University of Thessaly

hcmr ΕΛΚΕΘΕ

UNIVESRSITY OF THESSALY
BACHELOR PROGRAMME OF
BIOCHEMISTRY AND BIOTECHNOLOGY

BACHELOR THESIS

A PRELIMINARY CHECK-LIST OF TALITRID AMPHIPODS AND ONISCID ISOPODS FROM CRETAN BEACHES

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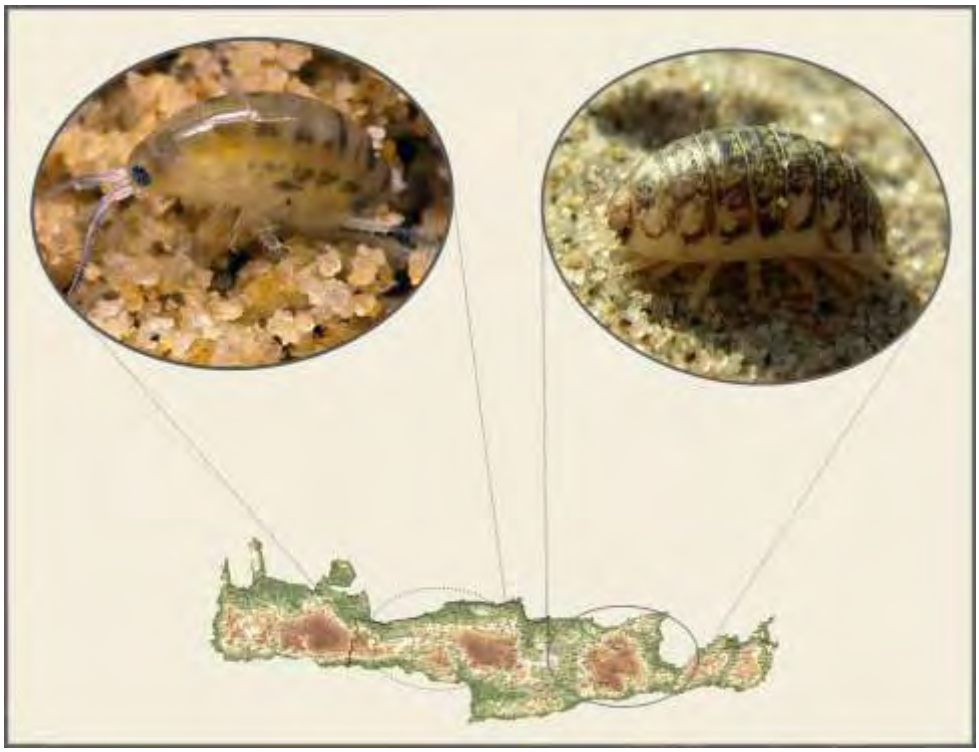

Crete 2015-2016

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Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor Prof. Katerina Moutou for enlightening me the first glance of research. Her teaching style and enthusiasm during my studies made a strong impression on me and she provided me with the inspiration to choose the field of marine biology for my thesis. I would also like to thank her for the continuous support of my bachelor study, for her patience, motivation, and immense knowledge. Her guidance helped me during the whole time and I could not have imagined having a better supervisor and mentor for my bachelor studies.

My sincere thanks also goes to Dr. Christos Arvanitidis, who provided me an opportunity to join his team, and who gave access to the laboratory and research facilities. Without his precious support, it would not be possible to conduct this research. I wish to express my grateful thanks to the Hellenic Center of Marine Research in Crete, for providing me with all the necessary facilities for the research.

Besides, I would like to thank Dr. Lucia Fanini and Dr. Jim Lowry for their insightful comments and continuous encouragement, but also for the hard question which incented me to widen my research from various perspectives. I am extremely thankful and indebted to them for sharing expertise, and sincere and valuable guidance to me.

Last but not the least, I place on record, my sense of gratitude to my partner for the endless patience and one and all, who directly or indirectly, have lent their hand in this venture.

INDEX

MISSION

The aim of this study is to compile a preliminary check-list of the most common peracarids (talitrid amphipods and oniscid isopods) inhabiting sandy coasts of Crete, identify them by morphology and barcoding, map their location (geographical distance) and provide an accurate description of the substrate they were found associated with. Finally, these data will contribute to the knowledge of distribution and composition of these taxa in the sandy shores of the Mediterranean basin.

***Keywords**: Crustacea, Amphipoda, Talitridae, Isopoda, Oniscidea, Sandy Beaches, Mediterranean Sea, Crete, taxonomy, DNA barcoding*

INTRODUCTION

This project is targeting to the establishment of baseline data, which are of great significance, especially nowadays, under conditions of rapid global change. It also represents a direct response to the call at international level for data from neglected ecosystems, including island beaches [Schoeman et al., 2014; Schlacher et al., 2016].

The study integrates environmental profiling, targeted sampling of main supralittoral peracarid taxa (amphipods and isopods), and barcoding. The focus is on the sandy beaches from different coasts of Crete, which are subject to disturbances mainly represented by natural and artificial bioturbation, mostly related by recreational seashore activities. During the summer, beach-goers commonly watch large numbers of amphipods and isopods feeding on seaweed washed ashore by wave action. Sampling took place during winter, when the beaches are less frequented and it is much easier to set the traps. Also, winter is indicated as the appropriated timing for sampling in order to obtain baselines [Schlacher et al., 2008].

There is a growing interest in assessing factors and processes that occur in sandy beaches, in order to target their functionality as systems. The Cretan beaches are not an exception, thus, an assessment of peracarids represents a first step in this direction. This taxon plays a key role within trophic networks. Living throughout coastal zones, they have a significant impact on the transfer of carbon into the food chain, they play a major role in the decomposition of organic matter, they are detritivores, herbivores or predators of small animals, eggs and

larvae. Both amphipods and isopods eat dead and decaying algae, seaweed and other plants and animals. In the community succession, they are the first macro-organisms to reach freshly stranded material [Colombini et al., 2000]. Bacteria and smaller organisms that consume the waste of amphipods and isopods continue the decomposition process, then after. Peracarids represent at the same time an important food source for a variety of animals at higher trophic levels, like fish, crabs and shorebirds. Therefore, their distribution along a coastline could be used as practical tool for sustainable management of coastal areas [Cuttriss et al., 2015].

Environmental profiling of sandy shores

The ecology of sandy shores is a recent discipline, dating back to the '80s [McLachlan, 1983; McLachlan & Erasmus, 1983]. Most of the data on which the discipline is built come from Oceanic shores [McLachlan & Brown, 2006]. As a consequence, examples are being drawn by oceanic habitats, confining those of enclosed seas such as the Mediterranean as outliers, where not all of the oceanic variables take the same values (e.g. the current classification into beach morphotypes based on wave height and wave period). Also, on the Mediterranean shores, phenomena occur at different spatial scales than on the oceanic ones, and a standard spatial unit such as one kilometer could have dramatically different features, depending on the context of application. The island of Crete − selected here as case-study − presents all the Mediterranean characteristics, paired to another peculiarity: the coastal habitat is characterized by different substrates, including fine sand, coarse sand, mixed sand, cobbles, which occur all together on a small spatial scale along the coast. This is a topic so far ignored in sandy beach ecology, since it is mainly dealing with fine and fine-to-medium substrates [McLachlan & Brown, 2006].

Beach systems consist of wave-deposited accumulations of sediment at the shore, a process which is complicated by the force of the tides, sediment size composition variability and an ever-varying wave regime, each of which will have an impact on the beach type and behaviour but the overall result will be a balance between their cumulative effects and interactions. Beaches can be classified into three broad types: wave-dominated, tide-dominated, and mixed or tide-modified between these types [Masselink & Short, 1993]. These three broad beach types can then be subdivided into a range of beach states with the addition of the role of wave period and sediment size [Gourlay, 1968]. Wave-dominated beaches occur in areas of micro-tides where waves dominate the morphology with essentially stationary shoaling, surf and swash zones. These three factors (sand, waves, tides) influence human use of the beaches both individually and directly but also collectively through their determination of beach morphodynamic type [Short 1996; McLachlan & Brown, 2006].

On a microtidal coast, such as the Mediterranean, tidal currents are relatively weak unless the slope of the seabed is very shallow. The inlet formed by the Strait of Gibraltar, acts as a dynamic filter, which reduces tidal fluctuations in the Mediterranean regimes. Here, the main driving force is likely to be the exposure to waves (wave-dominated beaches) and local winds, so that shores from North and South Crete are extremely different in this respect:

dominant N-W winds are likely to affecting the South shores to a smaller extent, and vice-versa. Previous studies also showed that the effects of onshore winds appear to prevail over daily-tide cycles [Sofianos, 2002]. The island of Crete is an excellent example of a major isolated topographic feature, which significantly modifies the regional airflow as well as the pressure and temperature fields. During the summer months, strong winds from northern directions, named Etesians, blow over the Aegean Sea. They are mainly northeasterly in northern Aegean, northerly in central and southern Aegean, while they become northwesterly in southeastern Aegean [Theocharis et al., 1999]. On the other hand, Southern winds feed the coastal dunes and beaches with Sahara sands, which are mainly coarse medians.

As a resultant of the energy shaping the beach surface, the combined information provided by beach width and beach face slope is a useful parameter to be considered. Theoretically, beaches with reduced width and steep slope are reflecting the incoming wave energy, while extended beaches with gentle slope are dissipating the incoming wave energy along the supralittoral. Consequently, "reflective" beaches are harsher environments while "dissipative" beaches provide a more benign habitat for inhabiting fauna [McLachlan & Brown, 2006]. Even though these parameters are calibrated on oceanic shores and cannot be applied to the Mediterranean due to the lack of comparable wave period, at each sampling site, we recorded beach width and beach slope to get an estimate of the results of exposure.

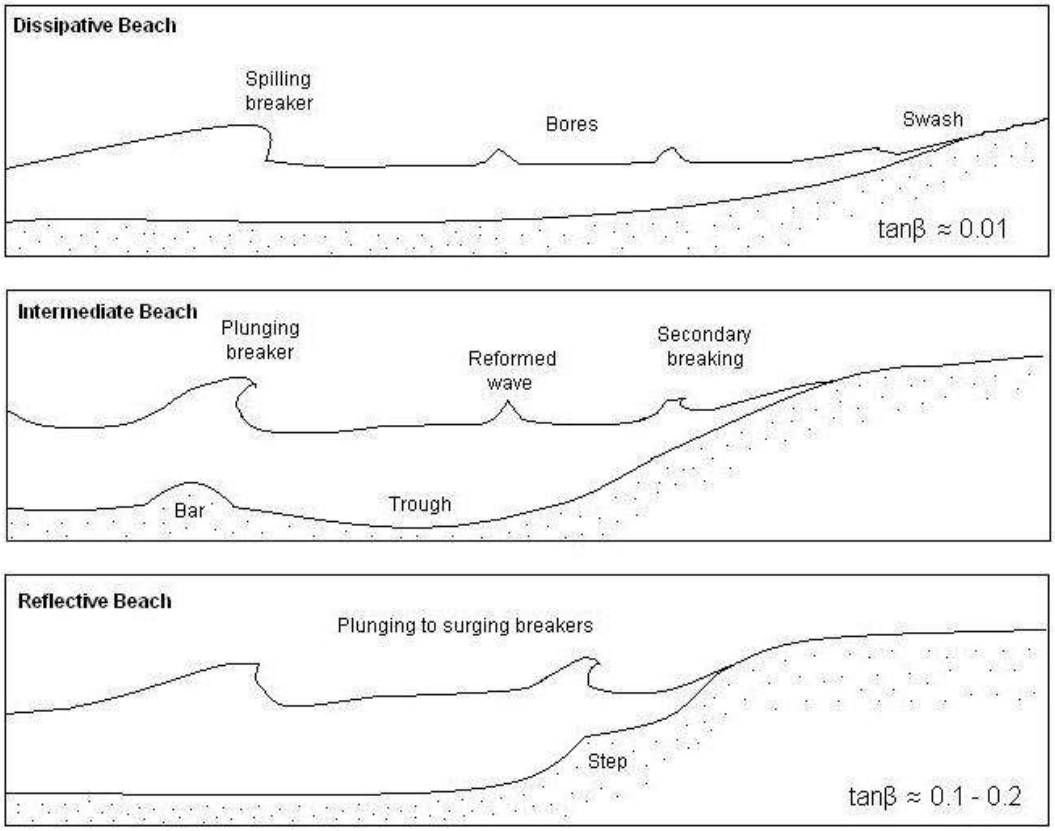


Figure 1: Beach classification by Wright and Short (1983) showing dissipative, intermediate and reflective beaches.

Sandy beaches are at the interface of land and sea, representing highly dynamic ecosystems which provide habitats for a diversity of fauna. Storms and large waves can deposit seaweeds on beaches, that provide nourishment for various inhabitant species in the supralittoral zone. Supralittoral species may have entire colonies torn away by strong waves, making way for other species to succeed in that area [Defeo & Gomez, 2005].

Peracarida display behavioral adaptations to cope with the harsh environment: mobility, burrowing capability, rhythmic activity and lack of larval dispersal since they hold the eggs into a brood pouch, called marsupium, until the juveniles are ready to hatch. These features are displayed and related to the meso-scale, i.e. at single beach level [McLachlan & Brown, 2006]. Due to the high vulnerability of beach habitats, action plans for their conservation should be a priority to the environmental managers; choosing a reliable group of species as a simple indicator taxon(a) to recommend preservation of biological diversity can be considered a fundamental task. Resident populations of peracarids were already proposed as indicators of changes, including the effects of global change [Defeo et al., 2005; Hubbard et al., 2014]. Oniscid isopods and talitrid amphipods can be found worldwide, dominant in abundance on the supralittoral zone, also on Mediterranean shores and on its different substrates.

Oniscidae (Isopoda)

Today, the order Isopoda contains 10,169 species, listed in WoRMS database (http://www.marinespecies.org/isopoda/) including marine, freshwater and terrestrial ones, with 3,527 species of the suborder Oniscidea (woodlice) belonging to them (http://www.marinespecies.org/isopoda/aphia.php?p=stats). The suborder Oniscidea is one of the most important taxon within the Isopoda and a few species are extensively studied and well-known, for instance *Armadillidium vulgare*, *Porcellio scaber*, *Porcellionides pruinosus*, and *Ligia oceanica* [Schmalfuss, 2003; Schmalfuss & Wolf-Schwenninger, 2002].

Description and Life Cycle [Hopkin, 1991]: In contrast to amphipods, isopods are flattened dorso-ventrally, are larger in size, and are bottom dwellers. As with all Arthropoda, the woodlouse is a segmented animal with a rigid exoskeleton and jointed limbs. The first segment is the head, the second is the pereon (thorax), the third is the pleon (abdomen). The head shape, eyes and antennae vary from one species to another and they are exemplar characters for their identification. The eyes can vary from compound ones to groups of up to three ocelli. There are two pairs of antennae, which are sensory organs; the first pair are vestigial and difficult to see. The mouthparts lie on the underside of the head; these are actually modified limbs of the head segments. The perion has 7 segments, each consisting of tergite (dorsal plate) and a sternite (ventral plate). There are 7 pairs of pereopods (legs), although when the young woodlouse emerges it has only 6 pairs, the 7th pair appears after the first moult. In females, there is also a brood pouch on the underside of the pereon. The pleon is always much shorter than the pereon and ends with the telson and the uropods. Their pleon has generally 5 free pleonites plus the pleotelson. The uropods are positioned on

either side of the telson at the posterior end of the woodlouse and can be held together to form a capillary channel that excretes excess water (e.g raindrop) into the ground. On the underside of the pleon there are the genitalia and, if present, the pleopodal lungs. In some species, there are no lungs, some have two pairs of lungs and some have five pairs. These lungs appear as white patches on the underside and can be seen with the naked eye. The number of lungs helps to determine the amount of time that the woodlouse can spend away from its damp shelter. Woodlice have four main life stages: egg, manca (which has two sub-stages) juvenile (which has several sub-stages) and adult. The eggs hatch into mancae. They undergo two molts -- they shed their skins -- in the manca stage, one into more independent mancae and the next into juvenile woodlice. As with many other arthropods, the juvenile woodlice continue molting periodically until they reach their full size a year or more later. In the adult stage, they can breed.

Terrestrialization: The suborder Oniscidea (woodlice), is the only suborder of Crustacea almost solely composed of strictly terrestrial species. From marine ancestors, woodlice are a key taxon to study the conquest of the land among arthropods because of their interesting gradation of adaptations (morphological/physiological: cuticle permeability, structure of pleopodal lungs or water conducting system; and behavioral: aggregation) allowing to apprehend mechanisms of terrestrialization [Vandel, 1943; Edney, 1968; Hornung, 2011]. Furthermore, woodlice are completely independent from the aquatic environment from which they originally arose. Indeed, no developmental stage (egg, juvenile, etc.) requires free water and all biological activities are able to be conducted on land. They present direct development, with the eggs developing inside the marsupium, where they hatch as mancas, and where they remain for a short period [Hoese & Jansenn 1989]. Woodlice frequently represent a large part of mesofauna and primary decomposers in soil [Shachak et al., 1976; Davis, 1984; Gongalsky et al., 2005]. Although their physiological adaptations to land life seem incomplete—notably the absence of waxy cuticle to prevent desiccation [Hadley & Quinlan, 1984]—we can consider them good land colonizers. Furthermore, although the morphological and physiological adaptations to terrestrial life have been extensively studied in living woodlice [Warburg, 1993; Hornung, 2011], the evolution of this monophyletic group [Schmalfuss, 1989f; Schmidt, 2008; Wilson, 2009] is still poorly known and its origin remains unclear. The Oniscidea are a key taxon for the study of terrestrialization processes in an evolutionary context.

Habitat: Oniscid isopods resemble land dwelling insects and are commonly found crawling and swimming among weeds, eelgrass, tide pools, dock pilings, and rocks. As mentioned above, they are essentially terrestrial, although certain species are restricted to moist conditions near the high tide line. In contrast to other terrestrial crustaceans, the terrestrial isopods are independent from open water, due to the fact that their early ontogeny takes place in a brood pouch (marsupium) on the ventral side of the female [Schmidt, 2008]. The majority of today's species might not be aquatic, but their skins are not fully waterproof, meaning they need damp habitats. In dry conditions, they dehydrate. Oniscideans have a very high species diversity and have exploited or colonized various terrestrial environments from supralittoral levels (e.g. *Ligia*; Vandel 1960) to high mountains (e.g. *Protracheoniscus nivalis*; Hegna and Lazo-Wasem 2010) and deserts (e.g. *Hemilepistus*; Linsenmair 1974). They occur also in other extreme habitats, such as salt marshes and arid grasslands. Here and in

other habitats they can reach extremely high local densities elevating them to the rank of the primary detritivore grazers and keystone group in regulating fungal communities [Crowther et al. 2013].

Distribution: The members of Oniscidea play an important role in terrestrial ecosystems. They became anthropophilous and they are currently cosmopolitan [Brereton, 1957]. At the same time, woodlice are naturally great colonizers of new localities, but many species may have been transported by human migration [Jass and Klausmeier, 2000]. A first attempt to review the distributional patterns of Oniscidea had been made by Vandel (1945), but the knowledge on the distribution and diversity of terrestrial isopoda has increased considerably in the last decades. Unfortunately, there are no recent monographs on the suborder. However, there are check-lists on Oniscidae from Oceania [Jackson, 1941] and south of the Sahara [Ferrara & Taiti, 1978]. Hence, it is desirable to give a summary of the littoral species in Crete in form of a check-list to fill in this gap.

Feeding: Despite a name reminiscent of woodworm and other nuisances, woodlice are not actually pests; they almost never consume living plants and are physically incapable of consuming solid wood. Like the preferred habitat, the woodlouse diet is also pretty damp, consisting nearly entirely of decaying plant material, often rotten wood. Woodlice are herbivorous animals and therefore they only eat organic plant matter. Terrestrial isopod species are not redundant members of soil community, but they have species-specific effects on decomposition of leaf litter, occupy different trophic levels in soil food web and feed on different food sources [Zimmer & Topp, 2002]. Isopods in general, play an important role in decomposition processes by the fragmentation of litter material and stimulating and/or ingesting fungi and bacteria that are very important in the cycling of nutrients [Loureiro et al., 2006]. The contribution of isopods to decomposition depends on leaf litter degradation and may be influenced by food preference [Van Wensem et al., 1993]. The feeding preferences of isopods may be related to leaf senescence, the nutrient content of food, microbial colonization and the presence of unpalatable or indigestible compounds [Ihnen & Zimmer, 2008]. Due to the small size of the woodlouse and despite the fact that the woodlouse can attempt to protect itself by curling up into a ball, the woodlouse is preyed upon by a number of animals around the world. Toads, centipedes, spiders, millipedes and the occasional wasp are the main predators of the woodlouse.

