably a first such an extensive study on the freshwater fauna of the Mediterranean Islands, providing a valuable and much-needed insight into the crustacean diversity in the insular fresh waters as well as the knowledge on its biogeographical affiliations. Noteworthy, amphipods are the most speciose group, being the most species-rich order on each of studied islands and archipelagos, with also one of the highest rate of endemism.

Tale of two islands – Crete and Sicily

Even though the Mediterranean islands share the same sea basin, their geological history is far from uniform, however in most cases it is strongly connected with the history of the basin itself. With the exception of Cyprus or the Adriatic Islands, which emerged well before the formation of the Mediterranean basin (Harland et al. 1982), most of the Mediterranean islands either emerged or were isolated from the continental plates in Oligocene and Miocene with Pelagie Islands and the Ionian Islands being the youngest archipelagos, developing as late as Pliocene. In my PhD thesis I investigated the freshwater amphipod communities from two large continental islands, Crete, along with other Aegean islands, and Sicily. Given that these two islands have different origin, date of isolation and history of connections with the mainland and they belong to different sea baśni within the Mediterranean, they provide a good framework for comparative analysis of its freshwater gammarid fauna.

Crete is the largest of the Aegean Islands and fifth largest of all the Mediterranean islands, covering an area of 8.336 km^2. At the beginning of Miocene, Crete along with all Aegean islands, was a part of the Aegeis landmass composed of the Balkan Peninsula and Asia Minor (23-12 million years ago; Meulenkamp 1971). The fragmentation of the Aegeis started from the collision of the African tectonic plate with the Eurasian plate in the Middle Miocene (ca. 16 Ma; Steininger & Rögl 1984) and the formation of the Mid-Aegean Trench (12–9 Ma; Dermitzakis & Papanikolaou, 1981), which resulted in subsequent events of gradual isolation of the separate landmasses representing current islands. About 11-8 Ma, the isolation of Crete from Peloponnese started, due to the movement of continental plates and the rise of sea levels. Then, during the Messinian Salinity Crisis, the hipersaline deserts formed and thus, many formerly isolated islands including Crete and several other Aegean islands, regained the connections with the mainland and/or the other islands. For Crete, it was the last known land connection to the mainland (Poulakakis et al. 2015). During Pliocene, some of the connections between some Aegean islands and the mainland were again temporarily re-established and lasted throughout Pleistocene, due to eustatic sea-level changes, with most of the islands, including Crete, gaining its present shape and the current level of isolation at the end of Pleistocene (Sondaar & Dermitzakis 1982, Sondaar et al. 1986). Up to now, the gammarid fauna in the fresh waters of Aegean islands was composed of three species of *Echinogammarus* from Crete, *E. kretensis, E. platvoeti* and *E. foxi*, where two former ones are considered endemic as well as three species of *Gammarus: G. komareki* from Gökçeada (Özbek & Özkan 2017), *G. uludagi* from Lesbos and Evia (Karaman & Pinkster 1977, Hou et al. 2011) and *G. pulex* from Crete, which turned out to be a new species, recently described as *G. plaitisi* (Karaman 2003, Hupało et al. 2018). None of them, except for *G. plaitisi,* were considered as endemic to the island.

Sicily is the largest island of all the Mediterranean islands and the largest in the Tyrrhenian basin, covering an area 25.711 km^2 and laying on the border of the eastern and western Mediterranean basins. Geologically, the island is far from being uniform and is composed of three tectonic units of different origin namely: the Hyblean foredeep, forming the southeastern part of the island, emerged as a part of the African continental plate, the Apenninic-Maghrebian orogen, forming the central and western part of Sicily and being also the largest part of Sicily, originating from Apennine Peninsula and the Calabrian-Peloritan arc, forming the northeastern part of Sicily, which used to be part of Sardinia (Broquet 2016). The respective parts of the island emerged or detached at different times with the Apenninic-Maghrebian orogen detaching in late Oligocene, Hyblean foreland detaching from African plate in middle Miocene and Calabrian-Peloritan arc isolating from Sardinia in middle/late Miocene (Broquet 2016). Later on, from the Pliocene up to the end of Pleistocene, due to recurrent sea level changes, Sicily was divided into two islands, one comprised of the northwestern and central part of the present Sicily and the other representing its present southwestern corner (Guglielmo & Marra 2011). Although Sicily remained isolated throughout the years with only temporal land connections with the continental, the island gained its present shape around 20.000 years ago and ever since was isolated from the mainland (Shackleton et al. 1984). The freshwater gammarid of Sicily is composed solely of members of *Echinogammarus*: *E. sicilianus, E. adipatus* and *E. tibaldii*. None of them are considered to be endemic to the island, as they were reported from the neighboring islands of Sardinia and the Maltese islands as well as the Apennine Peninsula (Pinkster 1993).

In the main core of my PhD thesis, I investigated the diversity and origin of the freshwater gammarids from both Sicily and Crete, including other Aegean islands, by gathering both morphological and molecular characteristics and by reconstructing the time-calibrated phylogenies using multimarket dataset. **Chapter II** provides a first evidence of the presence of freshwater populations of *Gammarus* on six Aegean islands with at least three endemic species, most probably new to science. The molecular methods were implemented to analyse their evolutionary history combining the information from the adjacent regions, including populations of recently described *Gammarus plaitisi* from Crete. Moreover, the divergence of all Aegean species is strongly connected with the geological history of the Aegean region and its islands. With that, this is arguably one of the first as thorough studies of the insular Aegean freshwater fauna. **Chapter III** reveals a substantial level of cryptic diversity within each of the Sicilian gammarid morphospecies. Moreover, the results support the different timescales and separate colonisation events of the gammarid fauna of Sicily. The evolutionary history of Sicilian freshwater gammarid fauna reflects the turbulent history of the island and the entire region.

Chapter I. Freshwater Malacostraca of the Mediterranean Islands – diversity, origin and conservation perspectives

1.1 Introduction

Malacostraca is a highly diversified and very speciose class of crustaceans. They exhibit a huge variety of body forms and ecological adaptations, allowing them to inhabit marine, freshwater and even terrestrial habitats. Out of the estimated 26,000 malacostracan species described so far, about 6,000 inhabit freshwater environment worldwide (Balian et al. 2008). In fresh waters, malacostracans adapted to colonise the plethora of available ecosystems including springs, rivers, estuaries, lakes, caves, all kinds of groundwater habitats, and even ephemeral water bodies (Fig.1.1) (Balian et al. 2008, Figueroa et al. 2013). The highest number of freshwater malacostracan species (2,165, ca. 35%) was recorded in the Palearctic (Balian et al. 2008). The diversity of malacostracans in fresh waters of the Mediterranean Basin is still largely unknown, particularly due to the lack of the reliable published information on isopods. According to various estimates, 25% to 40% of the Palearctic fauna occurs in the area (Balian et al. 2008, Figueroa et al. 2013).

The Mediterranean Basin has been recognized as one of the 25 most important biodiversity and endemism hotspots worldwide (Myers et al. 2000, Woodward 2009, Blondel et al. 2010). Even though the basin represents only 1.6% of the Earth's surface, it hosts nearly 10% of the global flora, 3% of all the discovered vertebrates and over 7% of world marine biodiversity, with a high level of endemism (Medail & Quezel 1997, Myers et al. 2000, Coll et al. 2010). Although the freshwater fauna of the Mediterranean Basin is still largely understudied, yet it is estimated that the region is inhabited by about 35% of all the Palearctic species. This corresponds to more than 6% of the world freshwater species. At least 43% of the freshwater Mediterranean fauna is considered to be endemic to the area with the majority inhabiting the Mediterranean islands (Figueroa et al. 2013).

The Mediterranean Sea is a semi-enclosed basin, covering an area of approximately 2.5 million km^2. The sea is connecting three continents, being bordered from the north by Europe, from the south by Africa and from the east by Asia. It has two narrow connections with other waters. The Strait of Bosphorus connects it with the Black Sea and the Strait of Gibraltar opens the basin to the Atlantic. The Mediterranean Sea is divided by the Siculo-Tunisian Strait (aka Strait of Sicily), a biogeographical barrier between Cape Bon (Tunisia) and Mazara

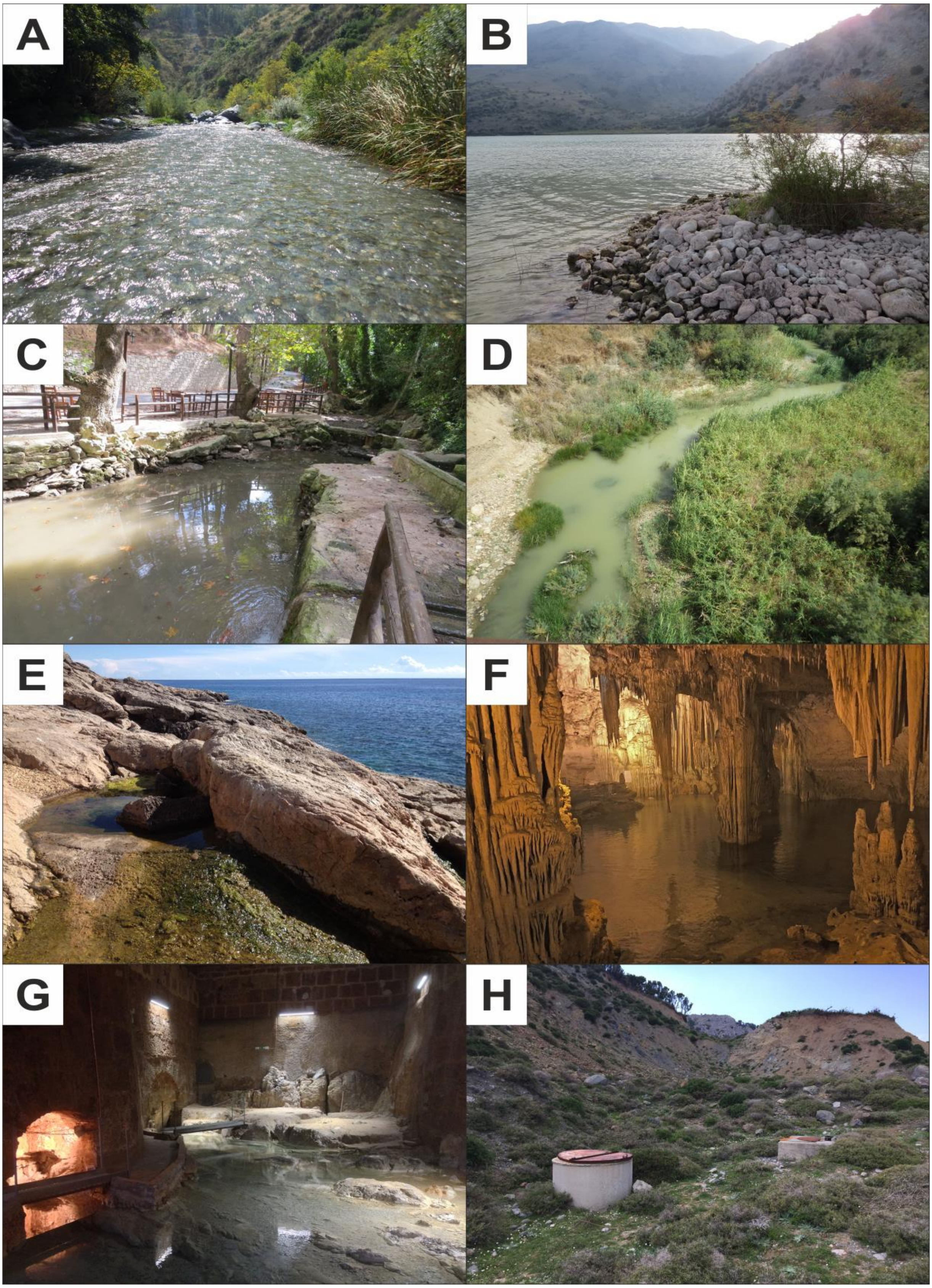
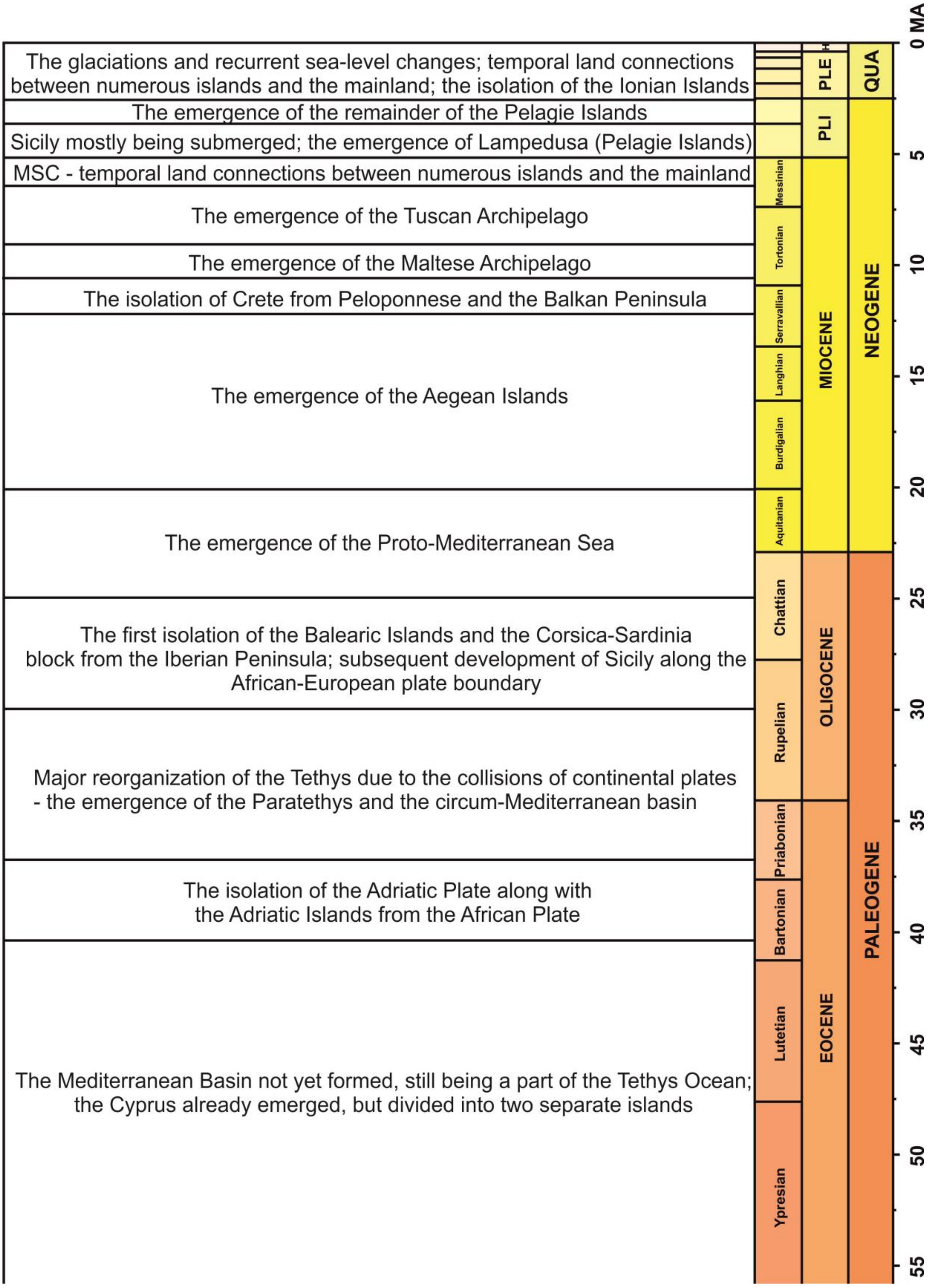


Fig.1.1 The example variety of habitats inhabited by freshwater malacostracans in the Mediterranean Islands. A) River – Rio Alcantara, Sicily B) Lake – Limni Kournas, Crete C) Artificial springs - Crete D) E) Karstic springs - Sardinia F) Phreatic cave – Grotta di Nettuno, Sardinia G) Artificial gallery – Sicily H) Wells – Evia.

del Vallo (Sicily), into two subbasins: the Western Mediterranean with Sardinia, Corsica, the Tuscan Archipelago as well as the Balearic Islands and the Eastern Mediterranean with Sicily, the Maltese Archipelago, the Pelagie Islands, the Adriatic, Ionian, Aegean Islands along with Crete, Cyprus and the islands of the Marmara Sea (Bianchi & Morri 2000). In total, there are more than 5,000 islands in the Mediterranean Basin, covering about 100.000 km^2, i.e. more than 5% of the surface of the entire region (Hopkins 2002, Vogiatzakis et al. 2008). They are either of oceanic or continental origin with some of them being the wedges of the oceanic plates, formed by volcanic activity, created by fragmentation of tectonic plates or by the sea level changes (Schüle 1993, Whittaker & Fernández-Palacios 2007). The Mediterranean Sea originated from the Tethys Ocean, which once separated supercontinents of Laurasia and Gondwana. Throughout the millions of years, due to the shifts of the continental plates, Tethys had gradually shrinked and at the end of Eocene, a major reorganisation of Tethys took place, resulting in its division into two residuals, Paratethys and the circum-Mediterranean basin.Then, at the end of Oligocene, the latter became the proto-Mediterranean Sea (Rögl 1999, Popov et al. 2004). Some of the islands like Cyprus or the Adriatic Islands emerged even before the formation of the circum-Mediterranean basin (Harland et al. 1982), however the majority of the Mediterranean Islands emerged or were isolated from the continental plates in Oligocene and Miocene (Fig.1.2). These include Corsica, Sardinia (Speranza et al. 2002; Advokaat et al. 2014), Sicily (Catalano et al. 1994), the Aegean Islands with Crete (Meulenkamp 1971, Poulakakis et al. 2015) as well as the Maltese (Savona Ventura 1975) and the Tuscan Archipelago (Barbato et al. 2018). One of the most crucial events in the history of the Mediterranean Basin was closure of the Strait of Gibraltar, which resulted in subsequent desiccation events, which eventually resulted in the drying out of the basin – the so-called Messinian Salinity Crisis (MSC, 5.96-5.33 Ma) (Boccaletti et al. 1990, Krijgsman et al. 1999). During that time, nearly all of the islands had temporal land connections with the mainland, resulting in the substantial faunal exchange at that time (eg. Ketmaier & Caccone 2013, Chueca et al. 2015, Poulakakis et al. 2015). For several islands (e.g. Sicily, many Aegean Islands), such situation repeated in Pleistocene, due to the recurrent glaciations and associated eustatic sea level oscillations; e.g. during the Last Glacial Maximum (LGM), the global sea level dropped down by ca. 120 m (eg. Arias et al. 1980, Perissoratis & Conispoliatis 2003, Marra 2009, Muscarella & Baragona 2017). The geologically youngest islands, that emerged only during Pliocene and Pleistocene, are the Pelagie Islands as well as the Ionian Islands (Sakellariou & Galanidou 2015, Muscarella & Baragona 2017) (Fig.1.2).



The glaciations and recurrent sea-level changes; temporal land connections between numerous islands and the mainland; the isolation of the Ionian Islands

The emergence of the remainder of the Pelagie Islands

Sicily mostly being submerged; the emergence of Lampedusa (Pelagie Islands)

MSC - temporal land connections between numerous islands and the mainland

The emergence of the Tuscan Archipelago

The emergence of the Maltese Archipelago

The isolation of Crete from Peloponnese and the Balkan Peninsula

The emergence of the Aegean Islands

The emergence of the Proto-Mediterranean Sea

The first isolation of the Balearic Islands and the Corsica-Sardinia block from the Iberian Peninsula; subsequent development of Sicily along the African-European plate boundary

Major reorganization of the Tethys due to the collisions of continental plates - the emergence of the Paratethys and the circum-Mediterranean basin

The isolation of the Adriatic Plate along with the Adriatic Islands from the African Plate

The Mediterranean Basin not yet formed, still being a part of the Tethys Ocean; the Cyprus already emerged, but divided into two separate islands

Fig.1.2 The stratigraphic table highlighting the major steps in the history of the Mediterranean Sea and the Mediterranean Islands.

Due to their isolation as well as climatic, topographic, and geological heterogeneity, the Mediterranean Islands are known as the natural laboratories of evolution with a high number of local endemics (Schüle 1993, Hopkins 2002, Vogiatzakis et al. 2008, Blondel et al. 2010). So far, however, the majority of studies upon biodiversity and origin of the Mediterranean insular fauna focused mostly on terrestrial, semi-terrestrial or marine coastal species, leaving the freshwater fauna relatively understudied (Figueroa et al. 2013). In addition, the extinction rate for freshwater biota is estimated to be even five times higher than for the terrestrial ones (Dudgeon et al. 2006), mainly due to the current rapid degradation caused by human activity (Cuttelod et al. 2008, Darwall et al. 2009). Freshwater ecosystems have been critical to sustaining life and establishing civilizations throughout history (Combes 2003). Given a long history of civilisation development in the Mediterranean Basin, combined with the limited local freshwater resources and consequences of the increasing tourist pressure, it is one of the most anthropogenically altered regions in the world with its islands being among the most severely threatened (Myers et al. 2000, Hopkins 2002, Mittermeier et al. 2004). Thus, gathering data on the diversity and distribution of the freshwater species in the area is of paramount importance to identify and prioritise the local biodiversity hotspots for planning conservation strategies.

The main aims of this study were (i) to assemble all the published records as well as the grey literature focusing on the freshwater Malacostraca from the Mediterranean islands; (ii) to provide information upon the species biogeographical affinities, evolutionary histories, and colonization of insular freshwater ecosystems; (iii) to summarise the data upon alien malacostracan species that colonised the area in focus; (iv) to discuss possible risks and perspectives for the conservation of the local malacostracan fauna.

1.2 Methods

This review of the Mediterranean insular malacostracan diversity focuses on the taxa that are able to complete their life cycles in fresh waters, including both epigean and hypogean biota. It also covers taxa of anchialine caves and aquifers. In this chapter however, the coastal brackish water malacostracans i.e., inhabiting marine caves, lagoons, euryhaline coastal lakes and estuaries, which cannot be considered as freshwater ecosystems, are excluded. In few cases, if the complete environmental data was not available, the selection of the excluded taxa was necessarily arbitrary. Only taxa described by the end of 2018 were counted, including the legitimate subspecies and the taxonomic nomenclature follows the World Register of Marine Species (WoRMS) database (as of November 2018).

An updated and exhaustive literature was used to include information not only on presence and distribution data, but also including the biogeographical affinities, evolutionary history and, where available, conservation remarks. The general reviews (i.e. Balian et al. 2008, Figueroa et al. 2013) and checklists (i.e. Ruffo & Stoch 2006, Stoch et al. 2006) of the freshwater malacostracans from the Mediterranean islands, web databases and repositories (e.g. WORMS, WADA) were consulted, as well as specialistic articles and books (reported in the each section of the review). Based on the gathered data, this review is divided into sections covering all major islands and archipelagoes in the Mediterranean, i.e. the Balearic Islands, Corsica and Sardinia, Sicily, the Maltese and Pelagie Islands, the Adriatic Islands, Crete, the Aegean and Ionian Islands, the islands of the Marmara Sea and Cyprus.

1.3 Results

1.3.1 The Balearic Islands

Geographical setting

The Balearic Islands are the most isolated archipelago of the Mediterranean Sea, located in its western part, off the Iberian Peninsula coast. It covers an area of approximately 4992 km^2. The archipelago is formed by four large islands: Mallorca, Menorca, Ibiza (in Catalan: Eivissa) and Formentera as well as by many minor islands and islets including Cabrera and Dragonera. Among the islands of the Mediterranean Sea, Mallorca, Menorca and Ibiza occupy the ranks of sixth (with 3640 km^2), eleventh (702 km^2), and fourteenth (572 km^2) largest, respectively (Cherry & Leppard, 2018). Based on the paleogeographical history, the Baleares are divided into two island units or “sub-archipelagos”: the western set, Pityusic, with Ibiza, Formentera and nearly 60 islets, and the eastern one, Gymnesic, with Mallorca, Menorca, Cabrera, Dragonera and nearly 30 islets. While the two Pityusic islands are separated only by a narrow, shallow channel, the two main islands of Gymnesic sub-archipelago, Mallorca and Menorca are more isolated and have different paleogeographical history (Bover et al., 2008, also see below). Physiographically, the Balearic Islands are low-lying, highly karstic and characterized by lack of surface waters, except for the Serra de Tramuntana mountain range on Mallorca, which reaches more than 1000 m (Palmer et al. 1999).

Malacostracan fauna

The Balearic malacostracan fauna seems to be quite rich with an outstanding number of endemics given the small area of the islands. So far, thirty two species are known from the archipelago: 29 from the Gymnesic islands (72% from Mallorca, 31% - Menorca, 25% -

Cabrera, 10% - Dragonera) and only 6 species from the Pityusic Islands. Most of the Balearic taxa are endemic with the majority being stygobionts (63%).

The order Amphipoda is represented by five families, associated with interstitial and subterranean karstic waters. Metacrangonyctidae Boutin & Messouli, 1988, the family of marine origin, is represented by *Metacrangonyx longipes* Chevreux, 1909, an endemic stygobiont known from both Mallorca and Menorca (Pons & Jaume, 1995). On Mallorca, it occurs in various types of groundwater habitats, from coastal anchialine caves with raised salinity to freshwater wells, caves and springs located inland. On Menorca, this species is restricted to the coastal anchialine caves and wells and is absent from fresh inland groundwaters. On both islands, the species is limited to lowlands and is absent from potentially suitable habitats located at the elevations higher than 125 m above sea level. The stygobiont family Bogidiellidae Hertzog, 1936 is represented on Mallorca by two species from genus *Bogidiella* Hertzog, 1933: *B. torrenticola* Pretus & Stock, 1990, an interstitial species known only from the type locality, the hyporheic zone of a stream on the west coast of Mallorca and *B. balearica* Dancau, 1973, a cave-dwelling anchialine species, present also on the neighbouring Cabrera (Pretus & Stock, 1990; Pretus 1991, Pons & Jaume, 1995, Jaume et al. 2007). Recently, a new genus and species of Bogidiellidae were described with a single representative, *Racovella birramea* Jaume, Gràcia & Boxshall, 2007, an endemic stygobiont discovered in a Mallorcan anchialine cave (Jaume et al. 2007; remark: in the abstract of the paper the invalid species name, *Racovella uniramea*, appears). According to Pretus (1991), the Balearic groundwaters are inhabited by at least three other, still undescribed, bogidiellid taxa. *Psammogammarus burri* Jaume & Garcia, 1992 is the sole representative of Eriopisidae Lowry & Myers, 2013. It is a Balearic endemic, so far known from a single location, Cova des Burrí, an anchialine cave on Cabrera (Jaume & Garcia, 1992). The family Gammaridae Leach, 1814 seems to be quite species-rich, especially on Mallorca and Menorca. Among five species of the genus *Echinogammarus* Stebbing, 1899, two taxa were described as endemics: *E. pungens minoricensis* (Margalef, 1952), inhabiting the springs on Menorca and *E. ebusitanus* Margalef, 1951 occurring on Mallorca and Ibiza (Pretus 1991, Pons & Jaume, 1995). Subsequently, Pinkster (1993) synonymised *E. pungens minoricensis* with *E. pungens* (H. Milne Edwards, 1840), however given the appearing signals from molecular studies on *E. pungens* morphospecies (Hou et al., 2013; A. Wysocka pers. comm), it may not be excluded that *E. pungens minoricensis* is a separate valid species. Similar situation concerns *E. ebusitanus*. Margalef (1951) described *E. klaptoczi ebusitanus* from Ibiza, to differentiate them from *Echinogammarus klaptoczi* Schäferna, 1908 present in North Africa. Karaman

(1977) upgraded it to a species rank, followed by Pinkster (1993), who synonymised *E. ebusitanus* with *E. klaptoczi*. The remaining non-endemic gammarid taxa are *Echinogammarus eisentrauti* (Schellenberg, 1937) and *Echinogammarus monomerus* Stock, 1978 found on Mallorca (Pretus 1991) as well as *Echinogammarus pungens* sensu lato, a species-complex common in the continental fresh waters of southern Europe and recorded in the permanent coastal lagoons on Menorca (Lucena-Moya et al., 2010). The genus *Rhipidogammarus* Stock, 1971 is represented by two species. The non-endemic *R. rhipidophorus* (Catta, 1878) is widely distributed in the Mediterranean Basin. It is known from the karstic springs close to the sea, on the northern slope of Serra de Tramuntana, as well as from the freshwaters of Menorca and Ibiza (Pretus 1991, Pons & Jaume, 1995). On Mallorca it is known to coexist with the endemic *Rhipidogammarus variicauda* Stock, 1978. The family Pseudoniphargidae Karaman, 1993 is represented in a plethora of cave environments by seven species, with most of them being endemic. One of the rarest endemic species is *Pseudoniphargus racovitzai* Pretus, 1990, known only from one, remote locality on Mallorca or *P. pityusensis* Pretus, 1990 along with *P. pedrerea* Pretus, 1990, both endemic to the Pityusic Islands (Ibiza, Formentera) (Pretus 1991, Pons & Jaume, 1995). *Pseudoniphargus daviui* Jaume 1991, a stygobitic species, endemic to the Balearic Islands (Mallorca, Cabrera), is sympatric with another Cabreran endemic, *P. triasi* Jaume 1991 (Pons & Jaume, 1995). The other species, *P. mercadali* Pretus, 1988 and *P. adriaticus* S. Karaman, 1955, are known from the groundwaters of Mallorca and Menorca, but their distribution extends much beyond the Balearic Islands (Pretus 1991; Pons & Jaume, 1995). The stygobiontic family Salentinellidae Bousfield, 1977 is represented by *S. angelieri* Ruffo & Delamare Deboutteville, 1952 displaying a wide circum-mediterranenan distribution (including the Ionian and Aegean archipelagoes) and being one of the most common macroinvertebrate in the anchialine caves of Mallorca. Another member of the family is *S. formenterae* Platvoet, 1984, endemic to Formentera, where it coexists with *P. pedrerea* (Pesce et al., 1979; Pesce 1985; Pretus 1991, Pons & Jaume, 1995).

Isopods are present on the Balearic Islands with four families: Asellidae Latreille, 1802, Cirolanidae Dana, 1852, Janiridae G.O. Sars, 1897 and Lepidocharonitidae Galassi & Bruce, 2016, including both epigean and stygobitic species. The family Asellidae is represented by only one, widely distributed taxon *Proasellus coxalis gabriellae* (Margalef, 1950). It inhabits the groundwaters (wells) as well as the channels close to the agricultural areas (Pons & Jaume, 1995) on the Gymnesic (Mallorca, Menorca, Cabrera, Dragonera) and the Pityusic (Ibiza, Formentera) Islands. Margalef (1950) questioned the taxonomic identity of this taxon and considered it to be *Proasellus coxalis banyulensis* Racovitza, 1919; the problem remains unresolved so far (F. Stoch, pers. comm). Amongst cirolanids, two endemic species are known from the Balearic Islands: *Typhlocirolana moraguesi* Racovitza, 1905 and *Metacirolana ponsi* Jaume & Garcia, 1992 (Pons & Jaume, 1995). The first one is widely distributed in the freshwater and anchialine ecosystems of Mallorca, Menorca, Cabrera and Dragonera (Palmer et al., 1999), whereas the second is found only in an anchialine cave on Cabrera (Jaume & Garcia, 1992). The epigean freshwater habitats on the islands are prone to be colonized by euryhaline marine species, which can migrate upstream and settle in the pure freshwater springs and brooks (Jaume & Garcia, 1988, Jaume & Queinnec, 2007). The isolated populations of two subspecies *Jaera nordmanni brevicaudata* Jaume & Garcia, 1990 and *J. n. balearica* Jaume & Garcia, 1990, from the generally marine and brackishwater family Janiridae, are found under the stones in freshwater streams, even 16 km upstream from the mouth, up to 500 m above sea level in the Serra de Tramuntana mountain range on Mallorca (Margalef 1952, Jaume & Garcia, 1988).

The stygobitic family Lepidocharonitidae is represented by two species: the endemic *Microcharon comasi* Coineau, 1968 (Mallorca) and the non-endemic, *M. marinus* Chappuis & Delamare-Deboutteville, 1954 (Mallorca and Menorca) (Pretus 1991).

For Thermosbaenacea only one endemic stygobitic species, *Tethysbaena scabra* (Pretus, 1991) of the family Thermosbaenidae Monod, 1927, has been reported from the Balearic Islands. It is well known from numerous localities on the Gymnesic Islands (Mallorca, Menorca, Cabrera, Dragonera) (Palmer et al., 1999, Jaume 2008). It occurs in anchialine waters of the coastal caves and wells adjacent to the seashore and never has been found in fresh inland groundwaters (Preteus 1991). Regarding the order Bathynellacea, the fauna of karstic areas on the Balearic Islands is represented only by *Paraiberobathynella fagei* (Delamare Deboutteville & Angelier, 1950), of the family Parabathynellidae Noodt, 1965, an interstitial and subterranean freshwater species, known also from numerous locations on the Iberian Peninsula (Serban, 1977; Guil & Camacho, 2001; Camacho, 2006; Camacho et al., 2014). This bathynellid was reported first from the Cueva de Genova on Mallorca (Margalef 1951), followed by a few other discoveries of *P. fagei* in the Serra de Tramuntana range (Pretus, 1991; National Biodiversity Data Portal, http://registros.gbif.es).

Only one decapod species has been reported from Mallorca, i.e. the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), of the family Cambaridae Hobbs, 1942 – a dangerous freshwater invasive species (Souty-Grosset et al. 2006; Barbaresi et al., 2007; Pinya et al. 2008). This crayfish prefers warm waters but occasionally is found also in small mountain streams. It tolerates low concentration of oxygen, high temperatures and salinity (Oscoz et al., 2011).

Geological history and malacostracan biogeography

The geological history of the Balearic Islands is a combination of complex interactions between the orogenic processes and the extensional tectonic activity observed in the entire western Mediterranean. The Balearic Islands were affected by extensional tectonics: the Liguro-Provençal Basin (to the west), the Tyrrhenian Basin (to the east) and the Algerian Basin (to the south), accompanied by the orogenesis in the Rif (Morocco) and the Baetic (Southern Spain) mountain chains, isolated by the Gibraltar Strait (Rosenbaum et al. 2002). It is speculated that during the Oligocene (30–25 Ma), when the origin and isolation of the Balearic archipelago is assumed, the internal parts of the Rif-Baetic belt (the Alboran Domain) were located near the present-day Balearic Islands (Rosenbaum et al. 2002; Rosenbaum & Lister, 2004).

During the Miocene (23–5.3 Mya) the Balearic Islands seem to be connected to the mainland, in the Langhian–Serravallian age (16–11.6 Mya), via the Baetic-Balearic corridor (Fontbote et al., 1990). In the Tortonian (11.3 Ma), this land connection was ceased, due to the transgression of the epicontinental sea, that flooded Mallorca and Menorca and reduced significantly their size to the area roughly corresponding to its present uplands (Pomar 1991). The Messinian Salinity Crisis (5.97-5.33 Mya), when the Mediterranean Basin was almost completely desiccated, created new temporal connections with the mainland, which allowed the faunal dispersal from Mallorca to Menorca and Ibiza (Hsü et al., 1973; Chueca et al. 2015). Such migration between islands is well documented for numerous taxa (e.g. Bover et al., 2008; Chueca et al. 2015), including the groundwater amphipod, *Metacrangonyx longipes* (Bauzà-Ribot et al., 2012). It is hypothesized that during the MSC the colonization of the Balearic Island from the mainland also took place (Delicado et al. 2014).

The final isolation of the Balearic Islands, which returned to their previous shape at the end of the MSC, allowed allopatric speciation of endemic taxa on each of the islands (Chueca et al. 2015). The islands have remained isolated from the continent and from the rest of the Balearic Archipelago since the beginning of the Pliocene and were connected with a marine transgression-regression cycle (Sosdian & Rosenthal, 2009; Bauzà-Ribot et al. 2011). The upper Pliocene transgression in the Western Mediterranean triggered the sea-level rise up to 100 m above the current sea level (Sosdian & Rosenthal, 2009; Bauzà-Ribot et al. 2011). Fluctuating sea levels have modified the shape and size of the emerged islands, allowing temporal connections between Mallorca (including the Cabrera archipelago) and Menorca and between Ibiza, Formentera and the Ses Bledes archipelago. These connections might have allowed species to migrate between islands within each of the Gymnesic and Pityusic islands of the Baleares (Bover et al., 2008; Brown et al., 2008). However, the sea level oscillations neither reconnect the western and eastern part of the Balearic Islands (separated by 640 m depth) nor linked the Baleares to the Iberian Peninsula (more than 800 m depth) (Chueca et al. 2015).

The paleogeographical history of the Gymnesic and Pityusic islands triggered the diversification of local endemic fauna as well as it affected the biogeographical affinities of species distributed across the islands within sub-archipelagos (Palmer et al, 1999). It seems that the islands sharing their palaeogeographical history (e.g. temporal land connections) have a similar pattern of endemic fauna. Malacostracan fauna on Mallorca is the species-richest amongst all the Balearic Islands. It seems to be most similar to the Menorcan fauna, what is evident with the presence of three shared species: *Typhlocirolana moraguesi, Tethysbaena scabra* and *Metacrangonyx longipes,* all being endemic to both islands (Pretus 1991, Pons & Jaume, 1995). However, the similarities within the Gymnesic fauna are also observed on Cabrera and Dragonera, that share two endemic species – *T. moraguesi* and *T. scabra* (Pretus 1991, Pons & Jaume, 1995). The malacostracan fauna of the Pityusic Islands is relatively poor and greatly differs in comparison with that of the Gymnesic sub-archipelago (Pretus 1991, Pons & Jaume, 1995). Thus, the faunistic differences between the Gymnesic and the Pityusic Islands are the consequence of the historical events. Based on vertebrate fossils (mammals and birds) from the upper Pleistocene/early Holocene, it seems the palaeoenvironmental conditions (e.g. vegetation and, thus, hydrology) of the Gymnesic Islands might have been different from that on the Pityusic Islands. Ibiza and Formentera share only two endemics: *Pseudoniphargus pityusensis* and *P. pedrerea*. The distinct character of Formentera manifests by presence of the endemic *Salentinella formenterae,* species closely related to *S. angelieri,* known from Mallorca (Platvoet, 1984; Pretus 1991).

Human colonization has brought expansion of widespread species and introduction of the high-impact alien invader *Procambarus clarkii* (Palmer et al. 1999). This species is native to the south-east North America and was intentionally introduced in 1973 in the Iberian Peninsula (Barbaresi et al., 2007). The red swamp crawfish is a species of commercial interests and Spain is one of its largest producers of. Nowadays, the species is considered as one of the hundred most dangerous alien aquatic invaders worldwide (Oscoz et al., 2011).

1.3.2 Corsica, Sardinia and circum-Sardinian islands

Geographical setting

Corsica and Sardinia are located on the Tyrrhenian Sea. Corsica covers an area of ca. 8,700 km^2, being as distant as ca. 90 km from Tuscany (Italy, Apennine Peninsula) and 170 km from France. Sardinia covers ca. 24,000 km^2 and is separated by 187 km from the Apennine Peninsula and 184 km from Tunisia. Corsica and Sardinia are separated by the Strait of Bonifacio (11 km wide). Several small islands surrounding Sardinia are collectively known as circum-Sardinian islands.

Corsica is made up of two major geological domains: (i) the crystalline western Corsica, which comprises two-thirds of the island, with the highest peaks and a steep coastline; and (ii) the alpine Corsica in the north-east (including cape Corsica), formed mainly by schists. Moreover, a coastal alluvial plain is present in the northwestern part, and a small karstic plateau in the southernmost part, around the town of Bonifacio. The island is mainly mountainous with 28 main watersheds. The average annual flow is ca. 105 m^3/s in an area of 6,204 km^2 that makes up 70% of the island's surface (Arrighi et al. 2005). These average values display a great seasonal variability due to the diverse landscape and the Mediterranean climate, e.g. the annual inflow reaches only 7 m^3/s during periods of low precipitation with 74% of total precipitations concentrated in the eight main watersheds (Arrighi et al. 2005). The Corsican Mountains are quite rich of lakes, while ponds are predominant in the coastal areas. The subsurface is a fractured environment which contains alluvial aquifers, small coastal sedimentary aquifers (Santoni et al. 2016) as well as aquifers in granitic and metamorphic rocks (which make up more than 80% of the groudwater resources in Corsica: Arrighi et al., 2005).

In Sardinia, two main geological complexes may be recognised: the Hercynian basement and the post-Hercynian covers and Quaternary deposits (Baiocchi et al. 2016). The Hercynian basement includes metamorphic rocks which outcrop extensively in the eastern and in the south-western sectors of the island (approximately 13,000 km^2). The karstic areas, hosting huge aquifers, extend for about 2,088 km^2, corresponding to the 8.7% of the entire island surface (Cassola, 1983). Supramonte is the most important karstic massif, located in the Central-Eastern part of the island and occupying an area of 170 km^2 (Cabras et al. 2008). Differently from Corsica, Sardinia has not a well-developed surface hydrographic network, albeit rich in springs, fed mainly from the karstic and volcanic aquifers, and from the alluvial aquifers (Baiocchi et al. 2016).

Malacostracan fauna

Forty-five malacostracan species are recorded up to now from Corsica, Sardinia and circum-Sardinian islands: 20 of them from Corsica and 35 from Sardinia and neighboring islands, while 10 of them (i.e. 22%) are common for both islands. Notwithstanding their common origin and proximity, malacostracan fauna greatly differs between the two islands, and Sardinia seems to be much more species-rich. This difference may be due to several reasons: (i) the greater surface of Sardinia with respect to Corsica (2.8x in areal extension); (ii) the fact that 31 out of 45 species (i.e. 69%) are stygobitic, and groundwater dwelling species in these islands are usually strict endemics (Stoch & Argano 2006; Ruffo & Stoch 2006); (iii) the presence of complex, extended and fragmented karstic aquifers in Sardinia (Cassola 1983), where endemic karstic stygobionts are present (Grafitti 2001; Casale et al. 2008), while the extent of carbonatic rocks in Corsica is restricted to its southern part close to Bonifacio (Santoni et al. 2016); and (iv) less research in Corsican freshwaters.

Amphipods are present on these islands with eight families. Nine, all stygobitic, species of Bogidiellidae (five endemic to Sardinia, and two to Corsica) are present, linked both to interstitial and subterranean karstic waters. The only non-endemic species, *Medigidiella chappuisi* (Ruffo, 1952), lives in interstitial anchihaline waters (Ruffo & Delamare Deboutteville 1952). The family Gammaridae is well represented on the islands, however in that terms Corsica is less studied than Sardinia. *Gammarus elvirae* Iannilli & Ruffo, 2002, three species of *Echinogammarus* (*E. sicilianus* Karaman & Tibaldi, 1972, *E. adipatus* Karaman & Tibaldi, 1972 and *E.* aff. *tibaldii*), and the subterranean *Tyrrhenogammarus sardous* Karaman & Ruffo, 1989 are endemic to Sardinia (Ruffo & Stoch 2006). *Rhipidogammarus rhipidiophorus* is present in coastal springs of both islands, while *R. karamani* Stock, 1971 is known up to now only from Corsica (its *terra typica*), where it inhabits interstitial habitats in streams not far from their estuaries. Another interesting subterranean family of marine origin, the Ingolfiellidae Hansen, 1903, is represented by *Ingolfiella cottarellii* Ruffo & Vigna-taglianti, 1989 – an endemic stygobiont, found up to now only in an anchialine cave on the small karstic island of Tavolara (Ruffo & Vigna Taglianti 1989). Another interesting family, the Ischyroceridae Stebbing, 1899, including almost exclusively marine species, was collected in central Sardinia in thermal, slightly sulphydic waters (54-58°C, Krapp et al. 2010). A single juvenile specimen found, named on the slide in the Museum of Verona as "*Thermojassa*", was reported by Krapp et al. (2010) as *Jassa* sp., and is still awaiting formal description. Only two stygobitic members of Niphargidae Bousfield, 1977, a speciose family widely distributed in eastern Mediterranean islands, are present in Corsica and Sardinia. *Niphargus corsicanus* Schellenberg, 1950 is widely distributed in the springs of Corsica (Stock 1972), springs and caves of northern Sardinia and on some islets (Ruffo & Stoch 1997). *Niphargus* aff. *speziae* Schellenberg, 1936 is present on Corsica and belongs to a species complex endemic to Italy (Stock 1972). Another species, *N. stefanellii* Ruffo & Vigna-Taglianti, 1968, was reported for Sardinia by Karaman (1985); this single specimen was recently re-examined, and turned out to be a juvenile of *N. corsicanus* (Fabio Stoch, unpublished data). Another family including only stygobitic species are the Pseudoniphargidae G. Karaman, 1993, represented by three species (*Pseudoniphargus obritus* Messouli, Messana & Yacoubi-Khebiza, 2006 and *P. adriaticus* S. Karaman, 1955 s.l. in Corsica, and P. *mercadali* Pretus, 1988 in the northwestern karstic area near Alghero in Sardinia). Even the small stygobitic family Salentinellidae Bousfield, 1977, represented by a single species complex - *Salentinella angelieri* Delamare-Deboutteville & Ruffo, 1952 s. l., is present on both islands. Similarly, the family Talitridae Rafinesque, 1815, mainly including marine or semi-terrestrial species, is represented by one species, *Macarorchestia remyi* (Schellenberg, 1950), inhabiting two anchialine caves on both islands (Schellenberg, 1950; Ruffo, 1960).

The order Isopoda is represented by three families including both surface and groundwater species. The family Asellidae is particularly speciose in Sardinia, where the genus *Proasellus* Dudich, 1925 is present in springs, caves and interstitial waters (Argano & Campanaro 2004). *Proasellus banyulensis* (Racovitza, 1919), widely distributed in western Europe, is the only common epigean species on both islands (Arcangeli 1942). Three stygobiotic species known from Sardinia and one known from Corsica are all strict endemics (Argano & Campanaro 2004). The representatives of the stygobitic genus *Stenasellus* Dollfus, 1897 (family Stenasellidae Dudich, 1924), present in both islands both in interstitial and karstic waters, are under taxonomic revision (Ketmaier et al. 2003). Finally, the minute representatives of the stygobitic family Lepidocharontidae Galassi & Bruce, 2016 are represented by four endemic species of the genus *Microcharon* S. Karaman, 1934, found up to now only in interstitial waters (Coineau 1966).

The order Bathynellacea is well represented on the islands, with the members of the group being quite common in interstitial waters, as well as in percolating waters in caves (Ruffo 2006a). The four known species belong to four genera. One of them, *Sardobathynella* Serban, 1973 of the family Bathynellidae Grobben, 1905, is endemic to Sardinia.

The order Decapoda is represented on the two main islands by four families. The species of Atyidae De Haan, 1849 (*Atyaephyra desmarestii* (Millet, 1831), whose identity was confirmed using DNA barcoding by Christodoulou et al. 2012) and Palaemonidae Rafinesque, 1815 (*Palaemon antennarius* H. Milne Edwards, 1837, identity confirmed by Tzomos & Koukouras 2015) are quite common and autochtonous inhabitants of coastal lakes, as well as rivers, ditches, artificial freshwater lakes, ponds, including even some brackishwater ponds (Froglia 2006). In contrary, the Astacidae Latreille, 1802 with *Austropotamobius fulcisianus* (Ninni, 1886), identity confirmed using molecular methods by Amouret *et al*. 2015 (which reported it as *Austropotamobius italicus meridionalis* sensu Fratini *et al*. 2005, currently *nomen nudum*) and the Cambaridae (*Procambarus clarkii*) were recently introduced by humans (Arrignon *et al*. 1999; Morpurgo et al. 2010; Bertocchi et al. 2010; Amouret et al. 2015). The highly invasive red swamp crayfish (*P. clarkii*) is now widespread in some areas of the two islands, and is the greatest threat for the surface native malacostracan fauna (Morpurgo et al. 2010).

Geological history and malacostracan biogeography

Corsica and Sardinia islands are a 30 km-thick crustal block bounded by two areas affected by extensional tectonics: the Liguro-Provençal basin to the West and the Tyrrhenian basin to the East. The Liguro-Provençal basin, located between the Provençal-Catalan coast and the Corsican-Sardinian block, opened during the Oligocene (the process began at least 29-30 Ma: Speranza et al. 2002; Advokaat et al. 2014). Eastward migrations of the Alpine belt, Corsican-Sardinian block and Calabrian-Peloritan block, accompanied by several micro-plates, were almost simultaneous (18.3 to 17.5 Ma: Edel et al. 2001; Malinverno & Ryan 1986). The question of coupling or decoupling between Corsica and Sardinia during the drift was resolved by Vigliotti et al. (1990), who showed that these two islands rotated as a single block.

Successively, during the Messinian Salinity Crisis (5.7-5.3 Ma, Boccaletti et al. 1990), the Mediterranean Sea was almost entirely desiccated, creating connections of dry land between Corsica-Sardinia, northern Italy and southern France. Sea level oscillations creating land bridges between Sardinia and Corsica continued during Pliocene and Pleistocene (5.3 – 0.23 Ma, Arias et al. 1980). During the Quaternary, Sardinia could have been in contact with peninsular Italy via the Elba island (Tuscan Archipelago), and the contemporaneous Corsican-Tuscan connection was possible as well (Cassian regression, 1.8 Ma: Baccetti 1983). During the Last Glacial Maximum (LGM: ca. 21,000 years ago), Corsica and Sardinia were connected to each other, but not to the continental landmasses (Antonioli & Vai 2004).

The complex paleogeography of these islands suggested a three-phase model (Baccetti 1983) of faunal colonization (pre-Miocenic, Messinian, and Quaternary: see Ketmaier & Caccone 2013 and bibliography cited therein); Baccetti (1983) suggested to add a fourth phase dealing with human-mediated species introductions.

The first phase would correspond to the detachment of the microplate from the Provençal-Catalan coast. Several of the endemic malacostracan species nowadays distributed in Corsica and/or Sardinia differentiated from ancestors that were supposedly distributed on the single landmass formed by the Corsican-Sardinian block, western Iberian Peninsula and southern France. The origin of these ancient lineages (sometimes called paleo-endemics: Ruffo & Stoch, 1987) is at least 29 Myr old, even though one cannot exclude that cladogenesis predated geological splits. Isopods such as *Proasellus* and *Stenasellus* conform to this model, together with Bathynellacea (Ketmaier et al. 2001, 2003; Camacho 2003).

The second phase took place during the Messinian Salinity Crisis; it allowed a number of species to reach the islands. Following Ketmaier & Caccone (2013), colonization proceeded along two major paths from south and east: thus Corsica and Sardinia share genera and species complexes with northern Africa and Sicily on one side, and with peninsular Italy on the other side. Although molecular phylogenetic studies are under way, it can be hypothesized that the amphipod genera *Gammarus* Fabricius, 1775 and *Niphargus* Schiödte, 1849 followed this colonization pathway from the Apennine Peninsula, while it is still uncertain if African or Sicilian affinities exist within Malacostraca.

The last connections between this insular Corsican-Sardinian system and the adjacent continent (especially the Apennine Peninsula) took place during the Quaternary ice ages. These connections were relatively short-lived, but allowed dispersal of species, which did not gave origin to endemics. The presence of *Proasellus banyulensis* on these islands is probably a good example of such a recent colonisation (although transport by humans cannot be excluded: Stoch et al. 1996). Also, the non-endemic representatives of the decapod families Atyidae and Palaemonidae, present in brackish waters as well as freshwaters, coud be such recent colonisers. Colinisation of crayfish could have been also enhanced by waterbird-mediated passive dispersal (Banha & Anastácio 2014).

