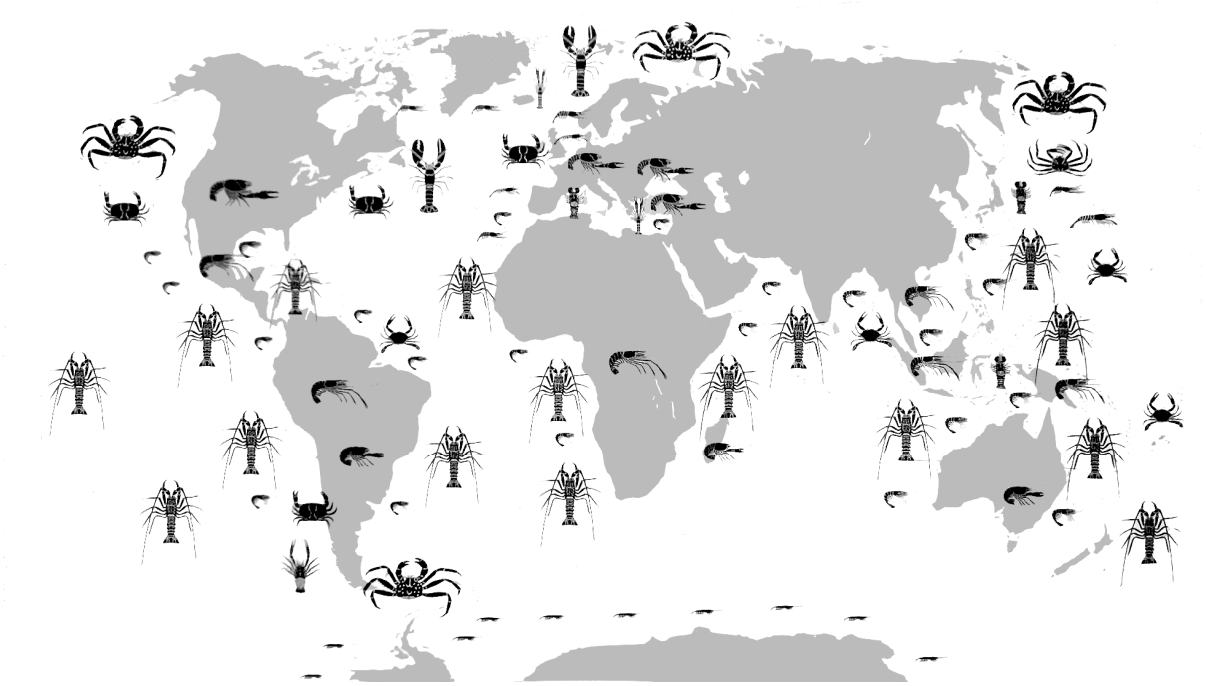
DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER NATURWISSENSCHAFTEN
(DR. RER. NAT.) DER NATURWISSENSCHAFTLICHEN FAKULTÄT III –
BIOLOGIE UND VORKLINISCHE MEDIZIN DER UNIVERSITÄT REGENSBURG

Comparative phylogeographic studies of three marine and one amphidromous decapod species clarifying the mechanisms of generation and maintenance of genetic diversity and identifying cryptic species



vorgelegt von
Silke Reuschel aus Kulmbach
Februar 2008

Promotionsgesuch eingereicht am 16.11.2007

Die Arbeit wurde angeleitet von Dr. C. Schubart

Prüfungsausschuss: Vorsitzender: Prof. Dr. S. Schneuwly

1. Prüfer: Dr. C. Schubart

2. Prüfer: Prof. Dr. J. Heinze

3. Prüfer: Prof. Dr. C. Oberprieler

TABLE OF CONTENTS

GENERAL INTRODUCTION

Phylogeography

In the era of molecular phylogenetics one of the first aims was to determine simultaneously the phylogenetic and geographical relationships among different mtDNA haplotypes or sequences (Hedrick 2005). Avise (2000) termed the joint use of phylogenetic techniques and spatial distributions phylogeography and defined it as the "field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages" and suggested that "time and space are the jointly axes of phylogeography onto which are mapped particular gene genealogies". This means that the most distant populations in space and populations that diverged the longest time ago should accumulate differences. The level of genetic differentiation between populations depends on different gene flow patterns (Hedrick 2005). Gene flow can be described by different models based on the population structure of a species: gene flow can occur from a continental to an island population (Continent-Island Model; Wright 1931, 1940) (Figure 1a); as random migration between many finite subpopulations (Island Model; Wright 1940) (Figure 1b); through gene flow between adjacent demes only (Stepping Stone Model; Kimura & Weiss 1964) (Figure 1c). The genetic divergence can change in a linear fashion inversely to geographic distance (Isolation by Distance; Wright 1943).