Ecologic Benefit: Since terrestrial isopods are macro-decomposers, they can significantly contribute to detritus processing (comminution, inoculation) and nutrient release. The nutritional morphology, physiology and ecology of terrestrial isopods (Isopoda: Oniscidea) is significant in two respects: (1) Most oniscid isopods are truly terrestrial in terms of being totally independent of the aquatic environment. Thus, they have evolved adaptations to terrestrial food sources. (2) In many terrestrial ecosystems, isopods play an important role in decomposition processes through mechanical and chemical breakdown of plant litter and by enhancing microbial activity. For the same reason, terrestrial isopods prefer feeding on decaying rather than fresh leaf litter. Due to their physiological adaptations to feeding on and digesting leaf litter, terrestrial isopods contribute strongly to nutrient recycling during decomposition processes. Yet, many of these adaptations are still not well understood.

A first comprehensive overview of the terrestrial isopods of Crete was published more than 30 years ago [Schmalfuss, 1972]. This publication contains records of 38 species. During the past decades, a number of new species descriptions and new records for the inland were published. Altogether, the number of terrestrial isopod species known from Crete by now has increased to 55, including 7 new species and 7 new records for Crete. In the present project, we summarise the current knowledge of the Cretan terrestrial isopod fauna, describe the new species, try to clarify systematic problems and discuss some ecological and biogeographical aspects.

There are 7 species found to be restricted to the littoral zone of Crete [Schmalfuss, 2004]. Six of them, namely *Ligia italica*, *Armadilloniscus ellipticus*, *Halophiloscia couchii*, *H. hirsuta*, *Stenophiloscia vandeli* and *Porcellio lamellatus* inhabit rocky coasts, while *Tylos ponticus* is found mainly in sandy beaches. In addition to these, several other species can also be found at the littoral zone, near the upper part of sandy beaches, without being restricted to it. These include *Agabiformius lentus*, *A. obtusus*, *Leptotrichus naupliensis*, *Proporcellio quadriseriatus*, and *Schizidium hybridum*, as well as most of the myrmecophilous species (*Platyarthrus spp* and *Porcellionides myrmecophilus*). *Armadillidium granulatum* is also present at the littoral zone, especially on hard calcareous substrate.

Talitridae (Ampipoda)

There are currently 9,628 accepted names of amphipod species, listed in WoRMS database (http://www.marinespecies.org/amphipoda/) including marine, freshwater and terrestrial ones, but there is no doubt that numerous species await formal description [Coleman, 2015]. The name "amphipod" means double, or two kinds of legs. Of their eight pairs of legs, the first five are used for walking and the last three pairs, in the tail region, are modified for swimming. Some amphipods also have modified tail appendages used for jumping, several pairs of antennae, and an appendage used for grasping. Amphipods generally swim on their sides, their bodies are flattened side-ways, and they have highly arched backs.

Description and Life Cycle [Lowry & Myers, 2013]: Talitrids typically have elongated body with a distinct head, a pereon (thorax) of 7 segments, and a six-segmented pleon (abdomen) and they are more or less compressed laterally. Eyes well developed or absent, if present then round or ovoid. Antennae 1–2 calceoli absent. Antenna 1 shorter than peduncle of antenna 2; peduncular article 1 shorter than, subequal to or longer than article 2; article 2 shorter than, subequal to or longer than article 3; article 3 subequal to or longer than article 1; peduncular articles 1–2 not geniculate; accessory flagellum absent. Antenna 2 peduncular article 1 not enlarged. Mandible molar triturative; palp absent. Maxilla 1 basal endite apically setose; palp present or absent, symmetrical. Maxilla 2 basal endite without oblique setal row. Coxal gills [not known]; not stalked; sternal gills absent; sternal blisters absent; oostegites fringing setae simple or curl-tipped. Gnathopod 1 simple, subchelate or chelate; similar in males and females (not sexually dimorphic); smaller (or weaker) than or similar in size to gnathopod 2; propodus palm without robust setae along palmar margin. Gnathopod 2 subchelate, minutely subchelate, chelate or simple; similar or dissimilar in males and

females (sexually dimorphic or not) carpus slightly produced along posterior margin of propodus or not produced along posterior margin of propodus, projecting between merus and propodus. Pereopods 3–4 not sexually dimorphic. Pereopod 4 with or small posteroventral lobe or without posteroventral lobe. Pereopod 5 shorter than pereopod 6; coxa equilobate or with posteroventral lobe or with posterodorsal lobe or with large anteroventral lobe. Pereopod 7 longer than pereopod 5. Pleonites 1–3 without dorsal carinae. Urosomites 1–3 free; without slender or robust dorsal setae. Urosomite 1 without large distoventral robust seta. Urosomite 2 without dorsal setae. Uropod 1 without basofacial robust setae. Uropod 3 not sexually dimorphic; uniramous, without plumose setae. Telson moderately cleft to entire; dorsal or lateral robust setae present or absent; apical robust setae present or absent. Adult talitrids range from 5 mm to 20 mm (3/16 to 3/4 inch) in length. Eggs are deposited within a brood pouch on the underside of the adult female amphipod's body. The eggs hatch in one to three weeks. Most species complete their life cycle (egg to adult) in one year or less.

Habitat: Coastal talitrids include species living by the sea on beaches, in estuarine areas, and even fully freshwater streams. Their distribution is cosmopolitan [Lowry & Myers, 2013]. Generally, their habitat includes supralittoral beaches, mangrove forests and terrestrial forests, being the only amphipod group that has colonized terrestrial habitats. They occur under stones or decaying vegetation and live on the surface of mulch and moist ground. They can also burrow into the soft substrate or into the sand down to a depth of 13 mm. Terrestrial talitrids do not have a waxy layer on their exoskeleton as do insects. They lose or gain moisture from their environment. Excessive water loss results in desiccation, while too rapid a gain is also lethal. This is the reason why they migrate out of rain-soaked soil to drier areas. Most species are active at night.

Ecologic benefit: Amphipods are one of the most successful marine taxa. Talitrids living in a coastal marine habitat, they best describe the community diversity and composition, and, in recent decades, they have been considered as reliable indicators for the analysis of changes in environmental quality. Due to specific adaptations to the local environment, they are frequently included in ecological studies. They are abundant from the tidal zone to abyssal depths occupying a variety of habitats. Their extraordinary morphological diversification is matched by a high level of eco-functional diversity. As consumers, they exhibit all sorts of feeding strategies, including carnivory, herbivory and detritivory, and a number of them are symbionts (parasites or commensals). Since they are, in turn, eaten by fish, birds and mammals, these crustaceans serve as an important intermediate trophic link between the primary and secondary production and higher trophic levels.

The genera in the family Talitridae are informally subdivided into four systematic-ecological units (not phylogenetic but taxonomically useful), as follows: (1) Marsh-hoppers: palustral talitrids, plesiomorphic, semi-aquatic (rarely terrestrial) in salt marshes, mangrove swamps, estuarine and some freshwater habitats of tropical and antipodean continental areas, (2) Beach-hoppers: semi-terrestrial and terrestrial (but non-substrate-modifying) in supralittoral and coastal rain forest habitats of tropical to boreal marine coastlines of the world, (3) Sand-hoppers: specialized fossorial (substrate-modifying), semi-terrestrial, supralittoral on sandy beaches of tropical and temperate marine shores, (4) Land-hoppers: supralittoral non-

substrate-modifying talitrids, specialized terrestrial in coastal continental and high-island angiosperm rain forests, mainly of tropical, Indo Pacific, and antipodean temperate regions. Land-hoppers, can be found near the sea or at high latitudes, but they inhabit the forest floor litter, and are considered truly terrestrial. Definitions of these four ecological groups were first proposed by Bousfield (1982, 1984) and are currently used in the literature.

The family Talitridae includes about 250 species distributed in 52 genera [Lowry & Myers, 2013]. The first genera established to include coastal talitrids were *Orchestia* (Leach, 1793), *Talitrus* (Latreille, 1802), *Orchestoidea* (Nicolet, 1849), and *Talorchestia* (Dana, 1852). Until Bousfield's revisions (1982, 1984) most coastal species were placed within these genera and in 1982 he revised part of the coastal talitrids based on material from the northeastern Pacific coast. The *Orchestia* complex was subdivided into six genera, and the *Orchestoidea* complex into four genera. *Uhlorchestia* was erected to include some of the marsh-hopper species from the Atlantic coast of North America [Bousfield & Heard, 1986].

There are currently 14 species of talitrids known from the Mediterranean Sea: *Britorchestia brito* (Stebbing, 1891); *B. ugolinii* (Bellan-Santini & Ruffo, 1991*); Cryptorchestia cavimana* (Heller, 1865); *C. kosswigi* (Ruffo, 1949); *Deshayesorchestia deshayesii* (Audouin, 1826); *Macarorchestia remyi* (Schellenberg, 1950); *Orchestia gammarellus* (Pallas, 1766); *O. mediterranea* (A. Costa, 1853); *O. montagui* (Audouin, 1826); *O. stephenseni* (Cecchini, 1928); *O. xylino* sp. nov.; *Platorchestia platensis* (Krøyer, 1845); *Sardorchestia pelecaniformis* (BellanSantini & Ruffo, 1986); and *Talitrus saltator* (Montagu, 1808).

The only previous records of talitrids from Crete are that of *O. stephenseni* from a Megalou Nerou beach just east of Heraklion [De Matthaeis et al. 1998, De Matthaeis et al. 2000]. Four species of talitrid amphipods (*Orchestia montagui* Audouin, 1826, *Orchestia stephenseni* Cecchini, 1928, *Orchestia xylino sp. nov.* and *Talitrus saltator* Montagu, 1808) were also reported from an ecological study of six beaches in the Kokkini Hani, Gournes, Gouves area of northern Crete [Lowry & Fanini, 2013].

Use of Barcoding (COI)

Previous studies have demonstrated that mitochondrial DNA (mtDNA) has a higher mutation rate than do nuclear genes, providing useful information for the genetic characterization and differentiation of morphologically similar talitrid species [Morrison et al., 2002; Lavrov et al., 2004]. DNA barcoding uses a short DNA sequence of the mitochondrial gene, cytochrome c oxidase (COI or COX1) and is a useful tool for the discovery of previously unrecognized species that are often morphologically cryptic [Hebert et al., 2003]. DNA barcoding has been successfully used in a great diversity of talitrid species [Hebert et al., 2004; Smith et al., 2008; Ward et al., 2005; Barrett and Hebert, 2005; Hajibabaei et al., 2006].

Identification of amphipods, and therefore talitridae species, has traditionally been based on morphological characters. The classical use of morphological characters for species identification has several limitations. Samples are often damaged during collection and storage. They cause, for example, the misidentification of a taxon due to the phenotypic

plasticity of the character studied or simply because a phenotypically homogenized species is in fact a number of cryptic taxa. Moreover, morphological keys are sometimes only effective for a particular life stage or gender, where species identification is mainly based on male genitalia. Thus, a high level of expertise is often required to correctly identify species with the accuracy required in ecological studies.

The DNA barcoding approach might currently represent the best solution for identifying species when their morphology is of limited use, even if DNA barcoding also presents its own limitations. Until now, biological specimens were identified using morphological features like the shape, size and color of body parts. In some cases, a trained technician could make routine identifications using morphological keys, but in most cases an experienced professional taxonomist is needed. If a specimen is damaged or is in an immature stage of development, even specialists may be unable to make identifications and therefore are able to assign the individuals only to genera, families of higher taxa. Specific features of amphipods—such as sexual dimorphism, ontogenic variations, and morphological uniformity among closely related species—further limit our ability to discriminate species based on morphological characters.

Barcoding solves these problems because barcodes can be obtained even from tiny amounts of tissue. This is not to say that traditional taxonomy has become less important. Rather, DNA barcoding can serve a dual purpose as a new tool in the taxonomists toolbox, supplementing their knowledge as well as being an innovative device for non-experts who need to make a quick identification. Thus, DNA barcoding turns to be a high value identification method because the molecular marker is present in all individuals but also has enough discriminatory power to separate them to species, whereas in traditional molecular identification one can use primers which are specifically designed to identify particular species, which are not required to work with all species. Another advantage of the DNA barcoding approach is that the basic data – the sequences – are not prone to subjectivity and can be reanalyzed in the future in accordance with improvements in taxonomic knowledge.

The growing application of standardized DNA barcodes [Hebert et al., 2003] to investigate taxonomic diversity has been exposing unforeseen layers of hidden diversity in numerous faunal groups, especially in less-studied taxa or geographic regions [Hajibabaei et al., 2006]. The hidden diversity is typically exposed by comparing morphology-based and DNA barcode-suggested species boundaries [Costa and Carvalho, 2010]. In some cases, this has led to the detection of numerous putative cryptic species, prompting a new appreciation of the diversity of entire faunal groups [Gomez et al., 2007]. DNA barcodes are therefore increasingly applied to probe and revise the taxonomic diversity of faunal groups, providing a quick screening method for highlighting mismatching morphological and molecular data, detection of putative cryptic species and taxonomic complexes, and also inaccurate or misleading identifications [Borges et al., 2012].

Mitochondrial Cytochrome oxidase subunit I (COI) is the gene most commonly employed for taxonomic identification purposes in animals. Its variability has been found to be ideal for

this task in a majority of cases; its use has therefore become standardized to the point it is considered as a genetic "barcode" [Hebert et al., 2003; http://www.barcodeoflife.org/].

COI sequence data has been found especially useful in providing new insights when used in combination with ecological or behavioural data. Concerning peracarids, COI sequence analysis has already been successfully used at local and larger geographical scales to study Mediterranean talitrids, and in intraspecific studies of amphipod populations [Pavesi et al., 2013]. Scapini et al. (1988) highlighted the inheritance of orientation towards the local seashore in the sand-hopper *Talitrus saltator*. Ketmaier et al. (2010) pointed out a correlation between molecular divergence and behavioral adaptations to changing environments across an erosion gradient on an extended sandy beach. Significant behavioral and molecular differences were found at distances as short as one kilometer. This seem however to be applicable only to the category "sand-hoppers", while the category "beach-hoppers" was found less differentiated on a large scale. Fanini & Lowry (2014) supported with behavioural data such hypothesis. The use of COI sequence analysis on beach isopods (Oniscidae) led so far to the identification of several species, including cryptic species complexes [Hurtado et al., 2014]. This is mainly due to the scarce mobility of this taxon. Up to now, there are no other studies available linking the behaviour of beach isopods to spatial and molecular changes.

MATERIALS AND METHODS

Sampling

The study was carried out on the island of Crete and the survey was based on a snapshot sampling design with as many sites as possible within a limited amount of time. The collection studied here followed a systematic two-months sampling program (October - December, 2015) in 38 sites selected from the Blue Crete guide from A. Roniotis *(Fig. 2)*. These supralittoral sites, display several types of habitat, like banquette of *Posidonia oceanica*, sand and algal/seagrass wrack, fine sand, coarse sand, mixed sand, cobbles and stones.

Figure 2. Map of sited around Crete. The map was created using Google Earth, 14/10/2016.

The two most commonly used sampling methods, to study community diversity and talitrid abundance on sandy beaches [Fanini and Lowry, 2016], are:

(a) Pitfall trapping *(Fig. 3)*, capable of collecting individuals during their active periods. Pitfall-traps are consisted of plastic cups which are 10 cm in diameter and 20 cm in height,

Figure 3. Pitfall traps (costellation).

positioned in the sand or in the banquettes. Constellations of pitfall traps were buried with their mouths flush with the surface of the substratum and connected by means of tubes of least one meter in length. A mixture of freshwater and leaves were placed in each trap with the aim of making the trap more palatable. The traps were deployed at nightfall and emptied at dawn in order to intercept individuals moving across the supralittoral zone and to mitigate any nuisance experienced by bathers.

(b) Corer/quadrat sampling and subsequent sieving, capable of collecting individuals buried in the substrate.

They are both widely used methods, however they are related to different behaviors: surface activity (pitfall traps) and burrowing in the substrate (quadrat sieving).

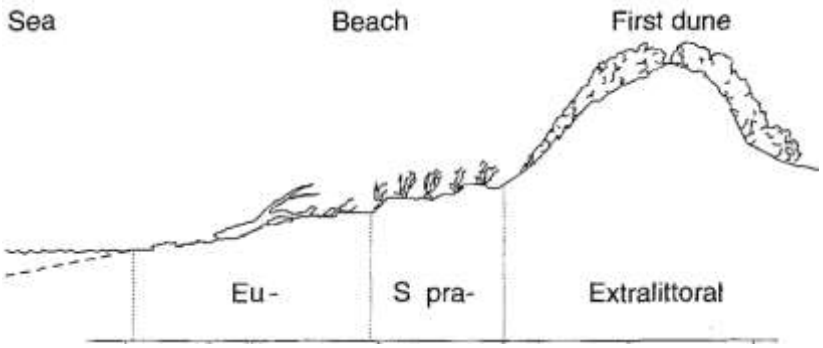


Figure 4. Study area: cross-section of the beach/dune system in non-perturbed system.

Figure 5. Transect along the beach.

On each beach, pitfall traps were placed along a transect *(Fig. 5)* running perpendicular to the shoreline, from the high-water mark (drift zone) to the first vegetation or, when vegetation was absent, to the substrate change in slope and texture *(Fig. 4)*. Pitfall traps were kept active overnight, to intercept individuals moving across the supralittoral [Colombini et al., 2003].

Specimens were preserved in alcohol, one tube per beach, with labels reporting beach names. Beach names were then related to GPS coordinates and date of collection. Collected amphipod specimens were sorted under a stereomicroscope, counted and stored in 70 % ethanol. Identification of the collected specimens was carried out in the beginning according to the morphological characteristics described by Ruffo (1993) and Lowry & Fanini (2013) for amphipods and by Schmallfuss et al. (2003) for isopods. Morphological identification was followed by DNA extraction and sequencing of selected amphipods, in order to proceed to DNA barcoding identification.

As was mentioned above, Bousfield (1982) proposed an ecological repartition of talitrids between sand-hoppers, or substrate modifiers, as they burrow in the sand, and beach-hoppers or non-substrate modifiers, as they shelter in stranded wrack. At night, they are

both expected to move across the supralittoral to forage on fresh wrack [Jaramillo et al., 2003; Colombini et al., 2013; Bessa et al., 2014a, 2014b]. Different zonations, related to different habitat features, were recorded when sand-hoppers and beach-hoppers were found in sympatry [Pavesi et al., 2007; Gambineri et al., 2008; Lastra et al., 2010; Colombini et al., 2013]. Such an allocated use of the supralittoral habitat might lead not only to different zonation, but also to different sensitiveness to impacts of these two ecological categories. In the case of co-occurrence of both sand-hoppers and beach-hoppers, the consideration of 'talitrids' as a general category might thus generate a bias in the study outputs. The two categories could be used, instead of multiple indicators [Dale and Beyeler, 2001], as they are linked to different niches on sandy shores.

Sampling strategy has to be carefully considered when dealing with sandy beach communities, because their arthropod component is affected by environmental constraints such as temperature, humidity and food availability, which determine the uneven distribution of the inhabiting fauna across the supralittoral over time and space. Therefore, the eulittoral zone *(Fig.4)* was targeted where conditions of sand compaction and humidity, as well as the presence of stranded detritus, allow for the presence of beach macrofauna.

A recent paper by Fanini and Lowry (2016) compared methodologies for biodiversity assessment, finding equal efficiency of sampling by sieving and sampling by trapping. We here applied pitfall trapping due to the substrate characteristics, preventing from sieving with the standard 1 mm mesh size.

Beach Characterization

The set of 38 beaches was selected to obtain a representative picture of the fragmented shoreline of Crete, its exposure to North and South and its variation in terms of substrate characteristics over a small spatial scale. Local standard measurements in correspondence of each sampling event were taken [McLachlan & Brown, 2006]. Given their microtidal characteristics and peculiar wave regimen, Mediterranean beaches do not fit into the oceanic framework describing a morphodynamical status. Consequently, the measurements did not allow for the allocation of beaches in Crete to categories such as "reflective" and "dissipative". Yet, the interaction of energy and substrate is shaping the littoral, defining the habitats for the fauna and driving patterns of biodiversity on the supralittoral.

Beach Profile

According to standard protocols for beach ecology [McLachlan & Brown, 2006] each beach (GPS coordinates) was characterized by measurements taken along a transect, running perpendicular to the shoreline: beach width (m); beach slope (◦, measured with a clinometer - *Fig. 6*); intertidal width (m); substrate penetrability (cm, as the penetrability of an

Figure 6. Clinometer set up on the beach.

iron rod falling straight into the substrate from 1 m height [Fanini et al., 2009], if on sand substrate, not applicable in case of cobbles); sand granulometry (Folk & Ward sieve series, taking sand samples/taking pictures of the cobbles); wrack cover (we measured the wrack with the Dugan 2005 method that does not allow to estimate if the wrack is permanent or not). Sand from the beach surface down to ca. 15 cm (the expected burrowing depth for most resident fauna) was collected from the area where pitfall traps were placed. Sand samples were preserved in plastic bags, to be dried and used to estimate granulometry.