In the fourth phase, humans started to introduce species to the islands intentionally or accidentally since historical times. Introduction of the alien species on the islands is an on-going process, and the decapods *Austropotamobius fulcisianus* and *Procambarus clarkii* are the only examples among Malacostraca known up to now (Arrignon 1996; Amouret et al. 2015).

Considering that stygobitic species can be both of ancient freshwater (limnicoid stygobionts) or marine (thalassoid stygobionts) origin (Coineau & Boutin 1992), some of the cited genera and families seem not to conform to the previous four-stage hypothesis, but may have had single or multiple origins directly from marine ancestors. The stygobitic species of the families Bogidiellidae, Ingolfiellidae, Ischyroceridae, Pseudoniphargidae and Microparasellidae are probably thalassoid stygobionts (Stock 1980; Coineau & Boutin 1992; Jurado-Rivera et al. 2017).

1.3.3 Tuscan Archipelago

Geographical setting

The Tuscan Archipelago is located in the northern Tyrrhenian Sea, between the coast of Tuscany (Peninsular Italy) and Corsica. It consists of seven main islands (Elba, Giglio, Capraia, Montecristo, Pianosa, Giannutri, Gorgona) and some islets, with a total area of more than 300 km^2. The archipelago once included an eighth island, Monte Argentario, now a promontory connected to mainland Tuscany. The carbonatic island of Pianosa is almost completely flat and devoid of a surface hydrographic system, while the other islands (mainly formed by volcanic, granitic and metamorphic rocks) are mountainous and, with the exception of the small Giannutri, rich in springs and/or temporary streams as well as small and isolated groundwater aquifers. Perennial water courses are few and restricted to Elba island.

Malacostracan fauna

Fifteen malacostracan species are known from the islands of the Tuscan archipelago, eleven of them (73%) being stygobitic. The scarcity of surface waters prevents the presence of a diversified epigean freshwater malacostracan fauna. Eleven species of amphipods and three of isopods are recorded; no representatives of the other orders are known up to now, although a rare, stygobitic thermosbaenacean species, *Tethysbaena argentarii* Stella, 1951, is recorded from a single cave of Monte Argentario (Ruffo 2006b).

Two interstitial species, the bogidiellid amphipod *Medigidiella chappuisi* and the microcerberid isopod *Microcharon marinus*, are widespread in the Mediterranean also in shallow marine waters. On Elba both species were collected in freshwater wells, not far from the coastline (Ruffo & Stoch 2006; Stoch et al. 2006). The janirid isopod *Jaera* cf. *nordmanni* (Rathke, 1837) was collected in a freshwater spring on the hills in the north-eastern part of the islands (Stoch et al. 2006).

The talitrid semi-terrestrial amphipod *Cryptorchestia garbinii* Ruffo, Tarocco & Latella, 2014, is very common in a subterranean freshwater reservoir on Montecristo island (Ruffo & Stoch 2006, under the old synonym *Orchestia cavimana* Heller, 1865). The presence of *C. garbinii* is strictly linked to freshwater springs and streams in its entire area of distribution (Ruffo & Stoch 2006).

The only epigean malacostracans present in surface waters on these islands are: (i) *Proasellus banyulensis*, widespread over Europe, recorded from Elba, Capraia and Giglio, associated with permanent standing waters (ponds and pools) or slowly flowing brooks (Stoch et al. 2006); (ii) the gammarid amphipod *Rhipidogammarus rhipidiophorus* (Catta, 1878), which was collected in some wells of Pianosa (Messana & Ruffo 2001). This species is also very common on the now semi-island Monte Argentario (Ruffo & Stoch 2006).

Except the stygobitic *Proasellus acutianus* Argano & Henry, 1972, widespread on Elba and present on the Apennine Peninsula in Tuscany and Latium (Stoch et al. 2006), all the other subterranean species are amphipods. The family Gammaridae is represented by *Ilvanella inexpectata* Vigna-Taglianti, 1971, present in interstitial environments of Elba and Tuscany (Ruffo & Stoch 2006) as well as by *Longigammarus planasiae* Messana & Ruffo, 2001, up to now found only in wells of Pianosa and considered an endemic of this island (Messana & Ruffo 2001). *Metacrangonyx ilvanus* Stoch, 1997 of the family Metacrangonyctidae Boutin & Messouli, 1988 is present in few wells in the central part of Elba, to which it is endemic (Stoch 1997). The family Niphargidae is represented by two species. *Niphargus longicaudatus* (A. Costa, 1851) s.l., a species complex under revision, is present on all the main islands (except Pianosa), including Monte Argentario. Another species complex, *Niphargus speziae* s.l., was recorded on Elba, Capraia, Montecristo and on Monte Argentario (Vigna Taglianti 1976; Ruffo & Stoch 2006). Representatives of the family Pseudoniphargidae are known from springs on Montecristo i.e. *Pseudoniphargus adriaticus* s.l., a further species complex in need of urgent revision (Messouli et al. 2006), as well as from the artificial wells of Pianosa, with the endemic *Pseudoniphargus planasiae* Messouli, Messana & Yacoubi-Khebiza, 2006. Finally, *Salentinella angelieri* Delamare-Deboutteville & Ruffo, 1952 was recorded from Monte Argentario (where it was describes as *Salentinella denticulata* Baschieri, 1952) and from Elba (Ruffo & Stoch 2006).

Geological history and malacostracan biogeography

The interaction between the Corsica–Sardinia block and the Apennines, which were then being formed, caused the emergence of the Tuscan Archipelago, including some islands that became incorporated later into the mainland (the so-called ‘fossil islands’: Lanza 1984).

Although small and well confined in the sea, the origin of the islands of Tuscan Archipelago and their geological history differ markedly. Capraia is the oldest island, emerged from the sea during volcanic eruptions (9-5 Ma), while the others emerged between 7 and 5 Ma, except Pianosa, which probably originated about 3 Ma as a consequence of the uplift of a marine ridge (Barbato et al. 2018). Elba has more complex geology, being the largest remaining stretch of land from the ancient tract that once (Cassian regression, 1.8 Ma: Baccetti 1983) connected the Apennine Peninsula with Corsica. Elba is formed of the slices of rocks, once being a part of the ancient Tethyan seafloor, which emerged through the Alpine and the Apennine orogeny (Marroni et al. 1998).

The biogeographical features of the Tuscan archipelago fauna are linked to the paleogeographic events that occurred after the disjunction and rotation of the Corsica-Sardinia block (Alvarez, 1972), the Messinian Salinity Crisis (Boccaletti et al. 1990) and the Pleistocene sea regressions. According to the most recent paleogeographical reconstructions, the lower sea level during the Pleistocene glacial maxima resulted in connection of Elba, Pianosa, Giannutri, Giglio and Monte Argentario to the mainland (Tuscany), while Capraia, Gorgona and Montecristo remained isolated (Bossio et al. 2000). During Holocene, Monte Argentario became a promontory, connected with the mainland by two narrow sandy isthmuses (Barbato et al. 2018).

A paleogeographical imprint on malacostracan fauna in the Tuscan Archipelago is evident. Surely the most intriguing species is *Metacrangonys ilvanus*. Stoch (1997) postulated that the ancestor of *M. ilvanus* colonized the brackish coastal waters of the Tethys sea and survived the Oligo-Miocenic fragmentation of the Western Mediterranean (Briançonnais) microplate that gave an origin to the Corsica-Sardinia block and, successively, caused the emergence of the Tuscan Archipelago. A recent molecular phylogeny of the family Metacrangonyctidae (Bauzà-Ribot et al. 2012) suggested that this species is part of the so called “insular clade” together with *Metacrangonyx longipes* (Balearic Islands), *M. repens* (Stock & Rondé-Broekhuizen, 1986) (Canary islands) and two species from the Caribbean islands. The initial diversification of the insular clade was estimated to have occurred between 60 and 108 Ma; of course at that time none of the archipelagoes hosting the “insular group” as well ad the Mediterranean sea existed. Bauzà-Ribot et al. (2012) hypothesized that Paleo-Macaronesian islands of the Tethys Ocean were located close to the present Western Mediterranean, and the existence of these vanished archipelagos made possible that the ancestor of the insular lineage was a shallow-water marine species. The origin of *M. ilvanus* was dated back to 50-90 Ma and the species can be considered a thalassoid stygobiont (*sensu* Coineau & Boutin 1992).

Other thalassoid stygobionts are the representatives of the genus *Pseudoniphargus* (Jurado-Rivera et al. 2017), including *P. planasiae*, and probably *Longigammarus planasiae* (Messana & Ruffo 2001). The colonization of Pianosa, which hosts these two endemics, cannot be older than the age of the island, i.e. 3 Myr (Barbato et al. 2018).

Another ancient, pre-Messinian colonizer, is the thermosbaenacean *Tethysbaena argentarii*, known only from the 'fossil island' of Monte Argentario, its closest relative being *T. scabra* (Pretus, 1991) from the Baleares (Canovas et al. 2016). The estimated age for the most recent common ancestor of the two *Tethysbaena* species varied, depending on the population model used, from 10.7 to 18.1 Myr, a datation compatible with the Oligocenic and early Miocenic vicissitudes of the Western Mediterranean (Canovas et al. 2016).

The Messinian Salinity Crisis and the incidence of the Corsica-Tuscany Pliocenic connection may be responsible for the presence of limnicoid stygobionts (like *Niphargus longicaudatus* s.l. and *N. speziae* s.l.) in the most remote islands, like Capraia, Gorgona and Montecristo, which remained isolated since those ancient paleogeographical events (Vigna Taglianti 1976).

The Pleistocene sea regressions could explain the relationships between the Italian mainland and the Tuscan block of islands that have had contacts with the neighboring areas during the LGM, and this seems to be true at least for *Proasellus banyulensis*, although the presence of the species on Capraia islands remains unexplained, with a human-mediated introduction being a plausible option. A Pleistocene origin can explain the presence of *Ilvanella inexpextata*, *Salentinella angelieri* s.l. and *Proasellus acutianus* on Elba as well. Of course this explanation remains highly speculative, as the on-going molecular research (Fabio Stoch, unpublished data) is being carried out to support or deny this hypothesis.

1.3.4 Sicily, circum-Sicilian, Maltese and Pelagie islands

Geographical setting

Sicily is the largest Mediterranean island (25,710 km^2) and one of the most biodiverse areas in the Mediterranean Basin (Médail & Quézel 1997). From a geomorphological point of view, about 62% of the Sicilian surface is covered with hills, 24% with mountains, and 14% with plains (Barbera & Cullotta 2012). Mount Etna volcano is the highest mountain of the island, 3,350 m.a.s.l. The northern and north-eastern parts are the most heterogeneous in terms of topography, climate, and hydrography due to the mountain ranges of Madonie (limestones, with huge karstic caves), Nebrodi (mailny sandstones and limestones), and Peloritani (mainly igneous and metamorphic rocks) (Barbera & Cullotta 2012), where a perennial surface hydrographic network is well developed. In the south-western part, the karstic Iblean massif hosts some perennial streams, as well as a rich karstic aquifer (Ruggieri & Grasso 2000) and is rich in caves (Cavallaro, 1995). Most of the central part of the island is occupied by gypsum formations, with a poor and intermittent surface hydrography, and some well-developed caves hosting subterranean streams (Madonia & La Manna 1986). Ponds and pools are common on the island, while all the lakes are represented by artificial reservoirs for drinking water supply (Marrone et al. 2006).

Sicily is surrounded by smaller islands (isolated or grouped in archipelagos) called circum-Sicilian islands, consisting of the Aeolian Islands, the Island of Ustica (volcanic, on the Tyrrhenian Sea) and the Aegadian Islands (carbonatic, to the west). Surface running waters are absent from all of these small islands; the carbonatic Aegadian islands host small karstic aquifers, while the groundwaters on the volcanic islands are reduced to shallow freshwater lenses occurring on top of the saltwater. Artificial ponds and rock pools are present (Marrone et al. 2006).

In the Sicilian channel, the isolated Pantelleria (80 km^2, an active volcanic island), the Maltese islands (316 km^2) and the Pelagie islands (25.5 km^2) are present. The Maltese Archipelago consists of three islands, i.e. Malta, Gozo and Comino, and lies 93 km south of Sicily and 288 km north of the African coast. Maltese islands have no large permanent river systems. Inland surface water systems are small and restricted to several dry river valleys, locally called ‘widien’ (Moore & Schembri 1986). Sea-level groundwater bodies developed in the limestones take the form of freshwater lenses floating over sea water, while unconfined perched aquifers sustained in the upper limestone formation overlay, on western part of Malta and on Gozo, the sea-level aquifers (AA.VV., 2015). The Pelagie Islands consist of three small islands of Lampedusa, Linosa, and Lampione, located between Malta and Tunisia. Surface hydrography is absent. A small aquifer in the limestones and a spring are present on Lampedusa. The volcanic island of Linosa has only a small lens of fresh groundwaters on saltwater, while no freshwaters are known on the small islet of Lampione.

Malacostracan fauna

Twenty-one malacostracan species are recorded up to now from Sicilia and circum-Sicilian islands, eleven from the Maltese Islands and only two from the Pelagie islands. No freshwater malacostracans are known for Pantelleria.

Amphipods are represented by four families with Gammaridae being the most speciose one. Six species of the genus *Echinogammarus*, three on Sicily and three on Maltese Islands, have been recorded (Ruffo & Stoch 2006; Moore & Schembri 1986; Pinkster 1993). Recent molecular analyses (Hupało et al. 2017, 2018) revealed an extraordinary cryptic diversity in freshwater *Echinogammarus* from Sicily, where twenty-two Molecular Operational Taxonomic Units (MOTUs) were identified as the neutral and tentative species equivalents (sensu Grabowski et al. 2017a): three within *Echinogammarus* aff. *tibaldii* Pinkster & Stock, 1970 morphospecies, three within *E. adipatus* and seventeen within *E. sicilianus* Karaman & Tibaldi, 1972 s.l.. The majority of the sites were inhabited by one MOTU only, except one spring where six different MOTUs co-occurred. According to the on-going molecular researches, the old report of *Echinogammarus veneris* (Heller, 1865) from Anapo River (Iblean area) by Stock (1968) has to be considered erroneous. Of the three species present on Malta Island, *Echinogammarus klaptoczi* (type locality in Tripolitania, Libya), was originally reported from Malta under the name of *E. ebusitanus* by Karaman, (1977) and Moore & Schembri (1986); later on it was synonymized with *E. klaptoczi* by Pinkster (1993). The second species, *Echinogammarus pungens*, was reported by Baldacchino (1973) and Moore & Schembri (1986). Finally, Pinkster (1993) reported *Echinogammarus sicilianus* from a well on Malta; given the large number of cryptic species found in Sicily, this record remains doubtful. Another gammarid species, *Rhipidogammarus rhipidiophorus*, was collected in wells and springs in Sicily, the Aegadian Islands (Marettimo and Favignana), the Maltese Islands (Gozo) and the Pelagie islands (Lampedusa) (Moore & Schembri 1986, Ruffo & Stoch 2006). The only stygobitic gammarid, *Tyrrhenogammarus catacumbae* Karaman & Ruffo, 1977, was recorded from the caves, catacombs, wells and a spring in the karstic area of the Iblean massif, in south-eastern Sicily (Ruffo & Stoch 2006). It is of interest to notice that all of the gammarid amphipods reported by Moore & Schembri (1986) were included later in the Red Data Book of the Maltese Islands (Schembri & Sultana 1989).

A single species of the family Ischyroceridae, *Jassa trinacriae* Krapp, Grasso & Ruffo, 2010, endemic to Sicily, was described from the cave Conza (175 m.a.s.l.) (Krapp et al. 2010). The species complex *Niphargus longicaudatus* s.l. (family Niphargidae) is present on western Sicily, where it is quite common in caves and springs (Ruffo & Stoch 2006), however the family is absent from all the other islands. Finally, Pseudoniphargidae is the most species-rich family on these islands; so far four species are known to be endemic to Sicily, where they never co-exist with Niphargidae (Ruffo & Stoch 2006). Another species, recorded as *Pseudoniphargus adriaticus* s.l., but undoubtedly representing an unknown new species, awaits formal description (Ruffo & Stoch 2006; Messouli et al. 2006). It is present in the groundwaters of Lampedusa and Linosa islands.

The isopod family Asellidae is present on Sicily with three species of the *Proasellus coxalis* (Dollfus, 1892) species complex (Stoch et al. 1996). The endemic *Proasellus montalentii* Stoch, Valentino & Volpi, 1996 is widespread on the island, where it inhabits springs and streams. Another species, *Proasellus wolfi* Dudich, 1925, is known only from some slow-flowing rivers at the foot of the Iblean massif. The third species, *Proasellus banyulensis,* is present in a small area in the surroundings of Palermo. This species was also recently found in the water reservoirs of the fortress on the volcanic island of Ustica (Stoch, personal observation), possibly introduced by man in the historical times (Stoch et al. 1996; Marrone & Naselli Flores 2015). One more species, named *Proasellus ragusani* by Galletti (2002), was never formally described and remains a ‘nomen nudum’. The members of the *Proasellus coxalis* species complex present on the Maltese islands were reported for the first time for Gozo (as “*Asellus (Proasellus) coxalia*”) by Baldacchino (1973); their taxonomic status is still unstudied. Another interesting stygobitic species endemic to south-eastern karstic areas of Sicily is the cirolanid isopod, *Typhlocirolana* cf. *moraguesi* (Ruffo & Stoch 2006). The species is morphologically very similar to *T. moraguesi* Racovitza, 1905 from the Balearic Islands, but well distinguished based on the molecular data (Baratti et al. 2004).

The order Thermosbaenacea is represented by a single, endemic stygobitic species, *Tethysbaena siracusae* Wagner, 1994, having the same distribution area as *Typhlocirolana* (Ruffo 2006b).

Six families of the order Decapoda are present on Sicily (five species) and on the Maltese islands (seven species). Apart from Atyiidae, Palaemonidae and Potamidae, all the others are introduced species (Deidun et al. 2018). *Palaemon antennarius* is quite common in the Sicilian brackish waters, especially in the estuaries, as well as in some inlands freshwater lakes and rivers, while *A. desmarestii* is present in freshwaters (Froglia 2006). *Potamon fluviatile* (Herbst, 1785) is present on Sicily and a distinct, endemic subspecies, *P. fluviatile lanfrancoi* Capolongo & Cilia, 1990 was thought to be present in the Maltese islands (Schembri 2003). However, recent molecular analyses demonstrated that a single taxon, of recent expansion, is present on the Apennine Peninsula, Sicily, Maltese Islands as well as on the Ionian islands, proving that the separation of the Maltese populations as a distinct subspecies was invalid (Vecchioni et al. 2017).

The first invasive alien species, *Procambarus clarkii* (family Cambaridae), was discovered on Sicily (Marrone & Naselli Flores 2015). Its distribution on Sicily likely derives from multiple deliberate and independent introduction events (Faraone et al. 2017). This species was recently also reported from Malta by Vella et al. (2017). The presence of another alien species from Sicily, *Cherax destructor* Clark, 1936 (the common yabby, family Parastacidae Huxley, 1879), was reported by Deidun et al. (2018) with three specimens collected in a stream in the close proximity to a crayfish culture. However, at the moment there is no data supporting the species' reproduction in the Sicilian fresh waters (Marrone, personal communication). Most recently, Deidun et al. (2018), reports the occurrence of five other alien decapods (see Table S1), important in the pet trade or in the aquaculture, from the freshwater localities on the Maltese Islands. According to the authors, the Malta's Environmental and Resources Authority (ERA) was alerted about the localities of the non-indigenous malacostracan species and a preliminary eradication programme has commenced.

Geological history and malacostracan biogeography

Sicily develops along the African-European plate boundary. During Oligocene, between 29-24 Ma, the central-western Sicily was part of the African Maghrebides – Southern Apennines accretionary wedge (Catalano et al. 1994). The major tectonic units of the island (Broquet 2016) are: (i) the Hyblean foreland (the emergence of the carbonatic Hyblean Plateau is dated to early Miocene, while during the Middle Miocene African subduction, ca. 15 Ma, it became the Hyblean-African plateau: Broquet 2016); (ii) the Gela foredeep, which extends from the northern margin of the Hyblean plateau to southwestern Sicily, which developed during Late Pliocene and filled with limestones, Messinian evaporites, and clays; (iii) the Apenninic-Maghrebian orogen forming the central and western part of Sicily, which represents the fold and thrust belt of the African subduction system; (iv) the Calabrian-Peloritan arc, which, being still attached to Sardinia in the Early Miocene, formed the northeastern part of Sicily (Peloritani Mountains). Some authors (e.g. Ruggieri 1973) hypothesized that at, the beginning of Pliocene, there was no emerged land of the Sicilian territory, and that the island began to raise up again from the sea during Pliocene (at ca. 4 Ma). At that time Sicily was divided into two islands, i.e. the Madonie-Nebrodi-Peloritani chain in the northern part, and the Hyblean plateau in the southeastern part (Guglielmo & Marra 2011). Finally, during the Pleistocene glaciations, Sicily had a temporal land connections with Calabria, the Maltese archipelago and most of the circum-Sicilian islands (Marra 2009). Nowadays all geologists agree that the African connection during Plio-Pleistocene, through the alleged African-Sicilian bridge (Sacchi 1955), never took place (Broquet 2016).

Among the circum-Sicilian islands hosting Malacostraca, the Aegadian Islands (except Marettimo) are, in fact, a fragment of Sicily, to which they were connected during the eustatic sea level variations in Pleistocene (Muscarella & Baragona 2017).

The Maltese Islands are geologically associated with the Hyblean Plateau of the southeastern Sicily. They emerged from the sea during the Alpine orogenesis at ca. 10 Ma (Savona Ventura 1975). The sea between the Maltese Islands and Sicily reaches a maximum depth of 200 m (mostly less than 90 m) and since the Messinian Salinity Crisis these islands were repeatedly connected to Sicily. In contrary, the sea depth between the Maltese Islands and the African coast is considered to be much deeper, making post-Messinian connections improbable (Savona Ventura 1975).

The Pelagie Islands are connected to each other only from a geographical point of view. Lampedusa (together with Lampione) is a part of the African continental shelf, being a stable carbonatic platform, raised up presumably during the Late Miocene or the Early Pliocene (Muscarella & Baragona 2017). The connections between Lampedusa and the Tunisian coast were quite frequent during Pliocene as well as during the Quaternary glaciations (Foglini et al. 2015), while any past connection with Sicily is considered unlikely (Muscarella & Baragona 2017). On the other hand, the volcanic islands, such as Linosa and Pantelleria, emerged during Pliocene and were never connected with the mainland (Muscarella & Baragona 2017).

The first scenario explaining the origin of Sicilian fauna, including the other islands, was formulated by La Greca (1957). Following the paleogeographical scenario accepted at that time, the author postulated five main categories of colonizers depending on their origin: widely distributed species, western Mediterranean species, eastern Mediterranean species, Sicilian-African species and the endemics. Among the endemics, he distinguished recent Pleistocene endemics from the relictual endemics, derived from the fragmentation of the "Tyrrhenidis", i.e. the post-Oligocenic vicissitudes of the western Mediterranean, which Sicily was considered to be a part of (Furon 1950). More recently, Massa et al. (2011), following the paleogeographic reconstruction by Ruggieri (1973), refused the hypothesis of the existence of ancient, pre-Pliocenic, endemics. Results of recent molecular analyses confirm both the existence of ancient endemic species (eg. *Typhlocirolana* cf. *moraguesi*: Baratti et al. 2004) and the presence of the Quaternary colonisers (eg. *Potamon fluviatile*) (Vecchioni et al. 2017).

Baratti et al. (2004) considered *T. moraguesi* (Balearic Islands) and *T.* cf. *moraguesi* (Sicily) as the sister group of the Moroccan *Typhlocirolana* species. The two clades could have separated when the hydrological continuity was interrupted in the Aquitanian (ca. 20–23 Ma), with the subsequent fragmentation of the 'Alboran–Kabylian–Calabrian Plate'. Thus, the interruption of gene flow between the populations colonising inland waters and their coastal, brackishwater ancestors was probably a vicariance event during the Tethian regression.

Moreover, Baratti et al. (2004) suggested that the divergence of *T.* cf. *moraguesi* from *T. moraguesi* took place about 15 Ma. Although this datation has to be taken with caution, it suggests that colonisation should have taken place well before the alleged re-emersion of Sicily hypothesized by Ruggieri (1973) and Massa et al. (2011). The same scenario could apply to *Tethysbaena siracusae*, considering that the congeners live in the Balearic Islands and on Monte Argentario in Tuscany, well known Miocenic relict lands (Ruffo 2006b).

In a recent, ongoing molecular study on the surface genus *Echinogammarus* on Sicily, Hupało et al. (2017, 2018), using both mitochondrial and nuclear markers, postulated an ancient (i.e. when Sicily lost its connections with Africa and Peninsular Italy) origin for both *E. adipatus* (late Serravallian/early Tortonian) and *E.* cf. *tibaldii*. *Echinogammarus sicilianus*, presenting a formidable level of cryptic diversity, forms a sister group to both the Apennine *Echinogammarus* and the other Sicilian congeners. Its separation from *E. adipatus* was dated at around late Oligocene/early Miocene. So, the presence of an ancient, endemic fauna in Sicily is confirmed, as postulated by La Greca (1957) more than 60 years ago. Other molecular studies, aiming to clarify the biogeographical affiliations, are being carried out on the isopod genus *Proasellus* and on the amphipod genera *Rhipidogammarus, Tyrrhenogammarus, Pseudoniphargus* and *Niphargus* (Fabio Stoch, unpublished).

On the other hand, the presence of recent colonizers is confirmed for the decapods *Potamon fluviatile* (Vecchioni et al. 2017), with eastern Mediterranean affinities, and *Atyaephyra desmarestii* (Christodoulou et al. 2012), widely distributed in the western Mediterranean, demonstrating the importance of recent, Quaternary connections between Peninsular Italy and the islands.

Regarding the Pelagian Islands, no old relict species are known up to now with the presence of only two species, *Rhipidogammarus rhipidiophorus* and *Pseudoniphargus adriaticus* s.l. (Ruffo & Stoch 2006), of a confirmed alleged marine origin; *P. adriaticus* s.l. is also present on Linosa, which was never connected to the mainland. These scarce data are insufficient to hypothesize any biogeographical scenario for this small archipelago.

1.3.5 Adriatic Islands

Geographical setting

The Adriatic Sea is located in the northernmost part of the Mediterranean sea. The basin is mostly shallow, elongated and enclosed by the mountain ranges of the Dinarides (Dinaric Alps) from the east and the Apennines from the west. Therefore it is the most continental basin of the Mediterranean Region after the Black Sea. The Adriatic Islands form the second largest archipelago of the Mediterranean Sea. The archipelago is composed of over 1,300 island and islets, with the majority of them (over 1,200) located in the northeastern Adriatic and belonging to Croatia. The largest islands of the Adriatic sea are Cres and Krk. These two islands are almost identical in size, each covering ca. 405 km^2 (Duplančić Leder et al. 2004). The islands located along the Croatian and Montenegrin coasts are of karstic character, composed of carbonate rocks, limestones and dolomites of Lower and Upper Cretaceous origins. The karstic running waters on these islands are characterised by water loss along their course or even by complete sinking underground, as well as by frequent seawater intrusions producing brackish conditions (Bonacci 2014). This geological feature combined with Mediterranean climate and relatively small size of the islands results in the scarcity of the epigean freshwater habitats. Only sparse springs, especially where marly arenaceous rocks meet limestones (e.g. on Krk island: Lončarić et al. 2011) and few temporary karstic lakes, together with a large number of pools and man-made ponds, can be found. The biggest natural lake on the islands is Lake Vrana (Vransko) on island Cres with surface 5,75 km^2. It is the only source of drinking water for the island (Katalinic et al. 2008). While the surface waters are scarce, the islands, similarly to the mainland of the Dinardies, are known to harbour considerable reserves of groundwater (Trincardi et al. 1996, Bonacci & Roje-Bonacci 2003, Duplančić Leder et al. 2004, McKinney 2007).

Malacostracan Fauna

The order Amphipoda is represented by 20 species present on the Adriatic Islands. Among them, two members of Gammaridae can be found both in fresh and brackish waters: *Echinogammarus pungens* and *E. veneris*. The latter was recorded from springs on the islands of Rab and Hvar (Karaman 1969; Karaman & Sket 1989). *E. pungens* was recorded from the springs on the islands of Cres and Hvar as well as in the streams of the Krk, Rab and Šipan islands (Karaman 1969, Zganec 2009). *Gammarus balcanicus* Schäferna, 1923 was reported from Krk Island by Sket (1988). Its presence on Krk was more recently confirmed by Zganec (2009), Mamos (2015) and Mamos et al. (2016) analysed molecular diversity of *G. balcanicus* Schäferna, 1923 in its European range, including the population from Krk, and proving it to be a complex of deeply divergent phylogenetic lineages, possibly representing a plethora of cryptic species. Therefore, the population from Krk should be addressed as *G. balcanicus* sensu lato and awaits revision of its taxonomic status. Finally, the gammarid *Rhipidogammarus karamani* is reported from coastal springs in Pag island (Sket 1988). The sole member of the family Crangonyctidae Bousfield, 1973 present on the Adriatic islands is

Synurella ambulans (F. Müller, 1846). First report of this species, under the name *Gammarus recurves* Grube (1861) came from the Vransko lake on the island of Cres (Grube 1861). Its presence on Cres island was confirmed by Karaman (1952), Karaman (1974) and Sket (1988). The species was also reported by Sket (1988) from the Pag island. Taking into account the wide distribution of *S. ambulans* (Ponto-Caspian region, Central and Eastern Europe) and the complex taxonomy manifested with the presence of numerous subspecies and local forms, *S. ambulans* can probably be considered as a species complex (Sidorov & Palatov 2012 and references therein). The most speciose amphipod family from the Adriatic Islands is the hypogean family of Niphargidae. There are 13 species reported from the Islands with five of them being endemics (Table S1, Karaman 1931; Karaman & Sket 1989; Sket & Karaman 1990; Fišer et al. 2007). Such diverse subterranean fauna is undoubtedly a result of the karstic character of the archipelago, creating an abundance of suitable groundwater habitats, even on the smaller islands (Bonacci & Roje-Bonacci 2003). Due to their narrow distribution ranges and habitat specialisation, five species of niphargids are classified as endangered (Ozimec & al. 2009): *N. hebereri* Schellenberg, 1933, *N. hvarensis* S. Karaman, 1952, *N. miljeticus* Straškraba, 1959, *N. pectencoronatae* Sket & G. Karaman, 1990 with *N. jadranko* Sket & G. Karaman, 1990 being critically endangered. Other hypogean amphipods from the Adriatic Islands are: *Salentinella angelieri* sensu lato (Salentinellidae), a species complex under revision (Stoch, pers. comm.), reported from an anchihaline cave on the Gangarol island of the Kornati Archipelago (Sket & Karaman 1990, Karaman 2011) and *Hadzia fragilis* S. Karaman, 1932 recorded from several islands and islets of the Kornati archipelago (Sket & Karaman 1990, Gottstein & al 2007), Krk, Cres and Lošinj (Karaman 2011 and authors cited therein).

Isopods are another malacostracan order well represented in fresh waters of the Adriatic Islands. Surface waters are inhabited by the family Asellidae, with *Asellus aquaticus* (Linnaeus, 1758) present only in Vransko jezero on the island of Cres (Sket 1988) and the *Proasellus coxalis* species complex widespread in several islands (Krk, Cres, Rab, Pag, and Molat: Sket 1988). Three stygobitic families were found in islands groundwaters. Sphaeromatidae are represented by *Monolistra pretneri* Sket, 1964*;* the subspecies *M. pretneri spinulosa* Sket 1965 was described from karstic springs in the southern part of the Cres island, later synonymized with the nominotypical species based on morphological and molecular analyses (Prevorčnik et al. 2010). Cirolanidae are present in the central and southern Adriatic islands with *Sphaeromides virei virei* (Brian, 1923) and *S. virei mediodalmatina* Sket, 1964 (Delić & Sket 2015). Finally, Sket (1988) reported an uknown representative of the genus *Microcharon* Karaman, 1934 (family Lepidocharontidae Galassi & Bruce, 2016) from the hyporheos of a small brook on the island of Krk.

The sole member of Thermosbaenacea from the Adriatic islands is the stygobitic *Tethysbaena halophila* (S.L. Karaman, 1953) (family Monodellidae Taramelli, 1954) recorded in anchihaline caves on the Korcula island and on the Kornati archipelago (Wagner 1994); it was previously reported also from Lošinj island (Sket 1988).

Finally, Decapoda are present on Adriatic islands with two families. Surface small streams of Krk and Vransko jezero on Cres islands are inhabited (Sket 1988) by *Austropotamobius fulcisianus* (family Astacidae). The species was later reported as *A. pallipes* by Maguire et al. (2003)., however, following Clavero et al. (2015) the populations of the area should be attributed to the subspecies *A. italicus carsicus* Karaman, 1962 a junior synonym of *A. fulcisianus orientalis* Karaman, 1929 following Crandall & de Grave, 2017. Karstic groudwaters host a stygobitic representative of the family Atyidae, *Troglocaris anophthalmus periadriaticus* Jugovic, Jalžić, Prevorčnik & Sket, 2012, reported by Jugovic et al. (2012) from the islands Ugljan and Brač; this subspecies is also widely distributed along the mainland Croatian coastal areas.

Geological history and malacostracan biogeography

The present shape of the Adriatic basin is a consequence of long and complex geological history. In Paleogene (66-23 Ma), the Adriatic Plate (aka Apulian Plate) decoupled from the African Plate and moved north, colliding with the Eurasian Plate, contributing to the Alpine orogeny that resulted in the uplift of the Alps, Dinarides and Hellenides. It led also to closing of the Tethys Ocean and formation of the north-eastern border of the Adriatic basin. In Early Neogene (ca. 20 Ma), movement of the Adriatic Plate led to the uplift of the Apennine Mts., establishing the western borders of the Adriatic basin, still, leaving the basin largely open in the south (Devoti et al. 2002, Pinter et al. 2016 and references therein) until Messinian (5.7-5.3 Ma). During the Messinian Salinity Crisis, the region equivalent to the present Adriatic basin almost completely evaporated leaving several small hypersaline waterbodies and probably only a scarce hydrological network was existing in the area. Such conditions changed in the Lago Mare episode at the end of Messinian, when the fresh/brackish waters of Paratethys flushed in to the Proto-Mediterranean See, partially refiling also the Adriatic basin. At ca. 5 Ma, with re-opening of the Gibraltar Strait, the Messinian ended and marine conditions re-established in the whole Mediterranean basin including the Adriatic sea (Orszag-Sperber 2006, Popov et al. 2004, 2006, Rögl 1999 and references therein). In

Pleistocene, major glaciation events caused recurrent eustatic sea level regressions in the Mediterranean and rearrangement of local hydrological networks. For example, it is evidenced that during the Last Glacial Maximum, the extended of Po River (aka Mega-Po), unified most of the Periadriatic hydrological networks (i.e. eastern Appenines, southern Alps and western Dinarides) within one huge river system. In Holocene (ca. 11 Ka) the Mega-Po valley was flooded due to the rise of sea level leading to formation of the current north Adriatic basin and isolation of the Adriatic islands from the Balkan Peninsula (Correggiari & al 1996, Colantoni and al. 1979, Stanley and Wezel 1985).

The geological history shows that colonization of the inland waters (including the those of the future islands) could start already in Eocene by the thalassoid malacostracans of Tethyan and Paratethyan origin. However, so far, studies employing molecular markers show rather younger patterns of colonisation and diversification. The most diverse crustacean group and probably one of the first inhabitants of the freshwater systems in the Adriatic region are Niphargidae. Molecular studies revealed that this group colonised the Periadriatic region in Late Oligocene (ca. 25 Ma) from the northern parts of Europe via land connection and hydrological networks developed during the Alpine orogeny (McInerney et al. 2016). Diversification of this hypogean group led to great diversification of species that survived through the dramatic geological and hydrological changes of Adriatic region in relatively stable underground habitats. Between 20 Ma and 10 Ma, in Miocene, freshwaters of the eastern Periadriatic were colonised by gammarids of Paratethyan (e.g. *Gammarus balcanicus* complex) and Tethyan (e.g. *Gammarus roeselii* Gervais, 1835 complex, *Echinogammarus* spp.) origins (Hou et al. 2013, Hou & Sket 2016, Mamos et al 2016, Grabowski et al. 2017b). So far, two species of *Echinogammarus*, presumably of Tethyan origin, and only one Paratethyan *Gammarus balcanicus* lineage was found on the islands. The population of *G. balcanicus* on Krk island diverged from the continental clade at ca. 7 Ma, in Messinian, possibly in result of isolation by hypersaline or arid environments during the salinity crisis (Krijgsman et al. 1999). Changes in this geological period could also influence diversification of isopods what is shown in the divergence time frame of the *A. aquaticus* complex in the western Periadristic; however no molecular data is available from the insular populations (Sworobowicz et al. 2015). During Lago Mare episode (5.3 Ma), due to oligohaline environment connecting the hydrological systems on both sides of the Periadriatic, freshwater hydrobionts had a possibility to migrate between the continent and the Adriatic Islands. However, molecular data suggest that most of such colonisations occurred as late as Pleistocene. Particularly, the most recent terrestrialization of almost entire Periadriatic region and formation of Mega-Po river connected river systems of Balkan and Apennine peninsulas (ca. ca.18 ka) opened a wide possibility for exchange of freshwater fauna. It can assumed that this opportunity could be used by species that show low molecular variability in the region, such as the genus *Austropotamobius* (Trontelj et al. 2005). Patterns of this exchange for malcostracan are currently studied using molecular approach (Grabowski et al 2017c). This opportunity ended with Holocene and rise of the sea level. Interestingly, so far no invasive or alien species freshwater Malacostraca were found on the Adriatic Island.

1.3.6 Crete, Aegean and Ionian Islands

Geographical setting

The Aegean Sea is one of four major basins of the East Mediterranean and covers an area of approximately 240,000 km^2. It stands in the center of the conjunction of three continents, Europe, Asia, and Africa and is situated between the coasts of mainland Greece, the coasts of Crete and Asia Minor. Around 7,500 islands and islets occur at a variety of isolation levels and topographic features placing the Aegean Sea amongst the archipelagos with the highest number of the islands worldwide (Triantis and Mylonas 2009; Poulakakis et al. 2015). Geographers and geologists have divided the Aegean Sea in three parts following the morphology of the coasts, the position of the islands and the formation of the seabed: North, Central and South Aegean. The South Aegean is considered the most important part of the Aegean Sea, since it comprises the majority of the islands, including the two big island complexes, Cyclades and the Dodecanese.

Crete is the fifth largest island of the Mediterranean, with a surface area of 8,261 km^2, and forms the border between the Aegean and Libyan seas. The island extents 260 km from west to east and its width varies from 12 to 60 km. Its coastline has a total length of 1,065 km and consists of both sandy beaches and rocky shores. The island is highly mountainous (52% of the Cretan land) and has four great mountain ranges; White Mountains (Lefka Ori), Psiloritis (Ida),

Dikti (Lasithian Mountains), Thryptis (Mountains of Siteia), with numerous peaks exceeding 2,000 m.a.s.l. Crete is gifted with a plethora of caves, gorges and plateaus and it has been estimated that around 5,200 caves and karst formations, rich in underground waters exist on the island (Fassoulas et al. 2007; Fassoulas, 2017), thus creating ideal habitats for subterranean animals.

The Ionian Islands are located in the Ionian Sea at the westernmost part of Greece and include the islands of Kerkyra (Corfu), Paxoi, Lefkada (Lefkas), Cephalonia (Kefallinia), Ithaki

(Ithaca), Zakynthos (Zante) and Kythera. They are traditionally called Heptanese or "the Seven Islands" but the group includes many smaller islands covering a total area of 2,318 km^2. The relief of the majority of the islands is mainly mountainous or semi-mountainous. The largest of the Ionian Islands is Cephalonia, covering a third of an area with 779.3 km^2.

The climate of the Aegean Sea islands (including Crete) is typical dry Mediterranean. The average yearly precipitation on Crete is about 900 mm, which correlates to approximately 7500 hm^3, while for the Aegean Islands the corresponding quantities are 585 mm and 5192 hm^3, respectively (Gikas, & Angelakis, 2009). However, less than 15% of the precipitation percolates through the ground in both the regions. Evapotranspiration and surface runoff to the sea accounts for 65% and 21%, respectively, for Crete, and 60% and 26%, respectively, for the Aegean Islands (Gikas, & Angelakis, 2009). The salinity of rainfall in the Aegean is high, due to airborne sea spray which has a strong effect on the chemical composition of the groundwater of the islands. During the last decades, Crete and many islands of the Aegean are subjected to increased water demands, due to tourism development and intense irrigation, resulting to surface as well as groundwater degradation and depletion which threaten aquatic biodiversity.

Natural water resources and water balance issues are of great importance for the Ionian Islands as well, due to their growing population, urbanization, and tourism development (Diamantopoulou and Voudouris 2008). As a result, the annual water demands are steadily growing and the groundwater abstraction during summer is estimated to further increase. Precipitation levels are higher in the Ionian Islands with mean annual precipitation of 1038 mm according to World Clim-Global climate data (http://www.worldclim.org).

Malacostracan fauna

Up to today, sixty-five malacostracan species have been recorded from the area in question: 19 from Crete, 27 from the Aegean Islands and 20 from the Ionian Islands. Based on the current knowledge, the malacostracan fauna greatly differs between the Ionian and Aegean islands, while Crete shares some only few common species with the Aegean islands. Out of the 20 Ionian malacostracan species, nine (45%) are stygobionts and eight (40%) are endemics; a similar situation is observed for Crete with nine (47%) stygobionts and ten (52%) endemics and the Aegean with 14 (51%) stygobionts and six (22%) endemics. Despite the significantly greater number of islands in the Aegean, the species richness is not markedly higher than in the Ionian Islands and Crete, as it would have been expected. It might be due to the fact that the malacostraca fauna of the Aegean Islands seem to be less explored compared to the Ionian Islands and Crete. Thus, one may expect that the number of malacostracan species in the Aegean islands is underestimated and may rise in the foreseeable future.

Amphipods are present on these islands with seven families. Four species of Bogidiellidae (one of them, *Medigidiella minotaurus* (Ruffo, 1976), endemic to Crete) are present, linked both to interstitial and subterranean karstic waters with all of them being freshwater stygobionts, except of *Medigidiella chappuisi* (Ruffo, 1952) that lives in interstitial anchihaline waters (Ruffo & Delamare Deboutteville, 1952). The current number of Gammaridae species known from the islands is relatively poor (7 species), compared to other families (e.g. Niphargidae), with one endemic from the Ionian (*Echinogammarus kerkuraios* Pinkster, 1993), two from Crete (*Echinogammarus platvoeti* Pinkster, 1993, *Gammarus plaitisi* Hupało, Mamos, Wrzesińska & Grabowski, 2018) and only one *Gammarus* representative from the Aegean (*Gammarus uludagi* G. Karaman, 1975) (Karaman, & Pinkster, 1977; Pinkster, 1993; Hupało et al., 2018). Hadziidae S. Karaman, 1943 is represented in Greece only by *Metahadzia helladis* Pesce, 1980, an endemic of Cephalonia (Pesce, 1980). The Ingolfiellidae, a subterranean family of marine origin, is also represented by one species, *Ingolfiella petkovskii* S. Karaman, 1957 that in Greece has been recorded only from Keramou, Euboea Island (Bou 1970). In consequence of complex geological history, geographical position, climate and hydromorphological conditions, the subterranean amphipod fauna in Greece is very rich with a high number of endemics. Many taxa are restricted to relatively small distribution areas, typically to springs, wells, caves and groundwater pools. Within the family Niphargidae, three genera are known from Greece: *Niphargus* Schiödte, 1849, *Exniphargus* G. Karaman 2016b and *Niphargobatoides* Karaman 2016b; the last two being endemic. The genus *Niphargus* is currently represented by 22 species from continental Greece and the Greek islands (Pesce, & Maggi, 1983; Ntakis et al., 2015; Karaman, 2015, 2016a 2016b, 2017). Fourteen of them occur in the Ionian, Aegean Islands and Crete, of which 75% are endemic. Sket (1990a) described *Niphargobates lefkodemonaki* from Lefka Ori in the west of Crete; recently Karaman (2016b) transferred the species to the genus *Niphargobatoides*. The majority of the Cretean niphargids have been discovered recently by Karaman (2016a), who described *Exniphargus tzanisi* from the area explored previously by Sket (1990) and *Niphargus impexus* from a location near Heraklion (Karaman, 2016a). Soon after, Karaman (2017a) described *Niphargus lakusici* from Pyrgos area. Another recently discovered species is *N. zarosiensis*, described from Lake Zaros (aka Limni Votomos) at the southern foothills of Psiloritis Mt (Zettler & Zettler, 2017). The Aegean Islands are colonized by five niphargids, three of which are endemic: *Niphargus* *spasenijae* G. Karaman, 2015 from the Thasos Island, *Niphargus lourensis skiroci* G. Karaman, 2018 from Skyros, and *Niphargus rhodi* S. Karaman, 1950 from Rhodes (Karaman 2015, 2016a, 2018). The remaining two niphargids from the Aegean Islands are *Niphargus adei* S.Karaman, 1934 that is endemic for Samothrace (Samothraki), and *Niphargus jovanovici* S. Karaman, 1931 recorded from two wells of Amarinthos, Euboea (Karaman 2015, 2017b). In the Ionian Islands, four species have been found; *Niphargus denarius* G. Karaman, 2017 endemic for Cephalonia (Karaman 2017c), *Niphargus* cf. *lourensis* Fišer, Trontelj & Sket, 2006, also from Cephalonia (originally described as *Niphargus orcinus* Fišer, Trontelj & Sket, 2006), *Niphargus skopljensis* S.Karaman, 1929 from Lefkada and *Niphargus versluysi* S. Karaman, 1950 from Zakynthos (Karaman 2015, 2017d). The small stygobitic family Salentinellidae is represented by a single species complex - *Salentinella angelieri* sensu lato, present on both Ionian and Aegean archipelagos (Pesce et al., 1979; Pesce, 1985). Similarly, the family Talitridae, that mainly includes marine or semi-terrestrial species, is represented by one freshwater species, *Cryptorchestia ruffoi* Latella & Vonk, 2017, endemic to Rhodes; collected from a freshwater spring on Monte Smith, and in the streams flowing out of the Epta Pyges springs (Davolos et al. 2017).

The order Isopoda is present on the studied islands with three families, Asellidae, Cirolanidae and Microparasellidae, including both epigean and groundwater species. Asellidae show high endemism in the Ionian, Aegean islands and Crete. The genus *Proasellus* is present in springs, caves and interstitial waters with eight species, six of which are endemic (Strouhal, 1966; Henry, 1975; Argano, & Pesce, 1979; Pesce, & Argano, 1980). The genus *Proasellus* is relatively speciose in the Ionian Islands with four endemics; *Proasellus coxalis cephallenus* (Strouhal, 1942), *P. coxalis corcyraeus* (Strouhal, 1942), *P. coxalis leucadius* (Strouhal, 1942) and *P. coxalis versluysi* (Strouhal, 1966), occuring on the islands of Cephalonia, Kerkyra, Lefkada and Zakynthos, respectively (Strouhal, 1966). In Crete, two subterranean endemics, *Proasellus cretensis* Pesce & Argano, 1980 and *Proasellus minoicus* Pesce & Argano, 1980, are present, while on the Aegean Islands, only one stygobiont, *Proasellus sketi* Henry, 1975, has been recorded from Euboea and the epigean *Proasellus coxalis s.l.* from Rhodes (Pesce at al., 1979; Pesce, & Argano, 1980). Arcangeli (1942) reported *Asellus (Proasellus) coxalis rhodiensis* from Rhodes, unfortunately without providing a description, so it remains as “nomen nudum”. Thus, the taxonomic status of epigean *Proasellus* from the island stays unknown. *Asellus aquaticus* has been reported from Crete by Chappuis (1949) and its presence was, in 2015, confirmed by Wysocka (unpublished data). The former author, suggested that the presence of *A. aquaticus* on Crete, may have occurred due to human-mediated introduction in the historical times and that humans played a key role in the island colonisation by this isopod. This seems to be plausible in light of the undergoing molecular research (Wysocka Sworobowicz & Grabowski, unpublished results). Perhaps the population of *A. aquaticus* in the cave of Zoodochos Pigi, Santorini Island (Verovnik et al. 2004) has the same origin (Gasparo 2009). Recently, Sworobowicz et al. (2015) discovered that *A. aquaticus* on this island is represented by two divergent lineages, one of them being possibly a local endemic and the other one widespread in Thrace and some other parts of the Balkans.

The genus *Turcolana*, of Cirolanidae, occurs in the freshwater and brackish groundwater environments around the eastern Mediterranean (Argano & Pesce, 1980). In the studied islands, only the endemic stygobitic *Turcolana rhodica Botosaneanu,* Boutin & Henry, 1985 has been discovered so far in the springs of Gadouras River on Rhodes (Botosaneanu et al. 1985). Finally, the minute representatives of the stygobitic family Lepidocharontidae are represented by five species of the genus *Microcharon*. Four of them have been recorded from the Aegean Islands and one from the Ionian Islands; none has been reported from Crete (Argano, & Pesce, 1979; Pesce 1981; Galassi et al., 1994). One species, *Microcharon hellenae* Chappuis & Delamare-Deboutteville, 1954, is endemic to the Greek mainland and Sporades. Within the family Microparasellidae, two representatives occur in Greece; *Microparasellus hellenicus* Argano & Pesce, 1979 and *Microparasellus puteanus* Karaman, 1933 from Cephalonia (Argano, & Pesce, 1979).

The order Decapoda is represented in the studied islands by the members of three families, Potamidae, Palaemonidae and Atyidae. The freshwater crabs from genus *Potamon* Savigny, 1816 (Potamidae) are most speciose with six species (Jesse et al. 2011). Genetic studies on this genus demonstrate high genetic diversity and the presence of cryptic lineages among previously identified species (Jesse et al., 2010, 2011). *Potamon hippocratis* Ghigi, 1929 inhabits the Aegean islands of Kos, Samos, Ikaria and Naxos but is also present on Crete. *Potamon rhodium* Parisi, 1913 occurs on Rhodes and Tilos and is also known to occur on southern Turkey (Özbek & Ustaoğlu 2006). *Potamon karpathos* Giavarini, 1934 was described from Karpathos, but it is distributed all along the southern Anatolian coast. *Potamon kretaion* Giavarini, 1934 is endemic to Crete, whereas *Potamon ibericum* (de Bieberstein, 1808) occurs in Chios and Lesbos (Jesse et al., 2011). *Potamon ibericum* has a relatively wide distribution range, with its easternmost limit in river systems draining into the southern Caspian Sea and extending to the northern Aegean Sea in the west (Brandis et al., 2000). Recently, the westernmost populations in the northern Aegean Sea, specifically in Chalikidiki, Thasos and Samothrace have been distinguished as a separately evolving lineage representing possibly a new, yet undescribed, species (Jesse et al., 2011). The widespread *P. fluviatile* is found on Euboea and Sporades. On the Ionian Islands, only *Potamon fluviatile* is present, occurring on Kerkyra and mainland Greece (Jesse et al. 2011). Nine freshwater shrimp species of Palaemonidae occur in the circum-Mediterranean area, with three of them being present on the studied islands; *Palaemon antennarius* reported from the Ionian Islands of Kerkyra and Zakynthos, *Palaemon minos* Tzomos & Koukouras, 2015 being endemic to Crete, and *Palaemon colossus* Tzomos & Koukouras, 2015 occuring in Rhodes and in the Antalya region of Turkey (Tzomos & Koukouras 2015). One species of the Atyidae family, *Atyaephyra thyamisensis* Christodoulou, Antoniou, Magoulas & Koukouras, 2012, has been recorded on the Ionian Islands Kerkyra and Lefkada (Christodoulou et al. 2012).