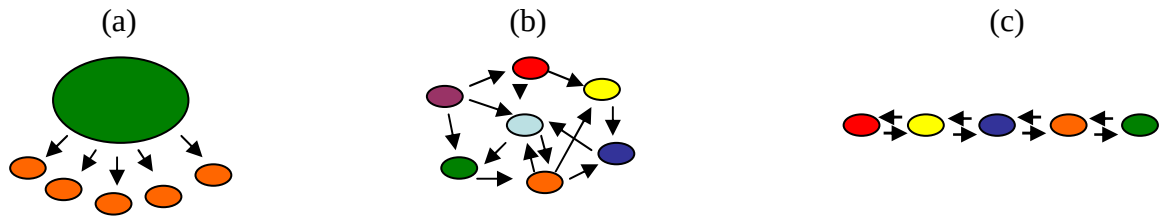


Figure 1. (a) Continent-Island Model; (b) Island Model; (c) Stepping Stone Model.

The differentiation of populations and often as a consequence the speciation process begins when gene flow is disrupted and populations become genetically isolated. It is assumed that the spatial differentiation of groups occurs either by dispersal or vicariance. A dispersal interpretation of a present-day distribution suggests that a new population would have been budding off from the ancestral species through active or passive dispersal (Briggs 1974) (Figure 2a). Vicariance means that a species was splitted into two or more isolated ranges by physical barriers that prevent gene flow, for example the rise of a mountain range, the

breakup of a continental landmass or the physical subdivision of a water body (Avise 2000) (Figure 2b). Dispersal and vicariant events create the conditions for genetic differentiation and allopatric speciation. The three non-geographic evolutionary mechanisms creating divergence are genetic drift, mutation and selection.

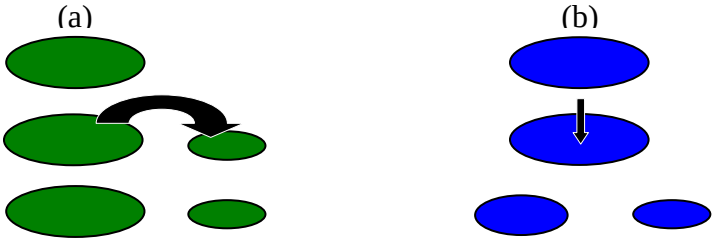


Figure 2. (a) Dispersal event; (b) Vicariant event.

Marine systems

Phylogeographic studies play an increasing role to understand how differentiation and speciation takes place in oceans. In marine systems, the population range could vary from thousands to tens of thousands of kilometres. Theoretically, only few absolute barriers to gene flow exist in oceans (Palumbi 1992). Thus, in marine species, high rates of gene flow are assumed to maintain panmictic reproduction and should slow down allopatric species formation. This has been shown for cephalopods (Garoia et al. 2004), fish (Heyden et al. 2007), sea cucumber (Arndt & Smith 1998) and crustaceans (Tolley et al. 2005). On the other hand, the high level of biodiversity in the oceans suggests a high speciation potential. Indeed a number of molecular studies have revealed that gene flow is restricted among many marine species resulting in moderate to high levels of genetic divergence. The interrelationships among these marine populations are the result of ecological traits, dispersal capabilities, isolation by distance, biogeographic history and oceanography (Palumbi 1994; Avise 1994; Queiroga 1996, 1998; Zane et al. 2000). In addition phylogeographic investigations have revealed the presence of cryptic species over large and smaller geographical scales which may go undetected by morphological investigations alone (Tarjuelo 2001; Gusmao et al. 2006; Mathews 2006). In the last decades, a high proportion of cryptic species was estimated: 5000 cryptic Porifera, 7000 cryptic Echinodermata and 52 000 cryptic Crustacea species are suggested in phylogeographic studies (Pfenninger & Schwenk 2007).