Figure 7. Sieving machine.

Granulometry Analysis

Grain size analysis is an essential tool for classifying sedimentary environments. It is the most fundamental property of sediment particles, affecting their entrainment, transport and deposition and also affecting the small invertebrates that live and burrow in the substrate. Grain size analysis therefore provides important clues to the sediment provenance, transport history, depositional conditions and the ecological impact on the fauna [Folk & Ward, 1957; Friedman, 1979; Bui et al., 1990].

Grain size was estimated by the Folk & Ward (1957) method, and grain classes followed Blott & Pye (2001) classification to make the results of granulometry comparable across published literature *(Fig. 8)*. One of the advantages of the Folk and Ward method is the opportunity to convert parameter values to descriptive terms for the sediment. Ca. 200 g of each sample were dry sieved for 20 minutes according to the recommendation of Syvitski (1991) and Mycielska-Dowgiałło (2007), using the sieve sizes: 4.0, 2.8, 2.0, 1.4, 1.0, 0.71, 0.5, 0.355, 0.25, 0.18, 0.125, 0.09 and 0.063 mm *(Fig. 7)*.

All techniques involve the division of the sediment sample into a number of size fractions, enabling a grain size distribution to be constructed from the weight percentage of sediment in each size fraction. In order to compare different sediments, grain size distributions have most frequently been described by their deviation from a prescribed ideal distribution and calculation of grain size statistics by Folk & Ward (1957). The results of granulometry estimate are expressed in terms of Mz (mean particle size in mm).

Finally, two fractions for the characterization of the substrate had to be considered: sand and sediments above 4 mm. For sand, we applied the Folk & Ward sieve classification explained above, while for the coarser fractions we estimated its relative weight as well, but it was expressed as percentage on the whole substrate sample [Schlacher et al., 2008]. This compromise was applied as the algorithm backing the Mz estimate cannot include in Mz calculation a fraction > 4 mm when its relative weight is above 5% of the total sample. Finally, the following comparative table was used as a reference point *(Fig. 8)*.

Grain size		Descriptive terminology			
phi	mm/μm	Udden (1914) and Wentworth (1922)	Friedman and Sanders (1978)	GRADISTAT program	
			Very large boulders		
−11	2048 mm		Large boulders	Very large	
−10	1024		Medium boulders	Large	
−9	512	Cobbles	Small boulders	Medium	Boulders
−8	256		Large cobbles	Small	
−7	128		Small cobbles	Very small	
−6	64		Very coarse pebbles	Very coarse	
−5	32		Coarse pebbles	Coarse	
−4	16	Pebbles	Medium pebbles	Medium	Gravel
−3	8		Fine pebbles	Fine	
−2	4	Granules	Very fine pebbles	Very fine	
−1	2	Very coarse sand	Very coarse sand	Very coarse	
0	1	Coarse sand	Coarse sand	Coarse	
1	500 μm	Medium sand	Medium sand	Medium	Sand
2	250	Fine sand	Fine sand	Fine	
3	125	Very fine sand	Very fine sand	Very fine	
4	63		Very coarse silt	Very coarse	
5	31		Coarse silt	Coarse	
6	16	Silt	Medium silt	Medium	Silt
7	8		Fine silt	Fine	
8	4		Very fine silt	Very fine	
9	2	Clay	Clay	Clay	

Figure 8. Size scale adopted in the GRADISTAT program, compared with those previously used by Udden (1914), Wentworth (1922) and Friedman and Sanders (1978). (table by Blott and Pye, p.1239)

Wrack Estimation

The wrack cover was estimated as the percentage of wrack cover along the total length of the transect [Dugan et al., 2003]. Wrack is mostly made up of seaweed, algae and surfgrass. No plants or seaweeds can grow in the unstable, wave-washed sand of the beach. As a result, beach animals rely largely upon sources of food, like seaweed wrack, that drift onto shore from other ecosystems. Common wrack-dependent species include sand-dwelling invertebrates, such as beach-hoppers, roly polies etc.

Since wrack provides food and shelter for a variety of beach inhabitants, it could be a good indicator for the existence of live animals on the beach, including talitrids and oniscids. Therefore, there was a significant necessity to notice and mention the amount of wrack deposited on the beaches we sampled. Although the sampled substrates did not contain a high density of wrack, so we can note only their presence or absence and there is no need to estimate the total density by dry weight or any other technique [Dugan et al., 2003].

The beaches were mainly covered by a strain of the endemic seagrass species *Posidonia oceanica* (L. Delile), dry wrack or no wrack at all. It should also be mentioned that the presence of wrack is unstable, since it depends highly on the wind and the waves. Thus, the data were not suitable for physical characterization of the beach and could not support comparable results.

Figures 9,10. Example of beaches with significant wrack presence (Left: Panormos, Right: Petres).

Human Indices

During the last few years, a general process of urbanization on the beaches of Crete, resulted in a massive coastal modification and an increase in beach use. For example, Matala, Sfakia and Malia beaches are crowded by bathers and receive a big number of entertainment events during the summer. These beaches have efficient public transportation and recreational facilities where many restaurants, bars and hotels are located. Crete has experienced a massive increment in the number of tourists visiting the island and attending the beaches. Although urbanization and tourism are placing 'escalating pressures' on sandy beaches at never experienced scales, studies on modifications caused by landfills, recreation and cleaning are still rare. During the sampling trips at winter time, we faced a very disappointing image with big amount of garbage and fouling in most of the beaches. In this context, we investigated how Urbanization and Recreation indices perform in predicting the absence/presence of crustacean species. Therefore, we tested the hypothesis that the abundances of the species are negatively affected by urbanization levels and recreation facilities, where higher abundances should occur on beaches with high conservation levels [McLachlan et al, 2013]. Afterwards, we adapted this hypothesis to the presence/absence scenario of the project.

Sandy beaches are the most common coastal environment around the world and harbor diverse and specialized biota. Having high socio-economic value and being intrinsic related with human culture, beaches are more frequented by people than any other type of shoreline. 'Coastal urbanization' resulted in widespread changing of sandy beach ecosystems [Defeo et al., 2009], modifying the morphodynamic characteristics of the beaches, their nutrient flux, habitat features, community structure and species richness. Massive coastal development across the globe is expected to be intensified over the coming decades. Therefore, more sandy beaches are becoming mechanically cleaned and used for recreation by increasing numbers of residents and tourists. Accurately determine the ecological effects caused by beach use is a critical step and presents complex management and conservation challenges.

Coastal urbanization is a global phenomenon and indices of beach health are related to the abundances of the crustaceans. Lower abundances are predicted for beaches with high levels of urbanization, whereas predictions of higher abundances occur on beaches with high conservation levels [McLachlan et al., 2013]. Generally, the ecological theory alleges that impacted sites are supposed to host r-strategist species in dense populations [Pearson & Rosenberg, 1978]. This model changes here, because high levels of activity on the beach, result to a more compacted substrate and to a more hostile environment for the inhabiting fauna.

The diversity and structure of the macrobenthic invertebrate fauna community are considered suitable ecological indicators of sandy beaches 'health', since human disturbances affect the distribution and life-history traits of the resident species. As reliable indicators of ecosystem stability, crustaceans have already been used as monitoring tools for coastal management of beaches and dunes [Colombini et al., 2003; Fanini et al., 2009]. Among them, the talitrid amphipods have received special attention as bioindicators because they are the ones of the dominant faunal species in terms of abundance, exhibit a variety of responses to human disturbances and have a high plasticity on their life-history characteristics. The sandhopper *Atlantorchestoidea brasiliensis* (Dana, 1853) is capable of maintaining populations from the supralittoral zone to the upper midlittoral zone across the entire morphodynamic spectrum on exposed sandy beaches [Cardoso & Veloso, 2001; Veloso et al., 2003; Gómez et al., 2013].

Human impacts on the world's shorelines are increasing due to expanding population, increasing affluence and increased demand for leisure on the coast [Defeo et al., 2009; Doney et al., 2012]. The resultant 'coastal squeeze' and need for science-based management of the sandy beaches as valuable natural resources has never been greater. Regional scale planning and management for multi-purpose use of beaches can consider three options for individual beaches at the local scale: management and use for recreation, management and use for conservation, management for multi-purpose use. Further, any management strategy needs not only to integrate these three suites of factors, but to be applicable across a wide range of spatial and temporal scales, and to be as objective as possible, preferably in a quantifiable way [McLachlan et al., 2013]. The strategy proposed by McLachlan, shown in the following tables, starts by considering the broad suite of physical, ecological and socio-economic factors that could influence beach use and management. Then, it develops from

this multiplicity of forcing factors two simple indices, each based on three key measures: one to assess conservation value and the other recreation potential. Finally, it proceeds to outline key principles and guidelines for application of these indices and consider case studies to illustrate application.

Category	Condition and score					
Dunes	**0** Absent, replaced by hard engineering structures	**1** Severely disturbed and limited in extent	**2** Extensive disturbance	**3** Disturbed but largely intact	**4** Well developed, little disturbance	**5** Pristine and extensive
Endangered and iconic species	**0** Absent	**1** Present in low numbers, not nesting	**2** Present in good numbers, may be nesting	**3** Nesting/spawning present in large numbers		
Macrobenthic diversity and abundance	**0** Low abundance, reflective and/or short beach	**1** Intermediate	**2** Species rich and abundant, dissipative and/or long beach			
Total score	**Minimum score is 0 + 0 + 0 = 0; maximum score is 5 + 3 + 2 = 10**					

Figure 11. Index of conservation value (CI). [McLachlan et al. 2013]

Category	Condition and score					
Infrastructure	**0** No infrastructure, difficult access	**1** No infrastructure, limited access	**2** Modest infrastructure reasonable access	**3** Good access, some amenities	**4** Good infrastructure and access	**5** Excellent access, parking and amenities, including lifesaving
Safety and health	**0** Extremely hazardous and/or polluted	**1** Hazardous and/or polluted	**2** Moderate hazards and clean	**3** Low bathing hazards, clean and totally pollution free		
Physical carrying capacity	**0** Limited, pocket beach, no backshore	**1** Intermediate	**2** Extensive beach with wide backshore			
Total score	**Minimum score is 0** plus; **0 + 0 = 0; maximum score is 5 + 3 + 2 = 10**					

Figure 12. Index of recreation potential (RI). [McLachlan et al. 2013]

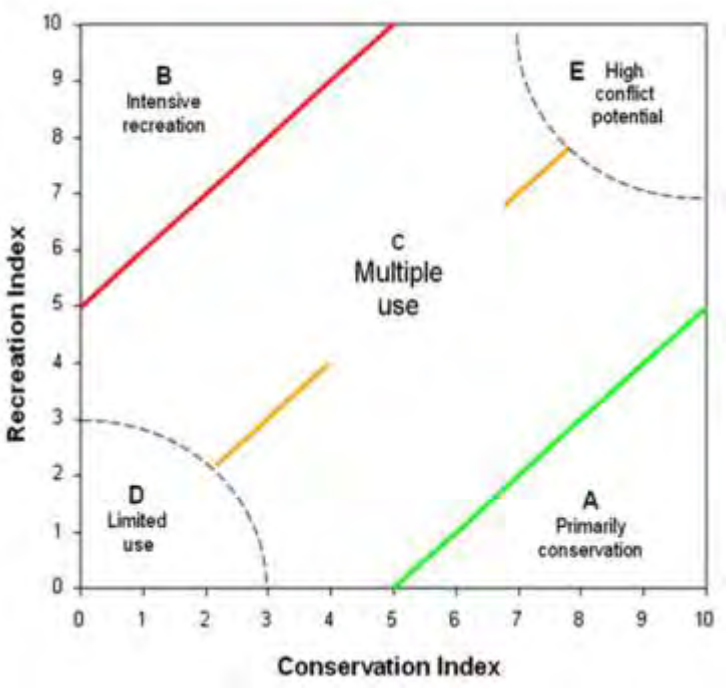


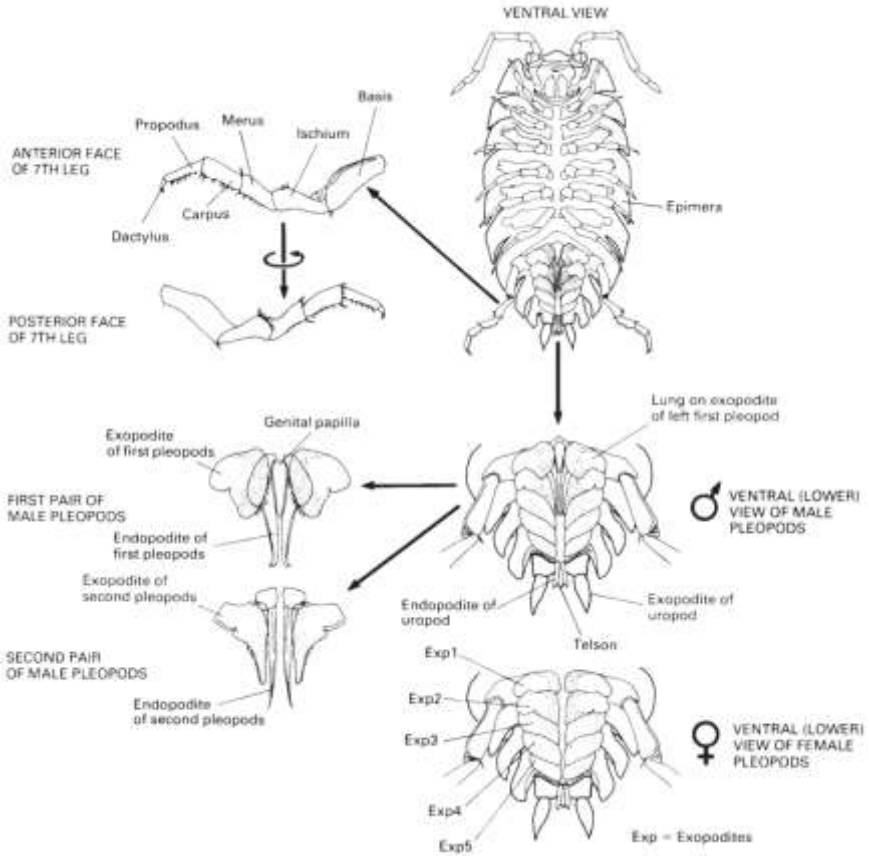
Figure 13. Plot of Recreation Index against Conservation Index scores to show demarcation of beaches for intensive recreation, conservation and multi-purpose use. [McLachlan et al. 2013]

Morphological Characterization of oniscidae species

For oniscidae isopods was possible to identify individuals to the species level via morphological characterization, using a stereomicroscope and identification keys. The expert judgment to confirm species was Dr. Stefano Taiti, National Research Council, Italy.

Identification key for Oniscid species of Crete [Schmallfuss et al. 2004]

1. Species able to conglobate……..2
– Species not able to conglobate……3
2. Pereion-epimera II–VII separated from tergites with visible sutures……*Tylos ponticus*
3. Antennal flagellum with more than 10 articles…… *Ligia italica*
4. Ridge forming upper margin of the frontal triangle not reaching the eyes ……5
5. Tergal parts granulated …… *Armadillidium granulatum*
6. pleopod-exopodite I as wide as long ……7
7. Tip of telson rounded ……*Armadillidium marmoratum*
8. Posterior margin of pereion-epimeron I at least slightly concave ……9
9. Hind margin of pereion-epimeron I without rounded concavity ……*Porcellio laevis*
10. Antennal flagellum with three distinct articles ……11
11. Pigment usually in distinct star-shaped blots under stereo-microscope; restricted to lit toral zone ……12
12. Small size (< 5 mm); short appendages; very narrow body; tergites covered with conical structures ……*Stenophiloscia vandeli*
– Larger size (> 5 mm); long appendages; tergites smooth, covered with setae ……13
13. Pleopod-endopodite I apically with spine at the exterior corner …… *Halophiloscia couchii*



Figures 14. Morphologic description of Oniscid Isopods (woodlice) [Stephen Hopkin, 1991].

Morphological Characterization of talitridae species

The individuals were photographed and morphologically identified to the lowest possible taxonomic level using an electronic microscope and identification keys. The generic-level descriptions were generated from a DELTA database [Dallwitz, 2005] to the talitrid genera and species of the world. The diagnostic descriptions were generated with the aid of the DELTA program Intkey. The characterization is based on diagnostic characters which distinguish each taxon in at least two respects from every other taxon. Material is lodged in the Australian Museum, Sydney (AM). Standard abbreviations on the plates are: EP, epimeron; G, gnathopod; HD, head; LMD, left mandible; MP, maxilliped; P, pereopod; PL, pleopod; T, telson; U, uropod.

The question of supraspecific level characters among talitrid amphipods is not settled nor is their homoplasy understood. We are currently using 26 characters (including eight sexually dimorphic characters) to define talitrid genera: **1.** eye size; **2.** antenna 1, length in relation to antenna 2 peduncle; **3.** antenna 2 male, development of incrassate peduncle; **4.** antenna 2 male, development of ventral plate on peduncular article 3; **5.** labrum, presence of robust setae; **6.** mandible, left lacinia mobilis, number of teeth; **7.** maxillipedal palp, presence of medial lobe on article 2; **8.** maxillipedal palp, condition of palp article 4; **9/10.** gnathopods 1 and 2 in males, chelation; **11.** gnathopod 1 males, number of palmate lobes along the posterior margin; **12.** gnathopod 1 males, merus/carpus free or fused; **13.** gnathopod 2 males, condition of the distal end of the dactylus; **14.** pereopods 3–7, simplidactylate or cuspidactylate; **15.** pereopods 5–7 with setae along posterior margin of the dactylus; **16.** pereopods 6 and/or 7 in males, sexual dimorphism in the merus and the carpus; **17.** pleonites 1–3 in males, development of dorsal spines; **18.** Pleopod condition; **19.** epimera, development of vertical slits; **20.** uropods 1–2, rami with apical spade-like robust setae; **21.** uropod 1, with or without marginal robust setae on outer ramus; **22.** uropod 1 sexually dimorphic, outer ramus inflated; **23.** uropod 3 well developed or vestigial; **24.** Uropod 3, length of ramus; **25.** telson, cleftness; **26.** telson, number of robust setae.

<u>Identification key to adult male Talitridae of Crete:</u>

1. Gnathopod 1 simple ……2
– Gnathopod 1 subchelate ……3
2. Eye small, less than 25% of head. Gnathopod 2 subchelate, with large proximal tooth on propodus……*Deshayesorchestia deshayesii*
– Eye large, about 50% of head. Gnathopod 2 mitten-shaped in male and female ……*Talitrus saltator*
3. Pereopod 6 merus and carpus slender, not swollen…… *Orchestia stephenseni*
– Pereopod 6 merus and carpus swollen ……4
4. Gnathopod 2 propodus palm with a strong distal excavation, dactylus with an opposing excavation on the inner margin…… *Orchestia montagui*
– Gnathopod 2 propodus palm entire, without distal excavation, dactylus with entire inner margin……*Orchestia gammarellus*

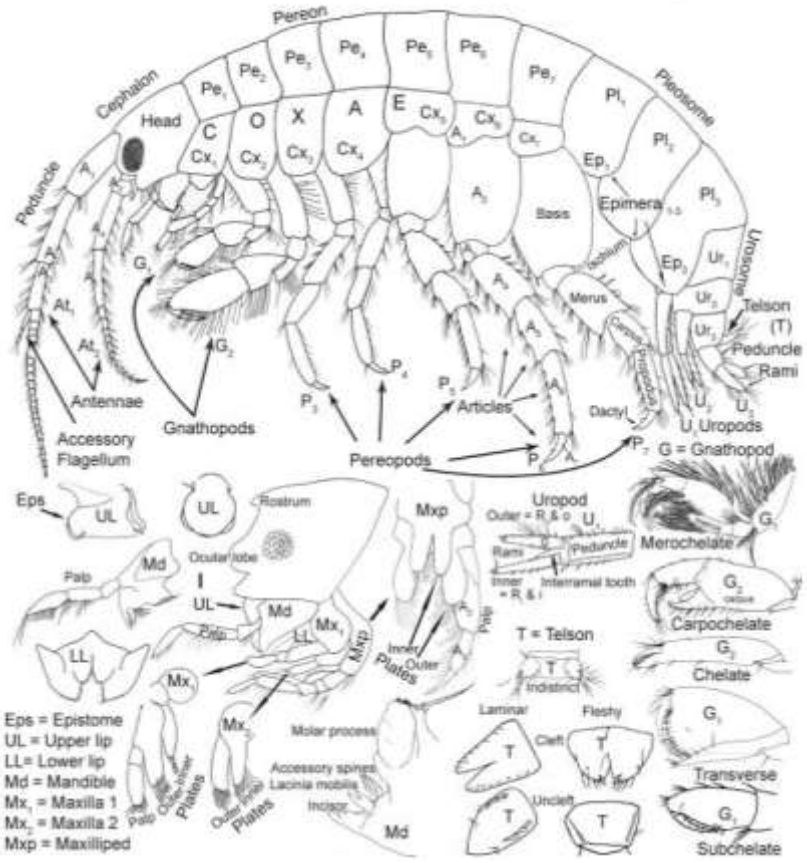


Figure 15. Analytical mapping of talitrid morphology used in taxonomic keys

Statistical Analysis

The presence/absence of the two categories of peracarids, isopods and amphipods, depending on beach characteristics were regressed against mutliple environmental variables. To this aim, we tested the pairwise correlation of the independent variables measured in correspondence of each sampling, namely beach width; beach slope; substrate penetrability. Also, the marginal association between the presence of isopods and amphipods was tested via the estimate of odds-ratio.