Geological history and malacostracan biogeography

The geological history of the Aegean started in Neogene, when the landmass of Aegeis emerged for the first time (23–12 Ma) (Meulenkamp 1971). The fragmentation of the Aegeis started from the collision of the African tectonic plate with the Eurasian plate in the Middle Miocene (ca. 16 Ma; Krijgsman, 2002; Steininger and Rogl, 1984) and the formation of the Mid-Aegean Trench (12–9 Ma; Dermitzakis and Papanikolaou, 1981). The subsequent sea expansion and the continent compartmentalization took place, dividing the former landmass into large number of islands of various sizes, which were connected and isolated repeatedly during the Messinian Salinity Crisis (5.96–5.33 Ma), when several desiccation events in the Mediterranean occurred (Krijgsman et al. 1999). Afterwards, some of the connections with the mainland were temporarily reestablished during the Pleistocene (2.58 Ma–11.70 ka), due to eustatic sea-level changes (Perissoratis and Conispoliatis, 2003). However, there is a strong evidence suggesting that during Pleistocene, the Cyclades were isolated and that a wide sea barrier existed between them, Crete and the Dodecanese (Dermitzakis, 1987; Dermitzakis 1990). The isolation of Cyclades started already at the end of Pliocene, while the Dodecanese, being much closer to Asia Minor and have remained as such only for the last few thousand years (Perissoratis and Conispoliatis, 2003). Intensive volcanic activity has also contributed to the formation of several islands; a few of which, such as Milos, Nisyros, Kimolos and Santorini, are purely volcanic (Francalanci et al., 2005).

Up to the Vallesian period of the Late Miocene (9 Ma), Crete was connected to the mainland of the Balkan Peninsula and Asia Minor, as shown by fossil remains of mainland fauna (van der Geer et al. 2006). Around 12 Ma, the split of the Balkan Peninsula (including Crete and Peloponnesus) from Asia Minor began. About 11-8 Ma, the isolation of Crete from

Peloponnesus started, due to the rise of sea levels. Later, between 5.96 and 5.33 million years ago, the desiccation of the Mediterranean Sea during the Messinian Salinity Crisis led to the formation of hypersaline deserts and semi-deserts around Crete and other islands, and this is the last known land connection between Crete and the mainland (Poulakakis et al. 2015). During the Pliocene, Crete was divided temporarily into at least four islands due to sea level rise associated with the Zanclean flood (Sondaar & Dermitzakis 1982). At the end of the Pliocene or in the Early Pleistocene, Crete gained its present configuration (Sondaar, et al., 1986).

Because of its complex paleogeographical history, high biodiversity and endemism, the Aegean archipelago as well as Crete have become key areas for the biogeographical studies (Sfenthourakis & Triantis 2017), especially upon the terrestrial taxa (Heller 1976; Sfenthourakis 1996; Fattorini 2002; Poulakakis et al. 2005; Jesse at al. 2009, 2011). They showed that the distribution of the Aegean species often mirrors palaeogeographical patterns and processes. During the last decades, the phylogeny of several freshwater malacostracan species has been addressed. For example, the divergence of the recently discovered endemic species, *Gammarus plaitisi,* from Crete from its continental relatives occurred most probably in the late Miocene, around 9.2 Ma, possibly due to the subsequent isolation of Crete from the mainland happening during the same time (Hupało et al. 2018). Although the molecular phylogenetic studies are still under way (unpublished data), it may be possible that the amphipod genera *Gammarus* and *Niphargus* followed this colonization pathway from the peninsular Greece, while it remains still uncertain if Asia Minor affinities exist within Malacostraca of the region. On the other side, the evolutionary history of insular *Echinogammarus* is apparently very different, presumable with direct Tethyan origins (Hou et al. 2013, Hou & Sket 2016).

Jesse et al. (2011) showed that the genus *Potamon* has no insular endemics in the Aegean archipelago, with exception of *P. kretaion* on Crete, indicating that the Pleistocene terrestrial connections, with some human-mediated dispersal in the historical times (e.g. in case of *P. hippocratis*), might have enabled dispersal within the Aegean area. In fact, most probably, the diversification of *Potamon* species happened during the Late Pliocene and Early Pleistocene in the eastern Aegean region. This coincides with climatic fluctuations and increased aridity in Anatolia, thus hypothesizing a correlation between these climatic oscillations and speciation events (Jesse et al. 2011). Most of the present distributional patterns of Malacostraca in the studied islands are possibly related to the Pleistocene glacial cycles, following similar patterns observed in the other taxa, such as reptiles, amphibians and beetles as well as the terrestrial malacostracans (Triantis & Mylonas 2009). The Pleistocene climatic fluctuations repeatedly caused invasions and retreats of numerous species. Several genera endemic to the Aegean area either went extinct during glaciations or, at least some of them, were able to survive in extreme habitats such as caves and the subalpine mountainous areas with isolated springs (Triantis & Mylonas 2009).

On the other hand, the geological evolution of the Ionian Islands was comparatively simpler if compared to the Aegean, with most islands becoming isolated from the western part of continental Greece during the Pleistocene or even in Holocene (Sakellariou & Galanidou 2015). The formation of the Ionian Islands took place only in the Quaternary, as a result of intense compressive tectonism and uplift, which started in the Lower Pliocene (Evelpidou 2012). Most of the islands of the Ionian archipelago were connected to each other and to the mainland during LGM (ca. 26.5 ka) and the earlier glaciations of the late Pleistocene resulting in lowered sea-level (Lykousis, 2009; Sakellariou & Galanidou, 2015). Ferentinos et al. (2012) who reconstructed the paleo-coastline of this area for the LGM (sea level at -120 m bpsl), showed that the islands of Cephalonia, Ithaki and Zakythos were connected to each other and separated from Lefkada by a narrow strait. The latter was connected to the mainland and to most of the smaller islets in the east (i.e. Meganissi, Skorpios, etc.). Lefkada is the only one of the Ionian Islands that was separated from the mainland throughout the Pleistocene. Kerkira was suggested to be connected to the mainland in early Holocene, about 8,000 years ago, when the sea level was lower (Perissoratis & Conispoliatis 2003).

While the history of biogeographic studies upon insular fauna in the Aegean basin is long, the similar studies in the Ionian Islands are limited, with the two island archipelagos rarely being compared. The biota of the Ionian Islands is very similar to those of the adjacent mainland, however, several endemic taxa are found, most of which live on the larger and more heterogeneous islands, such as Kerkyra and Cephalonia (Strouhal, 1966; Pesce 1980; Pinkster 1993; Karaman 2017c).

1.3.7 Marmara islands

Geographical setting

The Sea of Marmara is the northeasternmost part of the Mediterranean Basin. It is also the world smallest inner-continental sea (over 11.000 km^2), entirely located within the borders of Turkey. The Marmara basin is elongated in the east-west direction with a length of 275 km and width of ca. 80 km. From the east, it is connected with the Aegean Sea through the Strait of the Dardanelles and from the northeast with the Black Sea through the Bosporus strait. The water of the Marmara Sea has particular composition with the less saline upper water layer coming from the mesohaline Black Sea, while the lower water layer is of Aegean origin with the fully marine salinity. These layers do not intermix, but form a halocline at a depth of 25 m, what influence the faunal composition in the seashores of the Marmara Islands. The literature concerning the islands of the Sea of Marmara is scarce and usually in Turkish language. There are over 40 islands and islets on the sea. The Marmara archipelago is the only one from which freshwater malacostracans were recorded, and it is located in the southwestern part of the sea. The archipelago consists of 21 islands, of which the Marmara Island is the biggest, covering 118.9 km^2. It is mostly montaneous with the highest point of 700 m.a.s.l. There are only five villages, with a total human population being 6000. The climate is dry Mediterranean and the island has very poor surface water resources with few small rivers, streams and streams. Vegetation cover consists mostly of Mediterranean forest trees, maquis and frigana. The other islands of the Marmara archipelago are very similar in character (Akyol & al 2009, Bulut 2016, Ayfer & al 2017, Beşiktepe & al. 1994, Görür al. 1997 Wong & al 1995).

Malacostracan Fauna

While even the marine fauna of the seashores of the Marmara Islands is scarcely known (e.g. Ayfer & al 2017), there is only one study upon its fresh waters. Özbek et al. (2015) revealed the occurrence of two gammarid amphipods: *Gammarus uludagi* G.S. Karaman, 1975 and *Gammarus pulex* (Linnaeus, 1758) on the Marmara Island and on the neighboring (10 km distance) Kapıdağ peninsula. Both species were previously recorded from the mainland of Turkey (G.S. Karaman, 1975; Özbek, 2011). While *G. pulex* is widely distributed in Europe and is probably a complex of cryptic species (Hupało et al. 2018), *G. uludagi* was reported only from Turkey and from the adjacent Aegean Islands (Karaman & Pinkster, 1977; Özbek et al. 2017). Even if some widely distributed malacostracan species, such as *Potamon fluviatile* or *P. ibericum*, occur in the region there is a lack of records from the islands of the Marmara Sea (e.g. Güner 2009). Similarly, there are no data upon the subterranean fauna of the islands.

Geological history and malacostracan biogeography

The history of the Marmara Sea began in the middle Miocene (ca. 12 Ma) when the region was overflown by fully saline waters of the Proto-Mediterranean Sea. In Late Miocene (ca. 8.5 Ma) Paratethys waters prevailed with oligohaline conditions. Such conditions dominanated in the present Marmara Sea until the end of Pliocene (ca. 3 Ma) when another intrusion of waters from the Eastern Mediterranean Basin happened. In that period the connection between Paratethys and Mediterranean ceased what, with time, led to formation of an isolated basin that eventually turned into the present Black Sea. During Pleistocene, the connection was re-established and due to eustatic sea level changes, the salinity of water was greatly fluctuating in the present Marmara Basin (Görür et al. 1997 and references therein, Popov et al. 2004). During the last glaciation (ca. 115 to 11.7 ka), the Marmara basin was completely isolated both from the Mediterranean and the Black Sea. This isolation event of the Marmara Sea resulted in its transformation into an anoxic brackish lake (Görür et al. 1997). At the end of glaciation, the Mediterranean Sea once more intruded into the Marmara and ca. 7 ka broke through strait Bosporus overflowing the isolated and thus sweetish Black Sea. This led, eventually, to establishing the presently known dual flow regime in the Bosphorus Strait and in the Marmara Sea (Ryan et al. 1997). The freshwater fauna could colonize the islands in the brackishwater or lacustrine phases of the Marmara Sea. The Gammaridae recorded on the Marmara Island are presumably of the Paratethyan origins (Hou et al. 2011). The widely distributed *G. pulex* is a species complex occupying lowland and upland river system of Europe and Asia Minor and could colonize the Island waters already in late Miocene. On the other side, *G. uludagi* known only from Anatolia and Thrace could be a recent colonizer. However it is important to state that *G. uludagi* is a sister lineage to *G. pulex* (Hou et al. 2011) and is difficult to draw conclusions since no molecular studies were performed on the island population of the two species. It is important to emphasize the lack of knowledge on the hydrological regime and the crustaceans from the Marmara islands.

1.3.8 **Cyprus**

Geographical setting

Cyprus is located in the eastern part of Mediterranean Sea and it is as distant as 75 km south of Turkey and 100 km west of Syria. The island covers an area of 9,251 km^2 which makes it third largest island in the Mediterranean basin. It stretches 230 km in length and is up to 100 km wide. The shoreline is well developed, rocky and cliff-like in the northern part, while the southern one is more flat with numerous sandy beaches. Total length of the coastline is about 671 km (World Resource Institute).

Geomorphology of Cyprus consists of three main units. The northern part of the island is dominated by the ca. 160 km long Kyrenia Mountain range. It is primarily built of limestones and, occasionally, marbles, with the highest peak of Pentadaktylos (1,024 m.a.s.l.). The

Troodos Mountains are located in the southern and western part of the island, covering around half of its area. This massif is made of basalts, granits and gabbros originated as a part of the oceanic crust. The highest peak is Mt. Olympus (1,952 m.a.s.l.). The two aforementioned mountain ranges are separated by the wide alluvial plain of Mesaoria. Due to deforestation initiated already in the antiquity, much of the Mesaoria is covered with a hardpan (local name “Kafkalla"), formed by compacted calcium carbonate impervious to water.

Generally, freshwater resources are rather scarce on the island. However the surface hydrographic network is quite well developed, thus not even one river or stream on Cyprus is permanent on full length. The main and longest (98 km), yet intermittent, watercourse on Cyprus is the Pedieos River, that originates in the Troodos Mountains and flows, from late autumn till late spring, through the Mesaoria, to the Famagusta Bay. Numerous springs occur in the Troodos Mountains, and the upper parts of streams, above 1000 m.a.s.l., are often permanent. The Mesoaria plain is rich in underground waters, however they are heavily exploited due to agriculture and tourist industry (Charalambous 2001). Natural flows in streams and rivers are also disturbed by dams; their density is one of the highest in Europe (Zogaris et al. 2012).

Malacostracan fauna

Only nine malacostracan species are known from Cyprus, of which two are stygobitic. Comparing the extent of the local hydrological network, the island area and the local diversity of malacostracans to those of the other Mediterranean islands, the crustacean fauna of Cyprus seem to be either very poor or largely understudied. The former seems more likely. Only Amphipoda, Isopoda and Decapoda are represented, with three species from each order.

The sole subterranean representative of amphipods is the endemic *Stygogidiella cypria* (Karaman, 1989) of the Bogidiellidae family. This species was collected and described from a spring, located close to the coast, in Amathus, Limassol. It was also found in a spring located in a tunnel in Neofytos, Pafos (Karaman 1989). Two other amphipod species were described from Cyprus, the gammarid *Echinogammarus veneris*, widely distributed all around the Mediterranean and, most probably, being a species complex, and the semi-terrestrial talitrid *Cryptorchestia cavimana*, formerly presumed to be widely distributed all over Europe but recently identified as a Cyprian endemic (Ruffo et al. 2014, Davolos et al. 2017, 2018). Interestingly, the more recent extensive sampling in streams and springs on the Greek part of

Cyprus bring only records of talitrids (Stoch, unpublished data). Neither further gammarids nor niphargids were found.

Isopoda are represented by two families. Concerning Asellidae, the epigean *Proasellus coxalis s.l.*, widespread in the Mediterranean Region, was reported from springs in Arothes and Kritou Tera (Sket 1990b), while the endemic *Proasellus coxalis nanus* Sket, 1990 was found in a spring in Agia Mavri. The sole representative of Lepidocharontidae, *Microcharon luciae* Sket, 1991, was described from a well in the shallow gravel deposits in Amathous.

Freshwater decapods include three families, each with one species only. The crab *Potamon hippocratis* Ghigi, 1929 (Potamidae) was reported by Lewinsohn and Holthuis (1986), under the name of *Potamon potamios cyprion* Pretzmann, 1962, as Cyprian endemic. However, based on the molecular studies, Jesse et al. (2011) found out that the population from Cyprus belongs to *P. hippocratis*, described originally from the Kos Island, but widely distributed on the Aegean Islands and on the coast of Western Anatolia, more than 300 km from Cyprus. Given the distance and long isolation of Cyprus, such wide and disjunct distribution of the species, used as food source by ancient Greeks, may be attributed to anthropogenic activity (Jesse et al. 2011). The two other decapod species are the alien invasive crayfishes *Pacifastacus leniusculus* (Dana, 1852) (Astacidae) and *Procambarus clarkii* (Cambaridae), introduced probably, respectively, in 1980s and in 1990s (Hobbs et al. 1989; Holdich et al. 1999, Lewis 2002). The further monitoring of these species is required, as in many cases the two invaders pose a serious threat for the native invertebrate fauna in Mediterranean ecosystems (Morpurgo et al. 2010, Vella et al. 2017; Deidun et al. 2018).

Geological history and malacostracan biogeography

According to McCallum and Robertson (1990), the emergence of Cyprus began already in Mesozoic, between 85 and 92 Ma (Harland et al. 1982). On the other hand, Gass (1987) suggested that Mt. Troodos was an volcanic island, that had emerged at the break of Cretaceous and Paleogene periods, whereas Kyrenia Mts were another island or began as a part of the southern Taurus Mts, that originated in Eocene and subsequently split (Cavazza and Wezel 2003). During the Messinian Salinity Crisis (5.96-5.3 Ma) (Krijgsman et al. 2002), when the Mediterranean Sea largely desiccated, the Troodos island and the southern Tauruian-Kyrenian peninsula (present Kyrenia Mts) had land connection (Hsü et al. 1977; Cavazza and Wezel 2003). At the same time, Cyprus remained isolated from the continental mainland by the hypersaline deserts forming the floor of the Miocene death valleys, ca. 3,000 meters below the sea level (Hsü, 1983). Nowadays, together with Crete, Cyprus is among the most isolated Mediterranean Islands (Moores et al., 1984). After the refilling of the Mediterranean Sea with Atlantic waters during the Zanclean Flood, the new island, composed of two formerly disjunct landmasses, stayed isolated from the mainland ever since. It is now separated from the north by the Adana Strait and from the Levant by the Latakia Basin, reaching depths down to 1,000 m. Thus, even during the LGM, when the sea level dropped by ca. 120m, the closest mainland, nowadays the submerged Alexandretta Bay (Gulf of İskenderun), was still separated from Cyprus by ca. 30 km wide sea passage (Hadjisterkotis 2012, and the citations therein).

The widely distributed *Echinogammarus veneris* is reported from the Adriatic coast, the mainland Greece and Peloponnesus, the islands of Corfu, Zakinthos and Crete as well as from the Mediterranean coasts of Turkey, Lebanon and Israel (Pinkster 1993). The ongoing molecular studies points out that it holds a considerable level of cryptic diversity and is rather a complex of deeply divergent and narrowly distributed lineages, that could date back to the times preceding the Messinian Salinity Crisis. The hypogean species of the Bogidiellidae family, represented only by the endemic *Stygogidiella cypria* (G. Karaman, 1989), are probably thalassoid stygobionts of ancient marine origin (Coineau & Boutin 1992; Boutin 1997).

Two or even three of the nine malacostracan species present on Cyprus were introduced by humans, either intentionally or accidentally in the historical times. After the likely introduction of *P. hippocratis* in ancient times, this process is on-going. Given the growing economic and tourist activity in the area and favourable climatic conditions, one may expect that without the proper education and conservation actions, the recent introductions of *Pacifastacus leniusculus* and *Procambarus clarkii* may be soon followed by the arrival of other alien malacostracans, particularly those kept for ornamental purposes, such as the tropical/subtropical freshwater shrimps and crabs.

1.4 Discussion

Diversity and endemism

Up to date, the fresh waters of the Mediterranean islands houses 181 species belonging to five different orders of Malacostraca (Amphipoda, Isopoda, Thermosbaenacea, Bathynellacea, Decapoda), representing 50 genera and 26 families (Fig.1.3; Tab.1.1, Table S1). The Mediterranean insular fresh waters can be considered the malacostracan biodiversity hotspot, given that the islands cover only about 5% of the Mediterranean Region and about 0.2% of the whole Palearctic, but being inhabited by more than 25% of all the malacostracan species

Table 1.1 (right). Summary of described biodiversity of Mediterranean freshwater insular malacostracan fauna, divided by the islands. SAR – Sardinia and circum-Sardinian Islands; COR – Corsica; TUS – Tuscan Archipelago; SIC – Sicily; PEL – Pelagie Islands; MAL – Maltese Islands; ADR – Adriatic Islands; ION – Ionian Islands; AEG – Aegean Islands; CRE – Crete; MAR – Marmara Islands; CYP – Cyprus. *Numbers from TOTAL column is not a sum of the former columns due to the possibility of species overlap between the islands.

Table 1.2 (left). Summary of reported species from each of the Malacostraca order, divided by the islands. The abbreviations follow the pattern described in Table 1.1.

	Islands													**TOTAL***
	BAL	COR	SAR	TUS	SIC	PEL	MAL	ADR	ION	CRE	AEG	MAR	CYP	
Number of species + subspecies	32	20	35	15	21	2	12	29	20	19	27	2	9	181
Proportion of the Med. malacostracan insular fauna	18%	11%	19%	8%	12%	1%	7%	14%	11%	11%	15%	1%	5%	100%
Number of genera	17	15	21	12	14	2	9	15	11	11	12	1	8	51
Number of families	13	13	17	10	12	2	7	12	11	6	11	1	8	27
Number of endemics (proportion)	20 (63%)	4 (20%)	21 (60%)	3 (20%)	12 (57%)	0 (0%)	0 (0%)	6 (21%)	7 (35%)	12 (63%)	6 (22%)	0 (0%)	3 (33%)	93 (52%)
Number of hypogean species (proportion)	20 (63%)	12 (60%)	23 (66%)	11 (73%)	8 (38%)	1 (50%)	0 (0%)	21 (72%)	9 (45%)	10 (52%)	12 (44%)	0 (0%)	2 (22%)	111 (61%)
Number of alien, introduced species (proportion)	1 (3%)	2 (10%)	2 (6%)	0 (0%)	3 (14%)	0 (0%)	5 (42%)	0 (0%)	0 (0%)	1 (5%)	0 (0%)	0 (0%)	2 (22%)	9 (5%)

Malacostracan orders (**proportion of endemics**/aliens)	Islands													**TOTAL* (proportion)**
	BAL	COR	SAR	TUS	SIC	PEL	MAL	ADR	ION	CRE	AEG	MAR	CYP	
Amphipoda	22 (**64%**/0%)	12 (**25%**/0%)	18 (**61%**/0%)	11 (**27%**/0%)	11 (**82%**/0%)	2 (**0%**/0%)	4 (**0%**/0%)	20 (**25%/**0%)	10 (**33%**/0%)	12 (**67%**/0%)	11 (**36%**/0%)	2 (**0%**/0%)	3 (**33%**/0%)	106 – 58% (**58%**/0%)
Isopoda	7 (**71%/**0%)	4 (**25%/**0%)	10 (**70%**/0%)	4 (**0%**/0%)	4 (**50%**/25%)	0	1 (**0%**/0%)	6 (**0%**/0%)	7 (**57%**/0%)	3 (**67%**/33%)	8 (**25%**/0%)	0	3 (**67%**/0%)	47 – 26% (**53%/**4%)
Thermosbaenacea	1 (**100%**/0%)	0	0	0	1 (**100%**/0%)	0	0	1 (**0%**/0%)	0	0	0	0	0	3 – 2% (**67%**/0%)
Bathynellacea	1 (**0%**/0%)	1 (**0%**/0%)	3 (**67%**/0%)	0	0	0	0	0	0	0	0	0	0	4 – 2% (**50%/**0%)
Decapoda	1 (**0%/**100%)	3 (**0%/**67%)	4 (**0%/**50%)	0	5 (**0%/**40%)	0	7 (**0%/**71%)	2 (**50%/**0%)	3 (**0%**/0%)	4 (**50%/**0%)	8 (**0%**/0%)	0	3 (**0%/**67%)	21 – 12% (**14%**/33%)

reported from the region and more than 8% of the Palearctic Malacostraca (Balian et al. 2008, Figueroa et al. 2013). The species composition with respect to the Malacostraca orders seem to mirror the pattern previously observed in the entire Paleartic (Balian et al. 2008) with amphipods and isopods being the most speciose groups (58% and 26% of all freshwater malacostracans, respectively) and Thermosbaenacea and Bathynellacea having the lowest number of species (Tab.1.2).

The highest number of species were recorded on the large islands such as Sicily, Sardinia, Corsica and Crete as well as on the largest archipelagoes such as the Baleares, the Aegean Islands, the Ionian Islands and the Adriatic Islands (Tab.1.1). These findings could have been expected given both the age and the large area of the islands and the high number of islands forming the respective archipelagoes as well as taking into account the general correlation between the species number and the size of an area (Rosenzweig 1995). Equally expectedly, the lowest biodiversity was observed on the Marmara Islands and on the Pelagie Islands, being one of the smallest archipelagoes in the Mediterranean. Suprising, however, is the low number of species reported from Cyprus, the third largest island in the Mediterranean and on the Maltese Islands. This may indicate the scarcity of studies on the freshwater biota, which also may be a reason why no insular freshwater malacostracans have been recorded from Djerba or the Kerkennah Islands. On the other side, local environmental conditions may simply be unfavourable for the freshwater species. Sardinia exhibits the highest malacostracan biodiversity with 35 species belonging to 21 genera and 17 families (Tab.1.1, Fig.1.3). Such high levels of diversity may be a result of long and complex geological history of the island (Speranza et al. 2002), combined with the landscape heterogeneity (Vogiatzakis et al. 2008), also resulting in the highest number of endemic malacostracan taxa (Tab.1.1).

The Mediterranean islands are considered to be the natural laboratories of evolution housing a significant number of endemic species (Schüle 1993, Hopkins 2002, Vogiatzakis et al. 2008, Blondel et al. 2010). The insular freshwater malacostracan fauna seems to confirm that pattern with more than half of all reported species from the islands being endemic (Tab.1.1). Following Sardinia, Sicily, Crete and the Balearic Islands exhibit the highest rates of endemism, reaching and even exceeding 60% of all malacostracans reported from the area (Tab.1.1). This trend has been also confirmed for other freshwater organisms like insects, molluscs and annelids (Gómez-Campo et al. 1984, Stoch 2000, Ruffo & Stoch 2016, Sfenthourakis & Triantis, 2017). The proximity to the mainland and oftenly re-established land connections, combined with the scarcity of the freshwater studies, may provide an

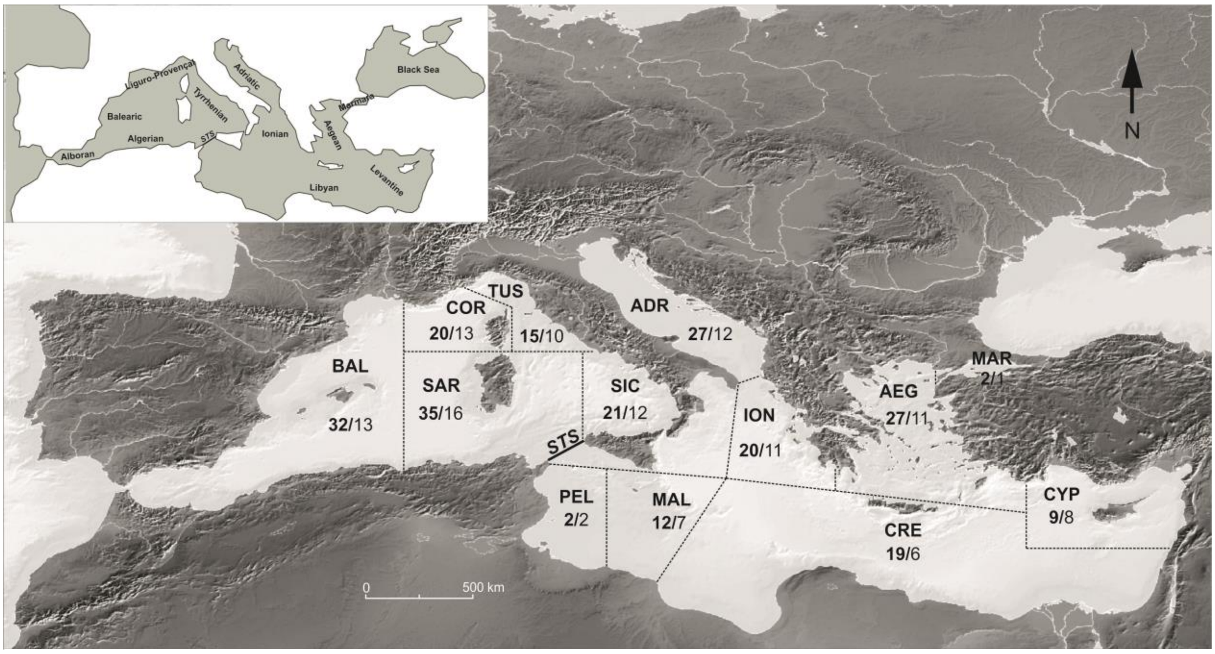


Fig.1.3 Distribution map of the freshwater malacostracans of the Mediterranean Islands. BAL – Balearic Islands; SAR – Sardinia and circum-Sardinian Islands; COR – Corsica; TUS – Tuscan Archipelago; SIC – Sicily; PEL – Pelagie Islands; MAL – Maltese Islands; ADR – Adriatic Islands; ION – Ionian Islands; AEG – Aegean Islands; CRE – Crete; MAR – Marmara Islands; CYP – Cyprus. Numbers in bold indicate the number of species, the regular number is the number of families per region. STS refers to Siculo-Tunisian Strait, dividing the Mediterranean Sea into western and eastern basin. The smaller map in the left upper corner indicates the major sea basin in the Mediterranean Region.

explanation for the lower rate of local endemism on islands like the Tuscan Archipelago, the Adriatic Islands and the Maltese Islands (Tab.1.1).

Generally, each of the malacostracan orders showed a high rate of endemism, exceeding the 50% of the reported taxa with an exception of decapods (Tab.2.1). This can be explained by the relatively higher, compared to members of other malacostracan orders, dispersal abilities, even enabling some of the decapods to disperse through land connections (e.g. Ponniah & Hughes 2004, Jesse et al. 2011). The highest number of endemics have been recorded in amphipods with 61 species being considered as insular endemics (Table S1).

With an exception of the Maltese Islands and Cyprus, amphipods were always the most species-rich malacostracan order in the insular fresh waters (Tab.2.1), with the family Gammaridae being the most speciose and comprising almost one third of all epigean freshwater malacostracans in the Mediterranean islands (Table S1). Gammarid amphipods are known to be the keystone species structuring freshwater epigean macroinvertebrate communities also being among the most abundant and biomass-dominant groups in lotic ecosystems of Europe as well as in the Mediterranean Region (MacNeil et al. 1997, Kelly et al. 2002). Following the pattern observed in the data from the insular freshwater of the Mediterranean, Gammaridae is also the most speciose epigean freshwater malacostracan family worldwide (Väinölä et al. 2007, Balian et al. 2008) with *Gammarus* being globally the largest epigean freshwater malacostracan genus. Even though, up to 2007 (Väinölä et al. 2008) there has been 204 *Gammarus* species known from inland waters, this number is still growing, mainly due to the implementation of the integrative taxonomy approach (e.g. Cannizzaro et al. 2017, Grabowski et al. 2017a, Hou et al. 2018, Hupało et al. 2018). The emergence and increasing use of the DNA-based identification of organisms has led to identifying plethora of cryptic and pseudo-cryptic species (Fišer et al. 2018). This is also the case of the freshwater malacostracans from the Mediterranean islands, with numerous reports on the complexes of cryptic species, e.g. *Gammarus balcanicus* (e.g. Mamos et al. 2014, 2016) or *Asellus aquaticus* (e.g. Sworobowicz et al. 2015) with many more studies ongoing (e.g. upon *Gammarus italicus*, *Echinogammarus veneris*, *Rhipidogammarus rhipidiophorus* (Stoch et al., unpublished data)).

Having already a substantially high level of freshwater malacostracan biodiversity in the insular epigean freshwaters, the majority of the species inhabits the subterranean habitats (Tab.1.1). This finding actually exceeds the supposed level of groundwater diversity, which is estimated to be lower than in the surface waters (Sket 1999, Väinölä et al. 2007), but on the regional scale it is known to be, in some cases, equal or exceed the epigean diversity (Stoch 1995). Again, the amphipods are leading the way with more than 60% of the hypogean species reported from the Mediterranean islands, represented by both members of stricly stygobitic families, such as Bogidiellidae, Niphargidae, Pseudoniphargidae, Salentinellidae and a few single members of Gammaridae or Ingolfiellidae (Table S1). The most speciose hypogean amphipod genus in the studied islands is *Niphargus,* represented by 27 species and subspecies, being also the globally largest known freshwater amphipod genus (Väinölä et al. 2007, Fišer et al. 2008), with many endemic insular species described very recently (Karaman 2017, 2018; Zettler & Zettler 2017). Interestingly, the subterranean malacostracan fauna on the Mediterranean islands show an extreme level of endemism with 60% of all the recorded stygobionts being endemic and representing overall 66 endemics out of the 110 malacostracans recorded from all the Mediterranean islands (Table S1). This exceed even the already very high estimates for all the European groundwaters (Deharveng et al. 2009). As for the epigean taxa, these number are most probably underestimated given that more and more cryptic diversity is being detected both within the hypogean amphipods (e.g. Jurado-Rivera et al. 2017) and the isopods (Ketmaier et al. 2003) with many more ongoing molecular studies upon the groundwater malacostracans (Stoch et al., unpublished).

Origin of insular freshwater malacostracans

The origin of the newly introduced fauna on the Mediterranean islands is known to be recent, within the last decades (e.g. Chappuis 1949, Holdich et al. 1999). However, the majority of the insular freshwater malacostracans have been inhabiting the islands already for millions of years, often being on the island since its very existence. Even though the islands largely differ in terms of their dates of origin and the geological history, the patterns of freshwater colonization by the malacostracan crustaceans remain similar throughout the islands. The insular freshwater Malacostraca are either of ancient marine origin (thalassoid, Coineau & Boutin 1992), which colonized the insular groundwater habitats, or they are of continental origin. The continental malacostracans were either inhabiting the island during the time when it was still a part of the mainland (e.g. Ruffo & Stoch, 1987, Ketmaier et al. 2001, 2003, Chueca et al. 2015) or they have migrated using the temporry land connections established either by the land movements or during the sea level fluctuations from Miocene to Pleistocene (e.g. Triantis & Mylonas 2009, Ketmaier & Caccone 2013, Delicado et al. 2014). The origin of the Mediterranean insular malacostracans has been one of the focal points of the biogeographical studies in the last decades. In these studies, several authors hypothesized possible biogeographical scenarios based mostly on the taxonomic and systematic evidence (e.g. La Greca 1957, Ruggieri 1973, Coineau & Boutin 1992). Nowadays, with the emegence and establishment of DNA-based molecular clock methodology (Ho & Duchêne 2014), the growing number of studies on origin and biogeography of insular faunas are testing the previous hypotheses based on molecular information. Although numerous recent studies on the Mediterranean insular freshwater fauna have shed some light on the biogeography of the freshwater malacostracans (e.g. Jesse et al. 2011, Ketmaier & Caccone 2013, Poulakakis et al. 2015, Hupało et al. 2018), more molecular evidence is needed to fully and reliably resolve the biogeographical entaglements of the Mediterranean insular freshwater Malacostraca.

Threats

The freshwater ecosystems, being among the most species-rich ecosystems in the world, are also the most endangered ones (Cuttelod et al. 2008, Darwall et al. 2009). In result of the ongoing climate change and heavy anthropogenic activities, the rate of extinction of the freshwater biota is believed to be even five times higher than in the terrestrial environment

(Dudgeon et al. 2006). The Mediterranean Region, including the islands, being recognised as a biodiversity hotspot, is inhabited by ca. 400 mln people and exposed to the growing mass tourism, already reaching 150 mln visitors a year and projected to double by 2025 (e.g. Vogiatzakis et al. 2008, Benoit & Comeau 2012). Even though, the tourism brings economic success for particular islands, it has a devastating impact on the environment, including fresh waters (Hopkins 2002). Besides the degradation of the freshwater ecosystems by pollution, daming, gravel exploitation and other activities leading to habitat alterations, humans are also responsible for introducing the alien, often invasive taxa (Hopkins 2002, Vogiatzakis et al. 2008), which in some cases has a devastating effect on the local fauna (e.g. Palmer & Pons 1996, Geiger et al. 2005). Although the number of alien malacostracan species present in the fresh waters of the Mediterranean islands is relatively low (nine species, Tab.1.1), it comprises a third of all the reported insular freshwater decapods (Tab.1.2). Notably, the islands with the highest percentage of the alien species present (the Maltese Islands, Cyprus, Sicily; Tab.1.1) are also the ones with the highest tourist activity (Hopkins 2002). Moreover, some of the non-indigenous insular freshwater malacostracans are considered highly invasive, posing a substantial threat to the native fauna (Hentonnen & Huner 1999). *Procambarus clarkii* is among the most invasive organisms globally and is also one of the most common malacostracan in the fresh waters of the Mediterranean islands, with its presence being confirmed on Corsica, Sardinia, Sicily, Cyprus, the Maltese and the Balearic Islands (Table S1). The invasiveness of *P. clarkii* in the Mediterranean Region is manifested by its strong impact on the entire ecosystems, often resulting in the extinction of coexisting macroinvertebrates (Geiger et al. 2005). The invaders along with the extensive human activity are posing the biggest ecological threat, especially to the endemic insular freshwater malacostracan fauna. Given that numerous Mediterranean endemics are known to inhabit only very limited areas, often restricted to a single site, one can imagine how big impact have the slightest alterations to their habitat. Even though the biodiversity of the freshwater malacostracan fauna is relatively high, it is most probably the remnant of even higher diversity with many recent extinctions occuring at the substantial rate, when the *loci typici* of some species, known now only from the museum specimens, are gone due to the human-mediated environmental changes (personal observations).

Conservation perspectives

The current situation points out to the necessity for planning reasonable conservation strategies for the fresh waters on the Mediterranean Islands. Although fresh waters are recognized globally as heavily affected by the anthropogenic pressure, with extremely high extinction rate and about 20.000 freshwater species being already extinct or threatened (Dudgeon 2006, Vörösmarty et al. 2010), the conservation policy regarding freshwater biota is poorly addressed. There are only few freshwater representatives included in the European Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (1992, also known as the Habitats Directive). It is even worse considering that only 0.1% of all European invertebrates is under protection and the freshwater invertebrate fauna is only a small fraction. It is a dramatic underestimation of the factual number of threatened invertebrate species in European fresh waters (Balian et al. 2008, Figueroa et al. 2013). Even though the freshwater habitats on the Mediterranean Islands have been identified as threatened, no island-specific, extensive conservation strategy has been planned nor implemented (Hopkins 2002). Clearly, the first step in protecting the freshwater ecosystems on the Mediterranean Islands has to be the summary of the knowledge on their factual freshwater biodiversity. Apart from the completion of the insular freshwater species assessments, the studies on their biology and their population structures must follow. It is necessary to recognize and establish the Freshwater Key Biodiversity Areas in the Mediterranean Islands, which could help in formulating long-term national protection plans and protecting the local freshwater biodiversity hotspots (Darwall et al. 2014). The second step would be to lower the anthropogenic pressure on the fresh waters. According to EU Water Framework Directive (and to the Groundwater Directive 2006/118/EC, which has been developed in response to the requirements of Article 17 of the Water Framework Directive), the EU Member States have to protect, enhance and restore all bodies of surface and subterranean water, aiming to achieve satisfactory water status. However, in reality, the fresh waters are being even more affected not only by the human activities causing the habitat degradation, water pollution, flow modification, overexploitation and introduction of alien fauna, but also by the global climate change (Dudgeon et al. 2006, Kernan et al. 2011). Thus, at the moment, the perspectives for maintaining the current biodiversity of freshwater malacostracan on the Mediterranean islands are not optimistic. That is why it is of huge importance to prepare similar biodiversity reviews of other freshwater biota inhabiting the Mediterranean Islands and initiate activities to raise public awareness and to help protecting the remaining insular freshwater habitats.

Chapter II. Origins and diversification of freshwater gammarids of the Aegean Archipelago.

2.1 Introduction

The Mediterranean islands are considered as natural laboratories of evolution, exhibiting high level of diversity and endemism, making them a vital part of one of the globally most precious biodiversity hotspots and a model system for studies of biogeography and evolution (Myers et al. 2000, Whittaker & Fernández-Palacios 2007, Woodward 2009, Poulakakis et al. 2015). However, the freshwater fauna of the islands is still heavily understudied, it is estimated that the Mediterranean region is inhabited by ca. 6% of the world’s freshwater species with at least 43% of the them considered to be local endemics (Figueroa et al. 2013).

The Aegean Sea is one of the major sea basins within the Mediterranean. The basin houses around 7500 islands and islets, which occur at a variety of isolation levels and exhibit different topographic features. With that, the Aegean region is recognized to have, globally, the highest number of islands in a single sea basin (Triantis & Mylonas 2009). The largest of the Aegean islands is Crete, being also the fifth largest in the Mediterranean region. The majority of the islands were once part of a united landmass called Aegeis, which emerged probably around 23 Ma (Meulenkamp 1971). In Middle Miocene, the movement of the African and Eurasian continental plates led eventually to the first fragmentation of Aegeis and to the isolation of Crete from the Peloponnese (van der Geer et al. 2006, Poulakakis et al. 2015). Further isolations of the land units within the Aegean basin were caused by the formation of Mid-Aegean Trench (around 12-9 Ma), which led to the separation of the central Aegean from the east Aegean islands (Dermitzakis & Papanikolaou 1981, Poulakakis et al. 2015). Another crucial event in the history of the entire region was the closure of the Mediterranean basin, which led to complete desiccation and as a result to mass extinction of the aquatic biota called the Messinian Salinity Crisis that took place at the end of Miocene (6-5 Ma). During that time, many formerly isolated islands regained their connections with the mainland and/or the other islands, which resulted in exchange of terrestrial and freshwater faunas (Poulakakis et al. 2015). Afterwards, some of the connections with the mainland were again temporarily re-established during the Pleistocene, due to eustatic sea-level changes, with most of the islands gaining their present shape and the current level of isolation at the end of Pleistocene (Perissoratis & Conispoliatis, 2003).

Gammarid amphipods are among the most dominant and abundant aquatic macroinvertebrates in fresh waters in Europe and, particularly, in the Mediterranean Region (MacNeil et al. 1997). They are known to shape the freshwater macrozoobenthic communities, being recognised as aquatic keystone species (Kelly et al. 2002). Given their exclusively aquatic life cycle and high level of diversity, the gammarids are considered to be particularly interesting evolutionary models (Bilton et al. 2001). The freshwater gammarid fauna is relatively poorly known with most of the studies on the Mediterranean amphipods being focused on the marine biota. At the moment, about 120 freshwater gammarid species have been reported from the Mediterranean region, while only 21 species, belonging mainly to two genera: *Gammarus* Fabricius, 1775 and *Echinogammarus* Stebbing, 1899, have been reported from the islands (Karaman & Pinkster 1977a,b, Karaman & Pinkster 1987, Pinkster 1993, Hupało et al. 2018). Given that recently a high rate of overlooked diversity was discovered in both mentioned genera (e.g. Hou et al. 2014, Mamos et al. 2014, 2016, Copilaş-Ciocianu and Petrusek 2015, 2017, Katouzian et al. 2016, Grabowski et al. 2017a,b), it may be argued that the current number of species already described from the Mediterranean islands is largely underestimated. To date, there have been five species reported from the Aegean Islands with three of them being Cretan endemics (*E. kretensis* Pinkster, 1993, *E. platvoeti* Pinkster, 1993 and the recently described *G. plaitisi* Hupało, Mamos, Wrzesińska & Grabowski, 2018) and with three lone records of *G. komareki* Schäferna, 1923 from Gökçeada and *G. uludagi* G.S. Karaman, 1975 from Lesbos and Evia (Karaman & Pinkster 1977a, Pinkster 1993, Hou et al. 2011, Özbek & Özkan 2017, Hupało et al. 2018).

In this paper, the main aim is to investigate the presence of freshwater populations of *Gammarus* on other Aegean islands. Given the high rate of endemism already observed in numerous biota from the Aegean archipelago (Poulakakis et al. 2015), including gammarids (Pinkster 1993, Hupało et al. 2018), one can hypothesise that there will be high level of local endemism on other Aegean islands with freshwater population of *Gammarus*. However, given that final isolation of particular Aegean islands happened at different times, with some of them being isolated since the end of Miocene (e.g. Crete) and some of them still bearing a land connection as late as in Pleistocene (e.g. Evia, Lesbos, Samothraki), it can be also hypothesised that the level of endemism will vary between the islands. In this case, the islands that still had land connections in Pleistocene, supposingly will not have endemic lineages, due to possible faunal exchanges with the mainland. These hypotheses are tested by performing DNA polymorphism analyses on four molecular markers: two mitochondrial (COI and 16S rDNA) and two nuclear (28S rDNA and EF1-alpha) in the freshwater populations of gammarids from the Aegean islands and neighboring, mainland regions. By doing so, the aim is to reveal their biogeographic affiliations and possible origin and provide the first insight into the phylogeography of the Aegean freshwater amphipod fauna.

2.2 Materials and methods

Sample collection and identification

The study material consisted of 50 individuals collected from 13 sampling sites, including nine sites from freshwater habitats from Aegean islands, namely Samothraki, Skyros, Evia, Andros, Tinos and Serifos and four sites from the mainland Greece (Tab.2.1). The material was collected during several sampling campaigns between 2008 and 2018. Gammarids were sampled using a variety of methods, including collection from gravel, rocks and aquatic vegetation with a hand net or using rectangular kick sample nets (aperture 25x25 cm and 0.5 mm mesh size). The samples were sorted at the site and gammarids were immediately fixed in 96% ethanol. Afterwards, the material was examined under a Nikon 800 stereomicroscope. Identification to the species level was done according to the newest available literature dealing with taxonomy of the Mediterranean freshwater gammarids (Karaman & Pinkster 1977a,b; 1987; Hupało et al. 2018).

DNA extraction, PCR amplification, sequencing

The DNA was extracted using the standard phenol/chloroform method (Hillis et al. 1996), implementing the protocol described previously by Hupało et al. (2018). The extracted DNA was stored at 4°C until amplification and finally long-term stored at -20°C. In the first step, a fragment of the cytochrome *c* oxidase subunit I gene (COI) was amplified using three different primer pairs, depending on the amplification success. In the second step, at least one individual per the delimited Molecular Operational Taxonomic Unit (MOTU) (see below) was amplified for additional markers that were used in phylogeny reconstruction – mitochondrial 16S rRNA and nuclear markers being 28S rRNA and EF1-alpha gene. All the primer sequences, PCR conditions and original references for all the molecular markers used in this study are provided in Table S2. Afterwards, all PCR products (5 μl) were cleaned up using exonuclease I (ThermoFisher Scientific) and alkaline phosphatase FastAP (ThermoFisher Scientific) according to the manufacturer’s guidelines. Direct sequencing was performed

Table 2.1. Collection sites and MOTU information for specimens of *Gammarus* used in this study.

MOTU	**Site Code**	**Locality (Island/Greece; exact location)**	**Latitude**	**Longitude**	**Number of individuals**
Gammarus plaitisi	YGR33	Island; Tinos, Komi	37.6001	25.1333	6
	YGR37	Island; Tinos, Kardiani	37.5998	25.0667	2
	YGR39	Island; Serifos, Kato Dipotama	37.1833	24.4667	5
Gammarus arduus	YGR2	Island; Samothraki, Katsabas	40.4961	25.5044	1
	GR43	Greece; Lissos river, near Arisvi	41.0531	25.6183	6
	GR44	Greece; Amfitriti	40.8883	25.9033	4
Gammarus sp.1	YGR24	Island; Evia, Stropones	38.6003	23.8908	4
Gammarus sp.*2*	YGR22	Island; Skyros, Loutro spring	38.8329	24.5492	4
Gammarus sp.*3*	YGR34	Island; Andros, Ano Menites	37.8167	24.8833	6
	YGR36	Island; Andros, Andros city	37.8333	24.9333	3
Gammarus uludagi	YGR9	Island; Lesbos, Ampeliko	39.0606	26.3142	3
Gammarus birsteini	GR29	Greece; Volos, near Chania	37.8333	24.9333	4
Gammarus crenulatus	GR26	Greece; Sofades, Sofadillos river; *locus typicus*	39.3247	22.0934	2

using the same forward primers as for amplification, using the BigDye terminator technology in Macrogen sequencing company. Since the nuclear 28S rRNA marker is over 1100 bp long it was sequenced both ways, which allowed for obtaining the full coverage, additionally providing better resolution and identification of polymorphic sites. Given that other nuclear marker used in this study, EF1-alpha, has shorter length and did not exhibit significant level of polymorphic sites, it was sequenced using only forward primer.

Sequence data authentication, editing, alignment and deposition

All obtained sequences were confirmed as belonging to *Gammarus* via BLASTn searches in GenBank (Altschul et al., 1990). Subsequently they were assembled, aligned and trimmed to 625 (COI), 391 (16S), 1107 (28S) and 602 (EF1-alpha) base pairs respectively, using the Geneious 10.0.9 software package (Kearse et al., 2012). Alignments were performed using the MAFFT plugin with G-INS-i algorithm in Geneious. In case of double peaks and low quality regions detected in some of the sequences of 28S, the two strands were compared and the dominant signal was chosen at each problematic site.

All the sequences were deposited in GenBank (accession numbers to be provided upon acceptance). Additionally, the sequences of all markers used in this study were compiled in the dataset and deposited in the public repository of the Barcode of Life Data Systems, (BOLD; Ratnasingham & Hebert, 2007), where all the relevant metadata information and sequence trace files, which will be publicly available upon the publication of this chapter.

MOTU delimitation, interspecific relationships and reference material

The Molecular Operational Taxonomic Units (MOTUs) were delimited using COI sequence dataset, according to the distance-based Automatic Barcode Gap Discovery (ABGD) methodology (Puillandre et al. 2012). The results of the genetic distance based, ABGD MOTU delimitation were cross-validated with other, phylogenetic tree based delimitation methods, namely Generalized Mixed Yule Coalescent (GMYC) single and multiple models (Pons et al., 2006; Monaghan et al., 2009) and multi-rate Poisson tree processes (mPTP; Kapli et al., 2017). Additionally, the patristic distances were measured using Patristic 1.0 (Fourment & Gibbs, 2006), according to a patristic distance threshold of 16% proposed for crustaceans at the COI locus (Lefebure et al. 2006a), based on the prior observations of morphological differences across multiple crustacean species. For tree based MOTU delimitation methods, a consensus tree was obtained using BEAST 2.4.7 after performing three MCMC runs of 10 M iterations, sampled every 1,000 iterations, using Tamura-Nei model of evolution and Yule tree model. MCMC runs were examined using Tracer v1.6 and all the sampled parameters for each studied MOTU achieved sufficient effective sample sizes (ESS > 200).

The dataset was also supplemented by reference sequences from the public repositories, representing the related *Gammarus* species from the Aegean islands, mainland Greece and adjacent regions were added to the dataset, along with a single sequence of isopod *Asellus aquaticus* used for rooting the tree (Tab.2.2). For final visualisation, the neighbour-joining tree of all COI sequences, using Tamura-Nei model of evolution with 1,000 bootstrap replicates, was created in MEGA7 software (Kumar et al. 2016).

Table 2.2. Reference sequences (mined from GenBank) used in the reconstruction of the phylogeny in this study.