Thus, investigations are needed to evaluate the role of geographical history, oceanography and ecology in generating marine diversity. Furthermore, phylogeographic studies are important to approach the real level of marine biodiversity. When necessary, these

phylogeographic data will also help to develop conservation strategies for threatened species, for example to design marine protected areas and reserves (Palumbi 2003). The present thesis was designed to clarify and compare the mechanisms of generation and maintenance of genetic diversity and to identify cryptic species in marine and freshwater habitats using phylogeographic approaches.

Geographic isolation

The Mediterranean marine fauna is receiving an increasing interest in phylogeographic studies to test the role of geological evolution of the Mediterranean Sea (Borsa et al. 1997; Bargelloni et al. 2003; Peijnenburg et al. 2004; Duran et al. 2004a). It has been suggested that the marine biota could be the result of different genetic mechanisms interacting with the geological history of the Mediterranean Sea (Almaça 1985). The geology of the Mediterranean Sea is complex, involving the break-up and subsequent collision of the African and Eurasian plates and several isolation events from the Atlantic, e.g. the well-known Messinian Salinity Crisis. The Messinian Crisis is widely regarded as one of the dramatic episodes of oceanic change (Krijgsman et al. 1999). In the Late Miocene (Messinium) the Mediterranean Sea became isolated from the Atlantic Ocean. In consequence, a full or partial desiccation of the Mediterranean Sea took place and large salty lakes recharged by rivers replaced the previously marine basins (McKenzie 1999). Krijgsman et al. (1999) date the beginning at 5.96 Myr and the end at 5.33 Myr ago. It is often discussed, whether marine species could have survived in the remaining salt-lakes during the Messinian Crisis or whether the Mediterranean basin dried out completely. *Carcinus aestuarii* is endemic to the Mediterranean Sea. Its sister species, *C. maenas*, occurs throughout the Atlantic Ocean. Demeusy (1958) suggested that the isolation between the two basins would have provided the geographic barrier permitting the allopatric speciation of *C. aestuarii*. At the beginning of the Pliocene, Atlantic water flooded the Mediterranean Basin again (Hsü 1972, 1983) allowing Atlantic species to re-colonize the Mediterranean. These species had to adapt to different conditions because the North Atlantic is considerably colder and more nutrient-rich than the Mediterranean (Hofrichter 2002). Also during the Quaternary glacial periods, sea level regressions limited the biotic exchanges through the Strait of Gibraltar (Vermeij 1978). The coolings between the Plio-Pleistocene had potentially an equally disastrous impact on the Mediterranean fauna as the Messinian Crisis (Néraudeau & Goubert 2002). The Mediterranean fauna could thus have originated by repeated or continuous multiple colonization events with adaptation to specialized habitats and adaptive

radiation in these habitats (Almaça 1985). In the last years, molecular population genetics have revealed historical separations as the cause for genetic differentiation in several marine species, for example the effect on the population structure of the killifish *Aphanius fasciatus* by the Messinian Crisis (Triantafyllidis et al. 2007). There is also strong support that the origin of the snail *Salenthydrobia ferrerii* correlates with the crisis (Wilke 2003). Population differentiation due to the Pleistocene regressions is suggested between the Atlantic and Mediterranean *Coryphoblennius galertia* (see Domingues et al. 2007), within the common sea bass *Dicentrarchus labrax* (see Lemaire et al. 2005) and within the calanoid copepods *Calanus helgolandicus* and *C. euxinus* (see Papadopoulos et al. 2005).