Once correlations and marginal association were falsified, variables included in models were: beach width; beach slope; substrate penetrability; substrate size Mz; North/South exposure; substrate type. Among them, continuous variables were: beach width, beach slope, substrate penetrability, Mz. Exposure to North/South was a discrete factor. The "substrate type" was considered as dummy variable, assigning value = 0 (meaning "sandy substrate") when the coarse fraction was below 5% in weight of the total substrate sample, and value = 1 (meaning "mixed and gravel substrate") when the coarse fraction was above 5% in weight of the total substrate sample. Models with presence of amphipods and isopods as dependent variable were calculated separately, assuming a binomial distribution of the data.

Models were selected stepwise, selection of the best model was based on the Akaike Information Criterion (AIC) [Akaike, 1973], i.e. the score expressing the maximum likelihood with the least number of parameters. Models for categories "isopods" and "amphipods"

were selected backwards. Furthermore, the variable “amphipods” was splitted into the two ecological categories “sand-hoppers” and “beach-hoppers”, each one used as dependent variable for logistic regression. Likewise, the variable “isopods” was splitted into the two Tylos species that were found “*Tylos ponticus*” and “*Tylos europaeus*”. The models for the categories were selected frowards to comply with algorythms robustness to our data distribution. The significance of single variables that resulted included in the best model was tested with chi-square test. R software was used for the analyses [R core team, 2014].

Molecular characterization of talitridae species

In this study COI barcoding was only applied to talitrids, as this taxa were found the most difficult to identify morphologically, due to the lower abundance and the presence of females and juveniles rather than adult males.

Reagents
Acetic Acid
Acrylamide
Agarose
Bis-Acrylamide
Ammonium persulfate
2-propanol
Bromophenol Blue
Boric acid
Xylene Cyanol
Chloroform
NaCl
SDS
dNTPs
EDTA
Ethidium bromide
EtOH
Proteinase K
Glycerol
HCl 37%
Formamide
NaOH
Formaldehyde
MgCl2 x 6H2O
NaBH4
Phenol
Potassium Acetate
TEMED
Tris Base
AgNO3
100bp Ladder
DNA polymerase
Taq (Bioline)

Samples

For the present study, 21 specimens of talitridae were used, one individual from every one of the beaches: Kalathas, Marathi, Platanias, Fragokastelo1, Frag2, Frag3, Frag3 cobbles, Pachia Ammos, Agia Galini, Loutraki, Skaleta, Geropotamos beach, Geropotamos river, Petres, Fodele, Diving Iera, Maleme shore, Paleokastro, Vamos, Stavros. We also used extra specimens from some of the beaches, as random examples to verify the repeatability of the result. We sampled two different parts of the site Geropotamos, the typical one on the supralittoral part and another one net to the river. The aim of this sampling was to examine if the fauna was different. A similar method was used in the Frag3 site, where we sampled the part of the beach that had a fine sand substrate (Frag 3) and the part with cobbles (Frag3 #2). This would also help me certify if the substrate can be such a strong factor to determine different types of fauna in one single site.

Table 1. DNA Extraction techniques that were used for specimens of every site and PCR concentrations.

No	Site	TNES-Urea Extraction	Mini Kit Extraction	PCR Concetration
1	Kalathas		Y	1 μL
2	Kalathas		Y	1 μL
3	Marathi		Y	1 μL
4	Platanias	Y		1 μL
5	Platanias	Y		2 μL
6	Frag1	Y		1.5 μL
7	Frag2	Y		1 μL
8	Frag3		Y	1 μL
9	Frag3		Y	1 μL
10	Frag3 #2(cobbles)		Y	1.5 μL
11	Pachia Ammos		Y	1 μL
12	Pachia Ammos		Y	1 μL
13	Agia Galini	Y		1.5 μL
14	Loutraki	Y		1 μL
15	Skaleta	Y		1.5 μL
16	Geropotamos beach	Y		1 μL
17	Geropotamos river	Y		1 μL
18	Petres		Y	1 μL
19	Petres	Y		1 μL
20	Fodele	Y		2 μL
21	Fodele	Y		2 μL
22	Diving Iera		Y	1 μL
23	Maleme shore		Y	1 μL
24	Paleokastro		Y	1 μL
25	Vamos		Y	1.5 μL
26	Stavros		Y	1.5 μL
27	Pachia Ammos		Y	1 μL

DNA Extraction

In order to study the haplotypes of mitochondrial DNA, which were used for the molecular analysis, it is necessary as a first step to isolate the cells from the genetic material, including genomic and mitochondrial DNA. In this study, DNA was extracted according to the protocol with TNES-Urea and also with the help of a Mini Kit, with appropriate modifications.

TNES-Urea Method:

About ≤25mg of tissue are used for each extraction, followed by the subsequent test procedures:

The animal is frittered in half and separated in two different eppendorf vials *(Fig. 16)*. One of the two will be dismembered and used for further analysis, while the other is stored in the freezer as stock. The animal is dissolved and the following components are added inside each eppendorf: 700 μL TNES-Urea Buffer, 15 μL proteinase K (stored in the freezer). The tubes are sealed with parafilm and placed in the oven at 55˚C, 5 verticils until the next day.

Figure 16. Preparation for section of the animal.

The samples are removed from the oven and so does the parafilm. Then they are processed as follows: addition of 700 mL phenol, vortex, centrifuge for 10', 4˚C, 13000rpm. After centrifugation, two different phases appear in the samples and the upper phase is collected into a new eppendorf. The samples are processed as follows: addition of 350 μL phenol and 350 μL chloroform, vortex, centrifuge for 10', 4˚C, 13000rpm. Two different phases are again obvious in the samples and the upper phase is now collected into a new eppendorf. The samples are processed as follows: addition of 700 μL chloroform, vortex, centrifuge for 5', 4˚C, 13000rpm. Again, two different phases occur in the samples and the upper phase is collected into a new eppendorf, in which 1 mL of ethanol 100% is added. The samples are stored in the freezer, where they can stay indefinitely. This is the point when the DNA precipitation is performed.

The samples are removed from the freezer and the isolation process proceeds with centrifuge for 5', 4˚C, 13000rpm. The liquid part is poured out and the sediment is kept. 1 mL of ethanol 70% is added to wash away the sediment and then it is stirred until the sediment comes off the bottom of the eppendorf. Centrifuge for 5', 4˚C, 13000rpm follows once more and the liquid part is poured out as much as possible (wiping on paper). The eppendorfs are placed in the oven with open lids until all the liquid evaporates completely. 100 mL of water for injection are added, followed by stirring until the sediment comes off the bottom of the eppendorf. The eppendorfs are named according to the sample and placed in the freezer.

The TNES-Urea Buffer solution was used to lyse the cells by adjusting the osmotic pressure of the cell, causing degradation of the cell membranes and homogenizing of the tissue. The proteinase K causes protein degradation, which protects DNA from nuclease activity. Phenol is used for denaturation of proteins and the separation of lipids, proteins and nucleic acids. The phenol solution used is equilibrated at pH> 7, so that DNA can be distributed in the upper aqueous phase. The addition of chloroform is aiming for a better phase separation

due to high density. It also denatures the proteins and removes the dissolved phenol from the aqueous phase. The precipitation of the DNA with isopropanol and subsequent washing with ethanol 70% is based on the fact that DNA is insoluble in these organic solvents.

Mini kit Method:

-Prepare lysate from mammalian tissues:
1. Set a water bath or heat block at 55C.
2. Place ≤25mg of mammalian tissue into a sterile microcentrifuge tube.
3. Add 180 µL PureLink Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to the tube. Ensure the tissue is completely immersed in the buffer mix.
4. Incubate at 55C with occasional vortexing until lysis is complete (1-4 hours). In my experiment an overnight digestion was performed to secure the lysis.
5. To remove any particulate materials, centrifuge the lysate at maximum speed for 3 minutes at room temperature. Transfer supernatant to a new, sterile microcentrifuge tube.
6. Add 20 µL RNase A (supplied with the kit) to the lysate, mix will by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink Genomic Lysis/Binding Buffer and mix well by vortexing.
8. Add µL 96-100% ethanol to the lysate. Mix well by vortexing for 5 seconds.

-Binding DNA
1. Remove a PureLink Spin Column in a Collection Tube from the package.
2. Add the lysate (~640µL) prepared with PureLink Genomic Lysis/Binding Buffer and ethanol to the PureLink Spin Column.
3. Centrifuge the column at 10.000 x for 1 minute at room temperature.
4. Discard the collection tube and place the spin column into a clean PureLink Collection Tube supplied with the kit.

-Washing DNA
1. Add 500 µL Wash Buffer 1 prepared with ethanol to the column
2, Centrifuge column at room temperature at 10.000 x for 1 minute.
3. Discard the collection tube and place the spin column into a clean PureLink collection tube supplied with the kit.
4. Add 500 µL Wash Buffer 2 prepared with ethanol to the column
5. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.

-Eluting DNA
1. Place the spin column in a sterile 1.5 mL microcentrifuge tube.
2. Add 100 µL PureLink Genomic Elution Buffer to the column.
3. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperatute. The tube contains purified genomic DNA.
4. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile, 1.5 mL microcentrifuge tube.
5. Centrifuge the column at maximum speed for 1.5 minutes at room temperature. The tube contains purifies DNA. Remove and discard the column.

The concentration for successful PCR that concerns samples, which were treated with both DNA extraction methods, is actually referring to the samples that were extracted with the Mini kit. Generally, the Mini kit method seemed to be more reliable and gave results when the TNES-Urea method didn't.

Determination of total DNA per sample

After the extraction procedure, we performed quality and quantity control upon the DNA, either photometrically or with an agarose gel 1% w / v.

Photometry takes place after the dilution of 1ml DNA solution into 49ml ddH_2O. Values of absorbance at 260nm are resolved to DNA concentration, which should be more than 150ng /ml. The ratio of the absorbance value at 260nm to the corresponding value at 280nm is a DNA purity index, which is expected to have a value around 2 to be suitable for use. The agarose gel procedure will be described below.

DNA amplification with Polymerase Chain Reaction (PCR)

We performed Polymerase chain reaction (PCR), in order to enhance a segment from the subunit I of the mitochondrial gene cytochrome oxidase (COI).

Table 2. Primers used for PCR [Leray et al, 2013]:

Primer	Sequence
Forward: mICOIintF	5'-GGWACWGGWTGAACWGTWTAYCCYCC-3'
Reverse: jgHCO2198	5'-TAIACYTCIGGRTGICCRAARAAYCA-3'

Table 3. The composition of the reaction solutions.

Template DNA	1-2 µL depending on the initial concentration of the sample that we saw on the agarose gel
dNTPs (10 mM each)	1 µl
MgCl2 (50mM)	2 µl
Buffer 10x	5 µl
Primer Fw 50 pmol/ml	1 µl
Primer Rv 50 pmol/ml	1 µl
Taq DNA Polymerase 5 U / ML	0,2 µl
ddH2O	37,8-38,8 µl depending on the DNA concentration

The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. The temperatures used and the length of time applied in each cycle depend on a variety of parameters including: the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature (Tm) of the primers.

<u>The amplification conditions and the stages are:</u>

Initial denaturation: 95° C for 4 min
Denaturation: 89 ° C for 40 sec ┐
Hybridization: 53° C for 30 sec ├ 35 circles
Elongation: 72° C for 35 sec ┘
Final Elongation: 72 ° C for 10 min
The PCR products were analyzed with agarose gel electrophorisis.

Agarose Gel Electrophorisis

Preparation of agarose gel was strictly conducted on drain areas, the electrophoresis table and UV table. We used the technique on two different types of samples. Firstly, for DNA samples that come directly from the extraction, in order to find out if it was successful. Secondly for DNA samples that come from PCR, in order to verify if the proliferation of the segment of interest was successful.

The agarose gel was used to separate DNA fragments according to their size. The size of the segments was estimated based on molecular sizes of control DNA fragments (ladder). The solutions used for this technique were:

TAE 50x (500ml)
Tris Base 121gr
Acetic Acid 28,5ml
EDTA 0,5M 50ml
ddH2O up to 500ml

Loading buffer 6x (10ml)
Bromophenol blue 1ml 1% w/v
TBE 20x 0,5ml
Glycerol 5ml
ddH2O up to 10ml

As an initial step, we prepared 1x TAE solution by diluting the 50x stock solution (20ml in final volume 1lt).

At first, we prepared the mold for the gel under the hood by placing the appropriate combs. Then we prepared the gel in a volumetric flask, in which we added 45 mL TAE 1x (final concentration of 2% w/v) along with 0.3 gr agarose in case we sampled DNA from extraction, while 0.6 gr in case we sampled DNA from PCR. The concentration of agarose gel varies depending on the size of the DNA fragments to be separated. We placed the sample in the microwaves until we get a homogenous solution. Once it was cooled down, we added 4.5 µL ethidium bromide (EtBr 10mg / ml). Ethidium bromide is added, so that the DNA bands can be apparent during observation of the gel under UV light. The gel is placed in a special mold for 10-15' where it gets polymerized.

Once the gel is ready we proceeded to DNA electrophoresis. We uploaded 3 µL DNA when it was originated from extraction, while 5 µL when it was originated from PCR together with 3 µL dye. We run the gel at approximately 100 volts for 20 minutes, until the dye reached almost halfway. The gel was examined under UV light. For the electrophoresis of the samples, addition of loading buffer is required. In 5ml of PCR product, 3ml loading buffer were added. Electrophoresis was performed at 100 volts followed by observation of the gel under UV light.

In order to ascertain whether the PCR generated the anticipated DNA fragment (also sometimes referred to as the amplimer or amplicon), agarose gel electrophoresis was employed for size separation of the PCR products. The size(s) of PCR products was/were determined by comparison to a DNA ladder (a molecular weight marker), which contained DNA fragments of known size, being run on the gel alongside with the PCR products. The agarose gel was carried out to quantify and control the quality of PCR products used in further experiments for the analysis.

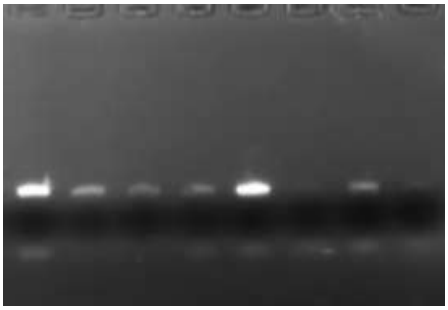


Figure 17. Agarose gel presenting the mtDNA band under UV light.

Single Strand Conformation Polymorphism

The SSCP analysis is based on the separation of single-stranded DNA fragments according to their different mobility on the gel and has a resolution of one nucleotide. Polyacrylamide gels can separate DNA that differs by 0.2% in length, well beyond the resolving capabilities of agarose (2% difference in DNA length). Another advantage to using polyacrylamide gels is that they can accommodate large amounts of DNA (up to 10 μg) without any loss in resolution. The SSCP analysis consists of three steps: denaturation of the PCR products, electrophoresis in polyacrylamide gel and staining of the gel to visualize the results.

1) Denaturation of PCR products

In order to denature the DNA fragments, we used denaturation buffer, the composition of which was as follows:

Denaturation buffer
95% formamide
0,05% Bromophenol blue
0,05% Xylene Cyanol
10mM NaOH

In 5-7μl of PCR product (depending on concentration) 10ml of denaturation buffer was added and the samples were incubated at 99^{o}C for 7 min. The purpose of denaturation was the transition of double-stranded DNA to single-stranded. The samples were then placed on ice broth and maintained in single-stranded state.

2) Preparation of polyacrylamide gel

For the preparation of polyacrylamide gels, the following solutions were used:

Acrylamide solution 38.5% (200ml): Acrylamide 75gr, Bis-acrylamide 2gr, ddH20 up to 200ml
TBE 10x (2lt): Tris Base 121 gr, Boric acid 81,5gr, EDTA 0.5M 80ml, ddH2O to the 2lt
Glycerol 50% v/v
APS 20% w/v
TEMED

For the electrophoresis of PCR products, polyacrylamide gel with 8% density is used.

Table 4. The concentration of the reagents used to prepare 8% polyacrylamide gels.

	8%
Acrylamide solutiuon 38,5%	10,6ml
Glycerol 50%	8ml
TBE 10x	5ml
TEMED	50μl
APS 20%	350μl
ddH2O	up to 50ml
Total volume	50ml

After the acrylamide polymerization, the denatured samples were electrophoresed using TBE buffer 0,5x. Electrophoresis was performed with 220 volts at room temperature for about 20 hours.

3) Polyacrylamide gel staining with silver nitrate (Silver Staining)

To display the electrophoresis results, the gel was stained with silver nitrate. This technique relies on the fact that silver binds on DNA and then reacts with formaldehyde in the presence of NaOH. The DNA bands appear brown with a yellow background [Sambrook et al., 2000]. For the silver staining the following solutions were used:

Solution 1 (400ml)
EtOH 8ml
Acetic Acid 0,5ml
ddH2O up to 400ml

Solution 2 (200ml)
Solution AgNO3 1gr / lt

Solution 3 (200ml)
NaOH 3gr
NaBH4 0,01gr
Formaldehyde 1ml
ddH2O up to 200ml

During the first step of the staining, the gel was soaked in 200ml of solution 1 and stirred for 3 min. Solution 1 was removed and the process was repeated, followed by washing the gel with distilled water for 1min. During the second step the AgNO3 solution was added and the gel was stirred for 20min. We washed again twice with distilled water, 1min duration each. During the third and last phase solution 3 was added, and stirring was effected until the appearance of visible bands on the gel.

When the bands became visible, we compared the patterns of different individuals to identify what specimens bring common patterns and which differ from each other. After grouping of specimens, we selected one representative individual of each pattern, which would be used to determine the nucleotide sequence of each group.

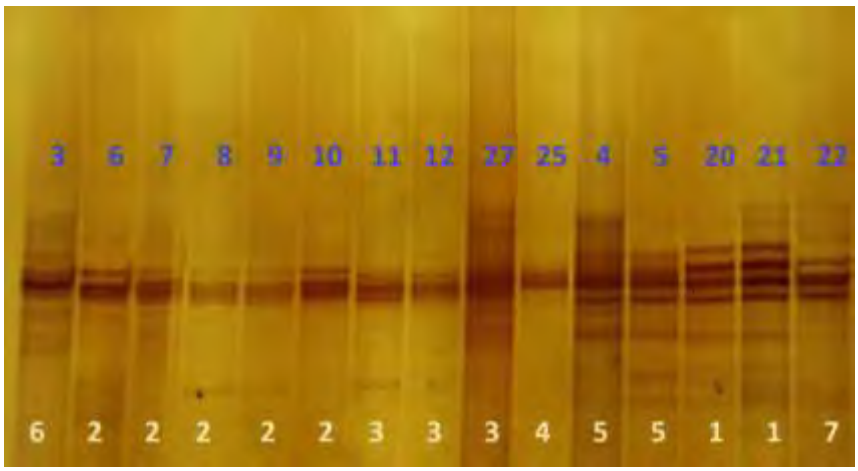


Figure 18. Acrylamide gel (1). Blue: identity number, White: different patterns

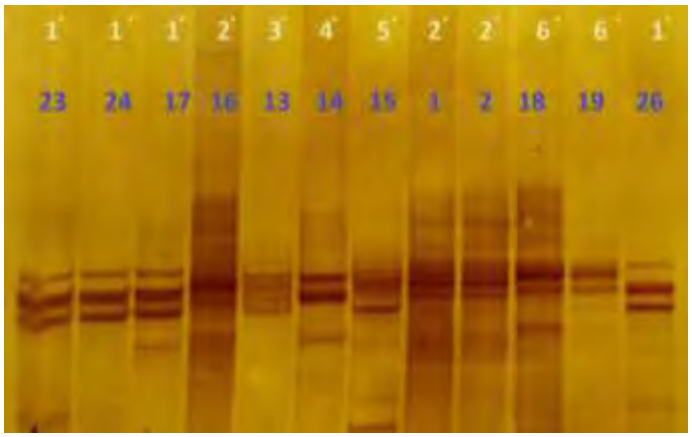


Figure 19. Acrylamide gel (2). Blue: identity number, White: different patterns.