Species	**Locality (Aegean Island/Country)**	**GenBank Accession Numbers**	**Reference study**
Gammarus alpinus	Switzerland	COI: KX283242	Alther et al. (2016)
Gammarus arduus	Bulgaria	COI: MH493848; 16S: MH496581; EF-α: MH493860	Copilaş-Ciocianu et al. (2018)
Gammarus balcanicus	Bulgaria	COI: KU056256; 16S: KU056052; 28S: KU056154	Mamos et al. (2016)
	Romania	COI: KU056394; 16S: KU056103; 28S: KU056154	Mamos et al. (2016)
Gammarus crenulatus	Greece	COI: KJ462752	Wysocka et al. (2014)
Gammarus fossarum	Romania	COI: KR061783; 16S: KR061718; 28S: KR061765	Copilaş-Ciocianu & Petrusek, (2015)
	Albania	COI: KJ462736	Wysocka et al. (2014)
	Bulgaria (1)	COI: KJ462737	Wysocka et al. (2014)
	Bulgaria (2)	COI: KJ462738	Wysocka et al. (2014)
Gammarus komareki	Turkey	COI: KJ462758; 16S: KJ462574; 28S: KJ462656	Wysocka et al. (2014)
	Greece	COI: KJ462739	Wysocka et al. (2014)
Gammarus lacustris	Finland	COI: KX283246	Alther et al. (2016)
Gammarus plaitisi	Crete (A); Spring in Sfinari beach	COI: MG784518	Hupało et al. (2018)
	Crete (B); Pelekaniotikos	COI: MG784477	Hupało et al. (2018)

	river		
	Crete; Fodele, locus typicus	COI: MG784515; 16S: MG784372 ; 28S: MG784428; EF-α: MG792358	Hupało et al. (2018)
Gammarus pulex	Greece (1)	COI: KJ462741, 16S: KJ462557; 28S: KJ462639	Wysocka et al. (2014)
	Greece (2)	COI: KJ462768; 16S: KJ462584; 28S: KJ462685	Wysocka et al. (2014)
	Peloponnese (1)	COI: MG784489; 16S: MG784379; 28S: MG784423; EF-α: MG792353	Hupało et al. (2018)
	Peloponnese (2)	COI: MG784478,MG784486,MG784481,MG784485; 16S: MG784354; 28S: MG784426; EF-α: MG792354	Hupało et al. (2018)
	Sweden	COI: JF965943,JF965939	Hou et al. (2011)
Gammarus roeselii	Greece	COI: JF965983; 28S: JF965817	Hou et al. (2011)
Gammarus rambouseki	Albania (1)	COI: KJ462742	Wysocka et al. (2014)
	Albania (2)	COI: KJ462744	Wysocka et al. (2014)
	Albania (3)	COI: KJ462746	Wysocka et al. (2014)
Gammarus uludagi	Evia	COI: JF965986; 28S: JF965817; EF-α: JF966112	Hou et al. (2011)
	Albania	COI: KJ462750	Wysocka et al. (2014)
Asellus aquaticus	Ukraine	COI: KR921859	Sworobowicz et al. (2015)

Phylogeny reconstruction, time calibration and history of the diversification

To assess the potential loss of phylogenetic signal, the substitution saturation was tested in DAMBE 7.0.28 (Xia, 2018), using the index proposed by Xia et al. (2003). No significant saturation was detected ($p<0.001$) for any of the applied molecular markers. Based on the best partitioning scheme selected by the PartitionFinder (Lanfear et al. 2012), the molecular data was divided into seven partitions: 16S, 28S with three codon positions of COI and two partitions of EF1-alpha, one comprising of codon positions 1. and 2. and other with 3. codon position. The time-calibrated phylogeny was reconstructed in BEAST 2.4.7 package (Bouckaert et al., 2014), performing three MCMC chains of 100 million iterations, with sampling every 2,000 iterations, using the best-fit substitution models determined by bModel test (Bouckaert & Drummond, 2017): for 16S - Tamura-Nei model (TN93) with gamma-distributed rate heterogeneity (G) and invariable sites (I), for 28S – TIM model with G and I, for first COI codon position – TN93 with G and I, second COI codon position – TIM model with G and I, third COI codon position – TN93 with G and I, for EF1-alpha first and second codon positions – HKY model with G and I and for third EF1-alpha codon position – TN93 with G and I. The optimal molecular clock was chosen via path sampling/stepping-stone procedures using three runs per clock model and Bayes factors (BF) (Baele et al. 2012), using BEAST 2.4.7. Provided the strict clock was rejected for each partition (BF>50), the uncorrelated log-normal relaxed clock was used (Drummond et al. 2006).

For molecular clock calibration, the five primary calibration points known from literature and related to geological events were used, as well as one secondary calibration point, which helped to validate the clock calibration based upon the primary calibration points (Tab.2.3). The most recent calibration point is based on the radiation of the endemic *Gammarus* species flock in Lake Ohrid, coinciding with the emergence of the lake itself (Wysocka et al., 2013, 2014), which most probably took place ca. 2 Ma. The second calibration point is based on the split between the Black Sea and the Caspian Sea populations of *Pontogammarus maeoticus*, estimated at about 4 Ma, connected with the shifts of continental plates, causing the of former Pontian Lake into Black and Caspian Sea, respectively (Cristescu et al. 2003; Nahavandi et al. 2013). The third calibration point reflects the estimated time of diversification of the *Gammarus fossarum* species complex that took place in the Carpathians, being caused by Middle Miocene subsidence event between around 15 and 17 Ma (Copilaş-Ciocianu & Petrusek, 2015). The fourth one marks the origin of the Acanthogammaridae family, endemic to Lake Baikal, estimated at 28-30 Ma (Sherbakov et al. 1999, Macdonald et al. 2005; Mats et al. 2011). The oldest calibration point reflects the connection between the Eocene regression of the Paratethys Sea at ca. 37 Ma and the divergence between *Sarothrogammarus* and *Rhipidogammarus* genera (Hou et al. 2011, 2014). The constraints of the calibration points were applied by imposing priors on the respective tree nodes, using of lognormal distribution of the MRCA priors, which allowed the incorporation of the possible uncertainty of the data. Besides using the sequences available for the molecular clock calibration, the reference sequences of *Gammarus* available from the literature were used in the reconstruction of phylogeny to provide further insights into the phylogenetic and biogeographical affinities within the Aegean gammarids (all individuals listed in Tab.2.2).

Table 2.3. Reference sequences used as primary calibration points for the calibration of the molecular clock for the reconstruction of the time-calibrated phylogeny.

Species	**Calibration time**	**GenBank Accession numbers**	**Reference study**
Gammarus salemaai	2 ± 1 Ma (f. *G. sketi*)	COI: JX899266	Wysocka et al. (2013)
		16S: JX899088	
		28S: KJ462668	Wysocka et al. (2014)
Gammarus sketi	2 ± 1 Ma (f. *G. solidus,G. salemai*)	COI: JX899272	Wysocka et al. (2013)
		16S: JX899094	
		28S: KJ462672	Wysocka et al. (2014)
Gammarus solidus	2 ± 1 Ma (f. *G. sketi*)	COI: JX899282	Wysocka et al., (2013)
		16S: JX899104	
		28S: KJ462673	Wysocka et al. (2014)
Pontogammarus maeoticus (Black Sea)	4 ± 2 Ma (f. *P. maeoticus* Caspian)	COI: AY189494	Cristescu et al. (2003)
		28S: AY529062	Cristescu & Hebert, (2005)
Pontogammarus maeoticus (Caspian Sea)	4 ± 2 Ma (f. *P. maeoticus* Black)	COI: AY189500	Cristescu et al. (2003)
		28S: AY529063	Cristescu & Hebert, (2005)
Gammarus fossarum G	15 ± 2 Ma (f. *G. fossarum* M)	COI: KR061783	Copilaş-Ciocianu & Petrusek, (2015)
		16S: KR061718	
		28S: KR061765	
Gammarus fossarum M	15 ± 2 Ma (f. *G. fossarum* G)	COI: KR061823	Copilaş-Ciocianu & Petrusek, (2015)
		16S: KR061694	
		28S: KR061753	
Acanthogammarus victorii	28 ± 2 Ma (f. *E. viridulus*)	COI: AY926652	MacDonald et al. (2005)
		16S: AY926695	
Eulimnogammarus viridulus	28 ± 2 Ma (f. *A. victorii*)	COI: AY926665	MacDonald et al. (2005)
		16S: AY926715	
Sarothrogammarus (Comatogammarus) ferghanensis	37 ± 2 Ma (f. *Rhipidogammarus*)	COI: JF965996	Hou et al. (2011)
		28S: JF965828	
Sarothrogammarus (Barnardiorum) shadini	37 ± 2 Ma (f. *Rhipidogammarus*)	COI: JF965994	Hou et al. (2011)
		28S: JF965826	
Rhipidogammarus karamani	37 ± 2 Ma (f. *Sarothrogammarus*)	COI: JF965993	Hou et al. (2011)
		28S: JF965825	
Rhipidogammarus rhipidiophorus	37 ± 2 Ma (f. *Sarothrogammarus*)	COI: JF965992	Hou et al. (2011)
		28S: JF965824	
		16S: AY926715	

Parameters of all three runs were examined in Tracer 1.7.1 and reached the ESS values above 200. The runs were combined and resampled with LogCombiner 2.4.7 with 30% burn-in, with the maximum clade credibility chronograms being annotated using TreeAnnotator 2.4.4 and visualised using FigTree 1.4.4 (Bouckaert et al., 2014).

The history of diversification was tested using the lineage through time (LTT) plot generated in Tracer 1.7.1 from the 1000, subsampled in Logcombiner, trees inferred from the Bayesian analysis performed in BEAST 2.4.7 software. The Bayesian analysis was performed using the same priors and MCMC settings as of the time-calibrated reconstruction of phylogeny, described above.

2.3 Results

Taxonomic assignment and geographical distribution

The studied material was composed exclusively of the genus *Gammarus* representatives. All were morphologically assigned to already known morphospecies of the so-called *Gammarus pulex* group (sensu Karaman & Pinkster, 1977a) reported from Greece and neighbouring areas. The samples from Andros, Evia and Skyros were morphologically identified as *Gammarus pulex* (Linnaeus, 1758) – the pan-European morphospecies The samples from Tinos and Serifos were identified as *Gammarus plaitisi*, supposed to be the Cretan endemic. The animals from Lesbos were ascribed to *Gammarus uludagi.* The sample from Samothraki contained *Gammarus arduus G.S. Karaman, 1975*, reported before from the mainland Greece and from the Balkan Peninsula, but never from any of the Aegean islands. *Gammarus arduus* was present in two samples from mainland Greece, namely Arisvi and Amfitriti. Material from the mainland Greece included samples from the Volos Peninsula and Sofades containing other members of *Gammarus pulex* group (sensu Karaman & Pinkster, 1977a) reported from adjacent regions - *Gammarus birsteini*, described and present only in Turkey and *Gammarus crenulatus*, described from Greece with its *locus typicus*, being same as sampled in this study. Although more samples from mainland Greece were collected, they have contained either *Gammarus roeselii* Gervais, 1835 or *Turkogammarus spandli* (S.Karaman, 1931) and thus, were excluded from further analyses.

MOTU delimitation, diversity, affiliations and distribution

The ABGD, along with MOTU delimitation based on the patristic distance, supported presence of eight distinct lineages of *Gammarus* present in the material collected from the islands and from the mainland Greece. Except for *Gammarus sp.1* from Evia, the patristic distance supported the distinction of remaining insular taxa (Fig.2.1). The tree based delimitation methods, namely GMYC single threshold model and mPTP indicated ten MOTUs, with two extra lineages within *G. plaitisi* and *G. arduus*, respectively, whereas GMYC multiple threshold model supported thirteen MOTUs, splitting *G. arduus* into three units, as well as two extra lineages within Gammarus from Evia and Andros. The ABGD was chosen as the main delimitation method due to its most conservative approach (Fig.2.1). All of the insular MOTUs grouped together with other *Gammarus pulex* sequences, accompanied by other members of the *Gammarus pulex* species group including *G. uludagi*, *G. lacustris* or *G. alpinus* (*sensu* Karaman & Pinkster, 1977a). However, the samples from mainland Greece with *G. birsteini* and *G. crenulatus* being grouped together with a reference sequence of *G. roeselii* with high bootstrap value, belonging to so-called *Gammarus roeselii* group (sensu Karaman & Pinkster, 1977b) (Fig.2.1).

Each of the studied islands is inhabited by a single MOTU only, with the exception of Evia, where both MOTUs of *Gammarus* sp.1 from this study and reference *G. uludagi* are present (Fig.2.2). In most cases, a MOTU found on one island is not present neither on any other island, nor on mainland Greece. The only exception is *Gammarus plaitisi* present on Tinos and Serifos as well as on Crete (Hupało et al., 2018) and *Gammarus arduus* present on both Samothraki and on the mainland (Fig.2.2).

Phylogeny reconstruction and history of the diversification

The time-calibrated phylogeny revealed that divergence within the *Gammarus pulex* group from the studied region started around 12 Ma (95% HPD: 18.9 – 8.8 Ma) (Fig.2.3, Fig.S1). All of the deeper divergence events between the insular taxa seem to take place between 12 to 8 million years ago. At that time, *G. platisi* seemed to diverge from the *G. pulex* from the mainland Greece, *G. uludagi* from Lesbos diverged from its conspecific from Evia as well as *G. pulex* from Evia and Peloponnese and *G. pulex* from Skyros and Andros probably diverged from other lineages (Fig.2.3). In the similar timescale, about 11.5 Ma (95% HPD: 20.8 – 5.5 Ma) *G. crenulatus* diverged from *G. birsteini*. These two species, along with *G. roeselii,* could have separated from *Gammarus pulex* group members already in Eocene, around 40 Ma. More recently, in Pliocene and Pleistocene diversification events probably took place only in the insular populations of *G. plaitisi,* about 2.5 Ma (95% HPD: 4.1 – 0.9 Ma) as well as in

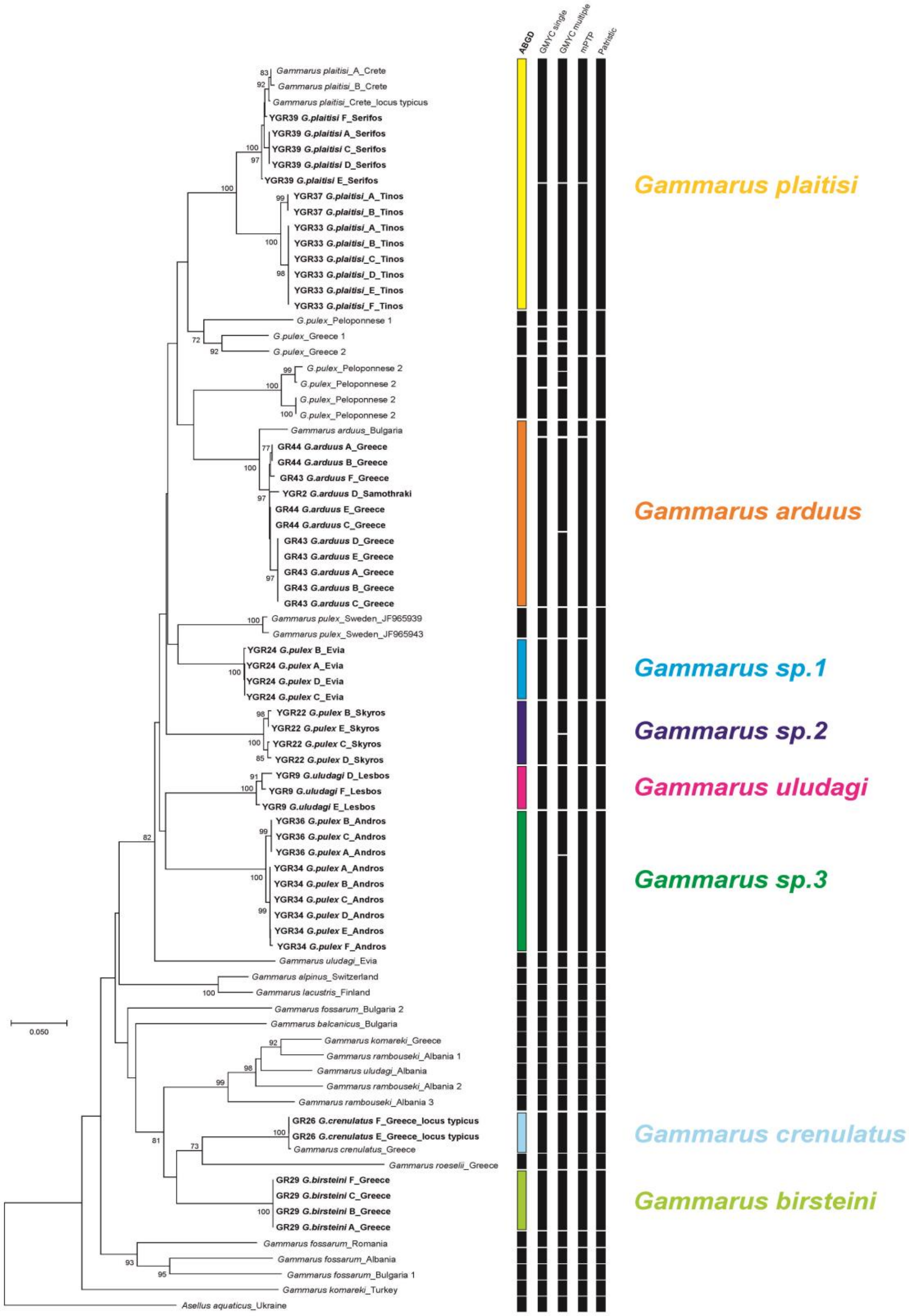


Fig.2.1 Neighbor-joining tree of the Aegean *Gammarus* obtained from the data along with reference sequences mined from NCBI GenBank. The numbers by respective nodes indicate bootstrap values ≥ 0.7. The scale bar corresponds to the number of substitutions per site. The rows of respective bars represent the delimitation of molecular operational taxonomic units (MOTU) by various methods of species delimitation used.

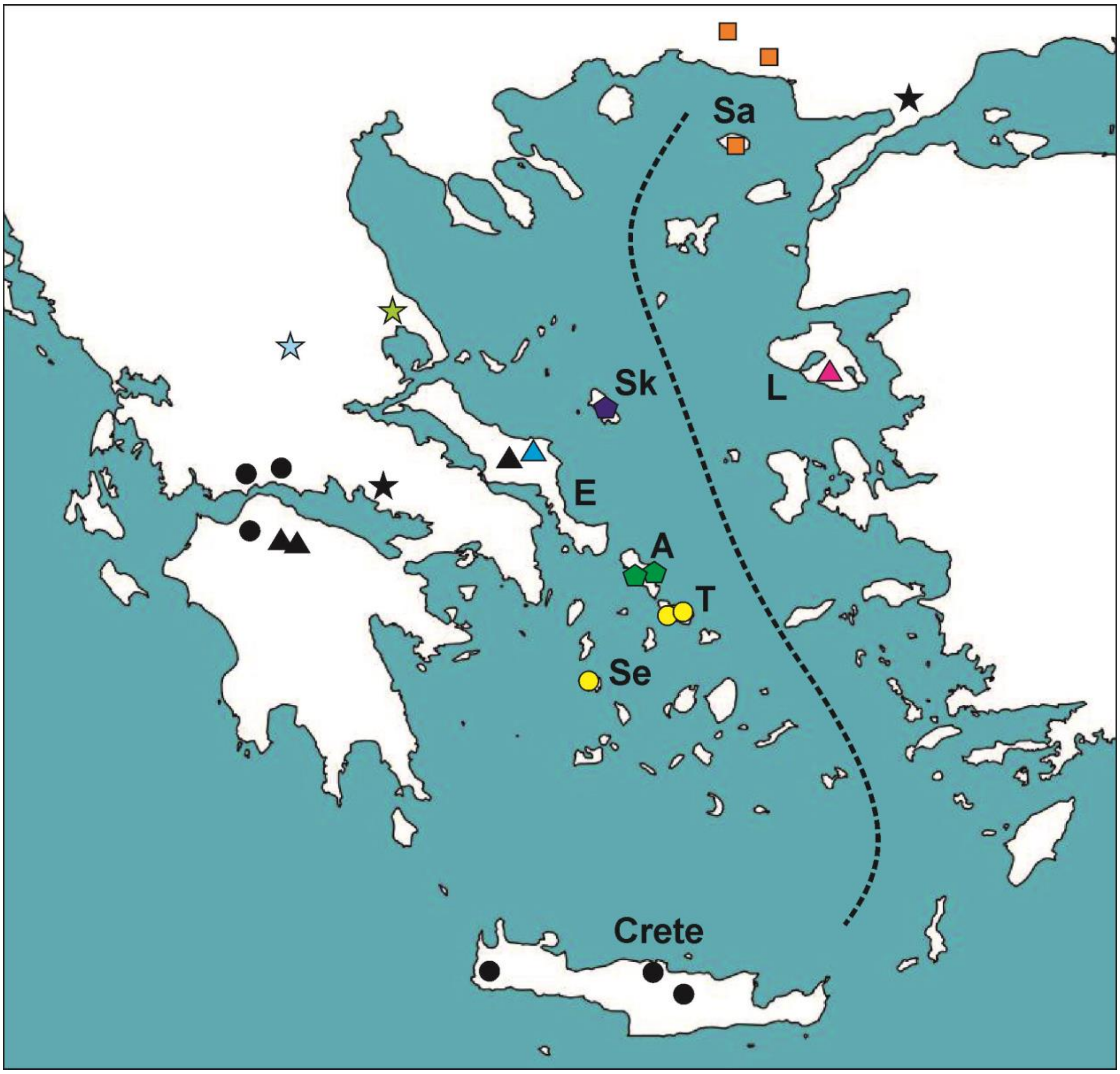


Fig.2.2 Map of the distribution of Aegean *Gammarus*. Coloured points represent data obtained in this study and black ones correspond to reference material. Colours correspond to the MOTUs presented on Fig.2.1. Circles represent *G. plaitisi* and related taxa; squares – *G. arduus;* triangles – *G.* sp1, *G. uludagi* and related taxa; pentagons – *G. sp2*, *G. sp3*; stars – *G. crenulatus*, *G. birsteini* as well as *G. roeselii* from Greece and *G. komareki* from Turkey. Symbols correspond to ones presented in Fig.2.3.

G. arduus, where the insular population from Samothraki most probably diverged from the mainland conspecifics, about 2 Ma (95% HPD: 2.8 – 0.7 Ma). The latest divergence seemed to take place in late Pleistocene, about 0.2 Ma (95% HPD: 0.45 – 0.03 Ma), when the population of *G. plaitisi* from Serifos seemingly diverged from the one from Crete (Fig.2.3).

Phylogeny reconstruction and history of the diversification

The time-calibrated phylogeny revealed that divergence within the *Gammarus pulex* group from the studied region started around 12 Ma (95% HPD: 18.9 – 8.8 Ma) (Fig.2.3). All of the deeper divergence events between the insular taxa seem to take place between 12 to 8 million years ago. At that time, *G. platisi* seemed to diverge from the *G. pulex* from the mainland Greece, *G. uludagi* from Lesbos diverged from its conspecific from Evia as well as *G. pulex* from Evia and Peloponnese and *G. pulex* from Skyros and Andros probably diverged from other lineages (Fig.2.3). In the similar timescale, about 11.5 Ma (95% HPD: 20.8 – 5.5 Ma) *G. crenulatus* diverged from *G. birsteini*. These two species, along with *G. roeselii*, could have separated from *Gammarus pulex* group members already in Eocene, around 40 Ma.

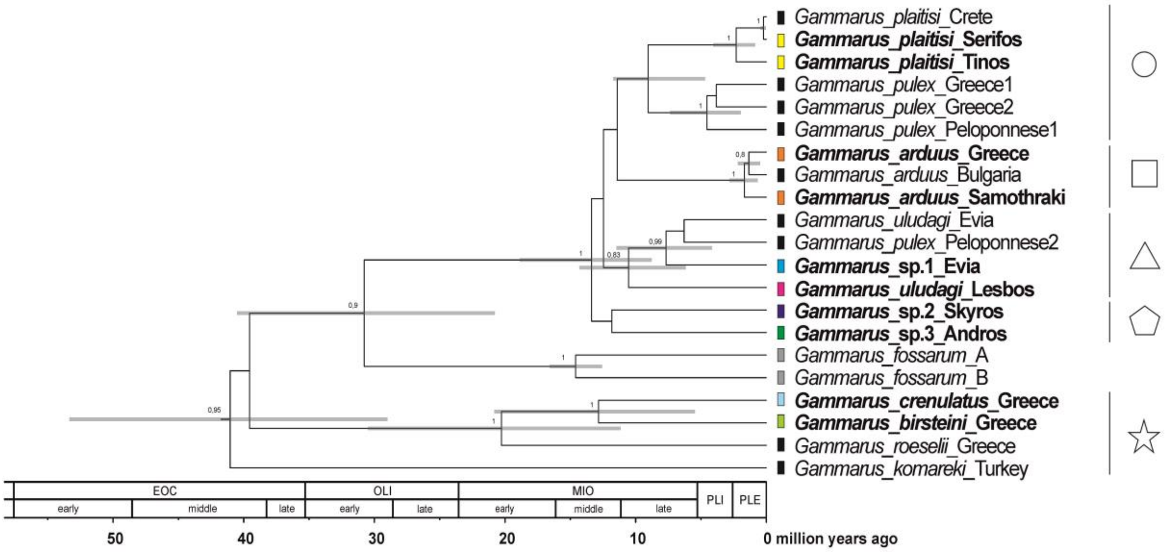


Fig.2.3 Maximum clade credibility, time-calibrated Bayesian reconstruction of phylogeny of Aegean *Gammarus* MOTUs. Phylogeny was inferred from sequences of the mitochondrial COI and 16S rRNA genes and nuclear 28S rRNA and EF1-alpha gene. The numbers by respective nodes indicate Bayesian posterior probability values ≥ 0.8. The coloured bars represent ABGD delimitation method, with colours and symbols correspond to those presented in other figures. Grey node bars represent 95% HPD.

More recently, in Pliocene and Pleistocene diversification events probably took place only in the insular populations of *G. plaitisi*, about 2.5 Ma (95% HPD: 4.1 – 0.9 Ma) as well as in *G. arduus*, where the insular population from Samothraki most probably diverged from the mainland conspecifics, about 2 Ma (95% HPD: 2.8 – 0.7 Ma). The latest divergence seemed to take place in late Pleistocene, about 0.2 Ma (95% HPD: 0.45 – 0.03 Ma), when the population of *G. plaitisi* from Serifos seemingly diverged from the one from Crete (Fig.2.3).

The lineage-through-time plot (Fig.2.4) shows that the accumulation of lineages remained rather constant over time with no significant increase in lineage accumulation throughout the time.

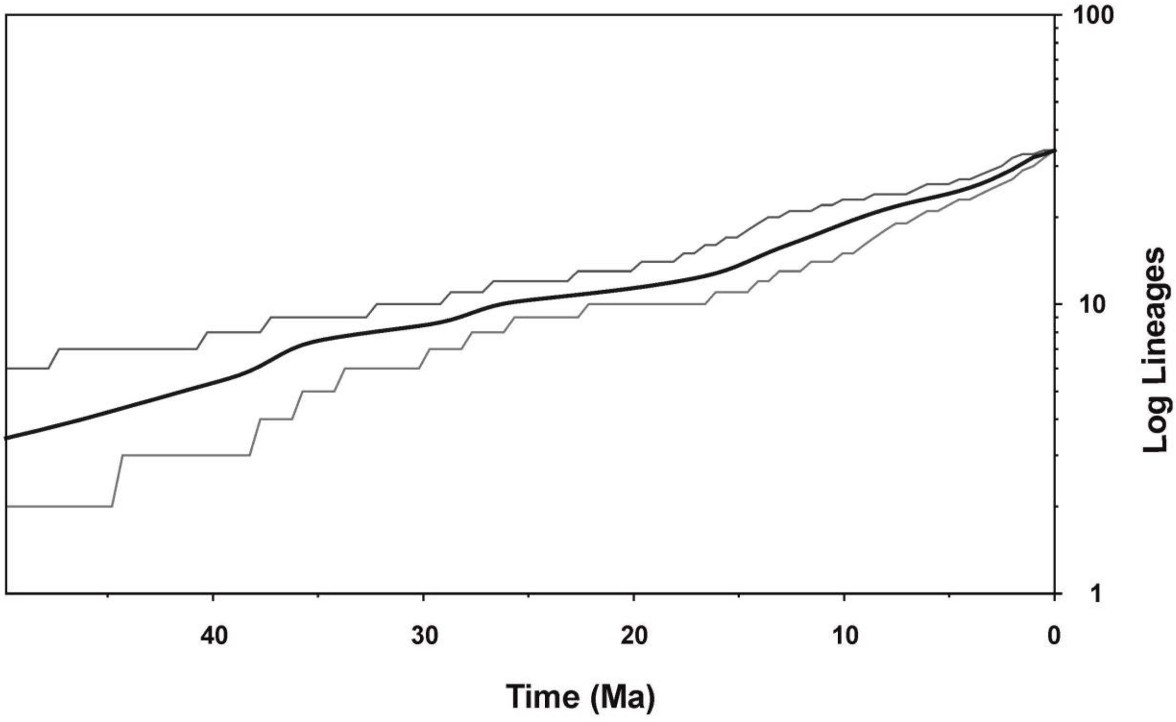


Fig.2.4 Lineage Through Time (LTT) plot for Aegean *Gammarus*. The black representing the median with grey lines representing 95% CI.

2.4 Discussion

Diversity and distribution of Aegean insular freshwater fauna

In this study, the first evidence of the presence of freshwater populations of *Gammarus* on five Aegean islands, namely Samothraki, Skyros, Andros, Tinos and Serifos is provided, with three of these populations most probably representing the new, distinct species supported by all delimitation methods used in this study. The ABGD approach used in this study as the main MOTU delimitation method is considered to be more conservative compared to tree-based methods like GMYC and closer to the species distinction provided by taxonomists (e.g. Puillandre et al. 2012, Hamilton et al. 2014). However, just as tree-based methods, it is in some cases prone to slight oversplit of the entities, depending on the overall genetic distances differential or sampling bias (Fontaneto et al. 2015, Yu et al. 2017). However, cross-validating it with the ultra-conservative approach of patristic distance, raises up the probability of the congruence of delimited taxa (Lefebure et al. 2006a). On the other hand, it still raises the question of unanimity of the phylogenetically delimited species with the biological species concept (De Queiroz, 2007). Recently, based on the experimental observations done on freshwater gammarids, it was argued that lineages separated by genetic distance exceeding 4% are less likely to form precopulatory pairs and thus, might be reproductively isolated (Lagrue et al. 2014). Even though, amphipods diverging by ca. 16% still formed precopulatory pairs under laboratory conditions, it was never observed in the field. It was then argued by the authors that lineages separated by ca. 16% living in sympatry seem to exhibit prezygotic barriers, preventing them from mating with divergent counterparts. In this case, the majority of the insular Aegean species, were isolated by more than 16% genetic distance from the closest related lineage, both when using commonly applied for calculating genetic distances Kimura-2-parameter model (K2p) as well as the patristic distance (comparison in Tab.2.4). According to the 4.3% K2p genetic distance threshold proposed for gammarids by Costa et al. (2009) and even more conservative, 16% patristic distance threshold proposed by Lefebure et al. (2006a), one may suppose that they are likely to represent separate and reproductively isolated lineages.

Table 2.4. Comparison of mean values of the genetic distance calculation methods.

MOTU	**Genetic distance to the closest MOTU (Patristic/K2p)**
MOTU1 *(Gammarus plaitisi)*	Patristic **17,6%/17,3%** K2p
MOTU2 *(Gammarus arduus)*	Patristic **17,2%/17%** K2p
MOTU3 *(Gammarus sp.1)*	Patristic **14,2%/14%** K2p
MOTU4 *(Gammarus* sp.*2)*	Patristic **16,5%/16,7%** K2p
MOTU5 *(Gammarus* sp.*3)*	Patristic **18%/18,1%** K2p
MOTU6 *(Gammarus uludagi)*	Patristic **18%/18,1%** K2p
MOTU7 *(Gammarus birsteini)*	Patristic **18,8%/20,5%** K2p
MOTU8 *(Gammarus crenulatus)*	Patristic **18,8%/20,5%** K2p

Up to now, in the Aegean basin, the members of the genus *Gammarus* were reported only from Lesbos, Gökçeada, Thassos, Evia and Crete (Karaman & Pinkster, 1977a; Karaman, 2003; Hou et al., 2011; Özbek & Özkan, 2017). Interestingly, none of these records indicated any endemic insular species. The results reveal that almost each lineage is endemic to one island only (Fig.2.2). Apart from the recent study by Hupało et al. (2018), describing *G.*

plaitisi and the dubious record of *G. uludagi* from Evia provided by Hou et al. (2011), there were no other molecular studies conducted on any Aegean gammarids. Given that molecular studies on the freshwater insular gammarids are scarce, one could expect a high number of overlooked diversity on the islands. Considering the fact that Aegean archipelago is characterized by exceptionally high percentage of endemism confirmed in numerous biota (Poulakakis et al., 2015), it is also probable that some of the overlooked Aegean taxa may, in fact, represent endemic lineages.

Miocene diversification of the Aegean freshwater insular gammarids

The results suggest that the diversification within the freshwater gammarids from the Aegean islands started already in the Middle Miocene, around 12 Ma (Fig.2.3). Up to that point, the Aegean region still formed a united landmass (Aegeis), comprising of not only all the present islands, but also the Balkan Peninsula and Asia Minor (Meulenkamp, 1971). Around 12 Ma, the fragmentation of the Aegeis landmass began, due to collision of the African tectonic plate with the Eurasian plate in the Middle Miocene (Steininger & Rögl, 1984). In fact, the movements of landmasses during that time supposingly induced divergence events in numerous freshwater crustaceans including amphipods, both epigean (Mamos et al. 2016, Copilaş-Ciocianu & Petrusek 2017, Grabowski et al. 2017a) and subterranean (Lefébure et al. 2006b), isopods (Sworobowicz et al. 2015) and crabs (Shih et al. 2009). Moreover, at this time, the formation of the Mid-Aegean Trench started and was fully accomplished by 10-9 Ma, resulting in separation of the western part of the Aegean region from the eastern one (Fig.2.5; Dermitzakis & Papanikolaou, 1981). These events have led to numerous isolation and diversification events in the Aegean fauna reflected in modern distribution patterns (Poulakakis et al. 2015). Interestingly, in the Aegean, Middle Miocene events are known to affect in general the divergence of mainly terrestrial taxa, including snails, beetles, isopods and a scorpion (Poulakakis et al. 2015). Seemingly, all of the Aegean freshwater biota diverging after the end of Miocene or more recently, in Pliocene and/or Pleistocene including species of crabs, crayfish and snails, used temporarily existing land connections (Trontelj et al. 2005, Jesse et al. 2011, Szarowska et al. 2016). The results suggest however, that the divergence of the Aegean insular freshwater gammarids was most probably affected by the

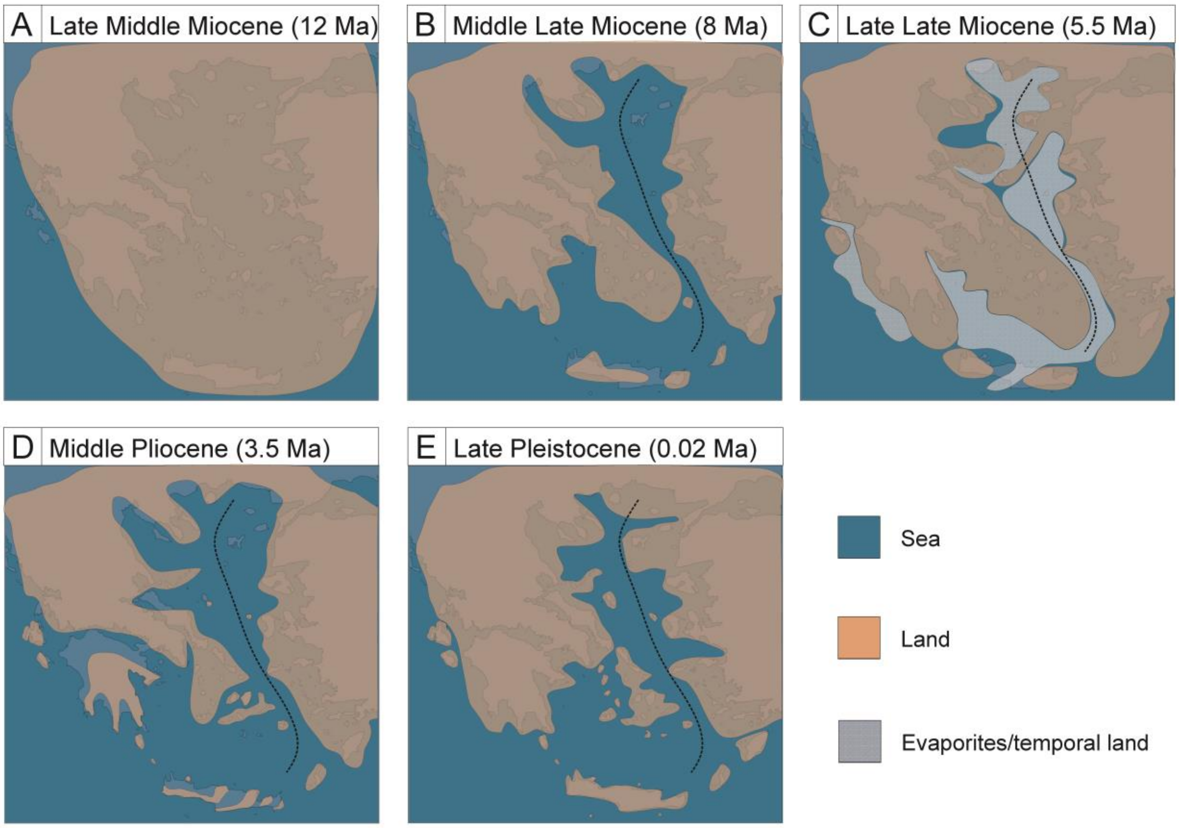


Fig. 2.5. The paleogeographical maps of the Aegean Basin (after Popov et al. 2004). The dotted black line represent Mid-Aegean Trench.

Middle Miocenic land movements and fragmentation, which also might have had significant impact on their current distribution. For example divergence of *G. uludagi* from Lesbos, belonging to the eastern Aegean islands, from its conspecific from Evia, as well as the divergence of *G. pulex* inhabiting the same island from that inhabiting Peloponnese, being on the western side of Aegean basin, coincides with the approximate time of the formation of the Mid-Aegean Trench (Fig.2.3, Fig.2.5), which supports the possible connectivity of the divergence with the geological history. It is also suggested that formation of Mid-Aegean Trench might have also played a role in the evolution and dispersal of freshwater *Pseudorientalia* snails (Szarowska et al. 2014). The authors suggest that limited distribution of *Pseudorientalia*, inhabiting currently only Aegean islands east of Mid-Aegean Trench might be associated with the formation of this biogeographical barrier and subsequent regional land fragmentation in Middle Miocene (Szarowska et al. 2014a). Similarly, the land fragmentation in the Middle Miocene most likely affected other divergence events shown in obtained data, such as the divergence of *G. plaitisi* from Crete, Tinos and Serifos from *G. pulex* inhabiting mainland Greece and Peloponnese, which coincides with the isolation of

Crete and the separation on the islands from Peloponnese (Fig.2.3, Fig.2.5). It also supports the previous results on the divergence of Cretan *G. plaitisi* (Hupało et al., 2018). The land separation at that time most probably resulted also in the divergence between the *Gammarus* from Andros and the one from Skyros (Fig.2.3, Fig.2.5). However, given the low posterior probability of that node, still more data is needed to determine the actual relationships between these taxa.

Most recent Plio-Pleistocene diversification events

Although major divergence events in Aegean freshwater gammarids took place in Middle Miocene, some of them happened more recently, in Plio-Pleistocene (Fig.2.3). The Pliocene water level fluctuations as well as Pleistocene glaciation events and subsequent falls of sea and inland waters strongly affected the river systems and promoted the diversification of numerous biota in the Mediterranean Region (Previšić et al., 2009; Poulakakis et al., 2015; Grabowski et al. 2017a). Several studies have confirmed that major European peninsulas, including Balkan Peninsula served as refugia for numerous taxa, which then served as starting point for species dispersal and diversification (Gonçalves et al. 2015, Mamos et al. 2016, Grabowski et al. 2017a). Obtained data suggests that the separation between *G. arduus* from Samothraki and the mainland conspecifics took place in the similar time, at the beginning of the Pleistocene (Fig.2.3). This makes Samothraki, based on the up-to-date molecular evidence, the only known Aegean island, not inhabited by gammarid endemics (Fig.2.1). Favorably, this event could be associated with the recurrent Pleistocene land connections between the island and the continent, with the last one being as recently as 20.000 years ago, which could then facilitate the gene flow between the populations (Perissoratis & Conispoliatis, 2003; Poulakakis et al., 2015). One could argue then for plausibility of similar scenario for the previous records, given that islands like Lesbos, Gökçeada and Thassos - all sharing the temporal land connections with the continent at similar time as Samothraki did (Perissoratis & Conispoliatis, 2003). However up to now, no molecular evidence is available to confirm or reject such hypothesis. On the other hand, although Evia was connected with the mainland in Pleistocene, the lineage most likely diverged earlier, in Miocene (Fig.2.3). However, this might be also be due to undersampling from the mainland and neighboring islands.

Even more recent is the diversification within *G. plaitisi* from the three Aegean islands: Crete, Tinos and Serifos (Fig.2.2, Fig.2.3). By confirming the presence of *G. plaitisi* on Tinos and Serifos, the proposed earlier alleged endemism of this species on Crete has to be rejected

(Hupało et al., 2018). This finding, along with the very low intraspecific haplotypic diversity on Crete, confirms late Pleistocenic dispersal of this species suggested by the authors (Hupało et al., 2018). Still, it is unclear how the species dispersed from Serifos to Crete, as there are no known temporal land connections between these two islands in Pleistocene (Fig.2.5; Poulakakis et al., 2015). It is equally puzzling to the evolutionary history and dispersal of the freshwater *Potamon* crabs, that diverged and dispersed to Crete as well as to Cyprus during Pleistocene, where no land connections were known to exist between the islands and the mainland (Jesse et al. 2011). It is suggested by the authors that early humans might have aided in the dispersal of certain taxa, including crabs, which would then mean that the arrival of these freshwater biota to Crete may be very recent. Another plausible scenario involves the passive dispersal by birds (e.g. Rachalewski et al. 2013), already suggested for freshwater *Daphniola* snails inhabiting distant eastern Aegean islands (Szarowska et al. 2014b). It is argued that the snail lineages probably diverged recently, in Pleistocene, where no land connection was supposingly present between those islands and thus, the dispersal was possible either due to the mediating factor or through the successive dispersal through neighboring islands (Szarowska et al. 2014b). It might be the case also for *G. plaitisi* with possible intermediate, yet still undiscovered, populations e.g. on Milos or Antikythera islands.

Taxonomic affiliations of the Aegean Gammarus

The results of this study provides new evidence for rejecting the monophyly of *Gammarus pulex* with three potentially new distinct taxa within this morphospecies (Fig.2.3). These findings confirm the recently observed high cryptic diversity and lack of monophyly in numerous widespread European freshwater gammarids (e.g. Mamos et al., 2014; Weiss et al., 2014; Copilaş-Ciocianu & Petrusek, 2015, 2017; Grabowski et al., 2017a,b, Hupało et al., 2019). Interestingly, this data also support the polyphyly of *Gammarus uludagi*, raising further questions about taxonomic congruence of other formerly described species (Fig.2.3). Moreover, the taxonomic affiliations of *G. birsteini* and *G. crenulatus* shown in these results (Fig.2.1, Fig.2.3) also suggest the incongruence of morphogroups, the so-called *Gammarus pulex*, *Gammarus roeselii* and *Gammarus balcanicus* groups, formerly described by Karaman & Pinkster (1977a,b; 1987). Even though both species were originally assigned to the *Gammarus pulex* group, these results suggest with high probability that they in fact are more closely related to *G. roeselii* than to *G. pulex* (Fig.2.3). It is also confirmed by thorough phylogenetic studies on gammarids, indicating that *G. birsteini* is probably outside of the *G. pulex* group (Hou et al. 2011, 2014). However, these studies did not include any molecular data on *G. crenulatus*, so more studies are needed to fully resolve this matter. The divergence between *G. roeselii* from mainland Greece, *G. birsteini* and *G. crenulatus* took place around 20 Ma, well before the fragmentation of Aegeis. It suggests that other processes might have played a role in their deep divergence, however again more data is needed to reveal more detailed evolutionary history of this group. These data, along with recent findings (Grabowski et al., 2017a) support the need for a comprehensive revision of *Gammarus pulex* and further studies on *Gammarus roeselii* morphospecies.

2.5 Conclusions

These results provide a first evidence of presence of the freshwater *Gammarus* species on Samothraki, Skyros, Andros, Tinos and Serifos, supporting the existence of at least three potentially new distinct species, providing a starting point for future species descriptions. Apart from Samothraki, the obtained results suggest high level of local lineage endemism, thus confirming the main hypothesis of this study implying that there will be high level of local endemism on the Aegean islands. The presence of *G. arduus* on Samothraki support the second hypothesis stating that the level of endemism will vary between the islands with lack of endemic lineages on the islands that were still connected with the mainland during Pleistocene. On the other hand, presence of distinct, separate MOTUs on Evia and Lesbos, favors rejecting this hypothesis, however, most probably still more data is needed both from the regions to Evia as well as from inland waters of Turkey, where from *G. uludagi* was reported.

The results of the time-calibrated phylogeny indicate multiple origins and different timescale of differentiation of the Aegean insular freshwater gammarids. The biogeographic affiliations of the studied insular taxa indicate its continental origin as well as the importance of the land fragmentation and the historical land connections of the islands that most probably influenced the evolutionary history of the Aegean biota. However, given the scarcity of available data and possible undersampling, one cannot exclude further discoveries of freshwater gammarids on other Aegean islands. More molecular data and a thorough sampling of the area is essential to provide a detailed picture on the evolutionary history of Aegean freshwater insular gammarids. The presented results provide not only another evidence indicating strong connectivity between the evolution of the freshwater biota and the geological history of the Mediterranean, but also broadens the still scarce knowledge on the evolutionary mechanisms of the diversification of the insular freshwater fauna.

Chapter III. Independent colonisations and high diversification in the insular freshwater gammarids

3.1 Introduction

Recently accumulating studies of the phylogeography of European freshwater organisms highlight the complex and turbulent geological and climatic history of the continent, particularly its southern part i.e. the Mediterranean region (e.g. Copilaş-Ciocianu & Petrusek 2017; Hou et al. 2011; Mamos et al. 2016). The Mediterranean ecoregion is recognized as one of the top biodiversity hotspots worldwide (Myers et al. 2000). Although the region is housing at least 6% of the global freshwater fauna, with nearly half of the species being endemic (Figueroa et al. 2013), the scarcity of studies investigatingthe biogeography and evolutionary history of freshwater organisms in the area (e.g. Casal-López & Doadrio 2018; Froufe et al. 2016) is striking, especially given that the Mediterranean freshwaters are subject to heavy anthropogenic pressure and are therefore critically endangered (Hopkins 2002). Even less is known about the origin of the freshwater fauna of the Mediterranean islands, which, given their isolation and habitat heterogeneity, are known as natural laboratories of evolution (Blondel et al. 2010; Hopkins 2002; Vogiatzakis et al. 2009).

The largest of the Mediterranean islands is Sicily. Geologically, Sicily is far from uniform, comprising three tectonic units of different origin (Fig.3.1), namely: 1) the Hyblean foredeep, forming the southeastern part of the island, emerged as a part of the African continental plate; 2) the Apenninic-Maghrebian orogen, forming the central and western part of Sicily and covering the largest part of the island, which is a belt formed by the collision of Southern Apennines and the Maghrebian chain belonging to the African plate; 3) the Calabrian-Peloritan arc, forming the northeastern part of Sicily, formerly part of the Corsican-Sardinian plate (Broquet, 2016; Di Maggio et al. 2017; Grasso 2001). Due to its heterogeneity, Sicily is known to house more than 14,000 freshwater and terrestrial species and, by that, is arguably the most speciose island in the Mediterranean (Médail & Quézel 1997; Ruffo & Stoch 2006). Still, little is known about the origin of the Sicilian freshwater biota. Given the complexity of the island’s geological composition and the turbulent changes in the geography of the

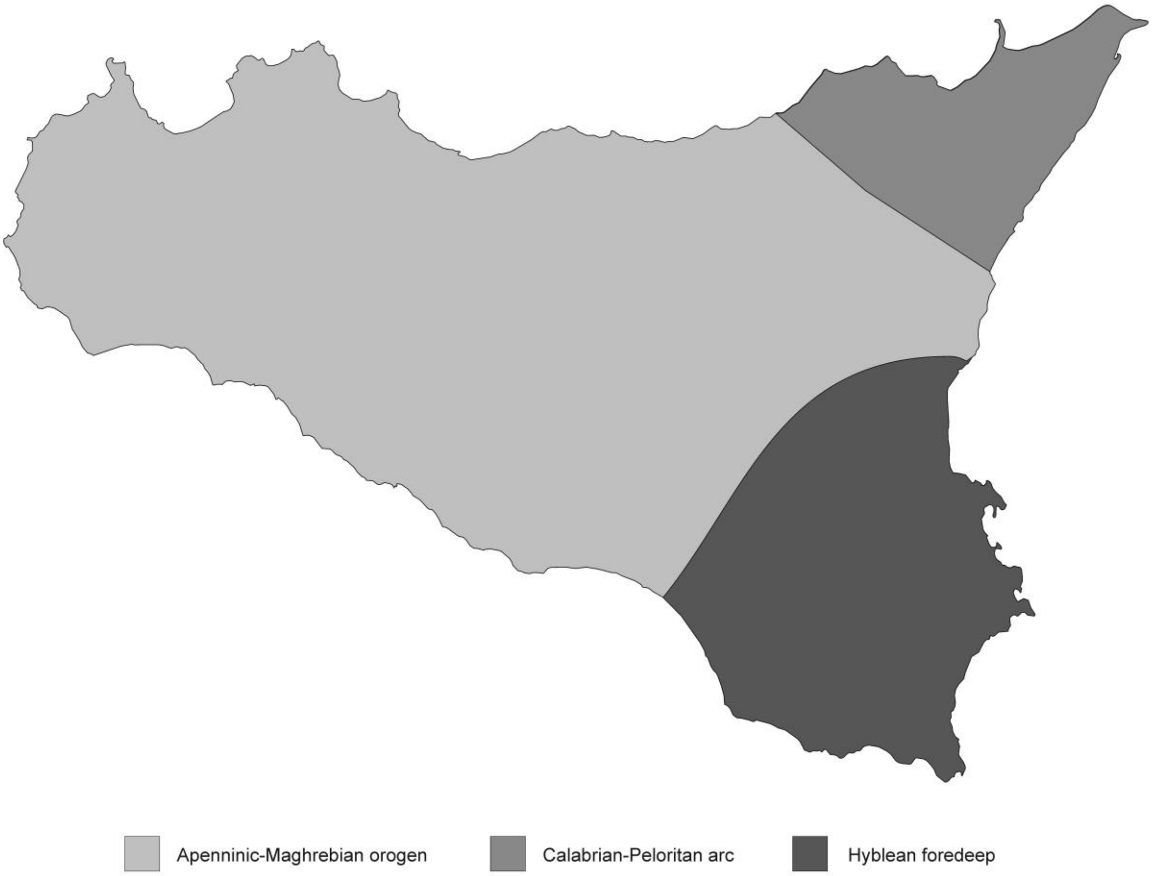


Fig. 3.1 Tectonic units of Sicily.

Mediterranean basin (Guglielmo & Marra 2011; Ruggieri 1973), several scenarios have been proposed for the origin of its freshwater fauna. First assumptions were formulated by La Greca (1957), who indicated for the first time that the Sicilian biota could have colonized the island during multiple, separate colonization events. It was also stated that the colonizations took place from different regions, including Apennine Peninsula and Northern Africa, which was agreed upon in several later studies (e.g. Baratti et al. 2004; Lázaro et al. 2011; Massa et al. 2011).

One of keystone aquatic taxon present in the majority of the lotic ecosystems of Europe and of the Mediterranean region are gammarid amphipods (Kelly et al. 2002). These crustaceans are one of the most abundant and biomass-dominant freshwater organisms, are considered to shape the local macroinvertebrate communities (MacNeil et al. 1997), and are also an important component of the freshwater biota of the Mediterranean Islands (Karaman & Pinkster 1977; Pinkster 1993). Given their limited dispersal abilities and exclusively aquatic life cycle, they are widely used as models for evolutionary studies (Bilton et al. 2001). Recent studies have revealed the complexity of phylogenetic patterns in freshwater gammarids, with one of the most speciose amphipod genus, *Echinogammarus* Stebbing 1899, turning out to be polyphyletic (Hou & Sket 2016; Hou et al. 2014). Moreover, further studies confirmed that most widespread freshwater gammarid morphospecies are actually complexes of local, distinct lineages or even cryptic species, with divergence events coinciding with major regional geological events (e.g. Copilaş-Ciocianu & Petrusek 2015, 2017; Grabowski et al. 2017; Hupało et al. 2018; Mamos et al. 2016; Rudolph et al. 2018; Weiss & Leese 2016).

In this study, the main aim is to investigate the origin and biogeographical affinities of some freshwater gammarids of Sicily. There are three prominent epigean gammarid morphospecies reported from the Sicilian freshwaters (Pinkster, 1993; Ruffo & Stoch, 2006) ascribed to the genus *Echinogammarus*: *Echinogammarus tibaldii* Pinkster & Stock, 1970, *E. adipatus* Karaman & Tibaldi, 1972 and *E. sicilianus* Karaman & Tibaldi, 1972. Recently, Sket & Hou (2018) proposed a new taxonomy of the *Echinogammarus* genus complex, attributing the clade to which *E. tibaldii* belongs to the genus *Homoeogammarus* Schellenberg 1937. Unfortunately, the other Sicilian morphospecies were not included in their analysis, and several genera and species remained “unclassified”. Pending an exhaustive taxonomic revision of *Echinogammarus*, the nomenclature adopted here follows the World Register of Marine Species (WoRMS) database (as of March 2019).

None of these morphospecies is considered endemic to Sicily, having populations in other Mediterranean islands, e.g. Sardinia and Malta, or on the Apennine Peninsula (Pinkster, 1993). Taking that into account, combined with the geological heterogeneity and the complex history of the Mediterranean region, main aim is to investigate the origin and biogeographical affinities of these freshwater gammarids of Sicily. It may be hypothesized that these three species, representing separate monophyletic lineages, have colonized the island during multiple, independent colonization events and by that, have different biogeographical affinities. Moreover, considering the continental character of the island, one can hypothesise that Sicilian freshwater gammarids should exhibit a low level of intraspecific genetic diversity. as in general continental islands are expected to have lower level of diversification and endemism comparing to oceanic ones due to their recurrent connections with the mainland (Whittaker & Fernández-Palacios 2007). Given that Sicily had numerous temporary land connections with Apennine Peninsula, Maltese Islands and North Africa, still sharing a land bridge with Apennine Peninsula in Pleistocene (Marra 2009), one could expect several possible faunal exchange episodes within the region.

These hypotheses are tested by performing DNA polymorphism analyses on three molecular makers: two mitochondrial (COI and 16S rDNA) and one nuclear (28S rDNA). By doing so, the first insight into the phylogeography of the Sicilian freshwater amphipod fauna is provided and their molecular diversity patterns interpreted in the framework of the paleogeography of Sicily.

3.2 Materials and methods

Sample collection and identification

The study material involved 174 individuals collected from 44 sampling sites and a variety of freshwater habitats on Sicily, including large rivers, streams and spring systems as well as from fresh waters of the Apennine Peninsula, Sardinia and Tunisia, visited during several sampling campaigns between 2010 and 2018 (Tab.3.1). Gammarids were sampled using a variety of methods, including collection from gravel, rocks and aquatic vegetation with a hand net or using rectangular kick sample nets (aperture 25x25 cm and 0.5 mm mesh size). The samples were sorted at the site and gammarids were immediately fixed in 96% ethanol. Afterwards, the material was examined under a Nikon 800 stereomicroscope. Identification to the species level followed the keys provided by Karaman (1993) and Pinkster (1993).

Table 3.1 Collection sites and MOTU information for specimens of Sicilian, Apennine, Sardinian and Tunisian *Echinogammarus* used in this study.

MOTU	**Site Code (number)**	**Locality (Province; Municipality; Exact site)**	**Longitude**	**Latitude**	**Number of individuals**	**Number of haplotypes (shared; shared site)**
E. tibaldii MOTU1	FS_11.129 **(1)**	Palermo; Monreale; Fiume Jato, Fosso della Chiusa, ponte strada Ginestra - Masseria La Chiusa	13.231509	37.992975	4	3 (0)
	FS_11.135 **(2)**	Palermo; Monreale; Abbeveratoio Masseria Strasatto,	13.251476	38.009727	2	2 (0)

		Poggio San Francesco				
	FS_11.138 **(3)**	Palermo; Marineo; Torrente Eleuterio	13.402153	37.958966	5	2 (0)
	FS_17.054 **(4)**	Palermo; Monreale; Sorgente a Strasatto	13.243091	38.008046	1	1 (0)
E. tibaldii MOTU2	FS_17.055 **(5)**	Palermo; Monreale; Sorgente di Villa Mirto	13.209903	38.033575	1	1 (0)
E. adipatus MOTU1	FS_11.130 **(6)**	Catania; Paternò; Scolina in cemento presso ex-Molino Vista	14.877122	37.566198	1	1 (0)
E. adipatus MOTU2	FS_18.002 **(7)**	Agrigento; Cattolica Eraclea; Cattolica Eraclea (AG) - Zubbia Camilleri	13.439258	37.441900	1	1 (0)
	FS_18.003 **(8)**	Agrigento; Cattolica Eraclea; Cattolica Eraclea (AG) - Zubbia del Cavallo	13.432847	37.483161	1	1 (0)
E. adipatus MOTU3	FS_14.208 **(9)**	Agrigento; Favara; Fiume Naro	13.663865	37.275839	3	1 (0)
E. adipatus MOTU4	FS_11.131 **(10)**	Caltanisetta; Santa Caterina Villarmosa; Fiume Salso o Imera Meridionale, Ponte Cinque Archi	14.131453	37.607169	1	1 (0)
	FS_18.004 **(11)**	Enna; Pietraperzia; Pietraperzia (EN) - Fiume Imera Meridionale	14.125617	37.463131	1	1 (1; SIT41)
	SIT41 **(12)**	Agrigento; Licata; Fiume Salso, a N di Licata	13.926400	37.157800	11	1 (1; FS_18.004)
E. adipatus MOTU5	SIT44 **(13)**	Catania; San Michele di Ganzaria; Cava dell'Elsa, a E di San	14.399800	37.298600	6	2 (0)

		Cono				
E. adipatus MOTU6	FS_18.026 **(14)**	Catania; Castiglione di Sicilia; Fiume Alcantara, Mitogio, S.P. 81	15.188002	37.875692	1	1 (0)
	SIT50 **(15)**	Catania; Motta Camastra; Fiume Alcantara, Gole dell'Alcantara	15.173600	37.877200	5	1 (0)
E. sicilianus MOTU1	FS_14.169 **(16)**	Palermo; Castronuovo di Sicilia; Rio nel Vallone di Sant'Antonio, a W di Monte Carcaci	13.481664	37.718404	1	1 (0)
E. sicilianus MOTU2	FS_18.027 **(17)**	Palermo; Segesta; Fiume presso la stazione di Calatafimi	12.852184	37.935750	1	1 (1; SIT34)
	SIT34 **(18)**	Palermo; Segesta; Fiume presso Calatafimi, Segesta	12.852200	37.935700	6	1 (1; FS_18.027)
E. sicilianus MOTU3	SIT44 **(13)**	Catania; San Michele di Ganzaria; Cava dell'Elsa, a E di San Cono	14.399800	37.298600	3	1 (0)
E. sicilianus MOTU4	SIT44 **(13)**	Catania; San Michele di Ganzaria; Cava dell'Elsa, a E di San Cono	14.399800	37.298600	1	1 (0)
E. sicilianus MOTU5	SIT44 **(13)**	Catania; San Michele di Ganzaria; Cava dell'Elsa, a E di San Cono	14.399800	37.298600	5	1 (0)
E. sicilianus MOTU6	FS_14.167 **(19)**	Ragusa; Ragusa; Sorgenti a Ragusa Ibla, riva destra Cava San Leonardo	14.748668	36.928743	6	3 (1; FS_18.024)

	FS_18.009 **(20)**	Ragusa; Ragusa; Cava Renna Rigagnolo (sotto grotta)	14.632886	36.870906	1	1 (0)
	FS_18.011 **(21)**	Ragusa; Ragusa; Grotta dell'Acqua	14.729139	36.988028	1	1 (0)
	FS_18.024 **(22)**	Ragusa; Ragusa; Fiume San Leonardo	14.720830	36.935469	1	1 (1; FS_14.167)
E. sicilianus MOTU7	FS_11.133 **(23)**	Siracusa; Siracusa; Fiume Ciane, 500 m a valle delle fonti	15.239626	37.040967	4	3 (0)
	FS_14.170 **(24)**	Siracusa; Siracusa; Fonte del Ciane	15.234864	37.041988	5	5 (0)
E. sicilianus MOTU8	FS_18.021 **(25)**	Siracusa Priolo Gargallo; Priolo Gargallo - Terzo Fontanile Diddino	15.149489	37.105403	1	1 (0)
	ITAJ1 **(26)**	Siracusa;Siracusa;Teatro Greco	15.274069	37.075686	4	1 (0)
	SIT48 **(27)**	Siracusa;Carlentini;Fiume lungo la SP9 Sortino-Lentini	15.043500	37.219700	3	3 (0)
E. sicilianus MOTU9	SIT48 **(27)**	Siracusa;Carlentini;Fiume lungo la SP9 Sortino-Lentini	15.043500	37.219700	13	7 (0)
E. sicilianus MOTU10	FS_18.015 **(28)**	Catania; Catania; Catania - Teatro Greco-Romano, Parodos Occidentale	15.083355	37.502663	3	3 (0)
E. sicilianus MOTU11	FS_11.136 **(29)**	Enna; Enna; Vallone della Furma, a N di Piazza Armerina	14.337759	37.448059	4	3 (0)
E. sicilianus MOTU12	FS_14.165 **(30)**	Enna; Enna; Abbeveratoio bivio SS121-SP28 per Enna	14.276855	37.571281	4	3 (0)
	FS_14.166	Enna; Enna;	14.324835	37.520972	4	3 (0)

	(31)	Abbeveratoio Acquacità				
E. sicilianus MOTU13	SIT44 **(13)**	Catania; San Michele di Ganzaria; Cava dell'Elsa, a E di San Cono	14.399800	37.298600	11	5 (0)
E. sicilianus MOTU14	**FS_11.132 (32)**	Siracusa; Sortino; Torrente Calcinara, necropoli di Pantalica, Monti Iblei **(locus typicus)**	15.025237	37.129838	2	2(0)
	FS_11.137 **(33)**	Siracusa; Noto; Sorgente presso la sponda del Torrente Manghisi, Ristorante la Trota, a SE di Palazzolo Acreide	14.977881	37.028090	4	3 (1; SIT47)
	FS_14.168 **(34)**	Ragusa; Ragusa; Torrente Dirupo Rosso, a valle sorgente Corchigliato	14.752915	36.980271	1	1 (0)
	FS_18.017 **(35)**	Siracusa; Noto; Noto antico - Cava del fiume Carosello, pressi Concerie	15.019839	36.940614	2	1 (0)
	SIT47 **(36)**	Siracusa; Noto; Fiume Manghisi, Villa Vela	15.027200	36.988400	11	7 (1; FS_11.137)
E. sicilianus MOTU15	**FS_11.132 (32)**	Siracusa; Sortino; Torrente Calcinara, necropoli di Pantalica, Monti Iblei **(locus typicus)**	15.025237	37.129838	4	3 (0)
Apennine outgroups						
E. veneris	SIT03	Viterbo; Bolsena; Lago di Bolsena	11.97680	42.6440	1	1 (0)
E. stammeri	SIT09	Roma; Percile; Torrente Licenza	12.90700	42.0956	1	1 (0)

E. veneris	SIT11	Roma; Castel Gandolfo; Lago di Albano	12.65430	41.7486	1	1 (0)
E. veneris	SIT12	Roma; Ardea; Fosso Grande	12.53300	41.6004	1	1 (0)
E. veneris	SIT15	Latina; Castelforte; Fiume Garigliano near Suio Terme	13.8893	41.3018	1	1 (0)
E. veneris	SIT20	Potenza; Armento; Fiume Agri, bridge on road SS598	40.2654	16.0580	1	1 (0)
E. pinksteri	SIT53	Potenza; Atella; Torrente la Levata, bridge on road SS93	15.6552	40.8675	1	1 (0)
E. pinksteri	SIT56	Avellino; Lacedonia; Torrente Calaggio, Contrada Chiancarelle	15.3643	41.0841	1	1 (0)
E. tabu mutus	SIT59	Campobasso; Bojano; Sorgenti del Biferno **(locus typicus)**	14.4779	41.4796	1	1 (0)
E. sp.	SIT60	Isernia; Rocchetta a Volturno; Sorgenti Capo Volturno	14.0781	41.6387	1	1 (0)
E. tibaldii	SIT61	Pescara; Popoli; Sorgenti di Capo Pescara	13.8214	42.1637	1	1 (0)
E. sp.	SIT70	Ascoli Piceno; Amandola; Torrente Vetremastro	13.3674	42.9660	1	1 (0)
E. sardus	SRD1	Oristano; Santu Lussurgiu; Riu Perda Lada **(locus typicus)**	8.6552	40.1580	1	1(0)
E. tibaldii	SRD3	Oristano; Cuglieri; Funtana Tummemmere	8.5764	40.1808	1	1(0)

E. sicilianus	SRD4	Nuoro; Isili; Riu San Sebastiano	9.1083	39.7625	1	1(0)
E. pungens	TN21	Béja; Ouechtata; river in Ouechtata	8.9867	36.9598	1	1(0)
E. afer	TN03	Gabès; Oudref; Oued Akarit	9.9820	34.1083	1	1(0)
E. tacapensis	TN09	Tozeur; As-Sabikah; spring in Oasis de Chebika	7.9392	34.3202	1	1(0)
E. simoni	TN26	Béja; Oued Zarga; small inflow to Oued Zarga, near Barrage Sidi Salem	9.3881	36.7065	1	1(0)
E. carthaginiensis	TN27	Bizerte; Fritissa; stream 12km south from Mateur	9.6289	36.9384	1	1(0)

DNA extraction, PCR amplification, sequencing

The DNA was extracted using the protocol described in Hupało et al. (2018). The eluted DNA was stored at 4°C until amplification and finally long-term stored at -20°C. Subsequently, a fragment of the cytochrome c oxidase subunit I gene (COI) was amplified employing different primer pairs depending on the amplification success. The primer sequences, PCR conditions and original references for all the molecular markers used in this study are described in Table S1. In UL, PCR products (5 µl) were cleaned up using exonuclease I (2 U, ThermoFisher Scientific) and alkaline phosphatase FastAP (1 U, ThermoFisher Scientific) according to the manufacturer's guidelines. Direct sequencing was performed using the same primers as for amplification and the BigDye terminator technology by Macrogen.

Subsequently, one individual per delimited Molecular Operational Taxonomic Unit (MOTU) (see below) was amplified for additional markers that were used in phylogeny reconstruction – mitochondrial 16S rRNA and a nuclear marker being 28S rRNA gene (Table S1).

Sequence data authentication, editing, alignment and deposition

All resulting sequences were positively checked to correspond to *Echinogammarus* DNA via BLASTn searches in GenBank (Altschul et al. 1990) and then assembled, aligned and trimmed to 622 (COI), 359 (16S) and 945 (28S) base pairs using the Geneious 10.0.9 software package (Kearse et al. 2012). Alignments were performed using the MAFFT plugin with G-INS-i algorithm in Geneious. In case of double peaks and low quality regions detected in some of the sequences of 28S, the two strands were compared and the most dominant signal was chosen at certain site. All sequences were deposited in GenBank. Additionally, the sequences of all markers used in this study were compiled in the dataset and deposited in the public repository of the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007), where all the relevant metadata information and sequence trace files will be accessible publicly after publication of this chapter.

MOTU delimitation, intra-MOTU diversity and historical demography

The Molecular Operational Taxonomic Units (MOTUs) were delimited using COI sequence dataset, according to the distance-based Automatic Barcode Gap Discovery (ABGD) methodology (Puillandre et al. 2012). The results of the delimitation results were cross-validated with other delimitation methods, namely Barcode Index Number (BIN) system (Ratnasingham & Hebert 2013), GMYC model (Pons et al. 2006; Monaghan et al. 2009) and mPTP implementation (Kapli et al. 2017). Additionally patristic distances were measured in Patristic 1.0 (Fourment & Gibbs 2006), according to a patristic distance threshold PDT of 16% proposed for crustaceans at the COI locus (Lefébure et al. 2006), inferred from prior observations of multiple morphologically distinguishable crustacean species. The phylogenetic relationships between MOTUs within each morphospecies were visualised by the phylogenetic networks computed with the neighbour-net algorithm and K2p-distances using the SplitsTree4 software (Huson & Bryant, 2005). The haplotype composition of particular MOTUs was illustrated by drawing median-joining networks (Bandelt et al. 1999) using PopART (Leigh & Bryant 2015). Since PopART does not consider indels as 5th characters, additional lines representing indel mutation steps were added by hand in some cases in the haplotype networks of nuclear markers. The haplotypes and intraspecific K2p distances were calculated using the DnaSP5 software (Librado & Rozas 2009) and MEGA 7.0 (Kumar et al., 2016), respectively. To provide insights into historical demography, such as the temporal changes of the effective population size of examined species in Sicily, Bayesian Skyline Plot (BSP) analysis was performed (Drummond et al. 2005) using the BEAST2 software package (Bouckaert et al. 2014). One most widely distributed MOTU per morphospecies was subjected to this analysis, i.e. MOTU1 of *E. tibaldii* (represented by 12 individuals from 4 localities), MOTU4 of *E. adipatus* (represented by 13 individuals from 3 localities) and MOTU14 of *E. sicilianus* (represented by 21 individuals from 5 localities). In each case the TN93 + G + I model of evolution was used as best fitting, according to bModel test (Bouckaert & Drummond 2017). Three MCMC runs of 10 M iterations were performed, sampled every 1,000 iterations. MCMC runs were examined using Tracer v1.6 and all the sampled parameters for each studied MOTU achieved sufficient effective sample sizes (ESS > 200).

Geographical patterns of molecular divergence

To illustrate the spatial distribution of the molecular divergence of the studied MOTUs, the tree models were built geographic using GenGIS 2.5 software package (Parks et al. 2013). This analysis was based on the complete COI sequence dataset, which was then combined with the geographical locations of the sampling sites. Only in case of *E. sicilianus,* containing a high number of MOTUs and COI sequences from a number of relatively nearby sampling sites, one sequence per MOTU was used for better clarity. To evaluate the best-fit geographic model of evolution for each morphospecies, both linear axes analysis and significance test was performed. The output of the analyses with the best supported phylogenetic trees were superimposed on the vector map of Sicily.

Phylogeny reconstruction and time calibration

Tests for substitution saturation were performed in DAMBE 7.0.28 (Xia 2018), using the index proposed by Xia et al. (2003) to assess the potential loss of phylogenetic signal, showed that no significant saturation was detected ($p<0.001$) for COI, 16S and 28S markers. Then, applying the best partitioning scheme proposed by PartitionFinder (Lanfear et al. 2012), the molecular data was divided into five partitions: 16S, 28S and the three codon positions of COI. The congruence between mitochondrial and nuclear data was checked using tanglegrams generated using Neighbor-Net algorithm in Dendroscope (Huson & Scornavacca 2012). The time-calibrated phylogeny was reconstructed in BEAST 2.4.7 package (Bouckaert et al. 2014), performing three MCMC chains of 100M iterations, with sampling every 2,000 iterations, using the best-fit substitution models determined by bModel test (Bouckaert & Drummond, 2017): for 16S and 28S – Tamura-Nei model (TN93) with gamma-distributed rate heterogeneity (G), for first COI codon position – TN93 with G and invariable sites (I), second COI codon position – TIM model with G and I, third COI codon position – TN93 with G. The optimal molecular clock was chosen via path sampling/stepping-stone procedures and

Bayes' factors, using BEAST 2.4.7. As the strict clock was rejected for each partition (lnBF>200), the uncorrelated log-normal relaxed clock was used (Drummond et al. 2006).

Five primary calibration points known from literature and related to geological events were used (Tab.2.2) as well as secondary calibration points, which served to validate the clock calibration based upon the primary calibration points (Tab.3.2). The most recent calibration point is based on the radiation of the endemic Gammarus species flock in Lake Ohrid, connected with the age of the lake itself (Wysocka et al. 2013, 2014), which is estimated to be maximum ca. 2 Ma. The second one is based on the split between the Black Sea and the Caspian Sea populations of Pontogammarus maeoticus, estimated at about 4 Ma, which coincided with the shifts of continental plates, leading to split of former Pontian Lake into Black and Caspian Sea (Cristescu et al. 2003; Nahavandi et al. 2013). The third calibration point marks the diversification of the Gammarus fossarum species complex that happened in the Carpathians due to Middle Miocene subsidence event between 15 and 17 Ma (Copilaş-Ciocianu & Petrusek 2015). The fourth one is based on the origin of the Acanthogammaridae family, endemic to Lake Baikal, estimated at 28-30 Ma (Macdonald et al. 2005; Mats et al. 2011; Sherbakov et al. 1999). The oldest calibration point deals with the assumed connection between the Eocene regression of the Paratethys Sea at ca. 37 Ma and the split between Sarothrogammarus and Rhipidogammarus genera (Hou et al. 2011, 2014). The constraints of the calibration points were applied by imposing priors on the respective tree nodes. The usage of lognormal distribution of the MRCA priors, enabled the incorporation of the possible uncertainty of the data. Besides using the sequences available for the molecular clock calibration, additional sequences of *Echinogammarus* from the literature and from the regions that had once land connections with certain geological compartments of Sicily, namely Apennine Peninsula, Sardinia and Tunisia were used to provide further insights into the phylogenetic and biogeographical affinities within *Echinogammarus* (all individuals listed in Tab.3.1 and Tab.3.2).

Parameters of all three runs were examined in Tracer 1.6 and reached the effective sampling size values above 200. The runs were combined and resampled with LogCombiner 2.4.7 with 30% burn-in, with the maximum clade credibility chronograms being annotated using TreeAnnotator 2.4.4 and visualised using FigTree 1.4.4 (Bouckaert et al. 2014).

Table 3.2 Reference sequences used for cross-validation of the calibration of the molecular clock used in the reconstruction of the time-calibrated phylogeny.

Outgroups	**Expected calibration time (based on literature)**	**Actual calibration time (based on our results)**	**GenBank Accession numbers**	**References**
Gammarus balcanicus A	2 ± 1 Ma (f. *G. balcanicus* B)	1.9 ± 1.3 Ma	COI: KU056256 16S: KU056052 28S: KU056154	Mamos et al., 2016
Gammarus balcanicus B	2 ± 1 Ma (f. *G. balcanicus* B)	1.9 ± 1.3 Ma	COI: KU056394 16S: KU056103 28S: KU056154	Mamos et al., 2016
Echinogammarus acarinatus	42 ± 10 Ma (f. Pontogammarids)	64.5 ± 20 Ma	COI: KF478548 28S: KF478458	Hou et al., 2013
Echinogammarus berilloni	46 ± 15 Ma (f. *Typhlogammarus*)	35 ± 18 Ma	COI: KF478583 28S: KF478497	Hou et al., 2013
Typhlogammarus mrazeki	46 ± 15 Ma (f. *E. berilloni*)	35 ± 18 Ma	COI: KF478586 28S: KF478500	Hou et al., 2013
Echinogammarus marinus	65 ± 15 Ma (f. freshwater *Echinogammarus*)	87 ± 22 Ma	COI: KF478580 28S: KF478494	Hou et al., 2013

History of diversification and reconstruction of the ancestral states

The history of diversification was tested using the lineage through time (LTT) plot generated in Tracer 1.6 from 1,000 trees inferred from the Bayesian analysis performed in BEAST 2.4.7 software. The Bayesian analysis was performed using the same priors and MCMC settings as of the time-calibrated reconstruction of phylogeny, described above.

Statistical Dispersal-Vicariance Analyses (S-DIVA; Yu et al. 2010) were used in order to reconstruct the possible geographic localisations of ancestors (ancestral nodes). S-DIVA statistically evaluates the alternative ancestral ranges at each node in a given phylogenetic reconstruction accounting for phylogenetic uncertainty and uncertainty in dispersal-vicariance analysis optimization identifying nodes at which episodes of dispersal, vicariance or extinction occurred. For the analysis each tip (individual) was coded with one of six geographic regions with each of three geological compartments of Sicily as well as Apennine Peninsula, Sardinia and Tunisia. Bayesian consensus tree generated during the phylogeny reconstruction and 1,000 trees resampled with LogCombiner 2 were used as an input. S-DIVA was run through RASP 4.1 (Reconstruct Ancestral State in Phylogenies) software package

(Yu et al. 2015), analysis was performed using 1,000 reconstructions for the final tree, allowing for a maximum of four areas at each node, without exclusion of any region combinations at nodes.

3.3 Results

Taxonomic identification and geographic distribution

The sorted material was morphologically assigned to the three species of *Echinogammarus* reported already from Sicily (Pinkster 1993): *E. tibaldii*, *E. adipatus* and *E. sicilianus*.

Out of 36 sampling sites, *E. tibaldii* was found only on five sites in a limited area of the northwestern part of Sicily, stretching latitudinally for ca. 19 km. *Echinogammarus adipatus* was identified in 10 sites, mainly in central and northeastern part of Sicily extending latitudinally for ca. 158 km. The most common species was *E. sicilianus*, present in 22 sites distributed in a stretch over ca. 233 km long, from northwest to southeast of Sicily. Interestingly, it was the only gammarid species found in the southeastern part of Sicily. Only one site, namely SIT44, was shared by two species, *E. adipatus* and *E. sicilianus* (Tab.1). Each of all the other sites contained single species only.

MOTU delimitation, diversity, distribution and demography

The ABGD MOTU (=lineage) delimitation method suggested presence of two MOTUs of E. tibaldii, six MOTUs of *E. adipatus* and 15 MOTUs of *E. sicilianus*. The other delimitation methods generally supported such numbers of distinct MOTUs, exhibiting even higher number of MOTUs for each species with BIN method delimiting in overall 27 MOTUs, GMYC delimiting 34 MOTUs, mPTP delimiting 27 MOTUs compared to 23 MOTUs delimited by ABGD (Fig.S2). The ABGD was chosen as the main delimitation method as it was most conservative approach. However, the MOTU delimitation based on patristic distance estimation with the ultraconservative 16% threshold, indicated the integrity of E. tibaldii and *E. adipatus*, though it distinguished two separate groups within *E. sicilianus*. On the other hand, the ABGD MOTUs were also backed up by the fact that none of them shared any nuclear haplotype with others, which supported the congruence and distinctness of delimited units.

Out of 13 sequenced individuals of *E. tibaldii* (Tab.3.1, Fig.3.2A), nine COI haplotypes were identified and none was shared between the locations. The maximum number of haplotypes per site was three (for FS_14.129). The highest K2p distance for MOTUs of *E. tibaldii* was 0.09, with the average genetic distance of 0.02. The MOTUs of *E. tibaldii* are confined to the northwestern part of Sicily, where four sites had one MOTU and the other one was present only in one separate site (Fig.3.3).

Out of 31 sequenced individuals of *E. adipatus* (Tab.3.1, Fig.3.2B), ten COI haplotypes were detected with one being shared between two sites (FS_18.004 and SIT41). None of the sites contained more than one haplotype of *E. adipatus*. The maximum K2p distance was 0.12 and the average genetic distance was 0.06. Each of the sites inhabited by *E. adipatus* contained a single MOTU only. One site (SIT44) was shared with several MOTUs of *E. sicilianus* (Fig.3.3).

Out of 109 sequenced individuals of *E. sicilianus* (Tab.3.1, Fig.3.2C), 75 COI haplotypes were identified with only two shared between the sites. The maximum number of haplotypes per site was 12 (SIT44), which was also the most diverse in terms of MOTUs with four distinct MOTUs co-occurring with one MOTU of *E. adipatus*. The maximum K2p distance was 0.2 with the average genetic distance of 0.13. Besides SIT44, only two other sites (FS_11.139 and SIT48) contained two co-occurring MOTUs of *E. sicilianus*. Each of other sites was inhabited by single MOTU only (Fig.3.3).

The highest COI haplotypic diversity and the most complex phylogenetic relationships between haplotypes was observed in *E. sicilianus* (Fig.3.3), whereas individuals of *E. adipatus* were grouped, at most, in two haplotypes per MOTU. In case of *E. adip*atus, with exception of the highly divergent MOTU2, the remaining MOTUs contained haplotypes separated by up to three mutation steps with one dominant haplotype (Fig.3.3). In contrary, the most diverse MOTUs of *E. sicilianus* included haplotypes separated by several substitutions, in some cases exceeding ten mutation steps. In particular, MOTUs of *E. sicilianus* inhabiting the southeastern part of Sicily (Hyblean foredeep; Fig.3.1) exhibited the highest number of haplotypes, including the highest intra-MOTU genetic distance (Fig.3.3). A similar pattern can be observed in MOTU1 of *E. tibaldii*.

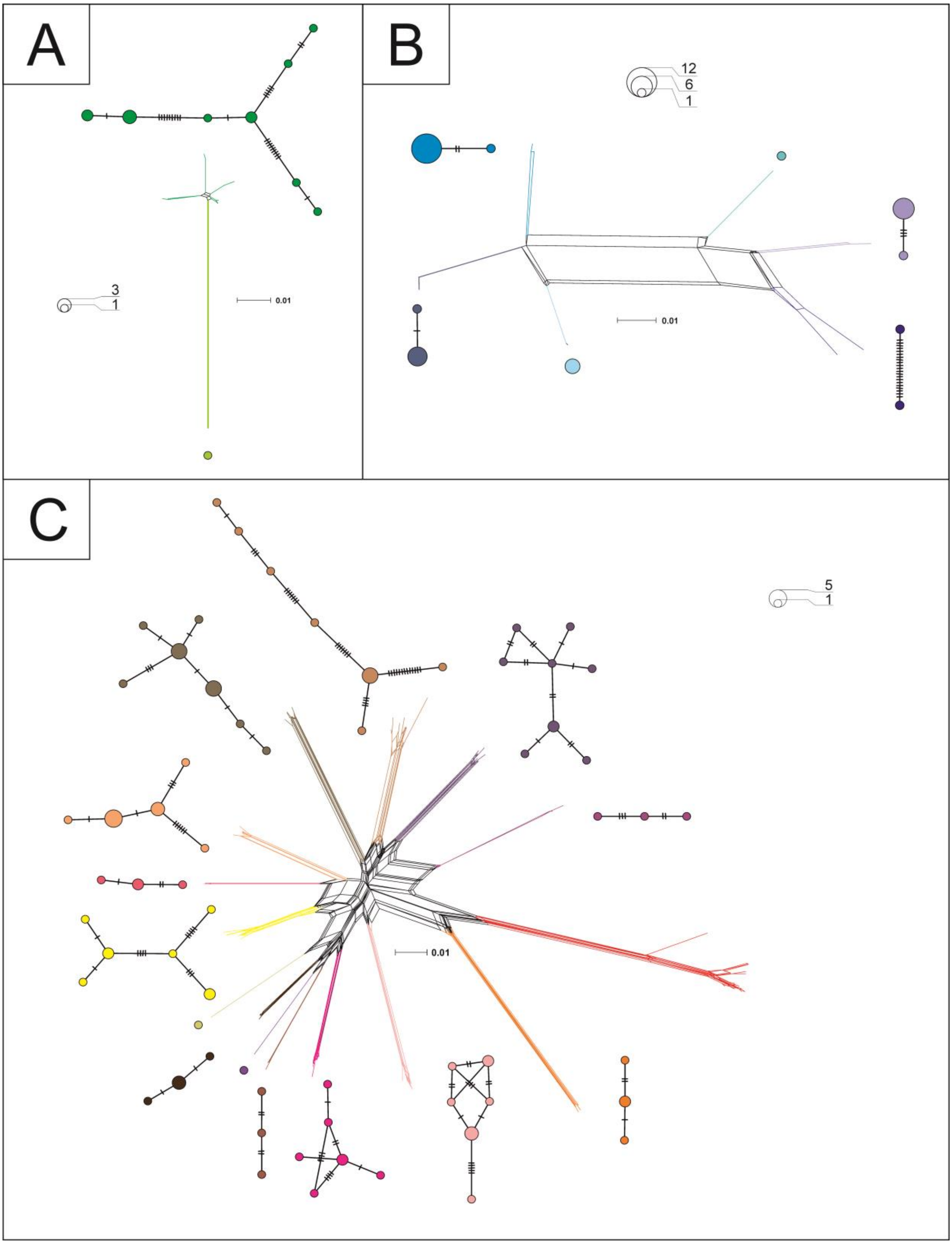


Fig.3.2 Phylogenetic network computed with the neighbour-net algorithm and K2p-distances with the Median Joining Networks for the respective MOTUs. The coloured dots correspond to the MOTUs presented in other figures. A) *Echinogammarus tibaldii*, B) *Echinogammarus adipatus*, C) *Echinogammarus sicilianus*.

Results of the BSP analyses (Fig.S3) suggests a recent slight population growth in MOTU14 of *E. sicilianus*, which took place around 100,000 years ago, whereas the MOTU1 of *E. tibaldii* as well as MOTU4 of *E. adipatus* did not exhibit a clear sign of expansion, suggesting that the effective population size of these lineages was quite stable over time.

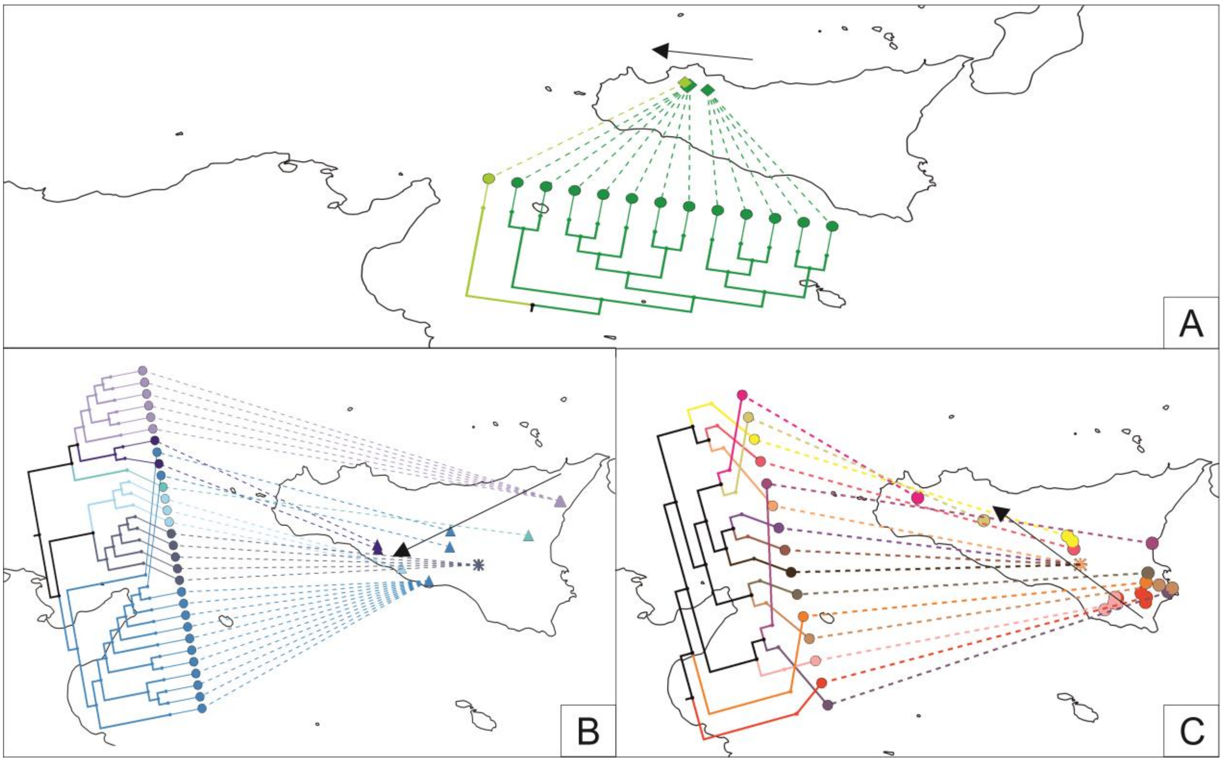


Fig.3.3 Geographic tree models superimposed on the vector map of Sicily. The colours correspond to those presented in other figures. A) *Echinogammarus tibaldii*, B) *Echinogammarus adipatus*, C) *Echinogammarus sicilianus*.

Geographic tree models

The geographic tree models indicated a significant correlation between the spatial distribution of MOTUs and their molecular divergence ($p<0.05$), regardless of the analysed morphospecies (=group of MOTUs within a described species) (Fig.3.3). The generated phylogenetic trees presented the statistically best-fit arrangements, reflecting the most supported geographic model of divergence, which differed between the studied morphospecies from Sicily (Fig.3.3). For *E. tibaldii*, the divergence following dispersal along the latitudinal axis is the most probable scenario (Fig.3.3A). Interestingly, both *E. adipatus* and *E. sicilianus* tree models supported the divergence along the diagonal axis with the nearly identical best-fit arrangements (Fig.3.3), which may indicate similar dispersal and diversification patterns for both morphospecies. The current distribution and molecular divergence of the MOTUs support the southwestward dispersal of *E. adipatus*, originating from the northeastern part (Calabrian-Peloritan arc), (Fig.3.3B) and the northwestern dispersal in case of *E. sicilianus*, originating from the southeastern part (Hyblean foredeep) (Fig.3.3).

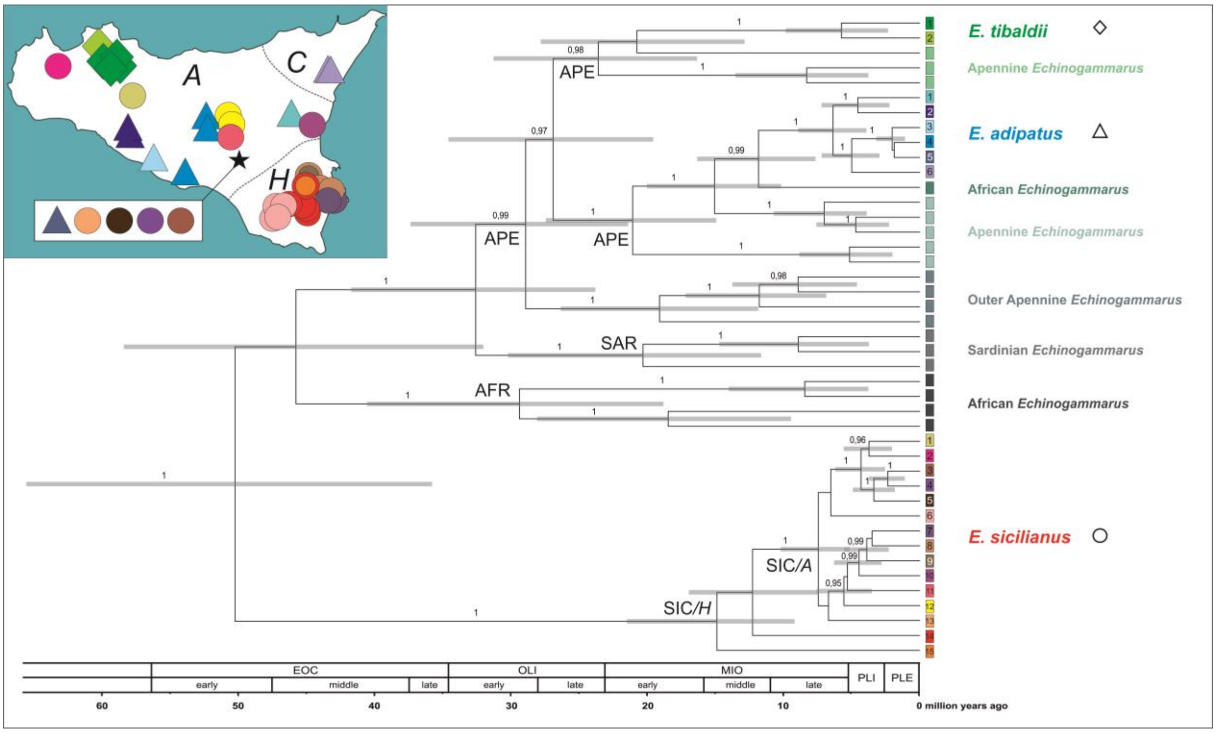


Fig.3.4 Maximum clade credibility, time-calibrated Bayesian reconstruction of phylogeny of Sicilian *Echinogammarus* MOTUs with the map of the distribution of particular MOTUs in the island. Phylogeny was inferred from a sequences of the mitochondrial COI and 16S rRNA genes and nuclear 28S rRNA gene. The numbers by respective nodes indicate Bayesian posterior probability values ≥ 0.9. The coloured bars represent ABGD delimitation method, with colours correspond to those presented in other figures. Violet node bars represent 95% HPD.

Phylogeny reconstruction

Based on the generated tanglegram, the mitochondrial and nuclear data provided generally congruent tree topology (Fig.S4). The time-calibrated phylogeny revealed the presence of two major clades, one comprising of *E. sicilianus* morphospecies and the other one including *E. tibaldii* and *E. adipatus* combined with various species of *Echinogammarus* from Africa, Sardinia and the Apennine Peninsula (Fig.3.4). These two clades share a common ancestor, which most likely diverged around 50 Ma (95% HPD: 65.7–35.6 Ma). The divergence between *E. tibaldii* and *E. adipatus* clade/morphospecies took place probably about 27.5 Ma (95% HPD: 37.4–21.4 Ma). Such divergence time overlaps both with the split of the Apennine *Echinogammarus* as well as with the split of *E. tibaldii* from its closest Apennine congeners (ca. 27–15 Ma). The split between *E. adipatus* clade and its closest relative, which inhabits northern Africa, took place probably ca. 14 Ma (95% HPD: 16.4–7.7 Ma), whereas the earlier divergence between these lineages from the closest Apennine congeners took place about 16 Ma (95% HPD: 20.1–10.2 Ma). The remainder of African and Apennine *Echinogammarus* along with the Sardinian ones formed the outgroups to *E. tibaldii* and *E. adipatus*, diverging from them between 20 and 60 Ma. The diversification within *E. tibaldii* and *E. adipatus* morphospecies started probably ca. 5 Ma (95% HPD: 9.1–2.3 Ma). On the other hand, the onset of diversification within *E. sicilianus* species complex commenced earlier, about 14.1 Ma (95% HPD: 21.5– 9.2 Ma). However, the highest peak of diversification most probably took place about 5 Ma (95% HPD: 8.3–3.7 Ma), similarly to both *E. tibaldii* and *E. adipatus* (Fig.3.4). All the reference sequences used to cross-validate the molecular clock reflected the divergence times known from literature (Tab.3.2; Fig.S5). Most of the MOTUs, belonging to all the studied morphospecies, inhabited the largest geological unit of Sicily, i.e. the Apenninic-Maghrebian orogen. The Hyblean foreland is exclusively inhabited by the oldest lineages of *E. sicilianus,* while in the northeastern Calabrian-Peloritan Arc only a single MOTU of *E. adipatus* was found (Fig.3.4).

History of diversification and reconstruction of the ancestral states

In general the lineages-through-time plot (Fig.3.5) shows that the accumulation of lineages remained mostly constant over time. Slight increase in lineage accumulation, hence diversification rate, can be noticed at the end of Miocene (ca. 5 Ma).

The reconstruction of ancestral states (Fig.S6) suggested that the ancestor of the clades including *E. tibaldii* and *E. adipatus* together with the Apennine lineages and the Tunisian *E. aff. adipatus*, inhabited the Apennine Peninsula, supporting their continental origin. The ancestor of *E. adipatus* lineages inhabited possibly the Apenninic-Maghrebic orogen, similarly to the ancestor of *E. tibaldii* MOTUs. On the other hand, the ancestor of *E. sicilianus* lineages most probably inhabited the Hyblean foredeep and subsequently dispersed to the Apenninic-Maghrebic orogen. Within *E. sicilianus* morphospecies, the analysis suggested secondary dispersal events from Apenninic-Maghrebic orogen back to Hyblean foredeep. With the exception of the Tunisian *E. pungens*, whose ancestor lived most probably in the Apennine Peninsula, the ancestry of the other Tunisian *Echinogammarus* is most probably African. Similarly, the ancestor of Sardinian taxa was likely to be restricted to this island. All the deep node splits are a result of multiple vicariance events (Fig.S6).

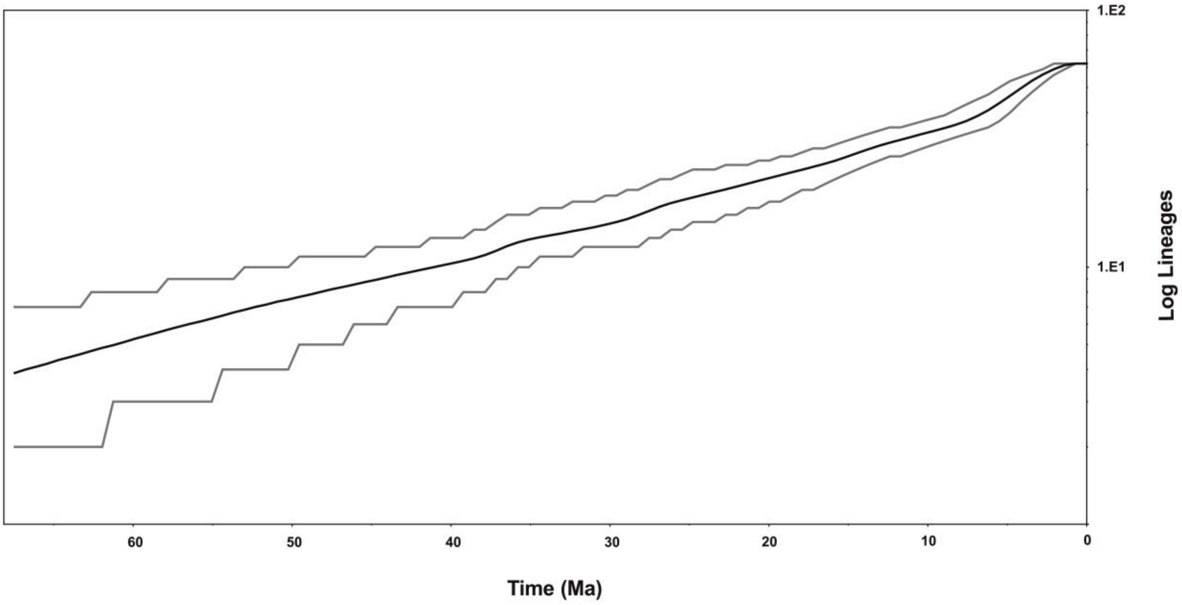


Fig.3.5 Lineage Through Time (LTT) plot for Sicilian *Echinogammarus*. The black representing the median with grey lines representing 95% CI.

3.4 Discussion

Biogeographical affinities of Sicilian freshwater fauna

The results of the time-calibrated phylogeny indicated that each morphospecies present in Sicily has a distinct evolutionary history and different biogeographical affinities (Fig.3.4), suggesting that they colonised the island independently at various time periods. There have been several recent studies aiming to provide biogeographical insights on the Mediterranean insular fauna, using available molecular genetic information (e.g. Bauzà-Ribot et al. 2011; Jesse et al. 2011; Ketmaier & Caccone 2013; Poulakakis et al. 2015). However, there have been only few recent studies focusing on the phylogeography of the Sicilian fauna, with a vast majority of them concerning terrestrial taxa, both vertebrates (e.g. Marzahn et al. 2016; Stöck et al. 2016; Senczuk et al. 2018) and invertebrates (e.g. Franck et al. 2000; Habel et al. 2010). In their thorough work upon the comparative phylogeny of Sicilian herpetofauna, Stöck et al. (2016) indicated that post-Messinian colonisation of the island by amphibians and reptiles took place, depending on species, either from the Apennine peninsula or from Africa. Less is known about the origin of Sicilian freshwater fauna, a topic subjected to a long-lasting debate. Over 70 years ago, La Greca (1957) formulated the first biogeographical hypotheses about the Sicilian fauna, supporting the existence of ‘ancient endemics’, which colonized the island following Oligocene and Miocene microplate migrations. The existence of such “ancient endemics” however, was rejected by Ruggieri (1973) and more recently, by Massa et al. (2011), who postulated that the extant Sicilian fauna comprises only Pliocenic and Quaternary colonisers. This conjecture was also supported by the study of microcrustacean communities of Sicilian temporary ponds (Marrone et al. 2009), suggesting that no insular freshwater crustaceans survived the Messinian Salinity Crisis (5.9-5.3 Ma) that resulted in the desiccation of the entire Mediterranean Basin (Hsü et al. 1977; Krijgsman et al. 1999).

The origin of Sicilian freshwater gammarids

Our data indicates that the common ancestor of all current Sicilian *Echinogammarus* species have diverged already in Eocene/early Oligocene (Fig.3.4). Thus, it would support the scenario that Sicilian *Echinogammarus* assemblages are de facto “ancient endemics” (sensu La Greca 1957) that survived the recurrent sea level changes throughout the turbulent history of the island. The ancestor of Sicilian freshwater *Echinogammarus* most likely had a marine origin as it diverged in the late Eocene, when the whole area comprising the current Sicily as well as the Apennine Peninsula and neighbouring islands was still submerged (Popov et al. 2004). These results are thus congruent with those by Hou et al. (2014), who postulated marine ancestry of Mediterranean *Echinogammarus* and the middle Eocene habitat shift. The colonisation of fresh waters by the widespread genus *Gammarus* also took place in middle Eocene, which suggests that the Tethyan regression and the growing number of freshwater habitats in the Eurasian plate due to the movement of landmasses favoured this evolutionary habitat shift in both genera (Hou et al. 2011, 2014). According to the data, at that time the common ancestor of the studied species diverged into two major groups – the *Echinogammarus sicilianus* morphospecies and another one that colonised the fresh waters of Sicily, Apennine Peninsula, Sardinia and Northern Africa. Thus, based on these findings, one may argue that the current *E. sicilianus* complex have evolved directly from marine ancestors.

On the other hand, within the other clade, consisting of *E. adipatus* together with some Apennine and African lineages, and including the Sicilian *E. tibaldii* and its Apennine lineages, the diversification started in Middle Oligocene (Fig.3.4). One can suppose that the Sicilian *E. tibaldii* shared a common ancestor with the Apennine *Echinogammaru*s taxa, from which it diverged at the beginning of Miocene (Fig.3.4). This divergence could have been connected with the land fragmentation events, which at that time led to the isolation of Peloritani chain and Calabria (Popov et al. 2004; Fig.6). At the same time, the other Apennine *Echinogammarus* species groups, related to *E. adipatus* started to diversify (Fig.3.4; Fig.3.6).