After the electrophorisis on acrylamide gel 8% at room temperature for 20 hours, we could observe the different patterns. The bands that occurred from silver staining revealed 7 different patterns on the first gel and 6 on the second gel. Some of the patterns between the two different gels may appear identical, but there is no certainty in saying that they match. Therefore, we selected one representative individual of each pattern for sequencing, after purified by means of a suitable kit (Nucleospin-gel and PCR clean up by Macherey-Nagel).

Table 5. Details of the samples that were used and their electrophoretic patterns.

No	Site	Acrylamide patterns	Sequencing
1	Kalathas	2'	
2	Kalathas	2'	
3	Marathi	6	Y
4	Platanias	5	
5	Platanias	5	Y
6	Frag1	2	
7	Frag2	2	
8	Frag3	2	
9	Frag3	2	
10	Frag3 #2(cobbles)	2	Y
11	Pachia Ammos	3	
12	Pachia Ammos	3	Y
13	Agia Marina	3'	Y
14	Loutraki	4'	Y
15	Skaleta	5'	Y
16	Geropotamos beach	2'	Y
17	Geropotamos river	1'	
18	Petres	6'	
19	Petres	6'	Y
20	Fodele	1	
21	Fodele	1	Y
22	Diving Iera	7	Y
23	Maleme shore	1'	
24	Paleokastro	1'	Y
25	Vamos	4	Y
26	Stavros	1'	
27	Pachia Ammos	3	

Sequencing of different standards SSCP

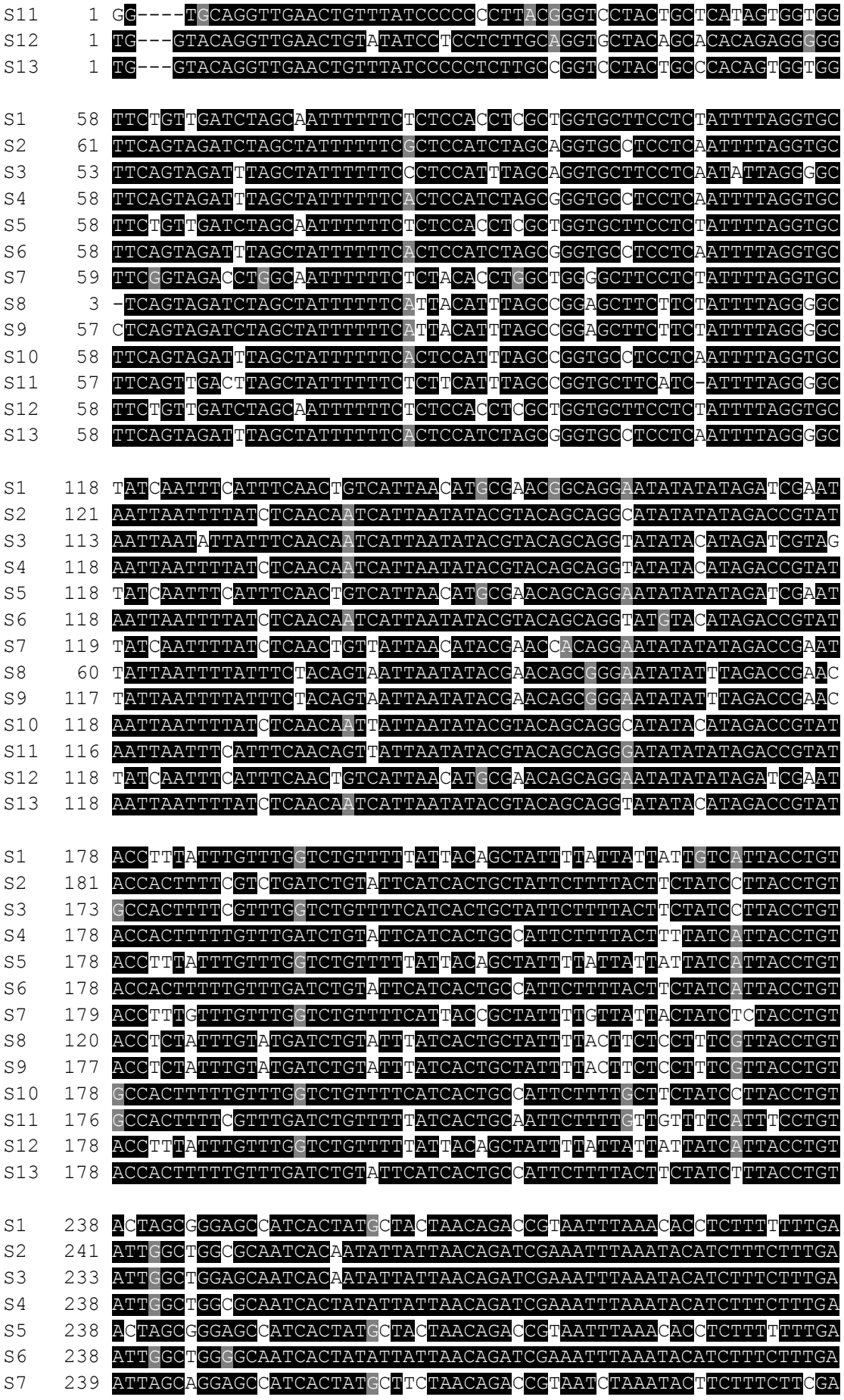
In order to identify the species, it was necessary to know the sequences of the individuals. Therefore, the PCR products were purified using a specific kit (Nucleospin-gel and PCR clean up by Macherey-Nagel) so they could be discharged from the presence of by-products, and then send to sequencing companies. The results were obtained in the form of a chromatogram and where mostly of high quality. The analysis of sequencing chromatograms was performed using BioEdit. In the graph, four curves were shown in different colors, each of which corresponds to a different nucleotide. Sequence trace files were carefully checked for the presence of ambiguous base calls and edited to remove poor quality terminal sections. The sequencing was performed for both strains (with different primer for each) and then the two sequences that occurred, were aligned using the appropriate bioinformatics program (ClustalX) in order to find possible errors arising during this process [Thompson et al., 1997]. After the end of this process, we have the complete sequence of the segment of interest. The total size of the segment we enhanced with PCR, was about 350 bp.

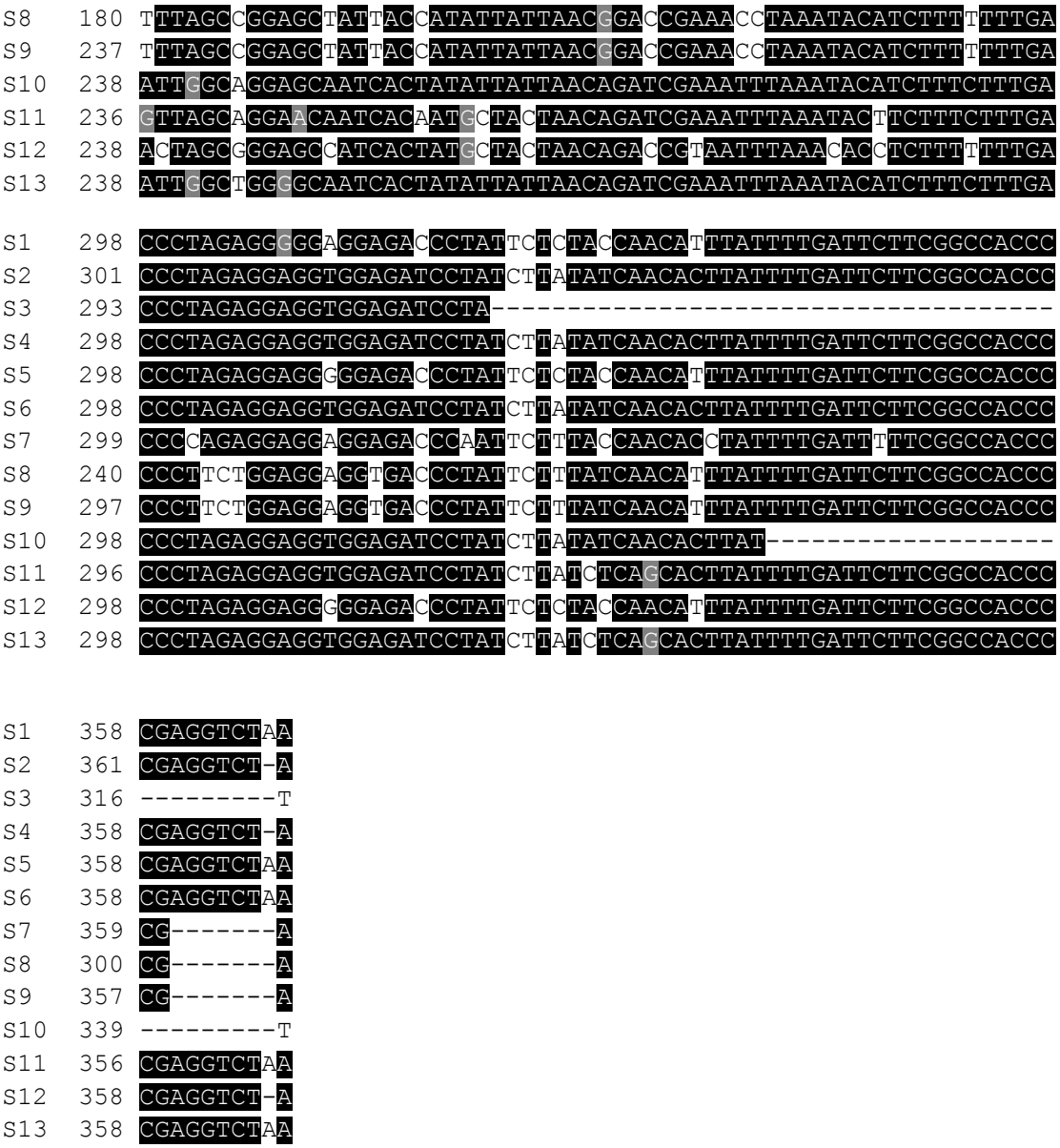
Table 6. Sequence names, SSCP patterns and their corresponding sample numbers.

Sequence Name	Pattern	Sample Number
S1	1	21
S2	2	10
S3	3	12
S4	4	25
S5	5	5
S6	6	3
S7	7	22
S8	1’	24
S9	2’	16
S10	3’	13
S11	4’	14
S12	5’	15
S13	6’	19

The multiple sequence alignment that follows was generated using T-Coffee and Boxshade. The sequences obtained were:

```
S1      1 TG---GTACAGGTTGAACTGTATATCCTCCTCTTGCAGGTGCTAGAGCACACAGAGGGGG
S2      1 TGTAGGTACAGGTTGAACTGTTTATCCCCCTCTTGCCGGTCCTACAGCCCACAGAGGGGG
S3      1 --------CAGGTTGAACTGTTTATCCCCCTCTTGCCGGTTCTACAGCTCACAGAGGTGG
S4      1 GG---GTACAGGTTGAACTGTTTATCCCCCTCTTGCAGGTCCTACTGCCCACAGAGGGGG
S5      1 TG---GTACAGGTTGAACTGTATATCCTCCTCTTGCAGGTGCTACAGCACACAGAGGGGG
S6      1 GG---GTACAGGTTGAACTGTTTATCCCCCTCTTGCAGGTCCTACTGCCCACAGAGGTGG
S7      1 TAG--GAACAGGATGAACCGTTTACCCCCCTTTAGCTGGTGCCACCGCCCATAGTGGAGG
S8      1 ------------------------------------------------------------
S9      1 GG----AACAGGTTGAACTGTATATCCTCCTTTAGCTTCAGCAACAGCTCACAGAGGTGG
S10     1 TG---GTACAGGTTGAACTGTTTATCCCCCTCTTGCAGGTCCTACTGCCCACAGTGGGGG
```





Sequence comparison - Phylogenetic analysis

By using MEGA, we performed multiple sequence alignment of the 13 sequences, the BLAST sequences that came up as identical and a sequence from distant species as a root. In order to visualize the clustering pattern for all aligned sequences, a Maximum Likelihood (ML) tree was built in MEGA v 6.0, using the best-fit model ~~K2~~ JC indicated by the same software according to the BIC (Bayesian Information Criteria) score. A Neighbor Joining tree was also constructed in order to rise the enhance the precision, using the K2 model. MEGA program helped the grouping of the sequences. Through the "grouping" trees, it can be possible to test whether the specimens are strongly related with the species they appear to be or the species that were identified morphologically. We used bootstrap control 1,000 for the constructed tree, which is indicative of the reliability of the final trees and GenBank BLASTn search [Altschul et al., 1990] to search which species of talitridae seem to be closer to every individual.

Table 7. BLAST results about the query and the identity of every sequence.

Sequence No	Pattern	Identification species	Query	Identity	Length (matched/ whole)	Represented SItes
1	1	*Deshayesorchestia deshayesii*	70%	92%	257/367	Fodele
2	2	*Talitrus saltator*	92%	90%	343/369	Frag1. Frag2, Frag3, Frag3(cobbles)
3	3	*Talitrus saltator*	100%	92%	316/316	Pachia Ammos
4	4	*Talitrus saltator*	92%	91%	339/366	Vamos
5	5	*Deshayesorchestia deshayesii*	70%	93%	257/367	Platanias
6	6	*Talitrus saltator*	92%	91%	339/367	Marathi
7	7	*Platorchestia sp.*	99%	83%	358/361	Diving Iera
8	1'	*Orchestia montagui*	93%	96%	283/302	Geropotamos river, Maleme shore, Paleokatsro, Stavros
9	2'	*Orchestia montagui*	94%	96%	340/359	Kalathas, Geropotamos beach
10	3'	*Talitrus saltator*	99%	93%	338/339	Agia Marina
11	4'	*Talitrus saltator*	92%	94%	338/365	Loutraki
12	5'	*Deshayesorchestia deshayesii*	70%	93%	257/366	Skaleta
13	6'	*Talitrus saltator*	92%	91%	339/367	Petres

The sequences obtained, represented each one of the different ~~haplotypes~~ patterns determined by SSCP analysis. For finding the phylogenetic relationships among haplotypes and in particular the different species of analysis, we used the MEGA program (Molecular Evolutionary Genetics Analysis) [Tamura et al, 2007]. By using this program, multiple sequence alignment is possible. The program offers the possibility of construction of a phylogenetic tree with the desired model (NJ, UPGMA, ML, MP), but also the bootstrap control for the constructed tree, which is indicative of the reliability of the final trees.

The construction of a phylogenetic tree requires far more genes and not only the sequences of one COI gene. Therefore, we used the final constructed tree only to remark the relationships between our specimens and the way they group, but not as a phylogenetic analysis. We created the tree using our own 13 sequences, the original sequences of the species that came out as a BLAST hit for every specimen and also a sequence from a different distant species as a root for the tree. The tree will prove how animals group together and how close or distant are between each other. According to the following model test, we used the Kimura 2 model [K2P, Kimura 1980] with bootstrap 1000 ~~[K2P, Kimura 1980]~~for the NJ tree.

Table 8. Model test occurred from MEGA.

Model	#Param	Gamma
T92+G+I	43	1,01145458
T92+G	42	0,216543197
HKY+G+I	45	1,31932203
HKY+G	44	0,233780407
TN93+G+I	46	1,371626795
TN93+G	45	0,231924579
T92+I	42	n/a
HKY+I	44	n/a
TN93+I	45	n/a
GTR+G+I	49	1,38528091
GTR+G	48	0,247976014
GTR+I	48	n/a
K2+G+I	42	1,488276183
K2+G	41	0,28205273
K2+I	41	n/a
JC+G+I	41	2,805281161
JC+I	40	n/a
JC+G	40	0,309342421
HKY	43	n/a
T92	41	n/a
TN93	44	n/a
GTR	47	n/a
K2	40	n/a
JC	39	n/a

RESULTS

Species Identification

Table 9. Morphological identification of Oniscidae and Talitridae and barcoding identification of Talitridae of every site that was sampled.

Site	Morphological identification for Oniscidae	Morphological identification for Talitridae	Barcoding identification for Talitridae
Paleokastro	*Armadillidium granulatum*	*Orchestia stephenseni*	*Orchestia montagui*
Fodele	—	*Deshayesorchestia deshayesii*	*Deshayesorchestia deshayesii*
Pachia Ammos	*Tylos europaeus; Stenophiloscia vandeli*	*Talitrus saltator*	*Talitrus saltator*
Voulisma	—	—	—
Arina	—	*Talitrus saltator*	*Not sequenced*
Amoudara	—		—
Kokkini Hani	*Lygia italica*	*Deshayesorchestia deshayesii*	*Not sequenced*
Panormos	*Lygia italica*	—	—
Geropotamos	—	*Not Identified*	*Orchestia montagui*
Geropotamos river	*Stenophiloscia sp.*	*Orchestia montagui*	*Orchestia montagui*
Skaleta	—	*Not Identified*	*Deshayesorchestia deshayesii*
Petres	*Tylos europaeus*	*Not Identified*	*Talitrus saltator*
Georgioupoli	—	*Talitrus saltator*	*Talitrus saltator*
Marathi	—	*Talitrus saltator*	*Talitrus saltator*
Loutraki	*Porcellio laevis; Tylos europeus*	*Not Identified*	*Talitrus saltator*
Stavros	—	*Orchestia gammarellus*	*Orchestia montagui*
Kalathas	*Lygia italica*	*Not Identified*	*Orchestia montagui*
Maleme	*Tylos ponticus; Stenophiloscia vandeli*	*Orchestia stephenseni*	*Orchestia montagui*
Maleme wrack	*Tylos ponticus*	*Orchestia stephenseni*	*Orchestia montagui*
Platanias	*Tylos ponticus*	*Deshayesorchestia deshayesii*	*Deshayesorchestia deshayesii*
Agia Marina	*Stenophiloscia vandeli; Tylos europaeus; Armadillidium marmoratum*	*Not Identified*	*Talitrus saltator*
Iera diving	*Tylos ponticus; Lygia italica*	*Deshayesorchestia deshayesii*	*Platorchestia sp*
Ierapetra 1	*Tylos ponticus*	—	—
Ierapetra 2	*Tylos ponticus*	—	—
Ierapetra 3	*Lygia italica; Tylos ponticus*	*Not Identified*	*Not sequenced*
Agios Panteleimonas	*Tylos ponticus*	—	—
Kalamokania	*Tylos ponticus*	—	—
Diaskari	—	*Talitrus saltator*	*Not sequenced*
Kaloi Limenes	*Tylos ponticus*	—	—
Kommos	*Tylos europaeus*	—	—
Matala	—	—	—
Red Beach	*Tylos europaeus*	—	—

Orthi Ammos sand	*Tylos europaeus*	*Talitrus saltator*	*Talitrus saltator*
Orthi Ammos cobbles	*Tylos europaeus*	*Talitrus saltator*	*Talitrus saltator*
Lakkoi	*Tylos europaeus*	—	—
Frangokastelo 2	—	*Talitrus saltator*	*Talitrus saltator*
Frangokastelo 1	*Tylos europaeus*	*Talitrus saltator*	*Talitrus saltator*
Ilingas	*Tylos ponticus;* *Halophiloscia couchii*	—	—

Table 10. Seperation of talitrids in beach-hoppers and sand-hoppers.

Site	*Talitridae*	*Ecological categories*
Paleokastro	*Orchestia sp.*	*Beach-hopper*
Fodele	*Deshayesorchestia deshayesii*	*Beach-hopper*
Pachia Ammos	*Talitrus saltator*	*Sand-hopper*
Arina	*Talitrus saltator*	*Sand-hopper*
Kokkini Hani	*Deshayesorchestia deshayesii*	*Beach-hopper*
Geropotamos river	*Orchestia montagui*	*Beach-hopper*
Georgioupoli	*Talitrus saltator*	*Sand-hopper*
Marathi	*Talitrus saltator*	*Sand-hopper*
Stavros	*Orchestia sp.*	*Beach-hopper*
Maleme	*Orchestia sp.*	*Beach-hopper*
Maleme wrack	*Orchestia sp.*	*Beach-hopper*
Platanias	*Deshayesorchestia deshayesii*	*Beach-hopper*
Iera diving	*Deshayesorchestia deshayesii*	*Beach-hopper*
Diaskari	*Talitrus saltator*	*Sand-hopper*
Orthi Ammos sand	*Talitrus saltator*	*Sand-hopper*
Orthi Ammos cobbles	*Talitrus saltator*	*Sand-hopper*
Frangokastelo 2	*Talitrus saltator*	*Sand-hopper*
Frangokastelo 1	*Talitrus saltator*	*Sand-hopper*

Table 11. Seperation of Tylids (T. ponticus, T. europeaus).