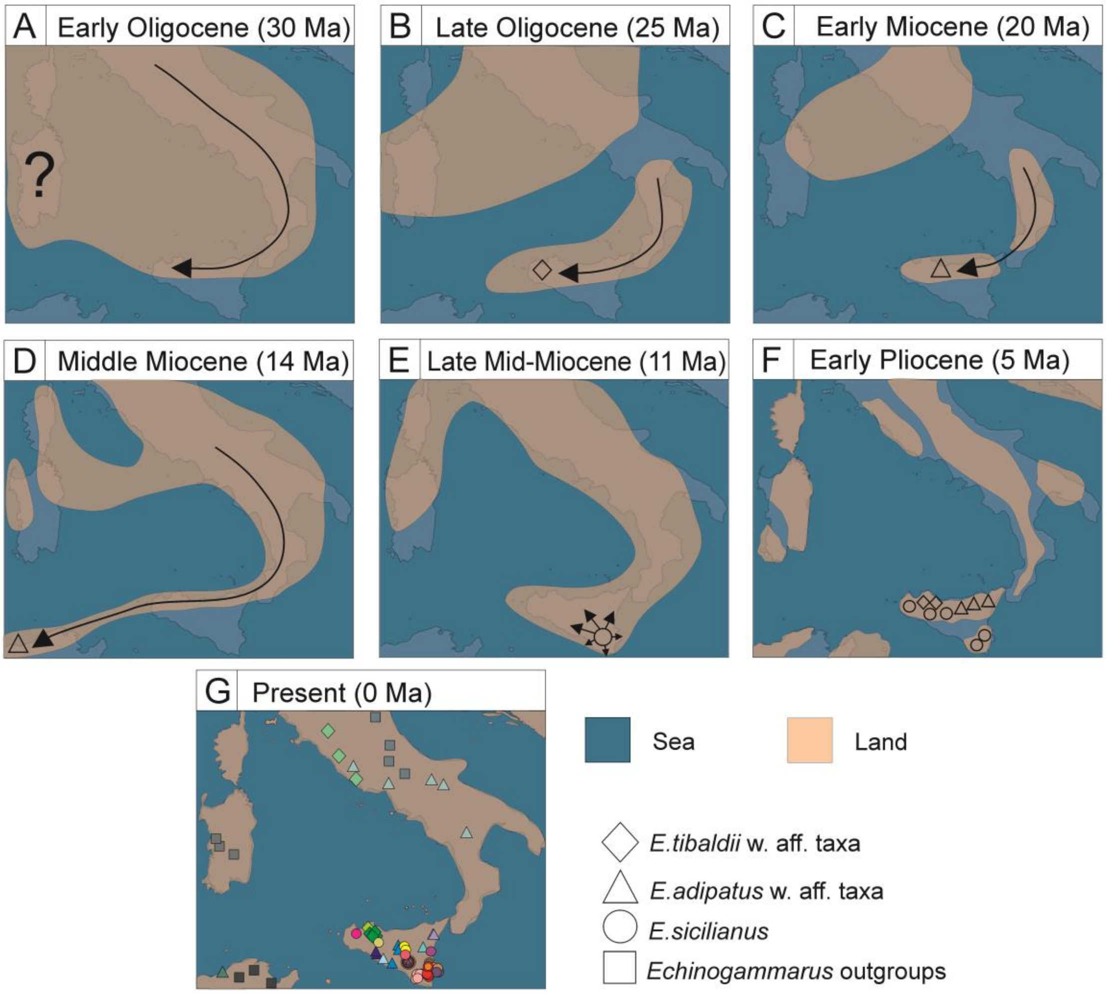


Fig.3.6 The paleogeographical maps of the Central Mediterranean Region including Sicily (after Popov et al. 2004). The arrows represent probable dispersal patterns confirmed by the geographic tree models and the symbols refer to particular MOTUs and morphospecies detected in this study. The colours correspond to those presented in other figures.

However one of the African lineages, which resembles E. pungens, thanks to interim mid-Miocene land bridge, that joined Apennine Peninsula, Calabria and Peloritanian Land with North Africa, diverged from its Apennine ancestors (Fig,3.4; Fig.3.6). These results suggest that this particular African lineage is of Apennine origin and has no biogeographical affinities with the remainder of North African gammarids (Fig.3.4). Thus, it confirms the African connection with the Sicilian biota, which was indicated already by La Greca (1957), and later on was hypothesised for various taxa (e.g. Massa et al. 2011; Stoch et al. 1996; Stöck et al. 2016). It may be hypothesized that both the Sicilian *E. tibaldii* and *E. adipatus* derived from the taxa currently inhabiting the Apennine Peninsula (Fig.3.4). Additionally, the time-calibrated phylogeny indicates that the species attributed to the Sicilian morphospecies inhabiting Sardinia and Apennine Peninsula are independent taxa, not related to the Sicilian counterparts. Similarly, most of the Tunisian taxa seem to be unrelated to the Sicilian biota, bearing no similar biogeographical affinities.

Diversification, dispersal and current distribution of Sicilian gammarids

The diversification within *E. sicilianus* morphospecies started in Middle Miocene (Fig.3.4). As indicated by the ancestral states reconstruction as well as by the geographical tree modelling, all the *E. sicilianus* MOTUs originated in the southeastern part of Sicily, i.e. the Hyblean foredeep (Fig.3.3, Fig.3.6, Fig.S6). The timing corresponds to the emergence of the Hyblean foredeep, which detached from the African continental plate during its collision with Calabrian Arc in Middle Miocene (Yellin-Dror et al. 1997). Although, all the MOTUs of *E. sicilianus* probably derived from the common ancestor, which inhabited the Hyblean area (Fig.3.3), the other MOTUs inhabiting the southeastern part of Sicily are seemingly a result of secondary colonisations by younger MOTUs from Apenninic-Maghrebian orogeny. In fact, the majority of the diversification events within all the three Sicilian *Echinogammarus* morphospecies took place in late Miocene/early Pliocene (Fig.3.4; Fig.3.5). Although these species had different evolutionary histories, the simultaneity of the major diversification events probably indicates the influence of major geological event that affected all of the species in the region. With low level of inland waters as a result of the dropping Mediterranean sea level at that time (Hsü et al. 1977; Krijgsman et al. 1999; Bianchi et al. 2012). Later on, at the beginning and in the middle Pliocene, due to the massive orogenetic events a large area of Sicily land has been uplifted above the sea level (Ruggieri 1973). From the Pliocene up to the end of Pleistocene, Sicily was divided into two islands; one comprised the northwestern and central part of the present Sicily and the other its present southwestern corner (Guglielmo & Marra 2011; Fig.3.6). Given the relative proximity of these geological events and the confidence interval of the molecular dating, it cannot be concluded with absolute certainty which of those events have affected the diversification, although it is likely that the general radiation event shared by all the Sicilian gammarids took place around 5 Ma, as indicated by the lineages-through-time analysis (Fig.3.5). This points to the particular importance of Pliocene water level fluctuations events that might have triggered diversification events in the Sicilian fauna. Moreover, the results of Bayesian Skyline Plot suggest that Pleistocene glaciation events and subsequent falls of sea and inland water might have promoted the population growth in *E. sicilianus* (Fig.S2) with no growth in *E. tibaldii* nor *E. adipatus* with the further intralineage diversification within all three morphospecies. These results may indicate that *E. sicilianus* had different demographic history that the other Sicilian gammarids or that Hyblean area was more affected by changes of inland water level than the other parts of Sicily. Moreover, considering the outcome of the geographic tree models, one may argue that the diversification and dispersal have started from the areas, which used to be two separate landmasses during Pliocene and Pleistocene (Fig.3.3; Fig.3.6). It seems that during the Pliocene split of the island, *E. adipatus* have originally inhabited the northern part, whereas *E. sicilianus* – the southeastern part of Sicily (Fig.3.3) and these are probable starting points of the further colonisation of the island, as also confirmed by the ancestral states analysis (Fig.S6). This might also explain why currently *E. adipatus* is distributed in the north and central parts of the island and why the highest concentration of MOTUs of *E. sicilianus* is located in the southeastern part with lack of any MOTU of *E. adipatus* (Fig.3.3; Fig.3.4). Thus, one may argue that the split of Sicily in Pliocene (Guglielmo & Marra 2011) could have also influenced the present biodiversity distribution of Sicilian gammarids.

Intraspecific diversity

A very high level of intraspecific diversity within Sicilian *Echinogammarus* morphospecies was also detected (Fig.3.3; Fig.S2). Even though recently there have been numerous studies detecting high level of overlooked diversity in the widespread freshwater gammarids (e.g. Copilaş-Ciocianu & Petrusek 2015; Grabowski et al. 2017; Mamos et al. 2016; Weiss & Leese 2016), it has never yet been confirmed for *Echinogammarus*. Furthermore, it is even more surprising considering a recent study on freshwater *Gammarus plaitisi* from Crete, another continental island of the Mediterranean, which showed that *G. plaitisi* Hupało, Mamos, Wrzesińska & Grabowski, 2018 has very low intraspecific diversity with genetic distances not exceeding the value of 0.005, despite being widely distributed in isolated river basins (Hupało et al. 2018). The morphological variability within *Echinogammarus* species has been debated for quite some time, causing many uncertainties in the taxonomic classification of certain taxa (e.g. Pinkster 1993). Due to this morphological variability, it was even hypothesized that the number of species described is exaggerated and the observed variability should be assigned to seasonal or regional intraspecific plasticity (Pinkster, 1988). Particularly striking then is the high number of MOTUs within *E. sicilianus*, which, with the conservative delimitation approach, reaches up to 15 (Fig.3.3; Fig.S2). Along with the highest haplotypic diversity (Tab.3.1; Fig.3.2), one can assume that several speciation events took place during Pliocene and Pleistocene, due to the lineage isolation and vicariance. Such radiation may be attributed to the geographical mosaic-like segregation, geological heterogeneity and the variety of microhabitats, that not only promoted the speciation events, but also might have served as the local refuges sustaining the freshwater biodiversity throughout the turbulent history of Sicily (Guarino & Pasta 2018). The widest distribution of *E. sicilianus*, compared to the rather limited areas of occurence of *E. adipatus* and especially, *E. tibaldii*, also suggests the behavioural and ecological differences between these species. The previous ecological observations gathered up by Pinkster (1993) indicate that *E. sicilianus*, contrary to *E. adipatus* and *E. tibaldii*, seems to have a wide range of environmental tolerance and high dispersal abilities within the river systems. Intriguingly, despite extensive sampling effort, there were no sites where any of the morphospecies co-occurred, with the notable exception of site SIT44 (Tab.3.1; Fig.3.4). Apart from being shared by both *E. sicilianus* and *E. adipatus*, the site is inhabited by four distinct, closely-related MOTUs of *E. sicilianus* which, based on other molecular studies on other freshwater gammarids, is an unusually high number (e.g. Copilaş-Ciocianu & Petrusek 2015; Grabowski et al. 2017; Mamos et al. 2016; Weiss & Leese 2016). Moreover, the MOTUs are separated by high level of mitochondrial genetic distance in the extreme case exceeding 10%. The reason behind such situation is yet unclear but may include introgression and/or incomplete lineage sorting (Fig.S4).

3.5 Conclusions

In conclusion, the phylogenetic patterns strongly indicate that that Sicilian freshwater gammarid fauna is biogeographically heterogeneous. The reconstruction of time-calibrated phylogeny supports different timescales and separate colonisation events that led to the presence of three morphospecies of *Echinogammarus* in Sicily. It seems that the turbulent geomorphological history of the island and entire region has left a phylogeographic footprint on the diversification and evolution of local amphipods. Moreover, the studies showed a high level of overlooked diversity and revealed taxonomic misidentifications of the Sardinian and Apennine conspecifics of Sicilian freshwater gammarids, indicating the need for further taxonomic studies on these *Echinogammarus* species. Given that the islands are among the most anthropogenically affected regions in the Mediterranean and are also one of the least studied in terms of its freshwater diversity (Hopkins 2002), there is an urgency for more studies revealing the actual biodiversity of insular freshwater biota, that may aid in planning a reasonable and sustainable strategy for its conservation.

The biogeographical complexity of the Sicilian freshwater fauna reflects the turbulent history of the island as well as of the entire region. Obtained results provide a valuable insight into the evolutionary history of the insular freshwater biota and indicate the importance of future studies on the Mediterranean islands for better understanding of the mechanisms of the diversification of the freshwater organisms as well as the geodynamics of the region.

General discussion

The studies enclosed in my thesis confirm the richness and the evolutionary complexity of freshwater crustacean biodiversity in the Mediterranean islands. I have supported the connectivity between the timescale of the evolution of freshwater gammarids and the geological history of the islands and the entire region. I have also detected a high level of overlooked biodiversity in the fresh waters of Crete and Sicily, resulting in describing a species new for science, being also arguably the first endemic *Gammarus* in the Mediterranean islands. I have confirmed the complexity of the colonisation and evolution of Sicilian freshwaters, indicating the independent timescale and biogeographical affiliations of each Sicilian freshwater gammarid species. The results of this thesis highlight the high level of overlooked diversity in the insular fresh waters and indicate the strong connection between the geological history of the islands and the evolution of its freshwater biota.

The Mediterranean insular fresh waters can be considered the malacostracan biodiversity hotspot, as up to date the fresh waters of the Mediterranean islands houses 181 species belonging to five different orders of Malacostraca (Amphipoda, Isopoda, Thermosbaenacea, Bathynellacea, Decapoda), representing 50 genera and 26 families (**Chapter I**). It is even more striking when considering that the islands cover only about 5% of the Mediterranean Region and about 0.2% of the whole Palearctic, but being inhabited by more than 25% of all the malacostracan species reported from the region and more than 8% of the Palearctic Malacostraca (Balian et al. 2008, Figueroa et al. 2013). Although the number of the reported insular species is already high, one might expect that this number will keep on growing, due to the implementation of the DNA-based taxonomic assessments with nearly 10% of all reported freshwater malacostracans from the Mediterranean islands being described in the last ten years. The insular freshwater malacostracan fauna seems to confirm that pattern with more than half of all reported species from the islands being endemic, with the highest number of endemics been recorded in amphipods (**Chapter I**). On the majority of islands, amphipods were always the most species-rich malacostracan order in the insular fresh waters, with the family Gammaridae being the most speciose, comprising of almost one third of all epigean freshwater malacostracans in the Mediterranean islands (**Chapter I**). Following this pattern, gammarids are considered to be the most speciose epigean freshwater malacostracan family worldwide with *Gammarus* being globally the largest epigean freshwater malacostracan genus (Väinölä et al. 2007, Balian et al. 2008). The evolutionary patterns and the origin of such high diversity on the islands still remain to be fully resolved with the origin of the Mediterranean insular malacostracans being of the focal points of the biogeographical studies in the last decades. However, presumably, even though the islands largely differ in terms of their dates of origin and the geological history, the patterns of freshwater colonization by the malacostracan crustaceans remain similar throughout the islands. The insular freshwater Malacostraca are either of ancient marine origin or they are dispersed to the island from the continent. On the other hand, most of the scenarios being hypothesized based mostly on the taxonomic and systematic evidence (e.g. La Greca 1957, Ruggieri 1973, Coineau & Boutin 1992). However, with the emergence of DNA-based methodology applied in many recent studies on the phylogeography of Mediterranean insular freshwater malacostracans (e.g. Jesse et al. 2011, Ketmaier & Caccone 2013, Poulakakis et al. 2015), there is high chance to reliably resolve the biogeographical entanglements of the freshwater biota of the Mediterranean islands. The results of this PhD thesis from Aegean Islands and Sicily (**Chapter II and Chapter III**) provide yet another piece of evidence helping to reach that goal.

The continental malacostracans were either inhabiting the island during the time when it was still a part of the mainland (e.g. Ketmaier et al. 2003) or they have migrated using the temporary land connections established either by the land movements or during the sea level fluctuations from Miocene to Pleistocene (e.g. Ketmaier & Caccone 2013). In this thesis, I provided evidence for the existence of new freshwater *Gammarus* species on Samothraki, Skyros, Andros, Tinos and Serifos, with three of them being the insular endemics (**Chapter II**). Along with *Gammarus plaitisi* described from Crete, the results of my thesis support the presence of four potentially new species in the Aegean islands. Moreover, the timescale of their evolution as well as their phylogeographic affiliations indicate they all have continental origin and by that, **confirming the first hypothesis of this PhD thesis**. In this case, the results supports the scenario where all those species have diverged from the continental lineages of members of *G. pulex* group around 12 million years ago. The timescale seems to be convergent with the estimated date of the first fragmentation of Aegeis landmass (Poulakakis et al. 2015). Thus, this findings indicate in most cases they most probably inhabited the islands before it detached from the mainland instead of migrating to them later. However, the time-calibrated phylogeny also support the further dispersal between the islands and back to the mainland during the Plio-Pleistocene water level shifts, which created the temporal land connections within the Aegean region. Interestingly, the results support the recent dispersal of *G. plaitisi,* formerly described from Crete (Hupało et al., 2018). Thus, by confirming the presence of *G. plaitisi* on Tinos and Serifos, the previously inferred endemism of this species on Crete has to be rejected. This finding, along with by low intraspecific haplotypic diversity discovered in the last paper, actually confirms late Pleistocenic dispersal of this species on Crete suggested before. It is yet unclear though, how the species dispersed from Serifos to Crete, as there are no known temporal land connections between these two islands in Pleistocene (Poulakakis et al., 2015). The most plausible scenario involve the passive dispersal by birds (Rachalewski et al., 2013), with possible intermediate populations on Milos or Antikythera islands.

Due to geological heterogeneity, the origin and biogeographical affiliations of Sicilian fauna remained under constant debate throughout the years with first assumptions formulated by La Greca (1957). The author stated that Sicilian biota could have colonized the island during multiple, separate colonization events, which took place from different regions, including Apennine Peninsula and Northern Africa, which was confirmed also in several later studies (e.g. Baratti et al. 2004; Lázaro et al. 2011). La Greca also supported the existence of ‘ancient endemics’, which colonized the island following Oligocene and Miocene microplate migrations. However, the existence of such ancient endemics was rejected by Ruggieri (1973) and more recently, by Massa et al. (2011), who postulated that the extant Sicilian fauna comprises of only Pliocenic and Quaternary colonisers, as supposingly no aquatic taxa in Sicily should have survived the Messinian Salinity Crisis. The results of my PhD thesis, however, support the existence of Sicilian freshwater ancient endemics (**Chapter III**). The time-calibrated phylogeny suggests that the common ancestor of all current Sicilian *Echinogammarus* species have diverged already in Eocene/early Oligocene, well before the dramatic events at the end of Miocene. This might support the survival of the Sicilian freshwater biota in the isolated refuges during the desiccation episodes at that time. Moreover, the phylogenetic patterns indicate the biogeographical heterogeneity of Sicilian freshwater gammarid fauna. The obtained results suggest the different timescale and separate colonisation events that led to the presence of three morphospecies of *Echinogammarus* in Sicily. Even though, they all inhabit the same island, the evolutionary timescale and phylogenetic relationships support the continental origin for two of them, namely *E. tibaldii* and *E. adipatus* and the evolution directly from the marine ancestors for *E. sicilianus*. Given the most probably mixed origin of *Echinogammarus* from Sicily, **the second hypothesis of this PhD study has to be rejected**. A very high level of intraspecific diversity within Sicilian *Echinogammarus* morphospecies was also detected, distinguishing up to 15 separate MOTUs within *E. sicilianus*. Even though recently there have been numerous studies detecting high level of overlooked diversity in the widespread freshwater gammarids (e.g. Weiss et al. 2014, Copilaş-Ciocianu & Petrusek, 2015, Grabowski et al., 2017), it has never yet been confirmed for *Echinogammarus.* Furthermore, it is even more surprising considering that *Gammarus plaitisi* from Crete has very low intraspecific diversity with genetic distances not exceeding the value of 0.005, despite being widely distributed in isolated river basins (Hupało et al. 2018). Such high level of intraspecific diversity within Sicilian gammarids may be attributed to the geographical mosaic-like segregation, geological heterogeneity and the variety of microhabitats, that not only promoted the speciation events, but also might have served as the local refuges sustaining the freshwater biodiversity not only during Messinian Salinity Crisis, but also throughout millions of years of turbulent history of Sicily.

From all these results taken together, I conclude that there is a certain level of overlooked diversity in the Mediterranean islands. The molecular evidence provided for the first time for freshwater insular gammarid amphipods indicate that the actual number of species present in the fresh waters on the Mediterranean islands might be at least twice as high comparing to what was previously reported. That raises a need for future integrative taxonomic studies on other Mediterranean islands. Given that the islands are among most anthropogenically affected regions in the Mediterranean and equally one of the least studied in terms of its freshwater diversity (Hopkins, 2002), there is an urgency for more studies revealing the actual biodiversity of insular freshwater biota, that may aid in planning a reasonable and sustainable strategy for its conservation. Moreover, the results of the time-calibrated phylogenies strongly indicate that the turbulent geomorphological history of the islands as well as the entire region has left a phylogeographic footprint on the diversification and evolution of local amphipods. The deep divergence times contradicts the former beliefs that the insular freshwater fauna of Mediterranean are a result of recent colonisations, but rather points out to the connectivity of the organisms' evolutionary history with the geological past of the region. I believe that these results deliver a valuable insight for better understanding of the mechanisms of the diversification of the freshwater organisms and provide evidence and framework for future studies on the insular freshwater Mediterranean biota.

References

AA.VV. (2015). The 2nd Water Catchment Management Plan for the Malta Water Catchment District 2015 – 2021. Sustainable Energy and Water Conservation Unit, Environment and Resources Authority, 571 pp.

Advokaat, E.L., van Hinsbergen D.J.J., Maffione M. et al. (2014). Eocene rotation of Sardinia, and the paleogeography of the western Mediterranean region. Earth and Planetary Science Letters, 401, 183–195.

Akyol, O., Ceyhan, T., Ertosluk, O. (2009). Marmara Adasi Kiyi Balikciligi ve Balikcilik Kaynaklari. Ege J FAS 2009; 26(2): 143-48.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of Molecular Biology, 215(3), 403-410.

Alvarez, W. (1972). Rotation of the Corsica-Sardinia microplate. Nature Physics Science, 235, 103–105.

Amouret, J., Bertocchi S., Brusconi S., Fondi M., Gherardi F., Grandjean F., Chessa L.A., Tricarico E. & Souty-Grosset C. (2015). The first record of translocated white-clawed crayfish from the *Austropotamobius pallipes* complex in Sardinia (Italy). Journal of Limnology, 74(3), 491-500.

Antonioli, F. & Vai G.B. (2004). Litho-paleoenvironmental maps of Italy during the last two climatic extremes. Climex Maps Italy, ENEA, Museo geologico Giovanni Capellini, Bologna, 80 pp.

Arcangeli, A., (1942). Il genere *Asellus* in Italia con speciale riguardo alla diffusione del genere Proasellus. Bollettino del Museo di Zoologia e Anatomia Comparata della Reale Università di Torino, 44 pp.

Argano, R., & Pesce, G. L. (1979). Microparasellids from phreatic waters of Greece (Isopoda, Asellota). Crustaceana, 37(2), 173-183.

Argano, R. & Pesce, G.L. (1980) A cirolanid from subterranean waters of Turkey (Crustacea, Isopoda, Flabellifera). Revue Suisse de Zoologie, 87 (2), 439–444. http://dx.doi.org/10.5962/bhl.part.85525

Argano, R. & Campanaro, A. (2004). Two new *Proasellus* (Crustacea, Isopoda, Asellidae) species from Sardinia: evidences of an old colonization wave of the island. Studi Trentini di Scienze Naturali, Acta Biologica, 81, 49-52.

Arias, C., Azzararoli A., Bigazzi G. & Bonadonna F.P. (1980). Magnetostratigraphy and Pliocene-Pleistocene boundary in Italy. Quarternary Research, 13, 65-74.

Arrighi, M.E., Khoumeri B., Ottavi V. et al. (2005). Report on Corsica. In: Donta, A.A., Lange, M.A. & Herrmann, A. (eds.), Water on Mediterranean Islands: current conditions and prospects for sustainable management. Centre for Environment Research, University of Muenster, 5: 97-226.

Arrignon, J.C.V., Martini, V. & Mattei J. (1999). *Austropotamobius pallipes pallipes* (Lereb.) in Corsica. Freshwater Crayfish, 12, 811-816.

Astrin, J. J., & Stüben, P. E. (2011). Molecular phylogeny of *Echinodera* and *Ruteria* (Coleoptera: Curculionidae: Cryptorhynchinae) and the parallel speciation of Canary Island weevils along replicate environmental gradients. Invertebrate Systematics, 24(5), 434-455.

Baccetti, B. (1983). Biogeografia sarda venti anni dopo. Biogeographia, 8, 859-870.

Baiocchi, A., Lotti F. & Piscopo V. (2016). Occurrence and flow of groundwater in crystalline rocks of Sardinia and Calabria (Italy): an overview of current knowledge. Italian Journal of Groundwater, AS16-195, 7-13.

Baele, G., Li, W. L. S., Drummond, A. J., Suchard, M. A., & Lemey, P. (2012). Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. Molecular biology and evolution, 30(2), 239-243.

Baldacchino, A.E. (1983). A preliminary list of freshwater crustaceans from the Maltese islands. The Central Mediterranean Naturalist, 1(2), 49-50.

Balian, E. V., Lévêque, C., Segers, H., & Martens, K. (Eds.). (2008). Freshwater animal diversity assessment (Vol. 198). Springer Science & Business Media.

Balkıs H. 1992. Marmara Adası littoralinin makrobentosu uzerine bir on arastirma. Istanbul Universitesi Deniz Bilimleri ve Cografya Enstitusu Bulteni 1992; 9: 309-27.

Banha, F. Anastácio P.M. (2014). Desiccation survival capacities of two invasive crayfish species. Knowledge and Management of Aquatic Ecosystems, 413, 01, 1-5.

Baratti, M., Yacoubi Khebiza M. & Messana G. (2004). Microevolutionary processes in the stygobitic genus *Typhlocirolana* (Isopoda Flabellifera Cirolanidae) as inferred by partial 12S and 16S rDNA sequences. Journal of Zoological Systematics and Evolutionary Research, 42, 27–32.

Barbaresi S., Gherardi F., Mengoni A., Souty-Grosset C. (2007). Genetics and invasion biology in fresh waters: a pilot study of *Procambarus clarkii* in Europe. In: Gherardi F., Biological invaders in inland waters: Profiles, distribution, and threats. Springer.

Barbato, D., Benocci A. & Manganelli G. (2018). The biogeography of non-marine molluscs in the Tuscan Archipelago reveals combined effects of current eco-geographical drivers and paleogeography. Organisms Diversity & Evolution, https://doi.org/10.1007/s13127-018-0378-2

Barbera, G. & Cullotta S. (2012). An inventory approach to the assessment of main traditional landscapes in Sicily (Central Mediterranean Basin). Landscape Research, 37(5), 539–569.

Barnes, R.S.K, 1988. The faunas of land-locked lagoons: chance differences and the problems of dispersal. Estuarine, Coastal and Shelf Science 26: 309-318.

Bathrellos, G. D., Antoniou, V. E., Skilodimou, H. D. 2009. Morphotectonic characteristics of Lefkas Island during the Quaternary (Ionian Sea, Greece). Geologica Balcanica 38(1—3), 23—33.

Bauzà-Ribot M. M., Jaume D., Fornós J. J., Juan C., Pons J. (2011). Islands beneath islands: phylogeography of a groundwater amphipod crustacean in the Balearic archipelago. BMC Evolutionary Biology, 11: 221

Bauzà-Ribot M. M., Juan C., Nardi F., Oromı P., Pons J., Jaume D. (2012). Mitogenomic phylogenetic analysis supports continental-scale vicariance in subterranean thalassoid crustaceans. Current Biology, 22: 2069–2074.

Benoit, G., Comeau, A. (2012). A sustainable future for the Mediterranean: the Blue Plan's environment and development outlook. Routledge, London, UK.

Bertocchi, S., Brusconi S., Gherardi F. & Chessa L. (2010). Prima segnalazione del gambero minacciato *Austropotamobius pallipes* complex in Sardegna. XIII Congresso Nazionale Associazione Italiana Ittiologi Acque Dolci, Sansepolcro, Arezzo, 12-13 Novembre 2010, 52.

Beşiktepe, Ş.T., Sur, H.İ., Özsoy, E., Latif, M.A., Oğuz, T. & Ünlüata, Ü. 1994. The circulation and hydrography of the Marmara Sea. Progress in Oceanography 34, 285-334.

Bianchi, C. N. (2007). Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia, 580(1), 7.

Bianchi, C. N., Morri, C. (2000). Marine biodiversity of the Mediterranean Sea: situation, problems and prospects for future research. Marine Pollution Bulletin, 40(5), 367-376.

Bianchi, C. N., Morri, C., Chiantore, M., Montefalcone, M., Parravicini, V., & Rovere, A. (2012). Mediterranean Sea biodiversity between the legacy from the past and a future of change. Life in the Mediterranean Sea: a look at habitat changes, 1-55.

Bilton, D. T., Freeland, J. R., & Okamura, B. (2001). Dispersal in freshwater invertebrates. Annual review of ecology and systematics, 32(1), 159-181.

Blondel, J., Aronson J., Bodiou J.-Y., Boeuf, G. 2010. The Mediterranean Region. Biological Diversity in Space and Time. Oxford University Press, Oxford.

Boccaletti. M., Ciaranfi N., Casentino D. et al. (1990). Palinspastic restoration and palaeogeographic reconstruction of the peri-Tyrrhenian area during the Neogene. Palaeogeography, Palaeoclimatology, Palaeoecology, 77, 41-50.

Bonacci O., Roje-Bonacci T. 2003. Groundwater on small Adriatic karst islands. RMZ – Materials and Geoenvironment. 50(1): 41-44.

Bonacci O. (2014) Karst hydrogeology/hydrology of dinaric chain and isles. Environmental Earth Sciences, 74(1): 37–55.

Bossio, A., Cornamusini G., Ferrandini J., Ferrandini M., Foresi L.M., Mazzanti R., Mazzei R., Salvatorini G. & Sandrelli F. (2000). Dinamica dal Neogene al Quaternario della Corsica orientale e della Toscana. Progetto Interreg II Toscana- Corsica 1997–1999, ETS, Pisa, 87–95.

Botosaneanu, L., Boutin, C., & Henry, J. P. (1985). Deux remarquables cirolanides stygobies nouveaux du Maroc et de Rhodes. Stygologia, 1(2), 186-207.

Bou, C. (1970). Observations on the Ingolfiellidae (Amphipod crustaceans) of Greece, (in French). Biol Gallo-Hellenica. 3 (1): 57-70. 1970.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C. H., Xie, D., ... & Drummond, A. J. (2014). BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS computational biology, 10(4), e1003537.

Bouckaert, R. R., & Drummond, A. J. (2017). bModelTest: Bayesian phylogenetic site model averaging and model comparison. BMC evolutionary biology, 17(1), 42.

Boutin C. 1997. Stygobiologie et géologie historique: l'émersion des terres de Méditerranée orientale datée à partir des amphipodes Metacrangonyctidae actuels (micro-crustacés souterrains) [Stygobiology and historical geology: emersion of lands in Eastern Mediterranean Basin dated with present Metacrangonyctid Amphipods (subterranean micro-crustaceans.]. GEOBIOS, M.S. 21: 67-74.

Bover P., Quintanab J., Alcoverc J.A. 2008. Three islands, three worlds: paleogeography and evolution of the vertebrate fauna from the Balearic Islands. Quaternary International 182(1): 135-144.

Boxshall G. A., Jaume D. 2009. Exopodites, Epipodites and Gills in Crustaceans. Arthropod Systematics & Phylogeny. 67(2): 229–254.

Brandis D., Storch V., Türkay M. 2000. Taxonomy and zoogeography of the freshwater crabs of Europe, North Africa, and the Middle East. Senckenbergiana Biologica, 80 : 5-56

Britton R. H, Crivelli A.J. 1993. Wetlands of southern Europe and North Africa: Mediterranean wetlands. In Whigham D.F., Dykyjova D., Hejny S. (ed) Wetlands of the world I: Inventory, ecology and management. Kluwer Academic Publishers, Dordrecht, Netherlands, 129–194.

Broquet, P. (2016). Sicily in its Mediterranean geological frame. Boletín Geológico y Minero, 127 (2/3), 547-562

Brown R. P., Terrasa B., Pérez-Mellado V., Castro J. A ., Hoskisson P. A., Picornell A. Ramon M. M. (2008). Bayesian estimation of post-Messinian divergence times in Balearic Island lizards. Molecular Phylogenetics and Evolution, 48: 350–358.

Bulut G. (2016). Medicinal and wild food plants of Marmara Island (Balikesir – Turkey). Acta Soc Bot Pol. 85(2):3501.

Cabras, S., De Waele J. & Sanna L. (2008). Caves and karst aquifer drainage of Supramonte (Sardinia, Italy): a review. Acta Carsologica, 37(2-3), 227-240.

Camacho, A.I. (2003). Historical biogeography of *Hexabathynella,* a cosmopolitan genus of groundwater Syncarida (Crustacea, Bathynellacea, Parabathynellidae). Biological Journal of the Linnean Society, 78, 457–466.

Camacho I. A. 2006. An annotated checklist of the Syncarida (Crustacea, Malacostraca) of the world. Zootaxa. 1374: 1–54

Camacho A. I., Dorda B. A.,Rey I. 2014. Iberian Peninsula and Balearic Island Bathynellacea (Crustacea, Syncarida) database. ZooKeys, 386: 1-20.

Cannizzaro, A. G., Walters, A. D., & Berg, D. J. (2017). A new species of freshwater *Gammarus* Fabricius, 1775 (Amphipoda: Gammaridae) from a desert spring in Texas, with a key to the species of the genus Gammarus from North America. The Journal of Crustacean Biology, 37(6), 709-722.

Cánovas, F., Jurado-Rivera J.A., Cerro-Gálvez E., Juan C., Jaume D. & Pons J. (2016). DNA barcodes, cryptic diversity and phylogeography of a W Mediterranean assemblage of thermosbaenacean crustaceans. Zoologica Scripta, 45, 659–670.

Casagranda, C., Mohamed, S., Charles, D., Boudouresque, F., 2006. Abundance, population structure and production of macro-invertebrate shredders in a Mediterranean brackish lagoon, Lake Ichkeul, Tunisia. Estuarine, Coastal and Shelf Science, 66(3-4), 437-446.

Casal-López, M., & Doadrio, I. (2018). The Messinian imprint on the evolution of freshwater fishes of the genus *Luciobarbus* Heckel, 1843 (Teleostei, Cyprinidae) in the western Mediterranean. Journal of biogeography, 45(7), 1593-1603.

Casale, A., Grafitti G., Lana E., Marcia P., Molinu A., Mucedda M., Onnis C. & Stoch F. (2008). La Grotta del Bue Marino: cinquanta anni di ricerche biospeleologiche in Sardegna. Memorie dell'Istituto Italiano di Speleologia, s. II, 21, 197-209.

Cassola, F. (1983). L’esplorazione naturalistica della Sardegna. Biogeographia, 8, 5-34.

Catalano, R., Di Stefano P., Nigro F. & Vitale, F.P. (1994). The Sicily mainland thrust belt. Evolution during the Neogene. Bollettino di Geofisica Teorica e Applicata, 36, 141-144.

Cavallaro, F . (1995). Fenomeni carsici nei Monti Iblei (Sicilia sud - orientale). Atti Convegno Regionale di Speleologia della Sicilia, Ragusa, 14-16 Dicembre 1990, 2, 237-255.

Cavazza W, Wezel FC (2003) The Mediterranean region – a geological primer. Episodes 26: 160–168.

Chappuis, A. (1949). Les Asellides d’Europe et pays limitrophes. Arch. Zool. exp. gen. 86, 78-94.

Charalambous C.N. (2001). Water management under drought conditions. Desalination, 138: 3-6

Cherry J. F., Leppard T. P (2018). The Balearic Paradox: Why were the islands colonized so Late? Pyrenae, 49 (1): 49-70.

Christodoulou, M., Antoniou A., Magoulas A. & Koukouras A. (2012). Revision of the freshwater genus *Atyaephyra* (Crustacea, Decapoda, Atyidae) based on morphological and molecular data. ZooKeys, 229, 53–110.

Chueca L.J., Madeira M. J., Goomez-Moliner B. J. (2015). Biogeography of the land snail genus *Allognathus* (Helicidae): middle Miocene colonization of the Balearic Islands. 42: 1845–1857.

Coineau N. (1986). Isopoda: Asellota: Janiroidea. In: Stygofauna Mundi. A Faunistic, Distributional and Ecological Syntesis of the World Fauna Inhabiting Subterranean Waters. Botosaneaunu L (ed). Brill/Backhuys, Leiden.

Coineau, N., Boutin C. (1992). Biological processes in space and time. Colonization, evolution and speciation in interstitial stygobionts. In: Camacho A.I. (ed.), The natural history of biospeleology, Museo Nacional de Ciencias Naturales, Madrid, Monografias, 7, 423-451.

Colantoni, P., Gallignani, P. & Lenaz, R. 1979. Late Pleistocene and Holocene evolution of the North Adriatic continental shelf (Italy). Marine Geology 33, M41-M50.

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F. B. R., Aguzzi, J., ... & Danovaro, R. (2010). The biodiversity of the Mediterranean Sea: estimates, patterns, and threats. PloS one, 5(8), e11842.

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W. W., Christensen, V., ... & Palomares, M. L. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Global Ecology and Biogeography, 21(4), 465-480.

Combes, S. 2003. Protecting freshwater ecosystems in the face of global climate change. In: Hansen LJ et al. (eds) Buying time: a user's manual for building resistance and resilience to climate change in natural systems. WWF, Washington, UDA, pp. 175–214

Copilaş-Ciocianu, D., Petrusek, A. (2015). The southwestern Carpathians as an ancient centre of diversity of freshwater gammarid amphipods: insights from the *Gammarus fossarum* species complex. Molecular ecology 24(15): 3980-3992

Copilaş-Ciocianu, D., & Petrusek, A. (2017). Phylogeography of a freshwater crustacean species complex reflects a long-gone archipelago. Journal of Biogeography, 44(2), 421-432.Correggiari, A., Roveri, M. & F. Trincardi. 1996. Late Pleistocene and Holocene evolution of the north Adriatic sea. Il Quaternario Italian Journal of Quaternary Sciences, 9(2), 1996, 697-704.

Costa, F.O., Neuparth, T., Correia A.D., Costa, M.H. (2005). Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta*: II. Organism and population-level endpoints. Marine Environmental Research, 60(1), 93-110.

Crandall K.A. & De Grave S. (2017). An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. Journal of Crustacean Biology, 37(5), 615–653.

Cristescu, M. E., Hebert, P. D., & Onciu, T. M. (2003). Phylogeography of Ponto-Caspian crustaceans: a benthic–planktonic comparison. Molecular Ecology, 12(4), 985-996.

Cuttelod, A., N. García, D. Abdul Malak, H. Temple & V. Katariya, (2008). The Mediterranean: a biodiversity hotspot under threat. In Vié, J.-C., C. Hilton-Taylor & S. N. Stuart (eds), The 2008 Review of The IUCN Red List of Threatened Species. IUCN, Gland, Switzerland.

Darwall, W., Smith, K., Allen, D., Seddon, M., Reid, G. M., Clausnitzer, V., & Kalkman, V. J. (2009). Freshwater biodiversity: a hidden resource under threat. Wildlife in a changing world–An Analysis of the 2008 IUCN Red List of Threatened Species, 43.

Darwall, W., Carrizo, S., Numa, C., Barrios, V., Freyhof, J., & Smith, K. (2014). Freshwater Key Biodiversity Areas in the Mediterranean Basin Hotspot: Informing species conservation and development planning in freshwater ecosystems. IUCN, Cambridge.

Davolos D, De Matthaeis E, Latella L, Vonk R (2017) *Cryptorchestia ruffoi* sp. n. from the island of Rhodes (Greece), revealed by morphological and phylogenetic analysis (Crustacea, Amphipoda, Talitridae). ZooKeys 652: 37–54. https://doi.org/10.3897/zookeys.652.11252

Davolos, D., De Matthaeis, E., Latella, L., Tarocco, M., Özbek, M., Vonk, R. (2018). On the molecular and morphological evolution of continental and insular *Cryptorchestia* species, with an additional description of *C. garbinii* (Talitridae). ZooKeys, (783), 37.

De Queiroz, K. (2007). Species concepts and species delimitation. Systematic biology, 56(6), 879-886.

Deharveng, L., Stoch, F., Gibert, J., Bedos, A., Galassi, D., Zagmajster, M., ... & Giani, N. (2009). Groundwater biodiversity in Europe. Freshwater Biology, 54(4), 709-726.

Deidun, A., Sciberras A., Formosa J., Zava B., Insacco G., Corsini-Foka M. & Crandall K.A. (2018). Invasion by non-indigenous freshwater decapods of Malta and Sicily, central Mediterranean Sea. Journal of Crustacean Biology, doi:10.1093/jcbiol/ruy076

Delicado, D., Machordom, A. & Ramos, M.A. (2014). Vicariant versus dispersal processes in the settlement of *Pseudamnicola* (Caenogastropoda, Hydrobiidae) in the Mediterranean Balearic Islands. Zoological Journal of the Linnean Society, 171: 38–71.

Dermitzakis, M.D., (1990). Paleogeography, geodynamic process and event stratigraphy during the late Cenozoic of the Aegean area. Atti. Convegni Lincei 85, 263–288.

Dermitzakis, M.D., Papanikolaou, D.J., (1981). Paleogeography and geodynamics of the Aegean region during the Neogene. Annales Geologiques des pays Helleniques. 30, 245–289.

Desiderato, A., Costa, F. O., Serejo, C. S., Abbiati, M., Queiroga, H., & Vieira, P. E. (2019). Macaronesian islands as promoters of diversification in amphipods: The remarkable case of the family Hyalidae (Crustacea, Amphipoda). Zoologica Scripta.

Devoti, R., Ferraro, C., Gueguen, E., Lanotte, R., Luceri, V., Nardi, A., ... & Vespe, F. (2002). Geodetic control on recent tectonic movements in the central Mediterranean area. Tectonophysics, 346(3-4), 151-167.

Diamantopoulou P, Voudouris K (2008) Optimization of water resources management using SWOT analysis: the case of Zakynthos Island, Ionian Sea, Greece. Env Geol 54:197–211

Di Maggio, C., Madonia, G., Vattano, M., Agnesi, V., & Monteleone, S. (2017). Geomorphological evolution of western Sicily, Italy. Geologica Carpathica, 68(1), 80-93.

Drummond, A. J., Rambaut, A., Shapiro, B., & Pybus, O. G. (2005). Bayesian coalescent inference of past population dynamics from molecular sequences. Molecular biology and evolution, 22(5), 1185-1192.

Drummond, A. J., Ho, S. Y., Phillips, M. J., & Rambaut, A. (2006). Relaxed phylogenetics and dating with confidence. PLoS biology, 4(5), e88.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., ... Sullivan, C. A. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews, 81(2), 163-182.

Duplančić Leder, T., Ujević, T. & Čala, M. 2004. Coastline lenghts and areas of islands in the Croatian part of the Adriatic Sea determined from the topographic maps at the scale of 1 : 25 000. Geoadria 9, 28.

Edel, J.B., Dubois D., Marchant R., Hernandez J. & Cosca M. (2001). La rotation miocène inférieur du bloc corso-sarde. Nouvelles constraintes paléomagnetiques sur la fin du mouvement. Bulletin Societé Géologique France, 172, 275-283.

Fišer C., Bogdan A., Gal M., European Council Directive 92/43/EEC. (1992). On the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Union L. 206 (22.07. 1992).

Flot, J.-F., Wörheide, G., & Dattagupta, S. (2010). Unsuspected diversity of *Niphargus* amphipods in the chemoautotrophic cave ecosystem of Frasassi, central Italy. BMC Evolutionary Biology, 10, 171.

Horvat M., Keber R., Kereži V., Kovačec Ž., Luštrik R., Moškrič A., Novak S. Ogorevc E., Palandačić A., Petkovska V., Pustovrh M., Sečen T., Slana D., Škrget M., Turjak M., Potočnik H., Sket B. 2008. Teaching speleobiology for understanding biology: an ecology case study (interaction of surface and subterranean) amphipods in springs. Abstracts of the 19th International Symposium of Subterranean Biology, Fremantle, Western Australia, 22-25.

Evelpidou N. (2012) Modelling of erosional processes in the Ionian Islands (Greece), Geomatics, Natural Hazards and Risk, 3:4, 293-310, DOI: 10.1080/19475705.2011.604798

Faraone, F.P., Giacalone G, Canale D.E. et al. (2017). Tracking the invasion of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda Cambaridae) in Sicily: a “citizen science” approach. Biogeographia – The Journal of Integrative Biogeography, 32, 25–29.

Fassoulas, C., Paragamian, K., & Iliopoulos, G. (2007). Identification and assessment of Cretan geotopes. Bulletin of the Geological Society of Greece, 1780(1795).

Fassoulas, C. (2017). The geological setting of Crete: an overview. Minoan Earthquakes: Breaking the Myth through Interdisciplinarity. Leuven University Press, 5, 135.

Fattorini, S., 2002. Biogeography of the tenebrionid beetles (Coleoptera, Tenebrionidae) on the Aegean Islands (Greece). J. Biogeogr. 29, 49–67.

Ferentinos, G., Gkioni, M., Geraga, M. & Papatheodorou, G. 2012. Early seafaring activity in the southern Ionian Islands, Mediterranean Sea. Journal of Archaeological Science, 39, 2167–2176, http://doi.org/10.1016/j.jas.2012.01.032

Fišer, C., Trontelj, P., & Sket, B. (2006). Phylogenetic analysis of the *Niphargus orcinus* species–aggregate (Crustacea: Amphipoda: Niphargidae) with description of new taxa. Journal of Natural History, 40(41-43), 2265-2315.

Fišer, C., Robinson, C. T., & Malard, F. (2018). Cryptic species as a window into the paradigm shift of the species concept. Molecular Ecology, 27(3), 613-635.

Figueroa, J.M.T., López-Rodríguez, M.J., Fenoglio, S., Sánchez-Castillo, P., Fochetti, R. 2013. Freshwater biodiversity in the rivers of the Mediterranean Basin. Hydrobiologia, 719(1), 137-186.

Fontaneto, D., Flot, J. F., & Tang, C. Q. (2015). Guidelines for DNA taxonomy, with a focus on the meiofauna. Marine Biodiversity, 45(3), 433-451.

Fontbote, J.M., Guimera, J., Roca, E., Sabat, F., Santanach, P., Fernandez-Ortigosa, F. (1990) The Cenozoic geodynamic evolution of the Valencia Trough (western Mediterranean). Revista de la Sociedad Geologica de Espana, 3: 249–259.

Foglini, F., Prampolini M., Micallef A., Angeletti L., Vandelli V., Deidun A., Soldati M. & Taviani M. (2015). Late Quaternary coastal landscape morphology and evolution of the Maltese Islands (Mediterranean Sea) reconstructed from high-resolution seafloor data. In: Harff, J., Bailey, G. & Lüth, F. (eds.), Geology and Archaeology: Submerged Landscapes of the Continental Shelf. Geological Society, London, Special Publications, 411, http://doi.org/10.1144/SP411.12

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology, 3(5), 294-299.

Fourment, M., & Gibbs, M. J. (2006). PATRISTIC: a program for calculating patristic distances and graphically comparing the components of genetic change. BMC evolutionary biology, 6(1), 1.

Francalanci, L., Vougioukalakis, G.E., Perini, G. & Manetti, P. (2005) A west-east traverse along the magmatism of the South Aegean Volcanic Arc in the light of volcanological, chemical and isotope data. (ed. by M. Fytikas and G.E. Vougioukalakis), pp. 65–111. The South Aegean active volcanic arc: present knowledge and future perspectives. Elsevier, Amsterdam.

Franck, P., Garnery, L., Celebrano, G., Solignac, M., & Cornuet, J. M. (2000). Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). Molecular Ecology, 9(7), 907-921.

Froglia, C. (2006). Crustacea Malacostrca Decapoda. In: Ruffo S. & Stoch F. (eds.), Checklist and distribution of the Italian fauna. 10,000 terrestrial and inland water species. Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 17, 113-114.

Froufe, E., Prié, V., Faria, J., Ghamizi, M., Gonçalves, D. V., Gürlek, M. E., ... & Sousa, R. (2016). Phylogeny, phylogeography, and evolution in the Mediterranean region: news from a freshwater mussel (Potomida, Unionida). Molecular phylogenetics and evolution, 100, 322-332.

Furon, M. (1950). Les grandes lignes de la Paléogéographie del la Méditerranée (Tertiaire et Quaternaire). Vie et Milieu, 1, 131-162.

Galassi, D.M. P., De Laurentiis P. & Pesce G. L. (1994). Some remarks on the genus *Microcharon* Karaman in Greece, and description of *M. agripensis* n. sp. (Crustacea, Isopoda, Microparasellidae). International Journal of Speleology, 23: 133-155.

Galletti, I. (2002). L'attività biospeleologica del CIRS in Sicilia e all'estero. Speleologia Iblea, 10, 155-164.

Garcia Socias L. L. (1989). Daira speciosa Reuss, 1871 (Crustacea, Decapoda, Xanthidae) en el Mioceno de la isla De Mallorca (Baleares). Bolleti de la Societat d'Historia Natural de les Balears, 33: 81-85.

Gasparo F. (2009). La grotta di Zoodochos Pigi a Santorini (Cicladi, Grecia) e la sua fauna. Progressione, 55(1-2), 102-105.

Gass, I. A., (1980). The Troodos massif: Its role in the unravelling of the ophiolite problem and its significance in the understanding of constructive plate margin processes. In A. Panayiotou (Ed.), Ophiolites. Proceedings International Ophiolite Symposium. 1979. pp. 23-35 Nicosia : Geological Survey Department.

Geiger, W., Alcorlo, P., Baltanas, A., & Montes, C. (2005). Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. Biological Invasions, 7(1), 49-73.

Gentile, G., & Argano, R. (2005). Island biogeography of the Mediterranean Sea: the species–area relationship for terrestrial isopods. Journal of Biogeography, 32(10), 1715-1726.

Gerhardt, A., Bloor, M., Mills, L. (2011). *Gammarus*: important taxon in freshwater and marine changing environments. International Journal of Zoology, vol. 2011, Article ID 524276, 2 pages. doi:10.1155/2011/524276.

Gikas, P., & Angelakis, A. N. (2009). Water resources management in Crete and in the Aegean Islands, with emphasis on the utilization of non-conventional water sources. Desalination, 248(1-3), 1049-1064.

Guglielmo, M. & Marra A.C. (2011). Le due Sicilie dle Pleistocene Medio: osservazioni paleobiogeografiche. Biogeographia, 30, 11-25.

Goffredo, S., & Dubinsky, Z. (Eds.). (2013). The Mediterranean Sea: Its history and present challenges. Springer Science & Business Media.

Gómez-Campo, C., Bermudez-de-Castro, L., Cagiga, M. J., & Sánchez-Yélamo, M. D. (1984). Endemism in the Iberian peninsula and Balearic Islands. Webbia, 38(1), 709-714.

Gómez, A., & Lunt, D. H. (2007). Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. In Phylogeography of southern European refugia (pp. 155-188). Springer, Dordrecht.

Gooch, J. L., & Glazier, D. S. (1986). Levels of heterozygosity in the amphipod *Gammarus minus* in an area affected by Pleistocene glaciation. American Midland Naturalist, 57-63.

Gonçalves, H., Maia-Carvalho, B., Sousa-Neves, T., García-París, M., Sequeira, F., Ferrand, N., & Martínez-Solano, I. (2015). Multilocus phylogeography of the common midwife toad, *Alytes obstetricans* (Anura, Alytidae): contrasting patterns of lineage diversification and genetic structure in the Iberian refugium. Molecular phylogenetics and evolution, 93, 363-379.

Gottstein, S., Ivković, M., Ternjej, I., Jalžić, B. & Kerovec, M. 2007. Environmental features and crustacean community of anchihaline hypogean waters on the Kornati islands, Croatia. Marine Ecology 28, 24-30.