Site	*Tylid species*
Pachia Ammos	*Tylos europaeus*
Petres	*Tylos europaeus*
Loutraki	*Tylos europeus*
Maleme	*Tylos ponticus*
Maleme wrack	*Tylos ponticus*
Platanias	*Tylos ponticus*
Agia Marina	*Tylos europaeus*
Iera diving	*Tylos ponticus*
Ierapetra 1	*Tylos ponticus*
Ierapetra 2	*Tylos ponticus*
Ierapetra 3	*Tylos ponticus*
Agios Panteleimonas	*Tylos ponticus*
Kalamokania	*Tylos ponticus*
Kaloi Limenes	*Tylos ponticus*
Kommos	*Tylos europaeus*
Red Beach	*Tylos europaeus*
Orthi Ammos sand	*Tylos europaeus*

Orthi Ammos cobbles	*Tylos europaeus*
Lakkoi	*Tylos europaeus*
Frangokastelo 1	*Tylos europaeus*
Ilingas	*Tylos ponticus*

Given the snapshot sampling design of the survey, the present data cannot be used to estimate abundance or perform population studies. Consequently, data are here presented as presence/absence of species per site. The identification of talitrids resulted difficult due to the low number of adult males found in the traps. We therefore proceeded with molecular characterisation to be paired to the morphological analysis.

The following trees ~~are showing~~represent how the sequences group together and their relative distance. As it is obvious, both trees appear to show similar grouping, which gives us a slightly higher accuracy to trust this grouping. We can observe the bootstrap values next to the branches.

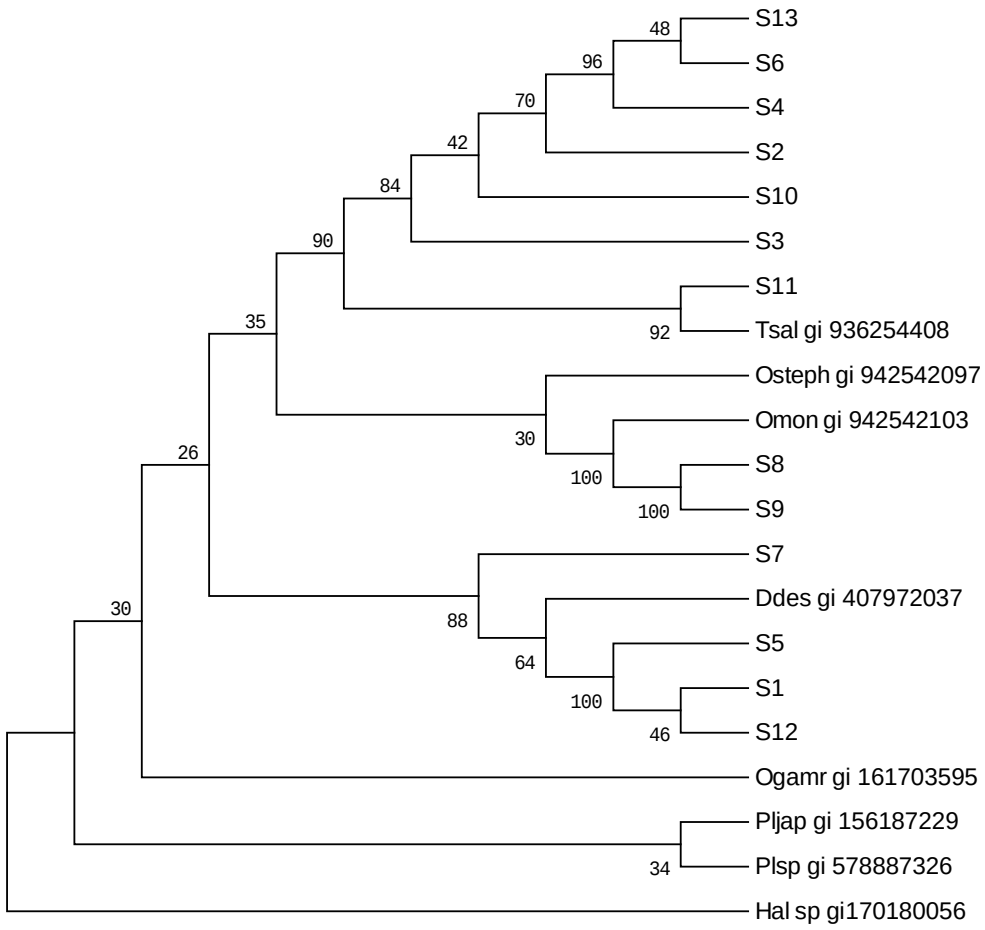


Figure 20. NJ Tree, Bootstrap 1000, Kimura 2-parameter model, Gamma distributed with gamma parameter 1.33, partial deletion

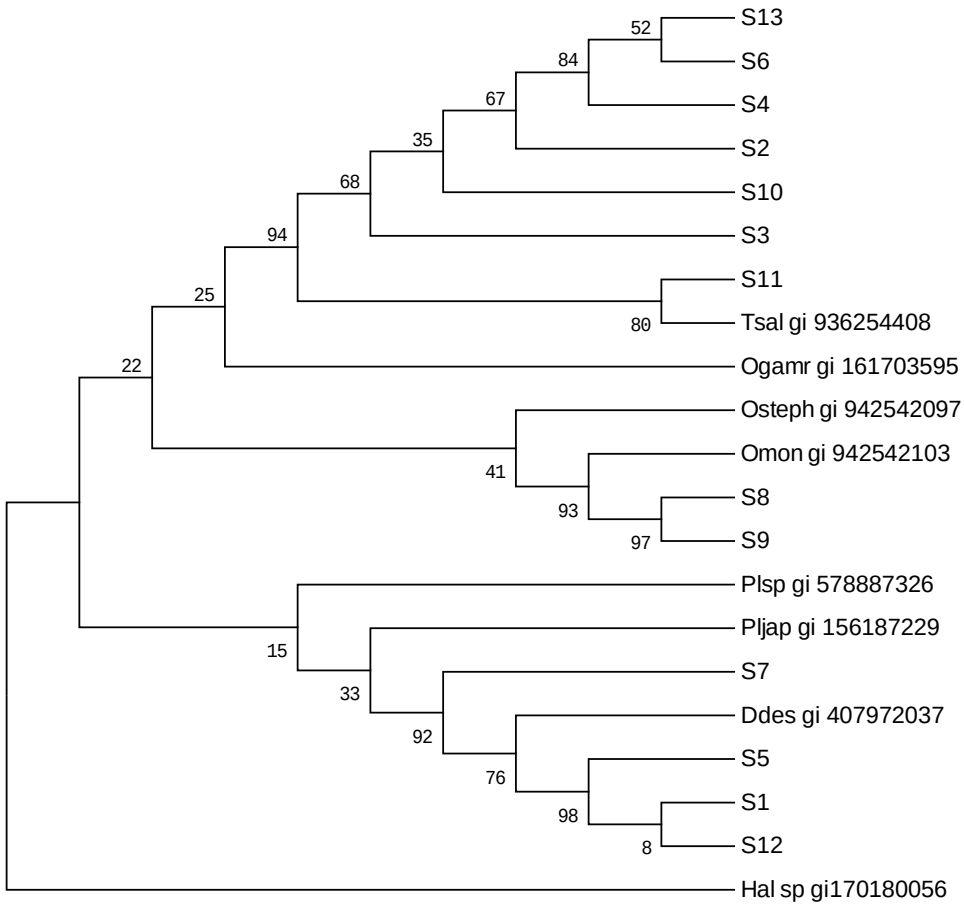


Figure 21. ML Tree, Bootstrap 1000, Jukes-Cantor model, complete deletion

Substrate characterization

The set of beaches included a variety of substrates, often with coarse fractions, which can be defined as "gravel" [Blott and Pye, 2001] *(~~ref. Table 1~~Fig. 8)*. The condition of stranded wrack was rare, and we did not find deposit freshly stranded, indicating an ephemeral presence of wrack and consequent organic input to the supralittoral. Nevertheless, the captures via pitfall traps were enough to allow statistical analyses on both oniscid isopods and talitrid amphipods.

Models for Presence/Absence of Isopods and Amphipods

The estimate of correlation identified trends of -0.5432~~negative~~ correlation of beach width with slope ~~(0.5432)~~; 0.2058 correlation of penetrability with slope ~~(0.2058)~~; 0.1139 correlation of penetrability with width ~~(0.1139)~~. Only the two variables width and slope were found significantly related (as for the oceanic model, beaches with shorter supralittoral zone are also characterised by steeper slopes), even though slightly as statistical value, but such correlation was coming out more and more when testing the models. We had to use "beach width" only as variable to model, knowing that whenever "width" resulted significant, this data also included the participation of slope in terms of information

provided. Also, the factor “North/South” coast resulted significant in further analysis, so it is included in the width and slope graph.

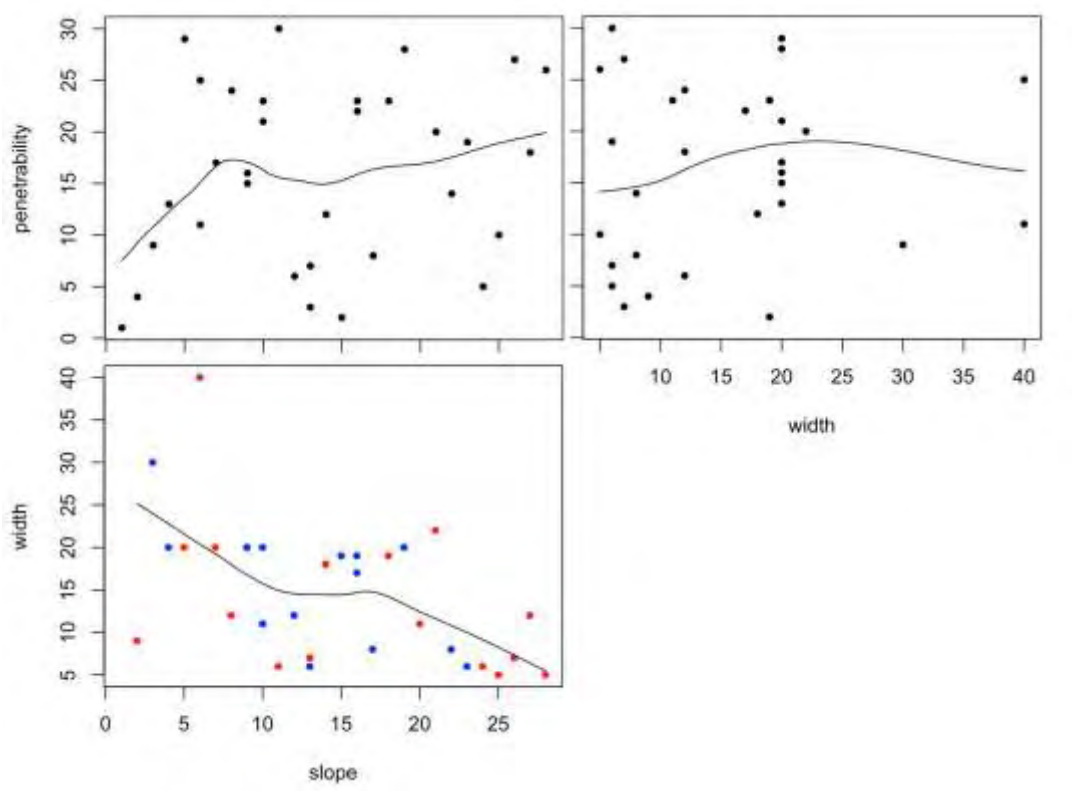


Figure 22. Correlation analysis (pairwise correlation) of physical variables measured at the time of the sampling. “North/South exposure” is here highlighted in color: blue dots indicate beaches exposed to North and red dots beaches exposed to South.

The presence of one taxon was not found to affect the other one: odds-ratio for the association between isopods and amphipods is about 0.5 (a significant association would be close to 1). On these assumptions of independence of the variables two different models were obtained, one for isopods and one for amphipods.

Model for talitrid amphipod presence/absence.

Starting model:
Amphipod presence ~ beach width + beach slope + penetrability + Mz + North/South exposure + substrate type AIC=38.55
Best model:
amphipod ~ beach width* + Mz + substrate type AIC=36.05
* = p = 0.05

The presence of amphipods is then associated with beach width: the larger the width the higher the probability of finding amphipods, for any value of Mz and any substrate type. Specifically, substrate type i.e. the presence of cobbles or sand appears to be not significant (yet they contribute to explain variability, because they were retained into the best model). This model includes both ecological categories of sand-hoppers and beach-hoppers and indicates the taxa as capable to inhabit a wide range of physical conditions, likely thanks to high mobility and plasticity [Brown 1996; Scapini, 2006]. Talitrids are a very successful group, being dominant in abundance on temperate beaches.

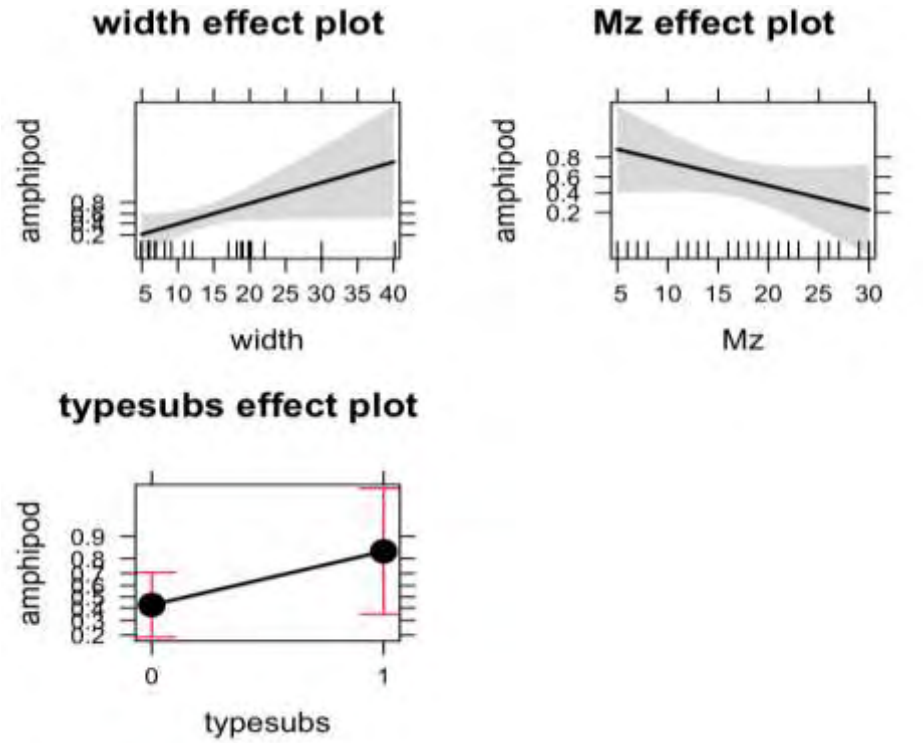


Figure 23. Plots of variables retained by the model for amphipods'presence. Substrate type = 0 indicates sandy substrate; substrate type = 1 indicates cobbles or mixed substrates.

Model for oniscid isopod presence/absence.

Starting model:
isopods ~ width + slope + penetrability + Mz + Nort/South exposure + substrate type AIC=33.08
Best model:
isopods ~ penetrability* + North/South exposure* AIC=27.32

* = 0.01 < p < 0.05

Presence of isopods resulted associated with penetrability and North-South orientation of the coastline: the probability of finding isopods decreases with increasing substrate penetrability (meaning lower compaction due to the combined effects of grain size and water content) and is larger in the South.

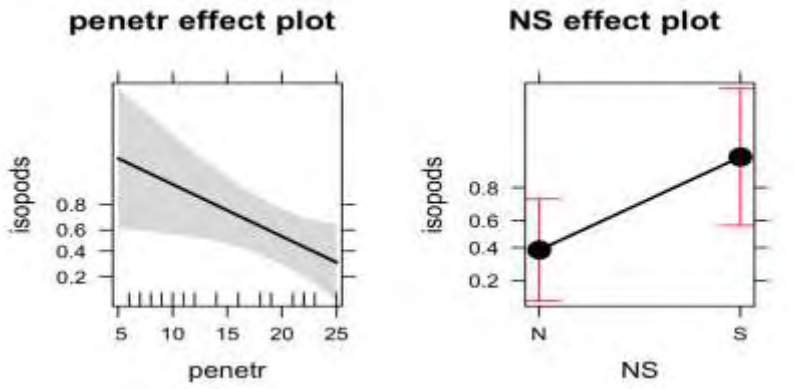


Figure 24. Plots of variables retained by the model for ispods'presence.

Different models for ecological categories (sand-hoppers and beach-hoppers for talitridae; *Tylos ponticus* and *T. europaeus* for oniscidae) were obtained:

Best model for sand-hoppers

Sand-hoppers ~ Mz* + North/South exposure

* = 0.01 < p < 0.05

That is, the presence of sand-hoppers is affected by mean grain size of the sand component of the substrate. Sand-hoppers' presence decreases when grain size gets coarser. Not significantly, but their presence also increases ~~in~~in the Southern coast.

Best model for beach-hoppers

Beach-hoppers ~ beach width* + North/South exposure

The presence of beach--hoppers is related to beach width, increasing on wider beaches. Not significant, but also decreases -in the Southern coast.

Best model for *Tylos ponticus*

Tylos ponticus ~ Mz + North/South + substrate type

None of the variables resulted significant, but this is still the best model. The species' presence tends to increase with coarser Mz, gravel substrate, in the South coast.

Best model for *Tylos europaeus*

Tylos europaeus ~ Mz* + -North/South*

* = 0.01 < p < 0.05

Both variables significantly define presence of *T. europaeus* associated with lower grain size and South coast.

Human Indexes

Finally, the biodiversity records obtained through this study allowed us to complete the information needed for the estimate of human use indices (CI and RI): part of CI score is related to species diversity which can be found on the beach, we consequently scored CIs on the basis of the species' number found.

Table 120. Calculating the factors that form the total value of CI and RI in every beach. (includes our contribution to "Macrobenthic diversity and abundance")

Site	(CI) Dunes	(CI) Endangered and iconic species	(CI) Macrobenthic diversity and abundance	(RI) Infrastructures	(RI) Safety and health	(RI) Physical carrying capacity
Paleokastro	0	0	2	4	2	0
Fodele	0	0	1	2	1	0
Pachia Ammos	0	0	2	1	1	0
Voulisma	2	0	0	4	2	1
Arina	1	0	1	5	3	1
Amoudara	0	0	0	2	1	1
Kokkini Hani	0	0	2	3	2	0
Panormos	0	0	1	4	2	0
Geropotamos	2	0	1	2	2	1
Geropotamos river	2	0	2	2	2	1
Skaleta	3	2	1	1	3	1
Petres	2	0	2	1	3	1
Georgioupoli	3	0	1	5	3	2
Marathi	0	0	1	4	3	0
Loutraki	0	0	1	4	3	0
Stavros	0	0	1	5	3	0
Kalathas	0	0	1	5	3	0
Maleme	0	0	2	5	3	1
Maleme wrack	0	0	1	5	3	1
Platanias	0	0	2	3	3	1
Agia Marina	0	0	1	4	3	1
Iera diving	0	0	2	3	3	1
Iera1	0	0	1	2	3	0
Iera2	0	0	1	0	3	0
Iera3	0	0	2	0	2	0
Agios Panteleimonas	0	0	1	3	3	1
Kalamokania	0	0	1	3	2	1
Dlaskari	3	0	1	1	2	1
Kaloi Limenes	1	0	1	1	3	1
Kommos	1	2	2	3	3	2
Matala	0	2	0	5	2	0
Red Beach	0	0	1	0	3	0
Orthi Ammos sand	1	0	2	1	3	1
Orthi Ammos cobbles	1	0	2	1	3	1
Lakkoi	0	0	1	2	2	0
Frangokastelo 2	1	0	1	3	2	1
Frangokastelo 1	2	0	2	2	3	1
Ilingas	0	0	1	2	3	0

Table 1~~1~~3. Total values of CI and RI and the outcome that occurs according to ~~Figure 2~~Figure 13. [McLachlan et al 2013]

Site	*Total CI*	*Total RI*	*Outcome*
Paleokastro	2	6	multiple use
Fodele	1	3	limited use
Pachia Ammos	2	2	limited use
Voulisma	2	7	intensive recreation
Arina	2	9	intensive recreation
Amoudara	0	4	multiple use
Kokkini Hani	2	5	multiple use
Panormos	1	6	intensive recreation
Geropotamos	3	5	multiple use
Geropotamos river	4	5	multiple use
Skaleta	6	5	multiple use
Petres	4	5	multiple use
Georgioupoli	4	10	intensive recreation
Marathi	1	7	intensive recreation
Loutraki	1	7	intensive recreation
Stavros	1	8	intensive recreation
Kalathas	1	8	intensive recreation
Maleme	2	9	intensive recreation
Maleme wrack	1	9	intensive recreation
Platanias	2	7	intensive recreation
Agia Marina	1	8	intensive recreation
Iera diving	2	7	intensive recreation
Iera1	1	5	multiple use
Iera2	1	3	limited use
Iera3	2	2	limited use
Agios Panteleimonas	1	7	intensive recreation
Kalamokania	1	6	intensive recreation
DIaskari	4	4	multiple use
Kaloi Limenes	2	5	multiple use
Kommos	5	8	multiple use
Matala	2	7	intensive recreation
Red Beach	1	3	limited use
Orthi Ammos sand	3	5	multiple use
Orthi Ammos cobbles	3	5	multiple use
Lakkoi	1	4	multiple use
Frangokastelo 2	2	6	multiple use
Frangokastelo 1	4	6	multiple use
Ilingas	1	5	multiple use

DISCUSSION

Biodiversity

This preliminary checklist led us to document the first record of the talitrid *Deshayesorchestia deshayesi* and of the oniscid *Tylos europaeus* on the island of Crete. Their distribution in the Mediterranean is known ~~and they are not considered to be invasive species~~. The type locality of *Deshayesorchestia deshayesi* is in Egypt (AphiaID: 236548 - marinespecies.org) and there is proof of its occurrence in Greece, but not in Crete so far. Similarly, type locality for *Tylos europaeus* is the Mediterranean (Taxonomic Serial No.: 597553 – itis.gov) [Hurtado et al, 2014].