Görür, N., Cagatay, M.N., Sakinc, M., Sümengen, M., Sentürk, K., Yaltirak, C. & Tchapalyga, A. 1997. Origin of the Sea of Marmara as Deduced from Neogene to Quaternary Paleogeographic Evolution of its Frame. International Geology Review 39, 342-352.

Grabowski, M., Wysocka, A., & Mamos, T. (2017a). Molecular species delimitation methods provide new insight into taxonomy of the endemic gammarid species flock from the ancient Lake Ohrid. Zoological Journal of the Linnean Society, 181(2), 272-285.

Grabowski, M., Mamos, T., Bącela-Spychalska, K., Rewicz, T., & Wattier, R. A. (2017b). Neogene paleogeography provides context for understanding the origin and spatial distribution of cryptic diversity in a widespread Balkan freshwater amphipod. PeerJ, 5, e3016.

Grabowski, M., Mamos, T., Hupało, K., Wrzesińska, W., Jelić, M., Žganec, K., ... & Wattier, R. A. (2017c). Don’t judge book by its cover: the case of freshwater gammarids of the Periadriatic region. Genome, 60: 938-939.

Grafitti, G. (2001). Osservazioni sulla fauna cavernicola della Sardegna. In: Piras G. &

Grasso, M. (2001). The Apenninic—Maghrebian orogen in southern Italy, Sicily and adjacent areas. In Anatomy of an orogen: the Apennines and adjacent Mediterranean basins (pp. 255-286). Springer, Dordrecht.

Randaccio F. (eds.), Atti Convegno “Biospeleologia dei sistemi carsici della Sardegna”, Cagliari, 10 giugno 2000, 13-33.

Grube, A.E. (1861). Ein Ausflug nach Triest und dem Quarnero. Beitrage zur Kenntniss der Thierwelt dieses Gebietes. Nicolaische Verlagsbuchhandlung, Berlin. 175 pp. 5 pls

Guarino, R., & Pasta, S. (2018). Sicily: the island that didn’t know to be an archipelago. Berichte der Reinhold-Tüxen-Gesellschaft. 30, 133-148.

Guil N., Camacho A. I. (2001). Historical biogeography of *Iberobathynella* (Crustacea, Syncarida, Bathynellacea), an aquatic subterranean genus of Parabathynellids, endemic to the Iberian Peninsula. Global Ecology & Biogeography, 10: 487–501.

Güner, U. (2009). Distribution of freshwater crab (*Potamon* sp.) in Turkish Thrace. Trakya Üniversitesi Fen Bilimleri Dergisi, 10(1).

Habel, J. C., Roedder, D., Stefano, S., Meyer, M., & Schmitt, T. (2010). Strong genetic cohesiveness between Italy and North Africa in four butterfly species. Biological Journal of the Linnean Society, 99(4), 818-830.

Hadjisterkotis E. The arrival of elephants on the island of Cyprus and their subsequent accumulation in fossil sites In: Aranovich M, Dufresne O, editors. Elephants: Ecology, Behavior and Conservation. Hauppauge (NY): Nova Science Publishers, Inc.; 2012. pp. 49–75.

Hamilton, C. A., Hendrixson, B. E., Brewer, M. S., & Bond, J. E. (2014). An evaluation of sampling effects on multiple DNA barcoding methods leads to an integrative approach for delimiting species: a case study of the North American tarantula genus *Aphonopelma* (Araneae, Mygalomorphae, Theraphosidae). Molecular Phylogenetics and Evolution, 71, 79-93.

Harland, W.B., Cox, A.V., Llewellyn, P.G., Pickton, C.A.G. Smit, A.G. & Walter, R. (1982). A geological time scale. Cambridge: Cambridge University Press.

Habsburgo-Lorena, A.S. (1983). Socioeconomic aspects of the crawfish industry in Spain. Freshwater Crayfish 5: 552–554.

Heller, C. (1865). Crustaceen. In: Reise der Osterreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859. Zoologischer Theil. 2(3):1-280, pls. 1-25.

Heller, J., 1976. The biogeography of enid landsnails on the Aegean Islands. J. Biogeogr. 3, 281–292.

Henry, J. P. (1975). Données sur les Asellides de Grèce et description de *Proasellus sketi* n. sp. (Crustacea, Isopoda, Asellota). Biologia Gallo-Hellenica, 6(1), 139-144.

Hentonnen P, Huner JV, 1999. The introduction of alien species of crayfish in Europe. An introduction. In: Gherardi F, Holdich DM, eds. Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation? Brookfiled, Rotterdam: AA Balkema, 13-22.

Hillis, D.M., Moritz, C., Mable, B.K.. 1996. Molecular systematics. Sinauer Associates. Sunderland, MA, USA.

Ho, S.Y., Duchêne, S., 2014. Molecular clock methods for estimating evolutionary rates and timescales. Molecular ecology, 23(24), 5947-5965.

Hobbs H.H, Jass J.P & Huner J.V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda: Cambaridae). Crustaceana, 56:299-316.

Holdich DM, Ackefors H, Gherardi F, Rogers WD, Skurdal J, 1999. Native and alien crayfish in Europe: some conclusions. In: Gheradi F, Holdich DM, eds. Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation? Brookfield, Rotterdam: AA Balkema, 281-291.

Hopkins, L. (2002). IUCN and the Mediterranean Islands: Opportunities for biodiversity conservation and sustainable use. International Union for Conservation of Nature: Gland, Switzerland.

Horton, T., Lowry, J., & De Broyer, C. (2017). World Amphipoda Database. WoRMS database.

Hou, Z., Sket, B., Fišer, C., Li, S., (2011). Eocene habitat shift from saline to freshwater promoted Tethyan amphipod diversification. Proceedings of the National Academy of Sciences, 108(35), 14533-14538.

Hou, Z., Sket, B., & Li, S. (2013). Phylogenetic analyses of Gammaridae crustacean reveal different diversification patterns among sister lineages in the Tethyan region. Cladistics, 30(4), 352-365.

Hou, Z., & Sket, B. (2016). A review of Gammaridae (Crustacea: Amphipoda): the family extent, its evolutionary history, and taxonomic redefinition of genera. Zoological Journal of the Linnean Society, 176(2), 323-348.

Hou, Z., Zhao, S., & Li, S. (2018). Seven new freshwater species of *Gammarus* from southern China (Crustacea, Amphipoda, Gammaridae). ZooKeys, (749), 1.

Hsü, K.J. (1983). The Meditterranean was a desert. A voyage of the Glomar Challenger. Princeton: Princeton University Press.

Hsü KJ, Montadert L, Bernoulli D, Cita MB, Erickson A, Garrison RE, Kidd RB, Mèlierés F, Müller C, Wright R (1977) History of the Mediterranean salinity crisis. Nature 267: 399–403. doi:10.1038/267399a0

Hupało, K., Rewicz T., Mamos T., Boulaaba S. & Grabowski M. (2017). Diversity and origin of freshwater gammarids from Sicily (Italy): preliminary results. Biodiversity Journal, 8(2), 515-516.

Hupało, K., Mamos, T., Wrzesińska, W., & Grabowski, M. (2018). First endemic freshwater *Gammarus* from Crete and its evolutionary history—an integrative taxonomy approach. PeerJ, 6, e4457.

Hupało, K., Teixeira, M. A. L., Rewicz, T., Sezgin, M., Iannilli, V., Karaman, G. S., ... & Costa, F. O. (2019). Persistence of phylogeographic footprints helps to understand cryptic diversity detected in two marine amphipods widespread in the Mediterranean basin. Molecular phylogenetics and evolution, 132, 53-66.

Huson, D. H., & Bryant, D. (2005). Application of phylogenetic networks in evolutionary studies. Molecular biology and evolution, 23(2), 254-267.

Huson, D. H., & Scornavacca, C. (2012). Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. Systematic biology, 61(6), 1061-1067.

Jaume D. (1991). Two new species of the amphipoda genus Psedoniphargus (Crustacea) from Cabrera (Balearic Islands). Stygologia.

Jaume D., Garcia, L. (1988). Revision de la especies politipica Jaera nordmanni (Rathke, 1837) (Isopoda, Asellota, Janiridae) de les aguas dulces de Mallorca. Miscellanea Zoologica (Barcelona) 12(1988): 79-88.

Jaume D., Garcia, L. (1992). A new *Metacirolana* (Crustacea: Isopoda: Cirolanidae) from anchihialine cave lake on Cabrera (Balearic Islands). Stygologia, 7: 179-186.

Jaume D., Garcia Lado F., Boxshall G. (2007). New genera of Bogidiellidae (Amphipoda: Gammaridea) from SW Pacific and Mediterranean marine caves. Journal of Natural History, 41: 419-444.

Jaume D., Queinnec E. (2007). A new species of freshwater isopod (Sphaeromatidea: Sphaeromatidae) from an inland karstic stream on Espíritu Santo Island, Vanuatu, southwestern Pacific. Zootaxa 1653: 41–55.

Jesse, R., Schubart, C. D., & Klaus, S. (2010). Identification of a cryptic lineage within *Potamon fluviatile* (Crustacea: Brachyura: Potamidae). Invertebrate Systematics, 24, 348–356.

Jesse, R., Grudinski, M., Klaus, S., Streit, B., & Pfenninger, M. (2011). Evolution of freshwater crab diversity in the Aegean region (Crustacea: Brachyura: Potamidae). Molecular Phylogenetics and Evolution, 59(1), 23-33.

Jugovic J., Jalžić B., Prevorčnik S. & Sket B. (2012) Cave shrimps *Troglocaris* s. str. (Dormitzer, 1853), taxonomic revision and description of new taxa after phylogenetic and morphometric studies. Zootaxa, 3421: 1-31.

Jurado-Rivera J.A., Álvarez G., Caro J.A., Juan C., Pons J. & Jaume D. (2017). Molecular systematics of *Haploginglymus*, a genus of subterranean amphipods endemic to the Iberian Peninsula (Amphipoda: Niphargidae). Contributions to Zoology, 86(3), 239-260.

Kapli, P., Lutteropp, S., Zhang, J., Kobert, K., Pavlidis, P., Stamatakis, A., & Flouri, T. (2017). Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics, 33(11), 1630-1638.

Karaman, G. S. (1969). 27. Beitrag zur Kenntnis der Amphipoden- Arten der Genera *Echinogammarus* Stebb. und Chaetogammarus Mart. an der Jugoslawischer Adriaküste . Glasnik Republičkog Zavoda Za Zaštitu Prirode i Prirodnjačke Zbirke, 2, 59–84.

Karaman, G. S. (1974) 58. Contribution to the Knowledge of the Apmhipoda. Genus *Synurella* Wrzes. In Yugoslavia with remarks on its all World known species, heir synonomy, bibliographi and distribution (fam. Gammaridae). Poljoprivreda i šumarstvo 20 (2/3): 49-60

Karaman G.S. (1975). 56. Contribution to the knowledge of the Amphipoda. Several new and very interesting *Gammarus* species from Asia Minor (fam. Gammaridae). Boll. Mus. Civ. Stor. nat. Verona. 1: 311-343.

Karaman, G.S. (1977). Contribution to the Knowledge of the Amphipoda 84. One interesting member of the genus *Echinogammarus* Stebb. from Malta island, E. ebusitanus (Marg. 1951) (fam. Gammaridae). Poljoprivreda i Šumarstvo, 23(3), 29-38.

Karaman, G.S. (1985). New data on genus *Niphargus* (fam. Niphargidae) in Italy and adjacent regions. Bollettino del Museo civico di Storia naturale di Verona, 12, 209-228.

Karaman, G.S. (1989). 190. Contribution to the knowledge of Amphipoda. *Bogidiella cypria*, new species of the family Bogidiellidae from Cyprus Island in the Mediterranean Sea. The Montenegrin Academy of Sciences and Arts, Glasnik of the Section of Natural Sciences. 7: 7-23.

Karaman, G.S. (1993). Crustacea: Amphipoda di acqua dolce (Vol. 31). Calderini.

Karaman, G.S. (2003). New data on some gammaridean amphipods (Amphipoda, Gammaridea) from Palearctic. Glasnik of the Section of Natural Sciences Montenegrin Academy of Sciences and Arts, 15, 20-37.

Karaman, G.S. (2015) New data of genus *Niphargus* Schiödte, 1849 (Fam. Niphargidae) from Greece (Contribution to the knowledge of the Amphipoda 284). Agriculture & Forestry, 61 (4): 43-60, Podgorica.

Karaman, G.S. (2016a) On two new or interesting species of the family Niphargidae from Greece and Croatia. (Contribution to the knowledge of the Amphipoda 286). Ecologica Montenegrina, 5, 1–17.

Karaman, G.S. (2016b). Two new genera of the family Niphargidae from Greece (Contribution to the Knowledge of the Amphipoda 287). Agriculture & Forestry, 62 (1), 7–27.

Karaman, G.S., (2017a). A new member of the genus *Niphargus* Schiödte, 1849 (Amphioda Gammaridea, fam. Niphargidae) from Crete Island, Greece (contribution to the knowledge of the Amphipoda 293). Ecologica Montenegrina, 10: 1-10.

Karaman, G.S. (2017b). *Niphargus cymbalus*, new species and *N. jovanovici* S. Kar. 1931 in Greece (contribution to the knowledge of the Amphipoda 298). Agriculture & Forestry, 63(4), 263-279.

Karaman, G.S. (2017c). New subterranean species of the family Niphargidae from Greece, *Niphargus denarius*, sp. n. (contribution to the knowledge of the Amphipoda 295). Agriculture & Forestry, 63, (1), 337-356

Karaman, G.S. (2017d). On the endemic subterranean amphipod *Niphargus versluysi* S. Karaman, 1950 (Fam. Niphargidae) in Greece (Contribution to the Knowledge of the Amphipoda 297). Biologia Serbica, 39(2).

Karaman, G.S. (2018). Further discovery of new or partially known taxa of the genus *Niphargus* Schiödte, 1849 (Fam. Niphargidae) in Greece (contribution to the knowledge of the Amphipoda 302). Agriculture and Forestry, 64(2), 5-31.

Karaman, 1950 (Fam. Niphargidae) in Greece (Contribution to the Knowledge of the Amphipoda 297). Biologia Serbica, 39(2).

Karaman, G.S. & Pinkster, S. (1977a) Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea-Amphipoda). Part I. *Gammarus pulex*-group and related species. Bijdragen tot de Dierkunde, 47(1), 1–97.

Karaman, G.S., Pinkster, S., (1977b). Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea,Amphipoda). PartII. *Gammarus roeseli*-group and related species. Bijdr. Dierk. 47, 165–196.

Karaman, G.S. & Pinkster, S. 1987. Freshwater *Gammarus* species from Europe, North Africa and adjacent regions of Asia (Crustacea-Amphipoda). Part III. *Gammarus balcanicus*-group and related species. Bijdragen tot de Dierkunde 57, 207-260.

Karaman G.S. & Sket B. (1989) *Niphargus* species (Crustacea: Amphipoda) of the Kvarner-Velebit Islands (NW Adriatic, Yugoslavia). Biološki vestnik 37(2): 19-36.

Karaman, S.L. 1931. Beitrag zur Kenntnus der Susswasseramphiopden. Bull. Soc. Scien. Skoplje IX, 93-107.

Karaman, S. (1952) Amphipoda i Izopoda Vranskog jezera na ostrvu Cresu. Prirodoslovna istraživanja 25: 87-95

Katalinic, A., Rubinic, J., & Buselic, G. (2008). Hydrology of two coastal karst cryptodepressions in Croatia: Vrana lake vs Vrana lake. In Proceedings of Taal2007: The 12th World Lake Conference (Vol. 732, p. 743).

Katouzian, A.R., Sari, A., Macher, J.N., Weiss, M., Saboori, A., Leese, F., Weigand, A.M. (2016). Drastic underestimation of amphipod biodiversity in the endangered Irano-Anatolian and Caucasus biodiversity hotspots. Scientific reports 6.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., ... & Thierer, T. (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28(12), 1647-1649.

Kelly DW, Dick JT, Montgomery WI. 2002. The functional role of *Gammarus* (Crustacea,Amphipoda): shredders, predators, or both? Hydrobiologia 485(1-3):199-203. DOI 10.1023/A:1021370405349.

Kernan, M., Battarbee, R. W., Moss, B. R. (Eds.). (2011). Climate change impacts on freshwater ecosystems. John Wiley & Sons.

Ketmaier, V. & Caccone A. (2013). Twenty years of molecular biogeography in the west Mediterranean islands of Corsica and Sardinia: lessons learnt and future prospects. In: Silva-Opps M. (ed.), Current Progress in Biological Research, IntechOpen, Chapter 4, 71-93.

Ketmaier, V., Argano R., Cobolli M., De Matthaeis E. & Messana G. (2001). A systematic and biogeographical study of hypogean and epigean populations of the *Proasellus* species group from Sardinia, Central Italy and Jordan: allozyme insights. Journal Zoological Systematic Evolutionary Research, 39, 53-61.

Ketmaier V., Argano R. & Caccone G. (2003). Phylogeography and molecular rates of subterranenan aquatic Stenasellid Isopod within a peri-Tyrrhenian distribution. Molecular Ecology, 12, 547-555.

Krapp, T., Grasso R. & Ruffo S. (2010). New data on the genus *Jassa* Leach (Amphipoda, Ischyroceridae). Zoologica Baetica, 21, 85-100.

Krijgsman, W. (2002). The Mediterranean: mare nostrum of earth sciences. Earth and Planetary Science Letters, 205(1-2), 1-12.

Krijgsman, W., Hilgen, F. J., Raffi, I., Sierro, F. J., & Wilson, D. S. (1999). Chronology, causes and progression of the Messinian salinity crisis. Nature, 400(6745), 652.

Krijgsman, W., Blanc-Valleron, M.M., Flecker, R., Hilgen, F.J., Kouwenhoven, T.J., Orszag-Sperber, F. & Rouchy, J.M. (2002) The onset of the Messinian salinity crisis in the eastern Mediterranean (Pissouri basin, Cyprus). Earth Planet. Sci. Lett., 194(3–4), 299–310.

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7), 1870-1874.

La Greca, M. (1957). Considerazioni sull'origine della fauna siciliana. Italian Journal of Zoology, 24(2), 593-631.

Lagrue, C., Wattier, R., Galipaud, M., Gauthey, Z., Rullmann, J. P., Dubreuil, C., ... & Bollache, L. (2014). Confrontation of cryptic diversity and mate discrimination within *Gammarus pulex* and *Gammarus fossarum* species complexes. Freshwater Biology, 59(12), 2555-2570.

Lanfear, R., Calcott, B., Ho, S. Y., & Guindon, S. (2012). PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular biology and evolution, 29(6), 1695-1701.

Lanza, B. (1984). Sul significato biogeografico delle isole fossili con particolare riferimento all’arcipelago pliocenico della Toscana. Atti Società Italiana Scienze Naturali, Museo Civico Storia Naturale Milano, 125, 145–158.

Lázaro, E. M., Harrath, A. H., Stocchino, G. A., Pala, M., Baguñà, J., & Riutort, M. (2011). *Schmidtea mediterranea* phylogeography: an old species surviving on a few Mediterranean islands?. BMC Evolutionary Biology, 11(1), 274.

Lefébure, T., Douady, C. J., Gouy, M., & Gibert, J. (2006a). Relationship between morphological taxonomy and molecular divergence within Crustacea: proposal of a molecular threshold to help species delimitation. Molecular phylogenetics and evolution, 40(2), 435-447.

Lefébure, T., Douady, C. J., Gouy, M., Trontelj, P., Briolay, J., & Gibert, J. (2006b). Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. Molecular Ecology, 15(7), 1797-1806.

Leigh, J. W., & Bryant, D. (2015). Popart: full-feature software for haplotype network construction. Methods in Ecology and Evolution, 6(9), 1110-1116.

Lewis S.D. (2002) *Pacifastacus*. In: Biology of Freshwater Crayfish (Ed. D.M. Holdich), pp. 511–540. Blackwell Scientific, Oxford.

Lewinsohn, Ch. & L.B. Holthuis, 1986. The Crustacea Decapoda of Cyprus. Zool. Verhand. Leiden, 230: 1-64.

Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11), 1451-1452.

Lobo, J., Ferreira, M. S., Antunes, I. C., Teixeira, M. A., Borges, L. M., Sousa, R., ... & Costa, F. O. (2016). Contrasting morphological and DNA barcode-suggested species boundaries among shallow-water amphipod fauna from the southern European Atlantic coast. Genome, 60(2), 147-157.

Lončarić, R., Magaš D. & Surić M. (2011). The influence of water availability on the historical, demographic and economic development of the Kvarner Islands (Croatia). Annales, Series Historica et Sociologica, 21(1), 1-12.

Lucena-Moya P., Abraín R., Pardo I., Hermida B., Domínguez M. 2010. Invertebrate species list of coastal lagoons in the Balearic Islands. Transitional Waters Bulletin 4: 1: 1-11.

Lykousis, V. 2009. Sea-level changes and shelf break prograding sequences during the last 400 ka in the Aegean margins: subsidence rates and palaeogeographic implications. Continental Shelf Research, 29, 2037– 2044.

Lymberakis, P., & Poulakakis, N. (2010). Three continents claiming an archipelago: the evolution of Aegean's herpetofaunal diversity. Diversity, 2(2), 233-255.

Macdonald, K. S., Yampolsky, L., & Duffy, J. E. (2005). Molecular and morphological evolution of the amphipod radiation of Lake Baikal. Molecular Phylogenetics and Evolution, 35(2), 323-343.

MacNeil C, Dick JT, Elwood RW. 1997. The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. Biological Reviews of the Cambridge Philosophical Society 72(03):349364.

Macneil, C., Dick, J. T., & Elwood, R. W. (1999). The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). Biological Reviews, 74(4), 375-395.

Madonia, P., La Manna M. (1986). Fenomeni carsici ipogei nelle evaporiti in Sicilia. Le Grotte d'Italia, 13, 163-189.

Maguire, I., Klobucar, G.I.V., Matocec, S.G. & Erben, R. (2003). Distribution of *Austropotamobius pallipes* (Lereboullet) in Croatia and notes on its morphology. Bulletin Francais De La Peche Et De La Pisciculture, 57-71.

Malinverno, A, Ryan W.B.F. (1986). Extension in the Tyrrhenian Sea and shortening in the Apennines as result of arc migration driven by sinking of the lithosphere. Tectonics. 5, 227-245.

Mamos T., (2015) Phylogeography and cryptic diversity of *Gammarus balcanicus* Schäferna, 1922 in Europe. PhD thesis, Faculty of Biology and Environmental Protection, University of Lodz.

Mamos, T., Wattier, R., Majda, A., Sket, B., & Grabowski, M. (2014). Morphological vs. molecular delineation of taxa across montane regions in Europe: the case study of *Gammarus balcanicus* Schäferna, (Crustacea: Amphipoda). Journal of Zoological Systematics and Evolutionary Research, 52(3), 237-248.

Mamos, T., Wattier, R., Burzyński, A., Grabowski, M. (2016). The legacy of a vanished sea: a high level of diversification within a European freshwater amphipod species complex driven by 15 My of Paratethys regression. Molecular ecology 25(3): 795-810

Margalef, R. (1952). Une *Jaera* dans les eaux douces des BalCares, *Jaera balearica* n. sp. (Isopoda, Asellota). Hydrobiologia 4, 209-13.

Marra A.C. (2009). Pleistocene mammal faunas of Calabria (Southern Italy): biochronology and palaeobiogeography. Bollettino della Società Paleontologica Italiana, 48 (2), 113-122.

Marroni, M., Molli G., Montanini A. & Tribuzio R. (1998). The association of continental crust rocks with ophiolites in the Northern Apennines (Italy): implications for the continent-ocean transition in the Western Tethys. Tectonophysics, 292(1-2), 43-66.

Marrone, F. & Naselli Flores L. (2015). A review on the animal xenodiversity in Sicilian inland waters (Italy). Advances in Oceanography and Limnology, 6(1/2), 2-12.

Marrone, F., Barone R. & Naselli Flores L. (2006). Cladocera (Branchiopoda: Anomopoda, Ctenopoda, and Onychopoda) from Sicilian inland aaters: an updated inventory. Crustaceana, 78(9), 1025-1039.

Marrone, F., Castelli, G., & Naselli-Flores, L. (2009). Sicilian temporary ponds: an overview of the composition and affinities of their crustacean biota. In International Conference on Mediterranean Temporary Ponds. Proceedings & Abstracts. Menorca: Consell Insular de Menorca, Recerca (Vol. 14, pp. 189-202).

Marrone, F., Alfonso, G., Stoch, F., Pieri, V., Alonso, M., Dretakis, M., & Naselli-Flores, L. (2019). An account on the non-malacostracan crustacean fauna from the inland waters of Crete, Greece, with the synonymization of *Arctodiaptomus piliger* Brehm, 1955 with *Arctodiaptomus alpinus* (Imhof, 1885)(Copepoda: Calanoida). Limnetica, 38(1), 00-00.

Marzahn, E., Mayer, W., Joger, U., Ilgaz, Ç., Jablonski, D., Kindler, C., ... & Žagar, A. (2016). Phylogeography of the *Lacerta viridis* complex: mitochondrial and nuclear markers provide taxonomic insights. Journal of Zoological Systematics and Evolutionary Research, 54(2), 85-105.

Massa, B., Sbordoni V. & Vigna Taglianti A. (2011). La biogeografia della sicilia: considerazioni conclusive sul XXXVII Congresso della Società Italiana di Biogeografia. Biogeographia, 30, 685-694).

Mats, V. D., Shcherbakov, D. Y., & Efimova, I. M. (2011). Late Cretaceous-Cenozoic history of the Lake Baikal depression and formation of its unique biodiversity. Stratigraphy and Geological Correlation, 19(4), 404.

McCallum, J.E. & Robertson, A.H.F. 1990. Pulsed uplift of the Troodos massif—evidence from the Plio-Pleistocene Mesaoria basin. In: Moores, E.M., Malpas, J., Panayiotou, A. & Xenophontos, C. (eds) Ophiolites: Crustal Analogues. Proceedings of the International Symposium ‘Troodos 1987’. Cyprus Geological Survey Department, Nicosia, 217–230.

Mcinerney, C.E., Maurice, L., Robertson, A.L., Knight, L.R., Arnscheidt, J., Venditti, C., Dooley, J.S., Mathers, T., Matthijs, S., Eriksson, K., Proudlove, G.S. & Hanfling, B. 2014. The ancient Britons: groundwater fauna survived extreme climate change over tens of millions of years across NW Europe. Molecular Ecology, 23, 1153-1166.

McKinney FK. 2007. The Northern Adriatic Ecosystem: Deep Time in a Shallow Sea. Columbia University Press

Medail, F., & Quezel, P. (1997). Hot-spots analysis for conservation of plant biodiversity in the Mediterranean Basin. Annals of the Missouri Botanical Garden, 112-127.

Mejri, R., Arculeo, M., Hassine, O. K. B., & Brutto, S. L. (2011). Genetic architecture of the marbled goby *Pomatoschistus marmoratus* (Perciformes, Gobiidae) in the Mediterranean Sea. Molecular phylogenetics and evolution, 58(2), 395-403.

Messana, G. & Ruffo S. (2001). A new species of *Longigammarus* (Crustacea Amphipoda, Gammaridae) from the Pianosa Island (Tuscany Archipelago). Italian Journal of Zoology, 68, 161-164.

Messouli, M., Messana G., Yacoubi-Kebiza M. (2006). Three new species of *Pseudoniphargus* (Amphipoda), from the groundwater of three Mediterranean islands, with notes on the Ps. Adriaticus. Subterranean Biology, 4, 79-101.

Meulenkamp, J. E. (1971). The Neogene in the southern Aegean area. Opera Botanica, 30, 5-12.

Mittermeier, R.A., Robles Gil P., Hoffmann M., Pilgrim J., Brooks T., Goettsch Mittermeier C., Lamoreux J., Da Fonseca, G.A.B. (2004). Hotspots Revisited: Earth’s Biologically Richest and Most Endangered Ecoregions, 2nd ed. Cemex, Mexico City.

Monaghan, M. T., Wild, R., Elliot, M., Fujisawa, T., Balke, M., Inward, D. J., ... & Vogler, A. P. (2009). Accelerated species inventory on Madagascar using coalescent-based models of species delineation. Systematic biology, 58(3), 298-311.

Moore, P.G. & Schembri P.J. (1986). Notes concerning the semi-terrestrial and freshwater amphipods (Crustacea: Peracarida) of the Maltese Islands. Animalia, 13(1/2), 65-75.

Moores, E.M., Robinson, P.T., Malpas, J. & Xenophontos, C. (1984). A Model for the Origin of the Troodos Massif, Cyprus, and other Mideast Ophiolites. Geology 12(8), 500-03.

Morpurgo, M., Aquiloni, L., Bertocchi, S., Brusconi, S., Tricarico, E. & Gherardi, F. (2010). Distribuzione dei gamberi d'acqua dolce in Italia. Studi Trentini di Scienze Naturali, 87, 125-132.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature, 403(6772), 853.

Nahavandi, N., Ketmaier, V., Plath, M., & Tiedemann, R. (2013). Diversification of Ponto-Caspian aquatic fauna: Morphology and molecules retrieve congruent evolutionary relationships in *Pontogammarus maeoticus* (Amphipoda: Pontogammaridae). Molecular Phylogenetics and Evolution, 69(3), 1063-1076.

Neuparth, T., Correia A.D., Costa, F.O., Lima, G., Costa, M.H. (2005). Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta*: I. Biochemical endpoints. Marine Environmental Research, 60(1), 69-91.

Ntakis, A., Anastasiadou, A., Zakšek, V. & Fišer, C. (2015) Three new species of *Niphargus* (Crustacea: Amphipoda) from Greece with their phylogenetic position and biogeographic characterization. Zoologischer Anzeiger

Orszag-Sperber, F. 2006. Changing perspectives in the concept of “Lago-Mare” in Mediterranean Late Miocene evolution. Sedimentary Geology 188-189, 259-277.

Oscoz J., Galicia D., Miranda R. (eds.) (2011). Identification Guide of Freshwater Macroinvertebrates of Spain. Springer Science & Business Media.

Özbek, M., 2011. An overview of *Gammarus* species distributed in Turkey, with an updated check-list and additional records. Zoology in the Middle East, 53: 71-78.

Özbek, M., & Ustaoğlu, M. R. (2006). Check-list of malacostraca (crustacea) species of Turkish inland waters. EU Journal of Fisheries & Aquatic Sciences, 23(1-2), 229-234.

Özbek, M., Öztürk, H. H., Özkan, N. 2015. Marmara ve Paşalimanı adaları ile Kapıdağ Yarımadası içsularının Gammaridae (Amphipoda) faunası. Ege J Fish Aqua Sci 32(4): 213-216

Özbek, M., & Özkan, N. (2017). Gökçeada içsularının Amphipoda (Crustacea: Malacostraca) faunası Amphipoda (Crustacea: Malacostraca) fauna of the inland-waters of Gökçeada Island. Ege Journal of Fisheries and Aquatic Sciences, 34(1), 63-67.

Ozimec R., Bedek J., Gottstein S., Jalžić B., Slapnik R., Štamol V., Bilandžija H., Dražina T., Kletečki E., Komerički A., Lukić M., Pavlek M. 2009. Crvena knjiga špiljske faune Hrvatske. 2009, Ministarstvo kulture, Državni zavod za zaštitu prirode

Palmer, M., & Pons, G. X. (1996). Diversity in Western Mediterranean islets: effects of rat presence on a beetle guild. Acta Oecologica, 17(4), 297-305.

Palmer M., Pons G.X., Cambefort I., Alcover J.A. (1999). Historical processes and environmental factors as determinants of inter-island differences in endemic faunas: the case of the Balearic Islands. Journal of Biogeography, 26: 813–823.

Palumbi, S., Martin, A., Romano, S., Mcmillan, W., Stice L., & Grabowski, G. (1991). The Simple Fool’s Guide to PCR. A Collection of PCR Protocols, Version 2. University of Hawaii, Honolulu

Papanikolaou, D., Alexandri, M., Nomikou, P., & Ballas, D. (2002). Morphotectonic structure of the western part of the North Aegean Basin based on swath bathymetry. Marine Geology, 190(1-2), 465-492.

Parks, D. H., Mankowski, T., Zangooei, S., Porter, M. S., Armanini, D. G., Baird, D. J., ... & Beiko, R. G. (2013). GenGIS 2: Geospatial analysis of traditional and genetic biodiversity, with new gradient algorithms and an extensible plugin framework. PloS one, 8(7), e69885.

Pavesi, L., Tiedemann, R., De Matthaeis, E., & Ketmaier, V. (2013). Genetic connectivity between land and sea: the case of the beachflea *Orchestia montagui* (Crustacea, Amphipoda, Talitridae) in the Mediterranean Sea. Frontiers in zoology, 10(1), 21.

Pérez-Moreno J. L., Iliffe T. M., Bracken-Grissom H. D. 2016. Life in the Underworld: Anchihialine cave biology in the era of speleogenomics. International Journal of Speleology, 45 (2): 149-170.

Palmer M., Pons G.X., Cambefort I., Alcover J.A. (1999). Historical processes and environmental factors as determinants of inter-island differences in endemic faunas: the case of the Balearic Islands. Journal of Biogeography, 26: 813–823.

Perissoratis, C., Conispoliatis, N., 2003. The impacts of sea-level changes during latest Pleistocene and Holocene times on the morphology of the Ionian and Aegean seas (SE Alpine Europe). Mar. Geol. 196, 145–156.

Pesce G. L., Maggi, D., Ciocca, A., Argano, R. 1979. Biological researches on the subterranean phreatic waters of Northern Greece. Biol. Gallo-Helen. 8: 109-126

Pesce, G. L., & Argano, R. (1980). Nouvelles données sur les Asellides de la Grèce continentale et insulaire (Crustacea, Isopoda). Bulletin Zoologisch Museum, 7(5), 49-54.

Pesce, G.L. & Maggi, D. (1983) Ricerche faunistiche in acque sotterranee freatiche della Grecia Meridionale ed insulare e stato attuale delle conoscenze sulla stygofauna di Grecia. Natura, Milano, 74(1-2), 15– 73.

Pesce, G. L. (1981). *Microcharon ullae* n. sp., a microparasellid from subterranean waters of Rhodes, Greece (Isopoda: Asellota). Fragm. Balcanica, 11 (7), 57-62.

Pesce, G. L. (1985). New records for *Salentinella* Ruffo (Crustacea Amphipoda) from phreatic waters of Italy and Greece. International Journal of Speleology 14, 19-29.

Pinkster, S. (1988). Problems in the taxonomy of the freshwater gammarids with special emphasis on the genus *Echinogammarus* in Italy. Crustaceana. Supplement, 245-255.

Pinkster S. (1993). A revision of the genus *Echinogammarus* Stebbing, 1899 with some notes on related genera (crustacea, Amphipoda). Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 19, 1-185.

Pinter N., Grenerczy G., Weber J., Stein S., Medak D., (eds.). 2016. The Adria Microplate: GPS Geodesy, Tectonics and Hazards. Nato Science Series: IV: 61, Springer Netherlands.

Pinya S, Guijarro B., Alberti R., Amorós L., Muňoz A. (2008) Sobre la presència del cranc de riu americà, *Procambarus clarkii*, a l'illa de Mallorca. Jornades de Medi de les Illes Balears.

Pinya, S., & Carretero, M. A. (2011). The Balearic herpetofauna: a species update and a review on the evidence. Acta Herpetologica, 6(1), 59-80.

Pipitone, C., & Arculeo, M. (2003). The marine Crustacea Decapoda of Sicily (central Mediterranean Sea): a checklist with remarks on their distribution. Italian Journal of Zoology, 70(1), 69-78.

Platvoet D. (1984). Observations on the genus *Salentinella* (Crustacea, Amphipoda) with description of *Salentinella formenterae* n. sp. Bijdragen lot de Dierkunde, 54: 178-184.

Plötner J., Baier F., Akın Ç., Mazepa G., Schreiber, R., Beerli, P., Litvinchuk, A. N., Bilgin, C. C., Borkin, L., & Uzzell, T. (2012): Genetic data reveal that water frogs of Cyprus (genus *Pelophylax*) are an endemic species of Messinian origin. Zoosystematics and Evolution, 88, 261–283.

Pomar L (1991). Reef geometries, erosion surfaces and high-frequency sea-level changes, upper miocene Reef Complex, Mallorca, Spain. Sedimentology, 38:243-269.

Ponniah, M., & Hughes, J. M. (2004). The evolution of Queensland spiny mountain crayfish of the genus *Euastacus*. I. Testing vicariance and dispersal with interspecific mitochondrial DNA. Evolution, 58(5), 1073-1085.

Pons G. X., Jaume D., Damians J. 1995. Fauna cavernicola de Mallorca. Bolletí de la Societat d'Història Natural de les Balears 3: 125-143.

Pons G. X., Palmer M. 1996. Fauna endèmica de les illes Balears. Mon. Soc. Hist. Nat. Balears. Palma.

Pons, J., Barraclough, T. G., Gomez-Zurita, J., Cardoso, A., Duran, D. P., Hazell, S., ... & Vogler, A. P. (2006). Sequence-based species delimitation for the DNA taxonomy of undescribed insects. Systematic biology, 55(4), 595-609.

Popov, S. V., Rögl, F., Rozanov, A. Y., Steininger, F. F., Shcherba, I. G., & Kovac, M. (2004). Lithological-Paleogeographic maps of Paratethys-10 maps Late Eocene to Pliocene.

Popov, S.V., Shcherba, I.G., Ilyina, L.B., Nevesskaya, L.A., Paramonova, N.P., Khondkarian, S.O. & Magyar, I. 2006. Late Miocene to Pliocene palaeogeography of the Paratethys and its relation to the Mediterranean. Palaeogeography, Palaeoclimatology, Palaeoecology, 238, 91-106.

Poulakakis, N., Lymberakis, P., Valakos, E., Zouros, E., Mylonas, M., 2005. Phylogenetic relationships and biogeography of *Podarcis* species from the Balkan Peninsula, by Bayesian and maximum likelihood analyses of mitochondrial DNA sequences. Mol. Phylogenet. Evol. 37, 845–857.

Poulakakis, N., Kapli, P., Lymberakis, P., Trichas, A., Vardinoyiannis, K., Sfenthourakis, S., & Mylonas, M. (2015). A review of phylogeographic analyses of animal taxa from the Aegean and surrounding regions. Journal of Zoological Systematics and Evolutionary Research, 53(1), 18-32.

Preus J.L. (1992). Contribució al coneixement de la fauna interstical litoral. Presencia de Mistacocarides (Crustacea) a i'lla de Mallorca. Butll. Inst. Cat. Hist. Nat. (Sec. Zool., 9) 60: 113-119.

Pretus J. L., Stock J. H. (1990). A new hyporheic *Bogidiella* (Crustacea, Amphipoda) from Mallorca. Endins, 16: 47-51.

Previšić, A., Walton, C., Kučinić, M., Mitrikeski, P. T., & Kerovec, M. (2009). Pleistocene divergence of Dinaric *Drusus* endemics (Trichoptera, Limnephilidae) in multiple microrefugia within the Balkan Peninsula. Molecular ecology, 18(4), 634-647.

Puillandre, N., Lambert, A., Brouillet, S., & Achaz, G. (2012). ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular ecology, 21(8), 1864-1877.

Rachalewski, M., Banha, F., Grabowski, M., & Anastácio, P. M. (2013). Ectozoochory as a possible vector enhancing the spread of an alien amphipod *Crangonyx pseudogracilis*. Hydrobiologia, 717(1), 109-117.

Rackham, O., & Moody, J. (1996). The making of the Cretan landscape. Manchester University Press.

Ratnasingham, S., & Hebert, P. D. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Molecular ecology notes, 7(3), 355-364.

Ratnasingham, S., & Hebert, P. D. (2013). A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PloS one, 8(7), e66213.

Raupach, M. J., Barco, A., Steinke, D., Beermann, J., Laakmann, S., Mohrbeck, I., ... & Segelken-Voigt, A. (2015). The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. PloS one, 10(9), e0139421.

Rögl, F. (1999). Mediterranean and Paratethys. Facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). Geologica Carpathica, 50(4), 339-349.

Rosenbaum, G., Lister, G.S., Duboz, C. (2002). Reconstruction of the tectonic evolution of the western Mediterranean since the Oligocene. Journal of the Virtual Explorer, 8: 107–130.

Rosenbaum, G., Lister, G.S. (2004). Formation of arcuate orogenic belts in the western Mediterranean region. Geological Society of America Special Papers, 383, 41–56.

Rosenzweig, M. L. (1995). Species diversity in space and time. Cambridge University Press.

Rudolph, K., Coleman, C. O., Mamos, T., & Grabowski, M. (2018). Description and post-glacial demography of *Gammarus jazdzewskii* sp. nov. (Crustacea: Amphipoda) from Central Europe. Systematics and Biodiversity, 16(6), 587-603.

Ruffo, S. & Delamare Deboutteville C. (1952). Deux nouveaux Amphipodes souterrains de France, *Salentinella angelieri* n. sp. et *Bogidiella chappuisi* n. sp. Comptes Rendus de l'Académie des Sciences, Paris, 234, 1636-1638.

Ruffo, S., & Stoch F. (2006). Crustacea Malacostraca Amphipoda. In: Ruffo S. & Stoch F. (eds.), Checklist and distribution of the Italian fauna. 10,000 terrestrial and inland water species. Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 17, 109-111.

Ruffo, S. & Vigna Taglianti A. (1989). Description of a new cavernicolous *Ingolfiella* species from Sardinia, with remarks on the systematics of the genus (Crustacea, Amphipoda, Ingolfiellidae). Annali Museo civico di Storia naturale di Genova, 87: 237-261.

Ruffo, S. (1960). Studies on Crustacean amphipods. A contribution to the knowledge of Crustacean amhpipods of subterranean waters of Sardinia and Baleari Islands. Atti dell’Istituto Veneto di Scienze, Lettere ed Arti, 118, 168-180.

Ruffo, S. (2006a). Crustacea Malacostraca Bathynellacea. In: Ruffo S. & Stoch F. (eds.), Checklist and distribution of the Italian fauna. 10,000 terrestrial and inland water species.

Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 17, 101.

Ruffo, S. (2006b). Crustacea Malacostraca Thermosbaenacea. In: Ruffo S. & Stoch F. (eds.), Checklist and distribution of the Italian fauna. 10,000 terrestrial and inland water species. Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 17, 103.

Ruffo, S., Tarocco, M., & Latella, L. (2014). *Cryptorchestia garbinii* n. sp.(Amphipoda: Talitridae) from Lake Garda (Northern Italy), previously referred to as *Orchestia cavimana* Heller, 1865, and notes on the distribution of the two species. Italian Journal of Zoology, 81(1), 92-99.

Ruggieri, G. (1973). Due parole sulla paleogeografia delle isole minori a Ovest e a Nord della Sicilia. Lavori della Società italiana di Biogeografia, 3, 5-12.

Ruggieri, R, Grasso M. (2000). Caratteristiche stratigrafiche e strutturali dell'Altipiano Ibleo Ragusano e sue implicazioni sulla morfologia carsica. Speleologia Iblea, 8, 19-35.

Ryan, W.B.F., Pitman, W.C., Major, C.O., Shimkus, K., Moskalenko, V., Jones, G.A., Dimitrov, P., Gorür, N., Sakinç, M. & Yüce, H. 1997. An abrupt drowning of the Black Sea shelf. Marine Geology 138, 119-126.

Sàbat F., Gelabert B., Rodríguez-Perea A., Giménez J. (2011). Geological structure and evolution of Majorca: Implications for the origin of the Western Mediterranean. Tectonophysics 510: 217–238.

Sacchi, C.F. (1955). Il contributo dei Molluschi terrestri alle ipotesi del "Ponte Siciliano". Archivio Zoologico Italiano, 40: 49-181.

Sakellariou, D., Galanidou, N., 2015. Pleistocene submerged landscapes and Palaeolithic archaeology in the tectonically active Aegean region. In: Harff, J., Bailey, G., Lüth, F. (Eds.), Geology and Archaeology: Submerged Landscapes of the Continental Shelf. Geological Society, London, Special Publications, 411, pp. 145–178

Santoni, S., Huneau F., Garel E., Vergnaud-Ayraud V., Labasque et al. (2016). Residence time, mineralization processes and groundwater origin within a carbonate coastal aquifer with a thick unsaturated zone. Journal of Hydrology, , 540, 50-63.

Savona Ventura, C. (1975). The Geographical Evolution of the Maltese Archipelago. The Maltese Naturalist, 2(1), 9-12.

Schellenberg, A. (1950). Subterrane Amphipoden korsikanischer Biotope. Archiv f. Hydrobiologie, 44 (Supplement 2), 325-332.

Schembri, P.J. (2003). Current state of knowledge of the Maltese non-marine fauna. In: Malta Environment and Planning Authority Annual report and accounts 2003, 33-65.

Schembri, J. & Sultana J. (1989). Red Data Book of the Maltese Islands. Department of Information, Ministry of Education, 142 pp.

Schüle, W. (1993). Mammals, vegetation and the initial human settlement of the Mediterranean islands: a palaeoecological approach. Journal of Biogeography, 399-411.

Selkoe, K. A., Aloia, C. C., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B., ... & Toonen, R. J. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. Marine Ecology Progress Series, 554, 1-19.

Senczuk, G., Harris, D. J., Castiglia, R., Litsi Mizan, V., Colangelo, P., Canestrelli, D., & Salvi, D. (2018). Evolutionary and demographic correlates of Pleistocene coastline changes in the Sicilian wall lizard *Podarcis wagleriana*. Journal of Biogeography.

Serban, E. (1977) Sur les péréiopodes VIII mâles de Iberobathynella cf. fagei de Majorque (Bathynellacea, Parabathynellidae). Crustaceana, 33, 1–16.

Sfenthourakis, S., 1996. A biogeographical analysis of terrestrial isopods (Isopoda, Oniscidea) from the central Aegean islands (Greece). J. Biogeogr. 23, 687–698.

Sfenthourakis S, & Triantis, K.A. (2017) The Aegean archipelago: a natural laboratory of evolution, ecology and civilisations, Journal of Biological Research-Thessaloniki, 24: 4, 1-13.

Shackleton, J. C., Van Andel, T. H., & Runnels, C. N. (1984). Coastal paleogeography of the central and western Mediterranean during the last 125,000 years and its archaeological implications. Journal of field Archaeology, 11(3), 307-314.

Sherbakov, D. Y., Kamaltynov, R. M., Ogarkov, O. B., Vainola, R., Vainio, J. K., & Verheyen, E. (1999). On the phylogeny of Lake Baikal amphipods in the light of mitochondrial and nuclear DNA sequence data. Crustaceana, 72(8), 911-919.

Shih, H. T., Yeo, D. C., & Ng, P. K. (2009). The collision of the Indian plate with Asia: molecular evidence for its impact on the phylogeny of freshwater crabs (Brachyura: Potamidae). Journal of Biogeography, 36(4), 703-719.

Sidorov, D.A. & Palatov, D. 2012. Taxonomy of the spring dwelling amphipod *Synurella ambulans* (Crustacea: Crangonyctidae) in West Russia: with notes on its distribution and ecology. 2012.

Sket B. (1988) Zoogeografija sladkovodnih i somornih rakov (Crustacea) v kvarnersko-velebitskem območju. Biološki vestnik 36 (2): 63- 76

Sket, B. (1990a) Is *Niphargobates lefkodemonaki* sp. n. (Crustacea: Amphipoda) from Kriti (Greece) a Zoogeographical Enigma? Zoologische Jahrbücher, Abteilung für Systematik, 117, 1–10.

Sket, B. (1990b). Isopoda (Crustacea: Isopoda: *Microcharon, Jaera, Proasellus*) and other fauna in hypogean waters of southern Cyprus. International Journal of Speleology 19(1-4):39-50.

Sket, B., 1999. The nature of biodiversity in hypogean waters and how it is endangered. Biodiversity & Conservation 8: 1319–1338.

Sket, B.; Karaman, G. (1990). *Niphargus rejici* (Amphipoda), its relatives in the Adriatic islands, and its possible relations to S.W. Asian taxa. Stygologia. 5(3): 153-172.

Sket, B., & Hou, Z. (2018). Family Gammaridae (Crustacea: Amphipoda), mainly its *Echinogammarus* clade in SW Europe. Further elucidation of its phylogeny and taxonomy. Acta Biologica Slovenica, 61(1), 93-102.

Sondaar, P.Y. & M.D. Dermitzakis 1982. Relation Migration Landvertebrates, Paleogeography and Tectonics. In: X.L. Pichon, S.S. Augustidis & J. Mascle, eds., International Symposium on the Hellenic Arc and Trench (H.E.A.T.), April 8-10 1981, vol 2, 283-308, Athens.

Sondaar, P. Y., De Vos, J., & Dermitzakis, M. D. (1986). Late Cenozoic faunal evolution and palaeogeography of the South Aegean island arc. Modern geology, 10(2-3), 249-259.

Sosdian S., Rosenthal Y. (2009). Deep-sea temperature and ice volume changes across the Pliocene-Pleistocene climate transitions. Science, 325: 306-310.

Speranza, F., Villa I.M., Sagnotti L., Florindo F., Cosentino D., Cipollari P. & Mattei M. (2002). Age of the Corsica-Sardinian rotation and Liguro-Provençal Basin spreading: new paleomagnetic and Ar/Ar evidence. Tectonophysics, 347, 231-251.

Stanley D. J., Wezel F-C 1985. Geological evolution of the Mediterranean Basin. New York : Springer-Verlag 589 p

Steininger, F. F., & Rögl, F. (1984). Paleogeography and palinspastic reconstruction of the Neogene of the Mediterranean and Paratethys. Geological Society, London, Special Publications, 17(1), 659-668.

Stoch, F. (1995). Diversity in groundwaters, or: why are there so many. Mémoires de Biospéologie, 22, 139-160.

Stoch, F. (1997). *Metacrangonyx ilvanus* n. sp., the first Italian representative of the family Metacrangonyctidae (Crustacea: Amphipoda). Annales Limnologie, 33(4), 255-262.

Stoch, F. (2000). How many endemic species? Species richness assessment and conservation priorities in Italy. Belgian Journal of Entomology, 2(1), 125-133.

Stoch, F., Valentino F. & Volpi E. (1996). Taxonomic and biogeographic analysis of the Proasellus coxalis-group (Crustacea, Isopoda, Asellidae) in Sicily, with description of *Proasellus montalentii* n. sp. Hydrobiologia, 317, 247- 258.

Stoch, F., Argano R. & Campanaro A. (2006). Crustacea Malacostraca Isopoda. In: Ruffo S. & Stoch F. (eds.), Checklist and distribution of the Italian fauna. 10,000 terrestrial and inland water species. Memorie del Museo Civico di Storia Naturale di Verona, 2.serie, Scienze della Vita, 17, 107-108.

Stock, J.H., (1967). A revision of the European species of the *Gammarus locusta* – group (Crustacea, Amphipoda). Zoologische Verhandelingen, 90, 1–56.

Stock, J. (1968). A revision of the European species of the *Echinogammarus pungens*-group (Crustacea, Amphipoda). Beaufortia, 16 (211), 13-78.

Stock, J.H. (1972). Les Gammarides (Crustacés Amphipodes) des eaux douces et saumâtres de Corse. Bulletin Zoologisch Museum Universiteit van Amsterdam, 2(19), 197-220.

Stock, J. T. 1976. On the identity of *Porrassia mallorquensis* Marcus, 1912, an amphipod supposedly endemic in Mallorca. Crustaceana, 30 (1): 110-111.

Stock, J.H. (1980). Regression model evolution as exemplified by the genus Pseudoniphargus (Amphipoda). Bijdragen tot der Dierkunde, 50 (1), 105-144.