The fragmentation of beaches and their diverse substrate was likely the driving force for diversity. The two taxa appear to be good indicators of ecological conditions when used as combined indicators [Gonçalves et al., 2013], which was also proven here through their contribution to calculate the Conservation Index. The study was planned to provide a checklist, but a first step was represented by the correct identification of species. Nevertheless, it is worth pointing the diversity of species found on the small spatial scale of Cretan beaches, likely due to high diversity of substrates, which is a Mediterranean characteristic [Gauci et al., 2005]. The sampling method (pitfall traps) may have selected species having mainly surface activity, i.e. beach-hopper, though Fanini & Lowry (2016) demonstrated that recreational use of the beaches can affect talitrid composition. In fact, sand-hoppers (substrate modifiers) appeared to be more sensitive than beach-hoppers (non-substrate modifiers) to such kind of bioturbation [Fanini & Lowry, 2016]. The above remark could explain why the human-frequented beaches of Crete Island preserve such a low presence of species. Notwithstanding this preliminary data collection, future investigations will need to assess the effective biodiversity of groups of organisms on the island. In this regard, this contribution is part of a study into species diversity of the North and South of Crete; subsequent research can only enhance our knowledge in this area.

An ecological study should be repeated across seasons, to see if the wrack or the physical characteristics of the beach are permanent. Although we did not proceed to such an approach in the current project, an extended yearly research could be a future plan.

Comparison between morphological and barcoding identification

It is not accurate to use the definition of species because the identity of barcoding results is less than 97% in all of the specimens. Therefore, we ~~will~~ can use the term OTU (operational taxonomic unit) when we refer to the identified individuals or group~~.~~. During Barcoding analysis, we also faced the problem that there were not enough data in the database to ensure the precision of our results.

The characterization based on the morphology of talitridae was highly difficult and inaccurate, as the animal must be male, mature and intact in order to be identified. The

logical conclusion was to try and increase the precision of the identification through molecular techniques, sequencing and barcoding. In this study, we compared DNA barcode-suggested species (biospecies) identification with morphology-based species (morphospecies) identification in the talirid fauna of Cretan coasts. Therefore, there is a need to examine how many specimens were found concordant in morphology and COI, how many discordant [Lobo et al. 2016]. We will also compare the resulted species of each method when they are different and conclude to the most possible one, if possible.

The final dataset *(Table 9~~12~~)* is comprised by 38 sites, in 25 of which there was presence of talitrids and in other 25 there was presence of oniscids.

There were 10 cases with concordant specimens, since the identification result of species was the same by using both techniques. In 7 of them occurred *T. saltator*, in 2 sites occurred *D. deshayesii* and in one site occurred *O. montagui*. These results indicate that *T. saltator* is really well established around several beaches of Crete.

Moreover, 5 specimens appeared to be discordant and the two methods argue in an identification level. In 3 of these specimens barcoding resulted in *O. montagui*, while *O. stephenseni* originated from morphology. According to the grouping trees *(Fig. ~~16~~20, ~~17~~21)* the sample S8 that represent these sites, are closer to *O. montagui*, which was a result of molecular identification.

One specimen came out as *O. montagui* by barcoding identification, instead of morpological identification that concluded in *O. gamarellus*. In correspondence with the grouping trees *~~(Fig. 16, 17)~~ (Fig. 20, 21)*, sample S8 that includes a specimen form this site is closer to *O. montagui*, which agrees again with the molecular identification results.

Additionally, one specimen was identified as *Platorchestia sp.* by the barcoding analysis and *D. deshayesii* by the morphological analysis. The sample S7 contains the relevant specimen, and it is obvious from the grouping trees *(Fig. 20, 21)~~(Fig. 16, 17)~~* that is closer to *D. deshayesii*, something that matches the morphological identification. In this particular example the result that occurred from Barcoding is not trustworthy at all, since the Identiy is 83% *(Table ~~10~~7)*.

Furthermore, there were 9 sites with singleton specimen identifications. Three of them were not sequenced, because none of the two extraction techniques (TNES-Urea and Mini kit) worked, thus we were not able to detect DNA after PCR. In this case, there may be some problem with the components of the extraction or the amplification procedure. The specimens of the other two sites were not identified with the morphological analysis, because there were no mature males. Finally, there was only one specimen that showed no significant results in any of the methods, since it was both impossible to be identified and to be sequenced.

These results by their own are not enough to inform us that one of these methods is better than the other, but practically molecular identification offered more opportunities to get a result, because the procedure could be repeated with the other half of the animal or even a new one. In the other hand, morphological identification does not offer this chance and if

the animals are juveniles or females then it is almost impossible to identify them down to species level.

T. saltator can be identified based solely on the presence of undeveloped granthopod in male species, which has the same size with the female second gnathopod, and can be a key character that makes the morphological recognition of this species relatively simple. On the other hand, the rest of the species found in Crete display bigger second gnathopods in males instead of females. However, according to our results, detailed morphological and molecular studies are necessary to assess whether these characteristics belong to one significant species. A morphological examination of the specimens may be required to discard possible misidentifications and compare the results from the two identification methods.

The sand-hopper *Talitrus saltator* displays an interesting and unique pattern of divergence within this dataset, so it is important to examine this species individually. Since *T. saltator* is a widespread and ecologically relevant species that is common in sandy beaches across Europe and is also an important environmental indicator [Fanini et al. 2005], a re-appreciation of its taxonomic (both morphological and genetic) diversity across Europe is important. *T. saltator* is also known for clustering at very little distance, such as 1 km on a continuous shore [Ketmaier et al., 2010], while other genera can be less differentiated [Pavesi et al., 2013]. Again, this relates to their ecology and the probability of being accidentally carried along surface currents [Fanini and Lowry, 2014]. This pattern could affect the trees, but there is no data indicating that it does.

By increasing the number of sequences in the DNA barcode library can help to discard congeneric misidentifications which, together with the ecological role of talitrids (e.g. important prey for many species), calls for a deeper investigation of the cryptic diversity. As far as the molecular identification is concerned, the high number of species in this genus, morphological identification also requires a good level of expertise. Due to the low number of specimens and populations investigated no definitive conclusions can be drawn by neither of the methods. All the species require deeper examination of their taxonomic status. More definitive conclusions about the species status and levels of hidden diversity requires not only a much wider spatial sampling, but also the focused collection of further morphological, molecular, and ecological evidence from the target species.

Models for Presence/Absence of Isopods and Amphipods

The presence of two main groups of peracarids considered, Talitrid Amphipods and Oniscid Isopods were found not related to each other, since we the odds-ratio for their association was about 0.5 This is in alignment with the overall hypothesis of autecology of sandy beach species, depicted at macroscale level [McLachlan et al., 1993; Defeo ~~and~~ & McLachlan, 2005]: physical constraints on beaches are the main drivers of diversity patterns, leaving limited possibilities for interaction between different species. Interestingly, what was observed and described across latitudes, here can be found within a single island.

The distribution of our data related to the physical environment tend to the relations observed in oceanic beaches: reflective shorter beaches with steeper slopes and dissipative

wide and flat beaches, however the patterns for perecarids presence are rather related to local substrate characteristics and North/South exposure of the coastline, with the only exception of beach-hoppers, found positively related to the width of the beach. This highlights the relevance, in our case, of other physical variables than the ones found to be main drivers on the oceanic beaches, especially in the macrotidal regime of the Mediterranean Sea. Furthermore, a finer description of presence/absence patterns was provided by the consideration of ecological categories of both talitrids and oniscidans, each one including different species as the case of talitrids, or a single species, as the case of oniscidean Tylids.

Talitrids are found independent on any of the immediate variables measured, although they were present on 25 out of 38 beaches *(Table ~~12~~9)*. This indicates a very successful group, an r-strategist species capable to cope with a variety of conditions. Beach-hoppers were found in 9 sites and sand-hoppers (containing only *T. saltator*) in 9 different sites *(Table 10)*. The deconstruction into the two ecological categories of sand-hoppers and beach-hoppers indicates mean grain size of sand substrates as limiting factor for the presence of sand-hoppers (substrate modifier, burrowing in the sand): with coarser grain size, the other category might be favoured. Wider beaches are likely to host beach-hoppers instead. The two categories were found to follow opposite trends with respect to beach exposure: sand-hoppers tend to be more present on Northern shores, while beach-hopper presence tends to increase on Southern shores. This might be related to their ecology as well: burrowing in the substrate could result more adaptive on beaches subject to frequent storms caused by North-western winds, while this stressor is not present on Southern shores, allowing beach-hoppers to establish populations.

~~*Table 12. Seperation of talitrids in beach-hoppers and sand-hoppers.*~~

~~*Site*~~	~~*Talitridae*~~	~~*Ecological categories*~~
~~Paleokastro~~	~~*Orchestia sp.*~~	~~*Beach-hopper*~~
~~Fodele~~	~~*Deshayesorchestia deshayesii*~~	~~*Beach-hopper*~~
~~Pachia Ammos~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Arina~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Kokkini Hani~~	~~*Deshayesorchestia deshayesii*~~	~~*Beach-hopper*~~
~~Geropotamos river~~	~~*Orchestia montagui*~~	~~*Beach-hopper*~~
~~Georgioupoli~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Marathi~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Stavros~~	~~*Orchestia sp.*~~	~~*Beach-hopper*~~
~~Maleme~~	~~*Orchestia sp.*~~	~~*Beach-hopper*~~
~~Maleme wrack~~	~~*Orchestia sp.*~~	~~*Beach-hopper*~~
~~Platanias~~	~~*Deshayesorchestia deshayesii*~~	~~*Beach-hopper*~~
~~Iera diving~~	~~*Deshayesorchestia deshayesii*~~	~~*Beach-hopper*~~
~~Diaskari~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Orthi Ammos sand~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Orthi Ammos cobbles~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Frangokastelo 2~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~
~~Frangokastelo 1~~	~~*Talitrus saltator*~~	~~*Sand-hopper*~~

Penetrability is the resultant of substrate size and its saturation in water [McLahlan and Brown, 2006]. Grain size and water saturation were found relevant for cirolanidae, that live on the low part of the supralittoral, around the swash zone [Yannicelli et al., 2002]. Even though the water content is less important on the supralittoral, the combined information offered by penetrability estimates resulted to be a good parameter to explain the presence of Tylidae. The presence of Tylidae was separated and we found *T. europaeus* in 10 sites, while *T. ponticus* in 11 sites *(Table 11)*. Substrate characteristics were found significant to explain isopod presence, consistently in the literature [Montesanto et al., 2014] reporting substrate specificity for fine sand for *T. europaeus* and cobbles for *T. ponticus*. Isopods' presence is otherwise independent of beach width and slope, given penetrability. These two species have different ecologies, which may enable their coexistence in the same regions, but not in the same microhabitat: *T. europaeus* occurs in fine grain sandy beaches, whereas *T. ponticus* inhabits coarse sand or pebble beaches (although sand is expected under cobbles). It is therefore possible that their divergence was associated with ecological speciation, and further studies on the topic are ongoing. *Tylos europaeus* appears to be competitively excluded from very coarse-grained beaches, whereas *T. ponticus* can tolerate a broader range of sediment grain sizes [Hurtado et al, 2014].

~~*Table 13. Seperation of Tylids (*T. ponticus, T. Europeaus*).*~~

~~*Site*~~	~~*Tylid species*~~
~~Pachia Ammos~~	~~*Tylos europaeus*~~
~~Petres~~	~~*Tylos europaeus*~~
~~Loutraki~~	~~*Tylos europeus*~~
~~Maleme~~	~~*Tylos ponticus*~~
~~Maleme wrack~~	~~*Tylos ponticus*~~
~~Platanias~~	~~*Tylos ponticus*~~
~~Agia Marina~~	~~*Tylos europaeus*~~
~~Iera diving~~	~~*Tylos ponticus*~~
~~Ierapetra 1~~	~~*Tylos ponticus*~~
~~Ierapetra 2~~	~~*Tylos ponticus*~~
~~Ierapetra 3~~	~~*Tylos ponticus*~~
~~Agios Panteleimonas~~	~~*Tylos ponticus*~~
~~Kalamokania~~	~~*Tylos ponticus*~~
~~Kaloi Limenes~~	~~*Tylos ponticus*~~
~~Kommos~~	~~*Tylos europaeus*~~
~~Red Beach~~	~~*Tylos europaeus*~~
~~Orthi Ammos sand~~	~~*Tylos europaeus*~~
~~Orthi Ammos cobbles~~	~~*Tylos europaeus*~~
~~Lakkoi~~	~~*Tylos europaeus*~~
~~Frangokastelo 1~~	~~*Tylos europaeus*~~
~~Ilingas~~	~~*Tylos ponticus*~~

Overall, our models indicate the relevance and peculiarity of every single beach, due to the suite of physical characteristics to the identification of diversity patterns. The very same

physical characteristics are also driving their human use, opening opportunities for the consideration of sandy beaches as social-ecological systems.

REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. J. Mol. Biol., 215(3), 403–410.

Akaike, H. (1973). Maximum Likelihood Identification of Gaussian Autoregressive Moving Average Models. Biometrika, 60(2), 255.

Barrett, R. & Hebert, P. (2005). Identifying spiders through DNA barcodes. Canadian Journal of Zoology, 83(3), 481-491.

Bessa, F., Baeta, A., Marques, J.C., (2014a). Niche segregation amongst sympatric species at exposed sandy shores with contrasting wrack availabilities illustrated by stable isotope analysis. Ecol. Indic. 36, 694–702.

Bessa, F., Marques, J.C., Scapini, F., (2014b). Behavioural adaptations of two sympatric sandhoppers living on a mesotidal European Atlantic sandy beach. Estuarine Coastal Shelf Sci 147, 17–24

Blott, S.J., Pye, K., (2001). Gradistat: a grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surf. Proc. Land. 26, 1237–1248.

Borges, L.M.S., Sivrikaya, H., le Roux, A., Shipway, J.R., Cragg, S.M., and Costa, F.O. (2012). Investigating the taxonomy and systematics of marine wood borers (Bivalvia: Teredinidae) combining evidence from morphology, DNA barcodes and nuclear locus sequences. Invertebr. Syst., 26(6): 572–582.

Bousfield, E.L., (1982). The Amphipod superfamily talitroidea in the Northeastern Pacific Region. I. Family Talitridae: systematics and distributional ecology. Natl. Mus. Nat. Sci. Ottawa Publ. Biol. Oceanogr. 11, 1–73.

Bousfield, E.L. (1982). The Amphipod superfamily Talitroidea in the Northeastern Pacific region (1st ed.). Ottawa: National Museums of Canada.

Bousfield, E.L. (1984) Recent advances in the systematics and biogeography of landhoppers (Amphipoda: Talitridae) of the Indo-Pacific Region. Bishop Museum Special Publication, 72, 171–210.

Bousfield, E. & Heard, R. (1986). Systematics, Distributional Ecology, and Some Host-Parasite Relationships of Uhlorchestia uhleri (Shoemaker) and U. spartinophila, New Species

(Crustacea: Amphipoda), Endemic to Salt Marshes of the Atlantic Coast of North America. Journal Of Crustacean Biology, 6(2), 264.

Brereton, J. (1957). The Distribution of Woodland Isopods. Oikos, 8(2), 85.

Brown, A.C., (1996). Behavioural plasticity as a key factor in the survival and evolution of the macrofauna on exposed sandy beaches. Rev. Chil. Hist. Nat. 69, 469–474.

Bui EN, Mazullo J, Wilding LP. (1990). Using quartz grain size and shape analysis to distinguish between aeolian and fluvial deposits in the Dallol Bosso of Niger (West Africa). Earth Surface Processes and Landforms 14: 157–166.

Coleman, C. (2015). Taxonomy in times of the taxonomic impediment – examples from the community of experts on amphipod crustaceans. *Journal of Crustacean Biology*, *35*(6), 729-740.

Colombini, I., Aloia, A., Fallaci, M., Pezzoli, G., & Chelazzi, L. (2000). Temporal and spatial use of stranded wrack by the macrofauna of a tropical sandy beach. Marine Biology, 136(3), 531-541.

Colombini, I., Fallaci, M., Milanesi, F., Scapini, F., Chelazzi, L., (2003). Comparative diversity analysis in sandy littoral ecosystems of the western Mediterranean. Estuarine Coastal Shelf Sci. 58, 93–104

Colombini, I., Fallaci, M., Gagnarli, E., Rossano, C., Scapini, F., Chelazzi, L., (2013). The behavioural ecology of two sympatric talitrid species, *Talitrus saltatory* (Montagu), and *Orchestia gammarellus* (Pallas) on a Tyrrhenian sandy beach dune system. Estuarine Coastal Shelf Sci. 117, 37–47.

Costa, F.O., and Carvalho, G.R. (2010). New insights into molecular evolution: prospects from the Barcode of Life Initiative (BOLI). Theory Biosci., 129(2-3): 149–157.

Crowther, T., Stanton, D., Thomas, S., A'Bear, A., Hiscox, J., & Jones, T. et al. (2013). Top-down control of soil fungal community composition by a globally distributed keystone consumer. Ecology, 94(11), 2518-2528.

Cuttriss, A., Maguire, G., Ehmke, G., & Weston, M. (2015). Breeding habitat selection in an obligate beach bird: a test of the food resource hypothesis. Marine And Freshwater Research, 66(9), 841.

Dale, V.H., Beyeler, S.C., (2001). Challenges in the development and use of ecological indicators. Ecol. Indic. 1, 3–10.

Dallwitz, M.J., and Paine, T.A. (1992). Definition of the DELTA format. http://delta-intkey.com

DAVIS, R. (1984): Effects of weather and habitat structure on the population dynamics of isopods in a dune grassland. – Oecologia (Berlin) 42: 387-395

De Matthaeis, E. (1998). Genetic divergence between populations and species of talitrids from Aegean islands. Journal of Heredity, 89(1), 37-43.

Defeo, O., McLachlan, A., Schoeman, D.S., Schlacher, T.A., Dugan, J., Jones, A., Lastra, M., Scapini, F., (2009). Threats to sandy beach ecosystem: a review. Estuarine Coastal Shelf Sci. 81, 1–12.

Defeo, O. & Gómez, J. (2005). Morphodynamics and habitat safety in sandy beaches: life-history adaptations in a supralittoral amphipod. Marine Ecology Progress Series, 293, 143-153.

Defeo, O. & McLachlan, A. (2005). Patterns, processes and regulatory mechanisms in sandy beach macrofauna: a multi-scale analysis. Marine Ecology Progress Series, 295, 1-20.

Doney, S.C., Ruckelshaus, M., Duffy, J.E., Barry, J.P., Chan, f., English, C.A., Galindo, H.M., Grebmeier, J.M., Hollowed, A.B., Knowlton, N., Polovina, J., Rabalais, N.N., Sydeman, W.J., Talley, L.D., (2012). Climate change impacts on marine ecosystems. Annual Review of Marine Science 4, 11e37

Dugan, J.E., Hubbard, D.M., McCrary, M.D., Pierson, M.O., (2003). The response of macrofauna communities and shorebirds to macrophyte wrack subsidies on exposed sandy beaches of southern California. Estuarine Coastal Shelf Sci. 58, 25–40

Edney E. (1968) Transition from water to land in isopod crustaceans. Am Zool 8(3):309–326

Fanini L. , Cantarino C.M. , Scapini F. (2005). Relationships between the dynamics of two Talitrus saltator populations and the impacts of activities linked to tourism. Oceanologia. 47 (1), 93-112.

Fanini, L., Defeo, O., Do Santos, C., Scapini, F., (2009a). Testing the Habitat Safety Hypothesis with behavioural field experiments: amphipod orientation on sandy beaches with contrasting morphodynamics. Mar. Ecol. Prog. Ser. 392, 133–141

Fanini, L., Marchetti, G.M., Scapini, F., Defeo, O., (2009b). Effects of beach nourishment and groynes building on population and community descriptors of mobile arthropodofauna. Ecol. Indic. 9, 167–178.

Fanini, L. & Lowry, J. (2014). Coastal talitrids and connectivity between beaches: A behavioural test. Journal Of Experimental Marine Biology And Ecology, 457, 120-127.

Fanini, L. & Lowry, J. (2016). Comparing methods used in estimating biodiversity on sandy beaches: Pitfall vs. quadrat sampling. Ecological Indicators, 60, 358-366.

Ferrara F. & Taiti S. (1978). *Pseudodiploexochus Madagascariensis*, New Species of Armadillidae (Terrestrial Isopoda) From Madagascar. Monitore Zoologico Italiano. Supplemento, 10:1, 81-83

Folk, R.L., Ward, W.C., (1957). Brazos river bar: a study in the significance of grain size parameters. J. Sediment. Petrol. 27, 3–26

Friedman GM, Sanders JE. (1978). Principles of Sedimentology. Wiley: New York.

Gambineri, S., Rossano, C., Durier, V., Fanini, L., Rivault, C., Scapini, F., (2008). Orientation of littoral amphipod in two sandy beaches of Brittany (France) with wide tidal excursions. Chem. Ecol. 24, 129–144.

Gauci M.J., Deidun A. and Scheimbri P.J. (2005). Faunistic diversity of Maltese pocket sandy and shingle beaches: are these of conservation value? Oceanologia 47(2), 219–241

Gomes Veloso, V., Soares Caetano, C., & Silva Cardoso, R. (2003). Composition, structure and zonation of intertidal macrofauna in relation to physical factors in microtidal sandy beaches at Río de Janeiro State, Brazil. Scientia Marina, 67(4), 393-402.

Gomez, A., Wright, P.J., Lunt, D.H., Cancino, J.M., Carvalho, G.R., and Hughes, R.N. (2007). Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. Proc. R. Soc. London, Ser. B, 274(1607): 199–207.

Gonçalves, S., Anastácio, P., & Marques, J. (2013). Talitrid and Tylid crustaceans bioecology as a tool to monitor and assess sandy beaches' ecological quality condition. Ecological Indicators, 29, 549-557.

Gongalsky, K., Savin, F., Pokarzhevskii, A., & Filimonova, Z. (2005). Spatial distribution of isopods in an oak–beech forest. European Journal Of Soil Biology, 41(3-4), 117-122.

Gourlay, M.R., 1968. Beach and Dune Erosion Tests. Delft Hydraulics Laboratory, Report No. M935/M936

HADLEY, H. & QUINLAN, M. (1984): Cuticular transpiration in the isopod Porcellio laevis: chemical and morphological factors involved in its control. – Symposia of the zoological Society of London 53: 97-107

Hajibabaei, M., Janzen, D., Burns, J., Hallwachs, W., & Hebert, P. (2006). DNA barcodes distinguish species of tropical Lepidoptera. Proceedings Of The National Academy Of Sciences, 103(4), 968-971.

Hebert, P., Cywinska, A., Ball, S., & deWaard, J. (2003). Biological identifications through DNA barcodes. Proceedings Of The Royal Society B: Biological Sciences, 270(1512), 313-321.

Hebert, P., Penton, E., Burns, J., Janzen, D., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. Proceedings Of The National Academy Of Sciences, 101(41), 14812-14817.

Hoese, B. & H.H. Janssen (1989). Morphological and physiological studies on the marsupium in terrestrial isopods. Monitore Zoologico Italiano 4: 153-173.

Hopkin, S. (1992). A key to the woodlice of Britain and Ireland (1st ed.). Shrewsbury: Field Studies Council.

Hornung E (2011) Evolutionary adaptation of oniscidean isopods to terrestrial life: structure, physiology and behavior. Terr Arthropod Rev 4:95–130

Hubbard, D., Dugan, J., Schooler, N., & Viola, S. (2014). Local extirpations and regional declines of endemic upper beach invertebrates in southern California. Estuarine, Coastal And Shelf Science, 150, 67-75.

Hurley, D. E. (1956). Bathypelagic and other Hyperiidea from California waters. Allan Hancock Foundation, Occas. Papers 18: 1-25.

Hurtado, L., Lee, E., Mateos, M., & Taiti, S. (2014). Global Diversification at the Harsh Sea-Land Interface: Mitochondrial Phylogeny of the Supralittoral Isopod Genus Tylos (Tylidae, Oniscidea). Plos ONE, 9(4), e94081.

Ihnen, K. & Zimmer, M. (2008). Selective consumption and digestion of litter microbes by Porcellio scaber (Isopoda: Oniscidea). Pedobiologia, 51(5-6), 335-342.

Jackson, H. (1941): Check-list of the terrestrial and fresh-water Isopoda of Oceania. – Smithsonian miscellaneous Collections 99, 1-35.

Jaramillo, E., Contreras, H., Duarte, C., Avellanal, M.H., (2003). Locomotor activity and zonation of upper shore arthropods in a sandy beach of north central Chile. Estuarine Coastal Shelf Sci. 58, 177–197

Jass, & Klausmeier,. (2000). Endemics and Immigrants: North American Terrestrial Isopods (Isopoda, Oniscidea) North of Mexico. Crustaceana, 73(7), 771-799.

Kimura, M. (1980). A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16(2): 111–120.

Ketmaier, V., De Matthaeis, E., Fanini, L., Rossano, C. & Scapini, F. (2010) Variation of genetic and behavioural traits in the sandhopper Talitrus saltator (Crustacea Amphipoda) along a dynamic sand beach. Ethology Ecology and Evolution, 22 (1), 17–35.

Lastra, M., Schlacher, T.A., Olabarria, C., (2010). Niche segregation in sandy beach animals: an analysis with surface-active peracarid crustaceans on the Atlantic coast of Spain. Mar. Biol. 157, 613–625.

Lavrov DV, Brown WM, Boore JL (2004). Phylogenetic position of the Pentastomida and (pan)crustacean relationships. Proc R Soc Lond Ser B 271: 537–544.

Leray, M., Yang, J., Meyer, C., Mills, S., Agudelo, N., & Ranwez, V. et al. (2013). A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers In Zoology, 10(1), 34.

Lobo, J., Ferreira, M. S., Antunes, I. C., Teixeira, M. A., Borges, L. M., Sousa, R., et al. (2016). Contrasting morphological and DNA barcode-suggested species boundaries among shallow-water amphipod fauna from the southern European Atlantic coast. Genome, (999), 1-11.

Loureiro, S., Sampaio, A., Brandão, A., Nogueira, A., & Soares, A. (2006). Feeding behaviour of the terrestrial isopod Porcellionides pruinosus Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination. Science Of The Total Environment, 369(1-3), 119-128.

Lowry, J. & Fanini, L. (2013). Substrate dependent talitrid amphipods from fragmented beaches on the norths coast of Crete (Crustacea, Amphipoda, Talitridae), including a redefinition of the genus *Orchestia* and descriptions of *Orchestia xylino* sp. nov. and *Cryptorchestia g*en. nov. Zootaxa, 3709 (3)

Lowry, J. & Myers, A. (2013). A Phylogeny and Classification of the Senticaudata subord. nov. Crustacea: Amphipoda. Zootaxa, 3610(1).

Masselink, G., and Short, A., D. (1993). The Effect of Tide Range on Beach Morphodynamics and Morphology: A Conceptual Beach Model. Journal of Coastal Research, 9 (3), 785-800

Matthaeis, E., Davolos, D., Cobolli, M., & Ketmaier, V. (2000). Isolation by distance in equilibrium and nonequilibrium populations of four Talitrid species in the Mediterranean Sea. Evolution, 54(5), 1606-1613.

McLachlan, A., Defeo, O., Jaramillo, E., Short, A.D., (2013). Sandy beaches conservation and recreation: Guidelines for optimising management strategies for multi-purpose use. Ocean Coast. Manage. 71, 256–268.

McLachlan, A. (1983). Habitat distribution and body size in rain-pool dwellers. Zoological Journal Of The Linnean Society, 79(4), 399-407.

McLachlan, A. & Erasmus, T. (1983). Sandy beaches as ecosystems (1st ed.). The Hague: W. Junk.

McLachlan, A., Jaramillo, E., Donn, T. E., Wessels, F., (1993). Sandy beach macrofauna communities and their control by the physical environment: a geographical comparison. Journal of Coastal Research, 15S, 27-38.

McLachlan, A., Brown, A., & Brown, A. (2006). The ecology of sandy shores (1st ed.). Amsterdam: Academic Press.

McLachlan, A., Defeo, O., (2013). Coastal beach ecosystems. In: Levin, S. (Ed.), Encyclopedia of Biodiversity, second ed. Elsevier, Oxford.

Morino, H. & Miyamoto, H. (1988). Redefinition of Talorchestia (Amphipoda: Talitridae) with Description of a New Species from the Tropical West Pacific. Journal Of Crustacean Biology, 8(1), 91.

Morrison CL, Harvey AW, Lavery S, Tieu K, Huang Y, Cunningham CW (2002). Mitochondrial gene rearrangements confirm the parallel evolution of the crab-like form. Proc R Soc Lond Ser B 269: 345–350.

Mycielska-Dowgiallo, E., (2007). Research methods for textural features of clastic deposits and the significance of interpretational results. In: Research into the textural features of Quaternary sediments and some dating methods (eds. E.Mycielska- Dowgiallo and J. Rutkowski): 95–180. The Family Alliance School of Higher Education Press, Warszawa.

Montesanto, G., Deidun, A., Sciberras, A., Sciberras, J., & Lombardo, B. M. (2014). Current distribution of two species of Tylos (Isopoda: Oniscidea) in the Central Mediterranean and the influence of beach sand grain-size parameters. Journal of Crustacean Biology, 34(1), 47-53.

Pavesi, L., Iannilli, V., Zarattini, P., De Matthaeis, E., (2007). Temporal and spatial distribution of three supralittoral amphipod species on a sandy beach of central Italy. Mar. Biol. 151, 1585–1595.

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.

Pearson, T.H. and R. Rosenberg (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. mar. Biol. A. Rev. *16*, 229–311

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Roniotis, A. (2012). Blue Crete: The Beaches of the Greek Island of Crete (1st ed.). Heraklion.

Sambrook,J. (2000). Molecular Cloning : Laboratory Manual (third edition)

Scapini, F., Buiatti, M. & Ottaviano, O. (1988) Phenotypic plasticity in sun orientation of sandhoppers. Journal of Comparative Physiology A, 163, 739–747.

Scapini, F. (2006) Keynote papers on sandhopper orientation and navigation. Marine and Freshwater Behaviour and Physiology, 39, 73–85.

Schmalfuss, H. (1972): Die Isopoden von Kreta.— Biologia gallo-hellenica 4: 33-60

Schmalfuss, H. (1989): Phylogenetics in Oniscidea. – Monitore zoologico italiano, Nuova Serie, Monografia 4: 3-27.

Schmalfuss, H. & Wolf-Schwenninger, K. (2002). A bibliography of terrestrial isopods (Crustacea: Isopoda: Oniscidea) (1st ed.). Stuttgart: Staatl. Museum für Naturkunde

Schmalfuss, H. (2003). World catalog of terrestrial isopods (Isopoda:Oniscidea) (1st ed.). Stuttgart: Staatliches Museum für Naturkunde.

Schmalfuss, H., Paragamian, K. & Sfenthourakis, S. (2004): The terrestrial isopods (Isopoda: Oniscidea) of Crete and the surrounding islands. – Stuttgarter Beiträge zur Naturkunde, Serie A, 662: 74 pp

Schlacher, T.A., Schoeman, D.S., Dugan, J., Lastra, M., Jones, A., Scapini, F., McLachlan, A., (2008). Sandy beach ecosystems: key features, sampling issues, management challenges and climate change impacts. Mar. Ecol. 29, 70–90.

Schlacher, T., Lucrezi, S., Connolly, R., Peterson, C., Gilby, B., & Maslo, B. et al. (2016). Human threats to sandy beaches: A meta-analysis of ghost crabs illustrates global anthropogenic impacts. Estuarine, Coastal And Shelf Science, 169, 56-73.

Schmidt, C. (2008) Phylogeny of the Terrestrial Isopoda (Oniscidea): A Review. Arthropod Systematics & Phylogeny, 66 (2), 191 – 226

Schoeman, D., Schlacher, T., & Defeo, O. (2014). Climate-change impacts on sandy-beach biota: crossing a line in the sand. Global Change Biology, 20(8), 2383-2392.

Shachak, M., Chapman, E., & Steinberger, Y. (1976). Feeding, energy flow and soil turnover in the desert isopod, Hemilepistus reaumuri. Oecologia, 24(1), 57-69.

Short, F. & Wyllie-Echeverria, S. (1996). Natural and human-induced disturbance of seagrasses. Environmental Conservation, 23(01), 17.

Smith, M., Rodriguez, J., Whitfield, J., Deans, A., Janzen, D., Hallwachs, W., & Hebert, P. (2008). Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proceedings Of The National Academy Of Sciences, 105(34), 12359-12364.

Sofianos, S., Johns, W., Lascaratos, A., Murray, S., Olson, D., & Theocharis, A. (2002). Draft Report of the Aegean Sea Workshop. Office of Naval Research.

Syvitski, J. (1991). Towards an understanding of sediment deposition on glaciated continental shelves. Continental Shelf Research, 11(8-10), 897-937.

Theocharis, A., Balopoulos, E., Kioroglou, S., Kontoyiannis, H., & Iona, A. (1999). A synthesis of the circulation and hydrography of the South Aegean Sea and the Straits of the Cretan Arc (March 1994–January 1995). Progress In Oceanography, 44(4), 469-509.

Vandel, A. (1943) Essai sur l'origine, l'évolution et la classification des Oniscoidea. Bull Biol France Belge 30:1–143

Vandel, A. (1945a): Isopodes terrestres récoltés par M. REMY au cours de son voyage en Corse. III. La famille des Buddelundiellidae. – Archives de Zoologie expérimentale et générale 84: 100-113.

Van Wensem, J., Verhoef, H., & Van Straalen, N. (1993). Litter degradation stage as a prime factor for isopod interaction with mineralization processes. Soil Biology And Biochemistry, 25(9), 1175-1183.

Veloso, V. & Cardoso, R. (2001). Effect of morphodynamics on the spatial and temporal variation of macrofauna on three sandy beaches, Rio de Janeiro State, Brazil. Journal Of The Marine Biological Association Of The UK, 81(03), 369.

Warburg, M. (1993): Evolutionary Biology of Land Isopods, 159 pp.; Heidelberg.

Ward, R., Zemlak, T., Innes, B., Last, P., & Hebert, P. (2005). DNA barcoding Australia's fish species. Philosophical Transactions of The Royal Society B: Biological Sciences, 360(1462), 1847-1857.

Wilson, G.D.F. (2007). Global diversity of Isopod crustaceans (Crustacea; Isopoda) in freshwater. Hydrobiologia, 595(1), 231-240.

Wilson, G.D.F. (2009). The Phylogenetic Position of the Isopoda in the Peracarida (Crustacea: Malacostraca). Arthropod Systematics & Phylogeny, 67 (2) 159 – 198

Yannicelli, B., Palacios, R., & Giménez, L. (2002). Swimming ability and burrowing time of two cirolanid isopods from different levels of exposed sandy beaches. Journal Of Experimental Marine Biology And Ecology, 273(1), 73-88.

Zimmer, M. & Topp, W. (2002): The role of coprophagy in nutrient release from feces of phytophagous insects. – Soil Biology and Biochemistry 34: 1093-1099.

APPENDIX

The following tables are summarizing the physical characterization of the beaches we sampled: Site (toponym), Coordinates, Exposure (North or South), Wrack, Width, Slope, Penetrability, Substrate. The sites are separated in two different tables from North to South.

SITE	EXPOSURE	AMPHIPOD/ISOPOD PRESENCE	WRACK PRESENCE	WIDTH	SLOPE^{0} (AVERAGE)	PENETRABILITY (AVERAGE)	MZ	%SUBSTRATE >4MM
IERA DIVING N35°00.075'E25°46.160'	S	Both	Y-20cm	40	1.9	4.2	1.820	>5%
IERA1 N35°00.050'E25°46.237'	S	Oniscids	N	22	5.9	5.7	1.016	<5%
IERA2 N35°00.016'E25°46.293'	S	Oniscids	N	5	7.0	3.7	1.057	>5%
IERA3 N35°00.003'E25°46.325'	S	Both	N	7	7.6	7	1.565	>5%
AGIOS PANTELEIMONAS N35°01.792'E25°54.719'	S	Oniscids	N	6	6.7	2.6	1.812	>5%
KALAMOKANIA N35°02.127'E25°58.306'	S	Oniscids	Y-40cm	12	8.8	5.4	NA	all substrate> 1,4mm
DIASKARI N35°01.989'E25°59.628'	S	Talitrids	N	40	1.9	6.7	0.453	>5%
KALOI LIMENES N34°56.169'E24°48.547'	S	Oniscids	Y-1m	18	3.5	4.5	1.741	>5%
KOMMOS N35°00.762'E24°45.603'	S	Oniscids	N	20	1.4	7.3	0.694	<5%
MATALA N34°59.561'E24°44.896'	S	None	N	5	9.5	6.8	0.494	<5%
RED BEACH N34°59.185'E24°44.962'	S	Oniscids	N	6	2.8	8.7	0.508	<5%
ORTHI AMMOS SAND N35°10'59.69'E24°14'39.49'	S	Both	N	9	-0.2	2.5	0.213	<5%
ORTHI AMMOS COBBLES N35°10'58.94'E24°14'44.95'	S	Both	N	7	3.3	2.4	0.263	>5%
LAKKOI N35°10.784'E24°16.248'	S	Oniscids	N	20	2.0	5.3	0.740	<5%
FRANGOKASTELO 2 N35°10.869'E24°14.118'	S	Talitrids	N	12	2.1	6.6	0.269	<5%
FRANGOKASTELO 1 N35°11.301'E24°13.266'	S	Both	N	19	4.6	6.4	0.610	<5%
ILINGAS N35°12.131'E24°07.429'	S	Oniscids	N	11	5.6	NA (cobbles)	1.751	>5%

SITE	EXPOSURE	TALITRID/ONISCID PRESENCE	WRACK PRESENCE	WIDTH (M)	SLOPE° (AVERAGE)	PENETRABILITY (AVERAGE)	MZ	%SUBSTRATE >4MM
PALEOKASTRO N35°21.963'E25°02.328'	N	Both	Y-2cm	19	4.1	1.2	0.722	>5%
FODELE N35°24.233'E24°57.673'	N	Talitrids	N	12	3.2	2.8	0.109	<5%
PACHIA AMMOS N35°06.564'E25°48.575'	N	Both	Y -spread	NA	NA	NA	0.315	<5%
VOULISMA N35°07.520'E25°44.571'	N	None	Y	7	NA (cobbles)	NA (cobbles)	NA (cobbles)	>5%
ARINA N35°19.824'E25°14.129'	N	Talitrids	Y	29	0.8	NA	NA	NA
AMOUDARA N35°20.279'E25°05.853'	N	None	Y-30cm	26	3.4	NA	NA	NA
KOKKINI HANI N35°19'55.31'E25°15'18.40'	N	Both	Y -2m	8	6.0	4.8	NA	NA
PANORMOS N35°25'7.68'E24°41'17.63'	N	Oniscids	Y -3 m	11	2.6	6.4	0.306	<5%
GEROPOTAMOS N35°24.803'E24°38.689'	N	Talitrids	Y -2m dry bamboo	30	0.7	3.56	2.236	>5%
GEROPOTAMOS RIVER N35°24.803'E24°38.689'	N	Both	NA	NA	NA	NA	NA	NA
SKALETA N35°23.475'E24°36.517'	N	Talitrids	Y -30 cm on strandline	17	4.2	6.11	0.741	<5%
PETRES N35°21'15.16'E24°21'52.81'	N	Both	NA	NA	NA	NA	1.122	<5%
GEORGIOUPOLI N35°21.402'E24°16.717'	N	Talitrids	N	20	2.4	5	0.464	<5%
MARATHI N35°30.333'E24°10.544'	N	Talitrids	N	6	6.3	5.62	0.243	<5%
LOUTRAKI N35°29.954'E24°09.843'	N	Oniscids	N	8	4.5	3.5	0.224	<5%
STAVROS N35°35.449'E24°05.769'	N	Talitrids	N	20	5.1	7.1	0.864	<5%
KALATHAS N35°33.300'E24°05.228'	N	Oniscids	Y -spread	6	3.3	3.25	0.205	<5%
MALEME N35°31.550'E23°50.941'	N	Both	Y-40cm	20	2.4	5.2	NA	>5%
MALEME WRACK N35°31.550'E23°50.941'	N	Both	NA	NA	NA	NA	NA	NA
PLATANIAS N35°31.256'E23°53.348'	N	Both	N	20	1.1	4.54	0.969	>5%
AGIA MARINA N35°31.157'E23°55.175'	N	Talitrids	Y -1m	19	4.2	6.4	1.296	<5%