Stöck, M., Grifoni, G., Armor, N., Scheidt, U., Sicilia, A., & Novarini, N. (2016). On the origin of the recent herpetofauna of Sicily: comparative phylogeography using homologous mitochondrial and nuclear genes. Zoologischer Anzeiger-A Journal of Comparative Zoology, 261, 70-81.

Strouhal, H. (1966). Ein weiterer Beitrag zur Süßwasser-und Landasselfauna Korfus. In Ein weiterer Beitrag zur Süßwasser-und Landasselfauna Korfus (pp. 257-315). Springer, Berlin, Heidelberg.

Sworobowicz, L., Grabowski, M., Mamos, T., Burzyński, A., Kilikowska, A., Sell, J., & Wysocka, A. (2015). Revisiting the phylogeography of *Asellus aquaticus* in Europe: insights into cryptic diversity and spatiotemporal diversification. Freshwater Biology, 60(9), 1824-1840.

Szarowska, M., Hofman, S., Osikowski, A., & Falniowski, A. (2014a). Divergence preceding island formation among Aegean insular populations of the freshwater snail genus *Pseudorientalia* (Caenogastropoda: Truncatelloidea). Zoological Science, 31(10), 680-687.

Szarowska, M., Hofman, S., Osikowski, A., & Falniowski, A. (2014b). *Daphniola* Radoman, 1973 (Caenogastropoda: Truncatelloidea) at east Aegean islands. Folia Malacologica, 22(4).

Szarowska, M., Osikowski, A., Hofman, S., & Falniowski, A. (2016). Do diversity patterns of the spring-inhabiting snail *Bythinella* (Gastropoda, Bythinellidae) on the Aegean Islands reflect geological history?. Hydrobiologia, 765(1), 225-243.

Tortonese, E. (1985). Distribution and ecology of endemic elements in the Mediterranean fauna (fishes and echinoderms). In Mediterranean marine ecosystems (pp. 57-83). Springer, Boston, MA.

Triantis, K. A., & Mylonas, M. (2009). Greek islands, biology. Encyclopedia of islands, 388-392.

Trincardi, F., Cattaneo, A., Asioli, A., Correggiari, A., Langone, L., 1996. Stratigraphy of the late- Quaternary deposits in the central Adriatic basin and the record of short-term climatic events. Mem. Ist. Ital. Idrobiol. 55, 39-70.

Trontelj, P., Machino, Y. & Sket, B. (2005). Phylogenetic and phylogeographic relationships in the crayfish genus *Austropotamobius* inferred from mitochondrial COI gene sequences. Molecular Phylogenetics and Evolution 34, 212–226.

Vader, W. (2005) How Many Amphipod Species? Poster Presented at XII International Amphipod Colloquium. Cork, Ireland.

van der Geer A., Dermitzakis M., & de Vos J. (2006). Crete before the Cretans: The reign of dwarfs. Pharos: J. Neth. Instit. Athens, 13:119-130.

Väinölä, R., Witt, J. D. S., Grabowski, M., Bradbury, J. H., Jazdzewski, K., & Sket, B. (2007). Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. In Freshwater Animal Diversity Assessment (pp. 241-255). Springer, Dordrecht.

Vecchioni, L., Deidun A., Sciberras J., Sciberras A., Marrone F. & Arculeo M. (2017). The late Pleistocene origin of the Italian and Maltese populations of *Potamon fluviatile* (Malacostraca: Decapoda): insights from an expanded sampling of molecular data. The European Zoological Journal, 84(1), 575–582.

Vella, N., Vella A. & Mifsud C.M. (2017). First scientific records of the invasive Red Swamp Crayfish, *Procambarus clarkii* (Girard, 1852) (Crustacea: Cambaridae) in Malta, a threat to fragile freshwater habitats. Natural and Engineering Sciences, 2, 58–66.

Verovnik R., Sket B., & Trontelj P. (2004). The colonization of Europe by the freshwater crustacean *Asellus aquaticus* (Crustacea: Isopoda) proceeded from ancient refugia and was directed by habitat connectivity. Molecular Ecology, 14, 4355–4369.

Vigliotti, L., Alvarez W. & McWilliams M. (1990). No relative motion detected between Corsica and Sardinia. Earth Planetary Science Letters, 98, 313-318.

Vogiatzakis, I. N., Pungetti, G., & Mannion, A. M. (Eds.). (2008). Mediterranean island landscapes: natural and cultural approaches (Vol. 9). Springer Science & Business Media.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., … Davies, P. M. (2010). Global threats to human water security and river biodiversity. Nature, 467(7315), 555.

Wagner, H.P. (1994) A monographic review of the Thermosbaenacea (Crustacea: Peracarida). Zoologische Verhandelingen, Leiden, 291, 1-338.

Whittaker, R. J., & Fernández-Palacios, J. M. 2007. Island biogeography: ecology, evolution, and conservation. Oxford University Press.

Weiss, M., & Leese, F. (2016). Widely distributed and regionally isolated! Drivers of genetic structure in *Gammarus fossarum* in a human-impacted landscape. BMC evolutionary biology, 16(1), 153.

Weiss, M., Macher, J.N., Seefeldt, M.A., Leese, F. (2014). Molecular evidence for further overlooked species within the *Gammarus fossarum* complex (Crustacea: Amphipoda). Hydrobiologia 721(1): 165

Williams, W. D. (1962). The geographical distribution of the isopods *Asellus aquaticus* (L.) and A. meridianus Rac. In Proceedings of the Zoological Society of London (Vol. 139, No. 1, pp. 75-96). Oxford, UK: Blackwell Publishing Ltd.

Wong, H.K., Lüdmann, T., Ulug, A. & Görür, N. 1995. The Sea of Marmara: a plate boundary sea in an escape tectonic regime. Tectonophysics 244, 231-250.

Woodward, J. (Ed.). 2009. The physical geography of the Mediterranean (Vol. 8). Oxford University Press on Demand.

Wysocka, A., Grabowski, M., Sworobowicz, L., Mamos, T., Burzyński, A., Sell, J. (2014). Origin of the Lake Ohrid gammarid species flock: ancient local phylogenetic lineage diversification. Journal of Biogeography, 41(9), 1758-1768.

Xia, X. (2018). DAMBE7: New and improved tools for data analysis in molecular biology and evolution. Molecular biology and evolution, 35(6), 1550-1552.

Xia, X., Xie, Z., Salemi, M., Chen, L., & Wang, Y. (2003). An index of substitution saturation and its application. Molecular phylogenetics and evolution, 26(1), 1-7.

Yellin-Dror, A., Grasso, M., Ben-Avraham, Z., & Tibor, G. (1997). The subsidence history of the northern Hyblean plateau margin, southeastern Sicily. Tectonophysics, 282(1-4), 277-289.

Yu, Y., Harris, A. J. & He, X. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. Molecular Phylogenetics and Evolution, 56, 848-850.

Yu, Y., Harris, A. J., Blair, C., & He, X. (2015). RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. Molecular Phylogenetics and Evolution, 87, 46-49.

Yu, G., Rao, D., Matsui, M., & Yang, J. (2017). Coalescent-based delimitation outperforms distance-based methods for delineating less divergent species: the case of *Kurixalus odontotarsus* species group. Scientific reports, 7(1), 16124.

Zganec K. 2009. Rasprostranjenost i ekologija nadzemnih rakušaca (Amphipoda: Gammaroidea) slatkih i bočatih voda Hrvatske. Doktorska disertacija, Prirodoslovno matematički fakultet, Zagreb.

Zogaris, S., Chatzinikolaou, Y., Koutsikos, N., Economou, A., Oikonomou, E., Michaelides, G., Hadjisterikotis, E., Beaumont, W.R.C., Ferreira, M.T. 2012. Freshwater fish assemblages in Cyprus with emphasis on the effects of dams. Acta Ichthyologica Et Piscatoria 42: 165–175.

Supplementary material

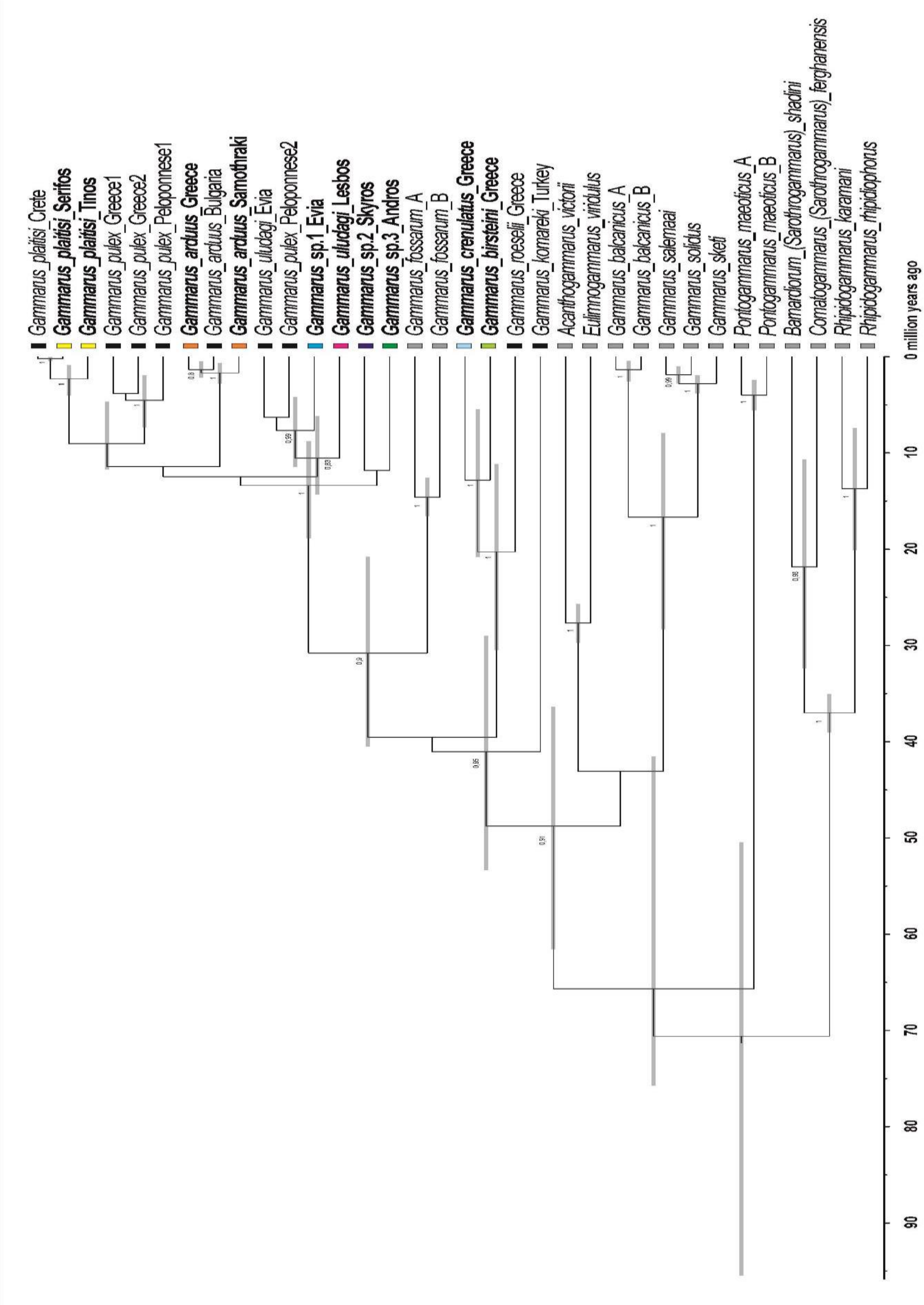


Fig.S1 Maximum clade credibility, time-calibrated Bayesian reconstruction of phylogeny of Aegean Gammarus MOTUs. Phylogeny was inferred from sequences of the mitochondrial COI and 16S rRNA genes and nuclear 28S rRNA and EF1-alpha gene. The numbers by respective nodes indicate Bayesian posterior probability values ≥ 0.8. The coloured bars represent ABGD delimitation method, with colours and symbols correspond to those presented in other figures. Grey node bars represent 95% HPD.

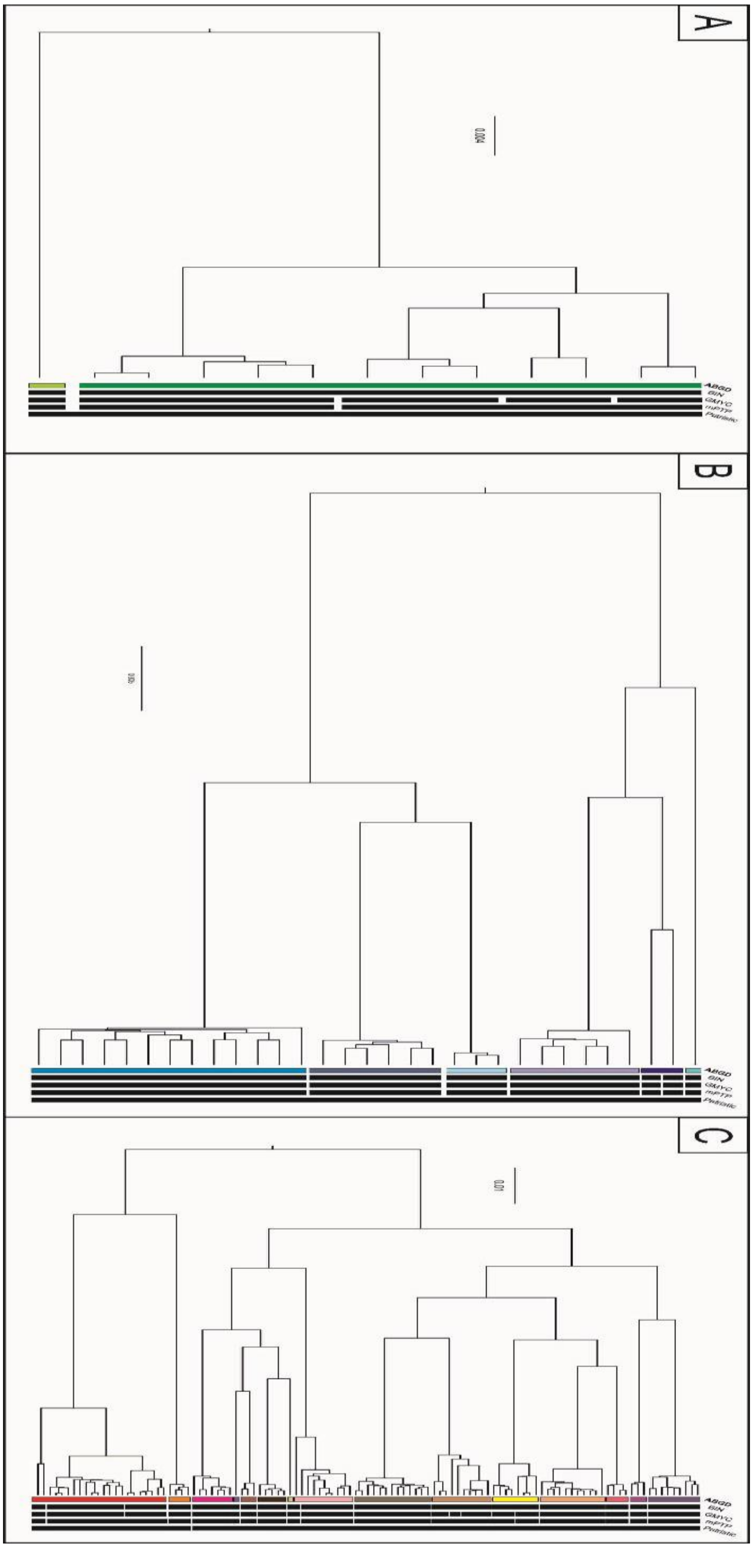


Fig.S2 Bayesian maximum clade credibility trees of Sicilian Echinogammarus species inferred from a sequences of the mitochondrial COI. Black bars indicate the respective MOTUs of Echinogammarus morphospecies, with the coloured ones representing ABGD delimitation method. The colours correspond to those presented in other figures. A) *Echinogammarus tibaldii*, B) *Echinogammarus adipatus*, C) *Echinogammarus sicilianus*.

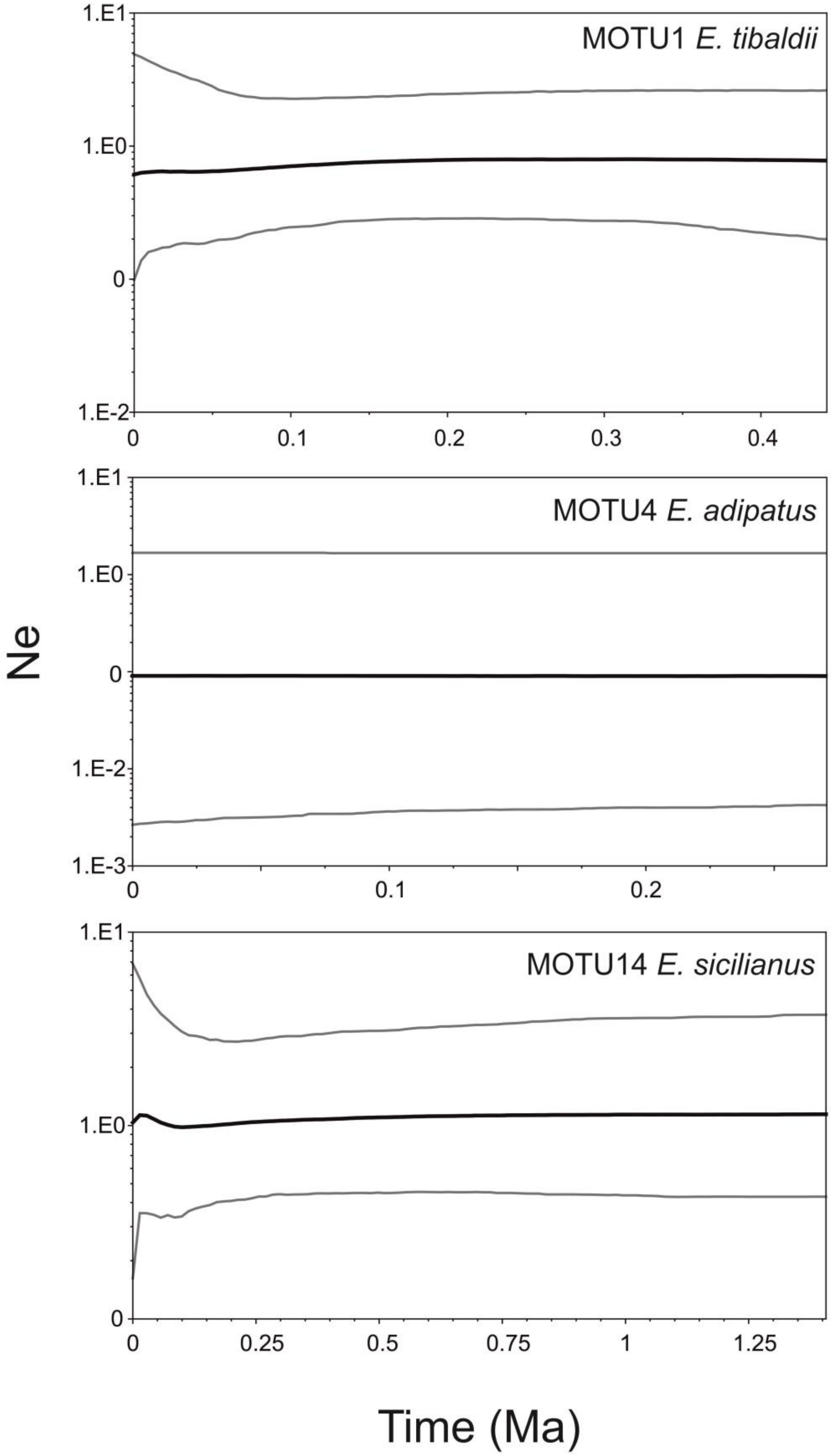


Fig.S3 Bayesian Skyline Plots for selected MOTUs of Sicilian *Echinogammarus*.

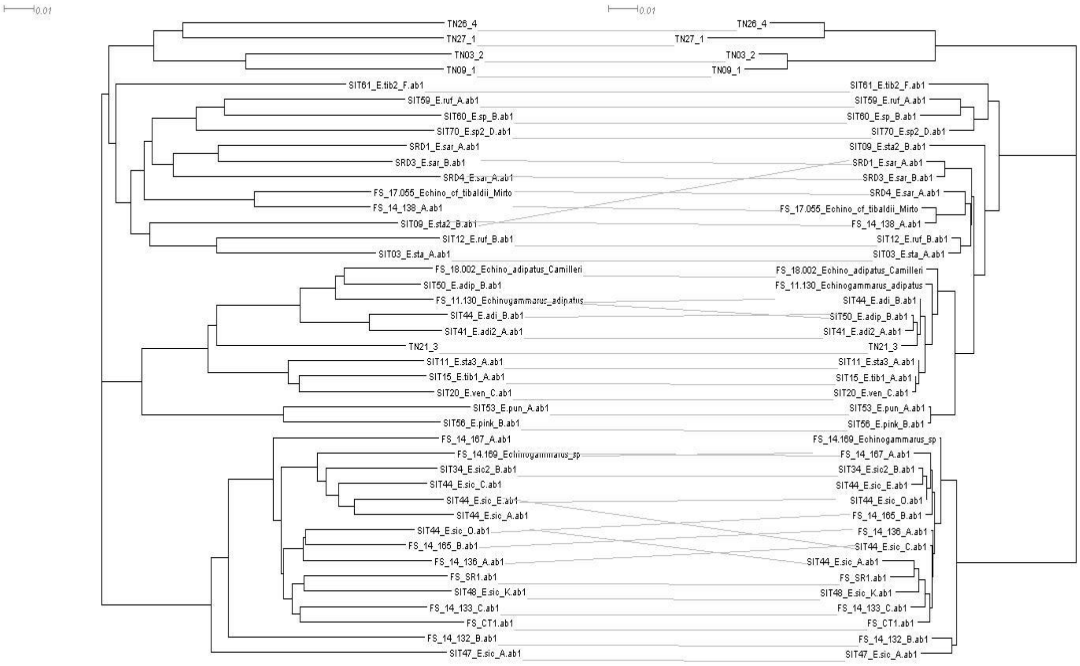


Fig.S4 The tanglegram generated for COI (on the left) and 28S rRNA data (on the right) of individuals used for phylogeny inferring.

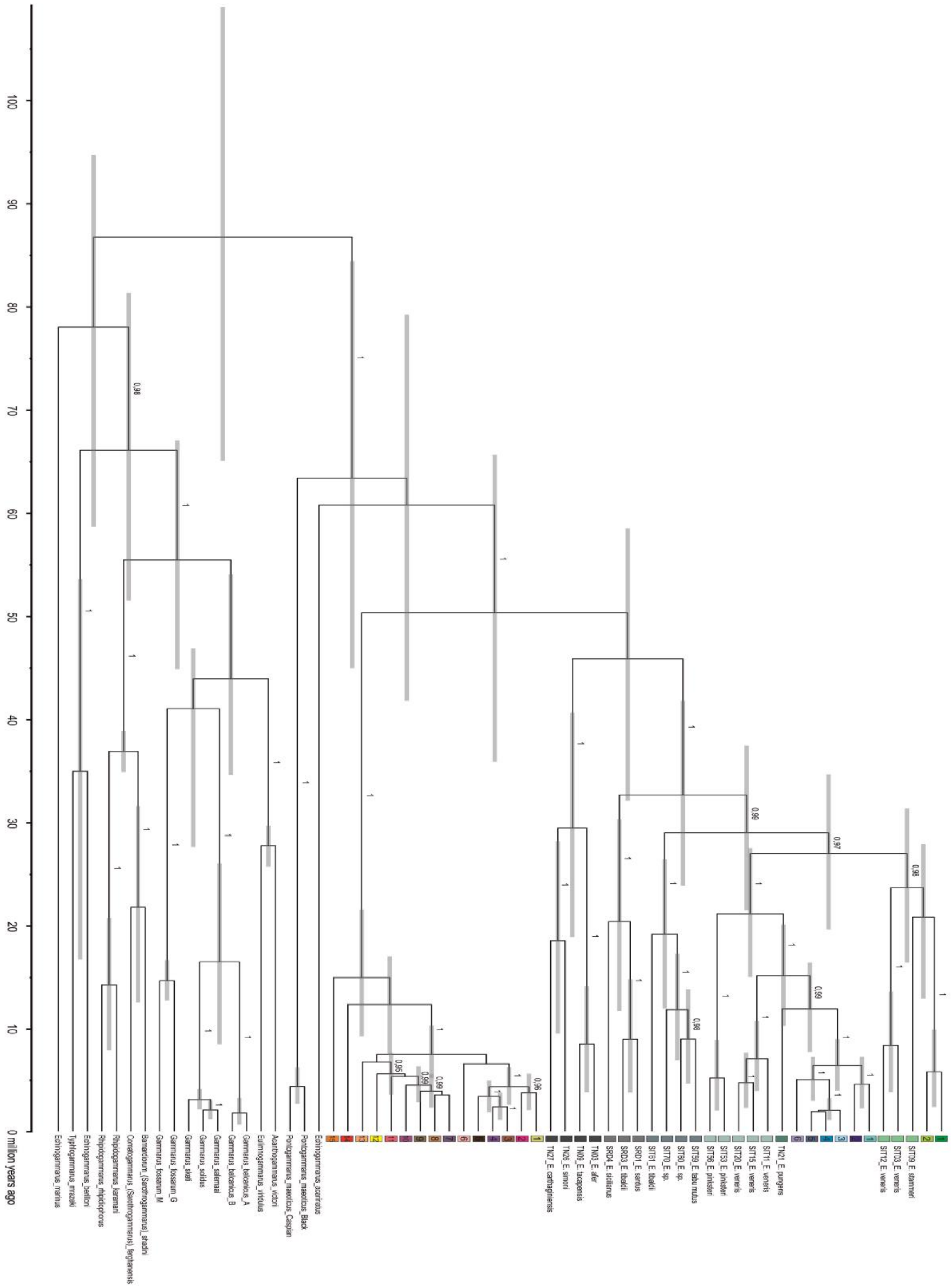


Fig.S5 Maximum clade credibility, time-calibrated Bayesian reconstruction of phylogeny of Sicilian Echinogammarus species including the outgroups used for molecular clock calibration and validation. Phylogeny was inferred from sequences of the mitochondrial COI and 16S rRNA genes and nuclear 28S rRNA gene. The numbers by respective nodes indicate Bayesian posterior probability values ≥ 0.9. The coloured bars represent ABGD delimitation method, with colours correspond to those presented in other figures. Violet node bars represent 95% HPD.

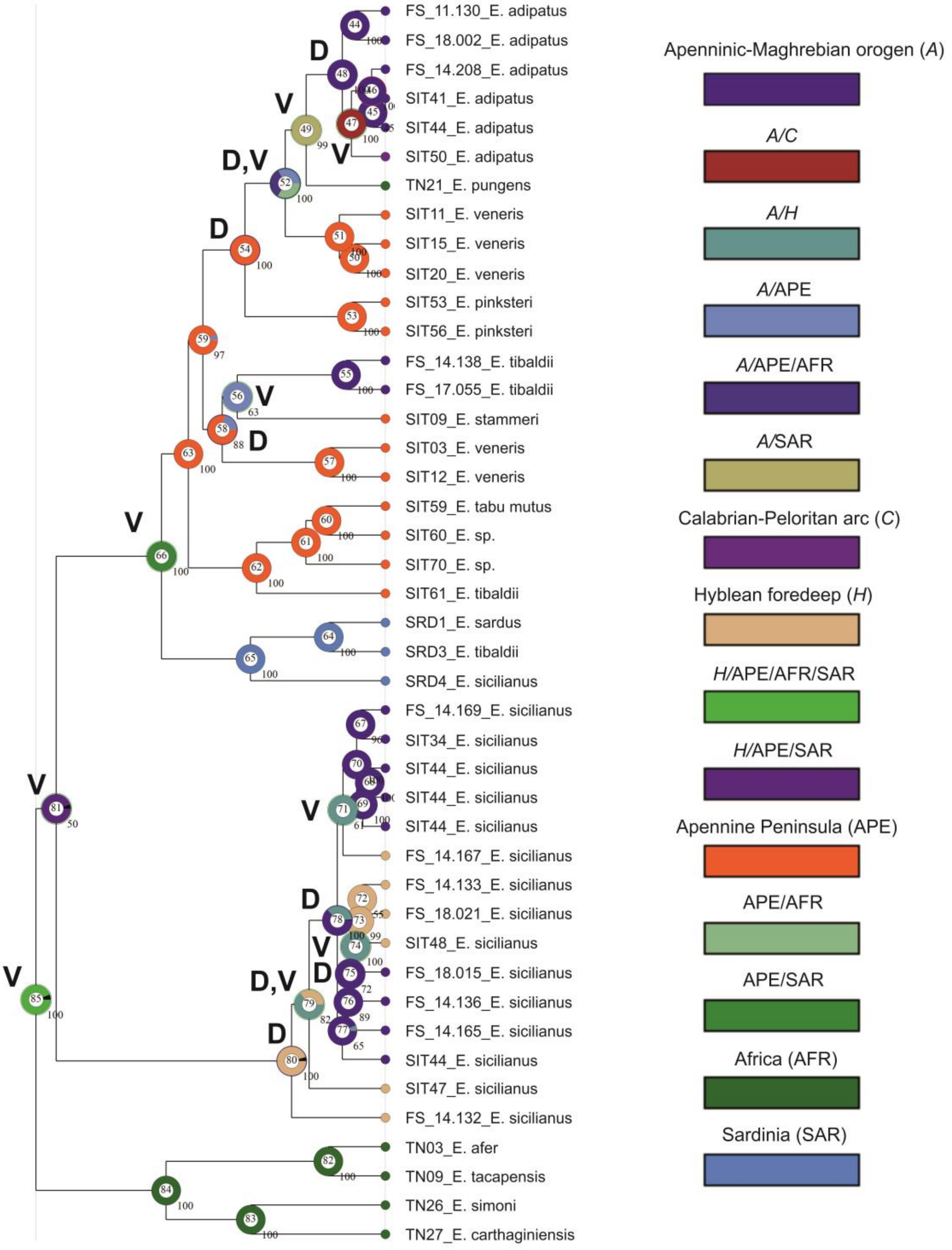


Fig.S6 Bayesian consensus tree with the ancestral node characteristics. The colours of the circles are independent of other figures and refer to the regions. The colours legend: Apenninic-Maghrebian orogen (A), Calabrian-Peloritan Arc (C), Hyblean foredeep (H), Apennine Peninsula (APE), Africa (AFR), Sardinia (SAR).

Islands (*species' name=hypogean; E=endemic; I=alien,introduced)	BAL	COR	SAR	TUS	SIC	PEL	MAL	ADR	ION	CRE	AEG	MAR	CYP
Order: Amphipoda Latreille, 1816													
Family: Bogidiellidae Hertzog, 1936													
*_Bogidiella balearica_ Dancau, 1973	E												
*_Bogidiella calicali_ G. Karaman, 1988			E										
*_Bogidiella cyrnensis_ Hovenkamp, Hovenkamp & Van der Heide, 1983		E											
*_Bogidiella ichnusae_ Ruffo & Vigna Taglianti, 1975			E										
*_Bogidiella longiflagellum_ S. Karaman, 1959									X	X			
*_Bogidiella silverii_ Pesce, 1981			E										
*_Bogidiella torrenticola_ Pretus & Stock, 1990	X	X											
*_Bogidiella vandeli_ Coineau, 1968			E										
*_Medigidiella aquatica_ (G. Karaman, 1990)										X			
*_Medigidiella chappuisi_ (Ruffo, 1952)			X	X							X		
*_Medigidiella minotaurus_ (Ruffo & Schiecke, 1976)										E			
*_Medigidiell paolii_ Hovenkamp, Hovenkamp & Van der Heide, 1983		E											
*_Medigidiella pescei_ (Karaman, 1989)			E										
*_Racovella birramea_ Jaume, Gràcia & Boxshall, 2007	E												
*_Stygogidiella cypria_ (G. Karaman, 1989)													E
Family: Crangonyctidae Bousfield, 1973													
Synurella ambulans (F. Müller, 1846)								X					
Family: Eriopisidae Lowry & Myers, 2013													
*_Psammogammarus burri_ Jaume & Garcia, 1992	E												
Family: Gammaridae Leach, 1813													
Echinogammarus adipatus Karaman & Tibaldi, 1972					E								
Echinogammarus ebusitanus Margalef, 1951	E												
Echinogammarus eisentrauti (Schellenberg, 1937)	X												
Echinogammarus kerkuraios Pinkster 1993									E				
Echinogammarus klaptoczi Schäferna, 1908							X						
Echinogammarus kretensis Pinkster 1993										X			
Echinogammarus monomerus Stock, 1978	X												
Echinogammarus platvoeti Pinkster 1993										E			
Echinogammarus pungens s.l. (H. Milne Edwards, 1840)	X						X	X	X				
Echinogammarus pungens minoricensis (Margalef, 1952)	E												
Echinogammarus aff. _tibaldii_ Pinkster & Stock, 1970					E								

Echinogammarus sardus Pinkster, 1993			**E**										
Echinogammarus sp. (= *fluminensis* Pinkster & Stock, 1970)		**X**											
Echinogammarus tibaldii bolo Karaman & Tibaldi, 1972			**E**										
Echinogammarus aff. *sicilianus* Karaman & Tibaldi, 1972			**X**				**X**						
Echinogammarus sicilianus Karaman & Tibaldi, 1972					**E**								
Echinogammarus veneris s.l. (Heller, 1865)								**X**	**X**	**X**			**X**
Gammarus balcanicus s.l. Schäferna, 1923								**X**					
Gammarus italicus Goedmakers & Pinkster, 1977			**E**										
Gammarus komareki Schäferna, 1923											**X**		
Gammarus plaitisi Hupało, Mamos, Wrzesińska & Grabowski, 2018										**E**			
Gammarus pulex s.l.(Linnaeus, 1758)												**X**	
Gammarus uludagi G. Karaman, 1975											**X**	**X**	
**Ilvanella inexpectata* Vigna-Taglianti, 1971				**X**									
**Longigammarus planasiae* Messana & Ruffo, 2001				**E**									
Rhipidogammarus rhipidiophorus s.l. (Catta, 1878)	**X**	**X**	**X**	**X**	**X**	**X**	**X**						
Rhipidogammarus karamani Stock, 1971		**X**						**X**					
Rhipidogammarus variicauda Stock, 1978	**E**												
**Tyrrhenogammarus catacumbae* (G. Karaman & Ruffo, 1977)					**E**								
**Tyrrhenogammarus sardous* Karaman & Ruffo, 1989			**E**										
Sarathrogammarus madeirensis (Dahl, 1958)	**E**												
Family: Hadziidae S. Karaman, 1943													
*Hadzia fragilis fragilis*S. Karaman, 1932								**X**					
Metahadzia helladis Pesce, 1980									**E**				
Family: Ingolfiellidae Hansen, 1903													
**Ingolfiella petkovskii* S. Karaman, 1975											**X**		
**Ingolfiella cottarellii*Ruffo & Vigna-taglianti, 1989			**E**										
Family: Ischyroceridae Stebbing, 1899													
Jassa sp. Leach, 1814			**E**										
Jassa trinacriae Krapp, Grasso & Ruffo, 2010					**E**								
Family: Metacrangonyctidae Boutin & Messouli, 1988													
**Metacrangonyx longipes* Chevreux, 1909	**E**												
**Metacrangonyx ilvanus* Stoch, 1997				**E**									
Family: Niphargidae Bousfield, 1977													
**Exniphargus tzanisi* G. Karaman, 2016										**E**			
**Niphargobatoides lefkodemonaki* Sket,										**E**			

1990													
**Niphargus adei* S.Karaman, 1934											E		
**Niphargus* aff. *aquilex* Schiödte, 1855								X					
**Niphargus alphaeus* Delić, Švara, Coleman, Trontelj & Fišer, 2017								E					
**Niphargus arethusa* Delić, Švara, Coleman, Trontelj & Fišer, 2017								E					
**Niphargus corsicanus* Schellenberg, 1950		X	X										
**Niphargus dalmatinus* Schäferna, 1932								X					
**Niphargus denarius* G. Karaman, 2017									E				
**Niphargus doli* Delić, Švara, Coleman, Trontelj & Fišer, 2017								X					
**Niphargus hebereri* s.l. Schellenberg, 1933								X					
**Niphargus hvarensis* S. Karaman, 1952								X					
**Niphargus impexus* G. Karaman, 2016										E			
**Niphargus jadranko* Sket & G. Karaman, 1990								E					
**Niphargus jovanovici* S. Karaman, 1931											X		
**Niphargus lakusici* G. Karaman, 2017										E			
**Niphargus liburnicus* G. Karaman & Sket, 1989								X					
**Niphargus longicaudatus* s.l. (A. Costa, 1851)				X	X			X					
**Niphargus* cf. *lourensis* Fišer, Trontelj & Sket, 2006									X				
**Niphargus lourensis skiroci* G. Karaman, 2018											E		
**Niphargus miljeticus* Straškraba, 1959								E					
**Niphargus pectencoronatae* Sket & G. Karaman, 1990								E					
**Niphargus rhodi* S. Karaman, 1950											E		
**Niphargus skopljensis* S.Karaman, 1929									X				
**Niphargus spasenijae* G.Karaman, 2015											E		
**Niphargus* aff. *speziae* Schellenberg, 1936		X		X									
**Niphargus versluysi* S.Karaman, 1950									X				
**Niphargus wolfi* Schellenberg, 1933								X					
**Niphargus zarosiensis* M. Zettler & A. Zettler, 2017										E			
Family: Pseudoniphargidae Karaman, 1993													
**Pseudoniphargus adriaticus* s.l. S. Karaman, 1955	X	X		X		X							
**Pseudoniphargus daviui* Jaume 1991	E												
**Pseudoniphargus duplus* Messouli, Messana & Yacoubi-Khebiza, 2006					E								
**Pseudoniphargus inconditus* Karaman & Ruffo, 1989					E								
**Pseudoniphargus italicus* Karaman & Ruffo,					E								

1989													
**Pseudoniphargus mercadali* Pretus, 1988	X		X										
**Pseudoniphargus obritus* Messouli, Messana & Yacoubi-Khebiza, 2006		E											
**Pseudoniphargus planasiae* Messouli, Messana & Yacoubi-Khebiza, 2006				E									
**Pseudoniphargus pedrerea* Pretus, 1990	E												
**Pseudoniphargus pityusensis* Pretus, 1990	E												
**Pseudoniphargus racovitzai* Pretus, 1990	E												
**Pseudoniphargus sodalis* Karaman & Ruffo, 1989					E								
**Pseudoniphargus triasi* Jaume 1991	E												
Family: Salentinellidae Bousfield, 1977													
**Salentinella angelieri* s.l. Ruffo & Delamare Deboutteville, 1952	X	X	X	X				X	X		X		
**Salentinella formenterae* Platvoet, 1984	E												
Family: Talitridae Rafinesque, 1815													
Cryptorchestia cavimana (Heller, 1865)													E
Cryptorchestia garbinii Ruffo, Tarocco & Latella, 2014				X									
Cryptorchestia ruffoi Latella & Vonk, 2017											X		
Macarorchestia remyi (Schellenberg, 1950)		X	X										
Order: Isopoda Latreille, 1817													
Family: Asellidae Latreille, 1802													
Asellus aquaticus (Linnaeus, 1758)								X		I	X		
**Proasellus acutianus* Argano & Henry, 1972				X									
Proasellus banyulensis Racovitza, 1919		X	X	X	I								
**Proasellus beroni* Henry & Magniez, 1968		X											
Proasellus coxalis s.l. (Dollfus, 1892)							X	X			X		X
Proasellus coxalis cephallenus (Strouhal, 1942)									E				
Proasellus coxalis corcyraeus (Strouhal, 1942)									E				
Proasellus coxalis gabriellae (Margalef, 1950)	X												
Proasellus coxalis leucadius (Strouhal, 1942)									E				
Proasellus coxalis nanus Sket, 1991													E
Proasellus coxalis versluysi (Strouhal, 1966)									E				
**Proasellus cretensis* Pesce & Argano, 1980										E			
**Proasellus ezzu* Argano & Campanaro, 2004			E										
**Proasellus minoicus* Pesce & Argano, 1980										E			
Proasellus montalentii Stoch, Valentino & Volpi, 1996					E								
**Proasellus patrizii* (Arcangeli, 1952)			E										
**Proasellus ruffoi* Argano & Campanaro, 2004			E										
**Proasellus sketi* Henry, 1975											X		

Proasellus wolfi Dudich, 1925					X								
Family: Stenasellidae Dudich, 1924													
**Stenasellus racovitzai* s.l. Razzauti, 1925		X	X										
**Stenasellus assorgiai* Argano, 1968			E										
**Stenasellus nuragicus* Argano, 1968			E										
Family: Sphaeromatidae Latreille, 1825													
**Monolistra (Microlistra) pretneri* Sket, 1964								X					
Family: Cirolanidae Dana, 1852													
**Metacirolana ponsi* Jaume & Garcia, 1992	E												
**Sphaeromides virei virei*(Brian, 1923)								X					
**Sphaeromides virei mediodalmatina* Sket, 1964								X					
**Typhlocirolana margalefi* Pretus, 1986													
**Typhlocirolana moraguesi* Racovitza, 1905	E												
**Typhlocirolana* sp. aff. *moraguesi Racovitza, 1905*					E								
**Turcolana rhodica* Botosaneanu, Boutin & Henry, 1985											E		
Family: Janiridae G.O. Sars, 1897													
Jaera (Jaera) nordmanni brevicaudata Jaume & Garcia, 1990	E												
Jaera (Jaera) nordmanni balearica Jaume & Garcia, 1990	E												
Jaera (Jaera) cf. *nordmanni* (Rathke, 1837)				X									
Family: Lepidocharontidae Galassi & Bruce, 2016													
**Microcharon agripensis* Galassi, De Laurentiis & Pesce, 1995											X		
**Microcharon comasi* Coineau, 1968	E												
**Microcharon hellenae* Chappuis & Delamare Deboutteville, 1954											E		
**Microcharon latus* Karaman, 1934									X				
**Microcharon luciae* Sket, 1991													E
**Microcharon marinus* Chappuis & Delamare-Deboutteville, 1954	X		X	X									
**Microcharon nuragicus* Pesce & Galassi, 1988			E										
**Microcharon prespensis* Karaman, 1954											X		
**Microcharon silverii* Pesce & Galassi, 1988			E										
**Microcharon sisyphus* Chappuis & Delamare-Deboutteville, 1954		E											
**Microcharon ullae* Pesce, 1981											X		
**Microcharon* sp. Karaman, 1934								X					
Family: Microparasellidae Karaman, 1933													
**Microparasellus hellenicus* Argano & Pesce, 1979									X				
**Microparasellus puteanus* Karaman, 1933									X				

Order: Thermosbaenacea Monod, 1927													
Family: Monodellidae Taramelli, 1954													
**Tethysbaena halophila* (S.L. Karaman, 1953)								**X**					
**Tethysbaena scabra* (Pretus, 1991)	**E**												
**Tethysbaena siracusae* Wagner, 1994					**E**								
Order: Bathynellacea Chappuis, 1915													
Family: Bathynellidae Grobben, 1905													
**Hispanobathynella* aff. *catalanensis* (Serban, Coineau & Delamare, 1971)			**E**										
**Sardobathynella cottarellii* Serban, 1973			**E**										
Family: Parabathynellidae Noodt, 1965													
**Hexabathynella knoepffleri* (Coineau, 1964)		**X**	**X**										
**Paraiberobathynella fagei* (Delamare Deboutteville & Angelier, 1950)	**X**												
Order: Decapoda Latreille, 1802													
Family: Astacidae Latreille, 1802													
Astacus (Pontastacus) leptodactylus (Eschscholtz, 1823)							**X**						
Austropotamobius fulcisianus (Ninni, 1886)			**I**										
Austropotamobius italicus carsicus (Karaman, 1962)								**X**					
Austropotamobius pallipes (Lereboullet, 1858)		**I**											
Pacifastacus leniusculus (Dana, 1852)							**I**						**I**
Family: Atyidae De Haan, 1849													
Atyaephyra desmarestii (Millet, 1831)		**X**	**X**		**X**								
Atyaephyra thyamisensis Christodoulou, Antoniou, Magoulas & Koukouras, 2012									**X**				
Atyopsis moluccensis (De Haan, 1849)							**I**						
**Troglocaris anophthalmus periadriaticus* Jugovic, Jalžić, Prevorčnik & Sket, 2012								**E**					
Family: Cambaridae Hobbs, 1942													
Procambarus clarkii (Girard, 1852)	**I**	**I**	**I**		**I**		**I**						**I**
Procambarus virginalis Lyko, 2017							**I**						
Family: Palaemonidae Rafinesque, 1815													
Palaemon antennarius Milne Edwards, 1837			**X**		**X**				**X**	**X**	**X**		
Palaemon colossus Tzomos & Koukouras, 2015											**X**		
Palaemon minos Tzomos & Koukouras, 2015										**E**			
Family: Parastacidae Huxley, 1879													
Cherax destructor Clark, 1936					**I**								
Cherax quadricarinatus von Martens, 1868							**I**						
Family: Potamidae Ortmann, 1896													
Potamon fluviatile (Herbst, 1785)					**X**		**X**		**X**		**X**		
Potamon hippocratis Ghigi, 1929										**X**	**X**		**X**
Potamon ibericum (Bieberstein, 1808)											**X**		
Potamon karpathos Giavarini, 1934											**X**		

Potamon kretaion Giavarini, 1934										**E**			
Potamon potamios (Olivier, 1804)											**X**		
Potamon rhodium Parisi, 1913											**X**		
TOTAL	**32**	**20**	**35**	**15**	**21**	**2**	**12**	**29**	**20**	**19**	**27**	**2**	**9**

Table S1. Complete species list of freshwater Malacostraca from Mediterranean islands. SAR – Sardinia and circum-Sardinian Islands; COR – Corsica; TUS – Tuscan Archipelago; SIC – Sicily; PEL – Pelagie Islands; MAL – Maltese Islands; ADR – Adriatic Islands; ION – Ionian Islands; AEG – Aegean Islands; CRE – Crete; MAR – Marmara Islands; CYP – Cyprus.

Molecular marker	Primer sequences	PCR conditions	References
COI	LCO1490: 5'-ggtcaacaaatcataaagatattgg-3' HC02198: 5'-taaacttcagggtgaccaaaaaatca-3'	60 s at 94ºC, 5x (30 s at 94 ºC, 90 s at 45ºC, 60 s at 72ºC), 35x (30 s at 94ºC, 90 s at 51ºC, 60 s at 72ºC), 5 min at 72ºC	Primer pairs: Folmer et al. 1994 PCR conditions: Hou et al. 2007
	LCO1490-JJ: 5'-chacwaaycataaagatatygg-3' HCO2198-JJ: 5'-awacttcvggrtgvccaaaraatca-3'		Primer pairs: Astrin and Stüben, 2008 PCR conditions: Hou et al. 2007
16S rRNA	16STf: 5'-ggtawhytracygtgctaag-3' 16SBr: 5'-ccggtttgaactcagatcatgt-3'	150 s at 94°C, 36x (40 s at 94°C, 40 s at 54°C, 80 s at 65°C), 8 min at 65°C	Primer pairs: Palumbi et al. 1991, MacDonald et al. 2005 PCR conditions: Weiss et al. 2014
28S rRNA	Niph15: 5'-caagtaccgtgagggaaagtt-3' Niph16: 5'-agggaaacttcggagggaacc-3'	3min at 94°C, 50x (30 s at 94°C, 60 s at 45°C, 1min at 72°C), 5 min at 72°C	Primer pairs: Verovnik et al. 2005 PCR conditions: Verovnik et al. 2005
EF-alpha	EF1aF: 5'-cactactggtcatctcatctac – 3' EF1aR: 5' – acttccaggagagtctcaaac – 3'	1min at 94°C, 35x (30 s at 94°C, 45 s at 50°C, 1min at 72°C), 5 min at 72°C	Primer pairs: Hou et al. 2011 PCR conditions: Hou et al. 2007

Tab.S2 Used molecular markers with the primer sequences, PCR cycling conditions and the original references.

Streszczenie

Wyspy są uznawane za 'naturalne laboratoria ewolucji' i miejsca o najwyższym poziomie endemizmu, a sam obszar śródziemnomorski uważany jest za jedno z 25 najcenniejszych przyrodniczo centrów bioróżnorodności w skali światowej. Pomimo tego, że różnorodność w wodach słodkich uznawana jest za jedną z najwyższych na świecie, a same organizmy słodkowodne za najbardziej zagrożone wyginięciem, wciąż niewiele wiadomo na temat różnorodności i pochodzenia słodkowodnej fauny wysp. Dotychczasowe badania nad różnorodnością i pochodzeniem fauny wysp Morza Śródziemnego skupiały się zwykle na organizmach lądowych, wodno-lądowych lub morskich. Jednymi z najbardziej dominujących organizmów słodkowodnych są skorupiaki obunogie, będące jednymi z kluczowych organizmów w zgrupowaniach makrobezkręgowców słodkowodnych. W trakcie moich studiów doktoranckich badałem różnorodność i pochodzenie słodkowodnych obunogów z wód słodkich wysp Morza Śródziemnego. W pierwszym rozdziale mojej rozprawy zebrałem oraz przeanalizowałem dostępne materiały źródłowe dotyczące różnorodności słodkowodnych pancerzowców wysp Morza Śródziemnego. Jest to pierwsza tak obszerna praca przeglądową na temat różnorodności wyspowej fauny słodkowodnej oraz jej powiązań biogeograficznych. Wyniki pracy potwierdzają, że skorupiaki obunogie są najbardziej różnorodną grupą pancerzowców, posiadającą największą liczbę gatunków na każdej z badanych wysp i archipelagów, cechującą się również wysokim poziomem lokalnego endemizmu. W pozostałych dwóch rozdziałach mojej pracy doktorskiej, przedstawiłem wyniki swoich badań nad różnorodnością molekularną i pochodzeniem słodkowodnych obunogów z kontynentalnych wysp Morza Śródziemnego o odmiennej historii geologicznej - wysp Morza Egejskiego wraz z Kretą oraz Sycylii. W rozdziale II, wykazałem obecność słodkowodnych populacji obunogów z rodzaju *Gammarus* na sześciu wyspach Egejskich, a wśród nich co najmniej trzy endemiczne gatunki, prawdopodobnie nowe dla nauki. Na podstawie danych molekularnych, zrekonstruowałem historię ewolucyjną tych gatunków. Dywergencja słodkowodnych gatunków z wysp Morza Egejskiego wydaje się być silnie powiązana z historią geologiczną tego akwenu. Ich powiązania biogeograficzne potwierdzają, że są to gatunki pochodzenia kontynentalnego. W rozdziale III przedstawiłem niespodziewanie wysoki poziom zmienności wewnątrzgatunkowej u każdego z trzech badanych sycylijskich morfogatunków obunogów. Moje wyniki, oprócz wysokiej różnorodności kryptycznej, wskazują również na odrębne kolonizacje Sycylii w odmiennym czasie przez każdy z badanych gatunków słodkowodnych obunogów. Wyniki mojej pracy

doktorskiej pozwalają powiązać historię geologiczną badanych wysp z pochodzeniem oraz ramami czasowymi ewolucji tamtejszych słodkowodnych obunogów. Wyniki moich badań poszerzają wiedzę na temat mechanizmów dywersyfikacji zwierząt słodkowodnych i wskazują na konieczność dalszych badań na słodkowodnych organizmach wyspowych Morza Śródziemnego.