Physical isolation

Present-day physical isolation of water bodies and hydrographical boundaries has been demonstrated to act as barrier to gene-flow and as an important trigger for differentiation of populations. Such physical barriers are the English Channel which isolates Atlantic from the English Channel populations Fiévet et al. 2007; Billot et al. 2003); the Gibraltar Strait and the Almería-Oran Front (AOF) which separate Atlantic and Mediterranean lineages (see below); the Siculo-Tunisia Strait which is a barrier between the western and eastern Mediterranean populations (Carlsson et al. 2004; Nikula & Väinölä 2004), and the hydrographic isolation of the Aegean-Ionian and Adriatic Seas with isolated Mediterranean subpopulations (see also Figure 3) (Bahri-Sfar et al. 2000). Particularly with regard to the AOF, there are an increasing number of molecular studies which reveal the influence of the AOF on the population structure of several marine species (Pérez-Losada et al. 2007; Gonzáles-Wangüemert et al. 2006; Rios et al. 2002; Zane et al. 2000; Naciri et al. 1999; Pannacciulli 1997). The Almería-Oran-Front is an effective boundary between Atlantic and Mediterranean surface waters. The cold and less saline Atlantic waters enter through the Strait of Gibraltar and induce a jet toward North Africa. A part of the Atlantic waters return westwards to form the Alboran gyre and another part flows eastwards along the coast of North Africa (Tintore et al. 1988). It appears plausible that the AOF constitutes a physical barrier for migration of larval stages between the Atlantic-Alboran and the Mediterranean Sea (Lemaire et al. 2005)

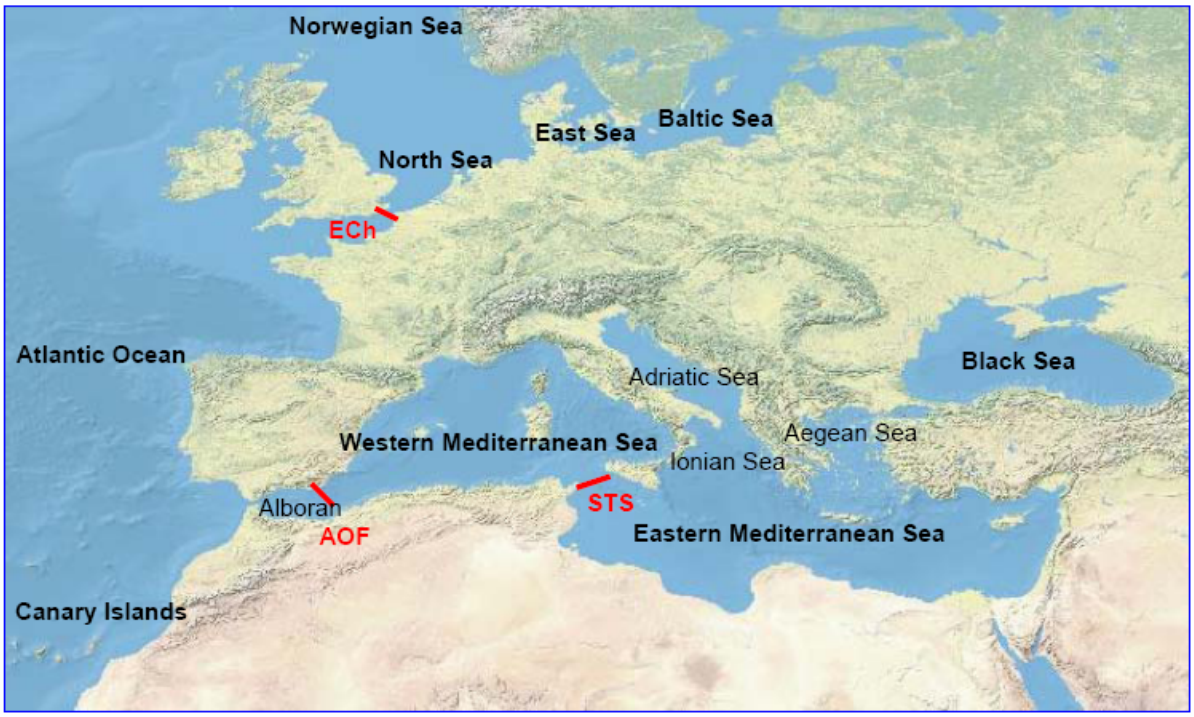


Figure 3. The Mediterranean Sea and potential physical barriers (ECh = English Channel, AOF = Almería-Oran-Front, STS = Siculo-Tunisia Strait).

Ocean currents could also have an influence on potential isolation by distance (IBD) in marine populations and subsequently on the genetic differentiation and population subdivision (Palumbi 2003). The plankters are advected by currents but there is only little information about their mean dispersal. The idea that average larval dispersal in marine systems may be lower than expected and that long-distance dispersal may be rare became more evident in the last years (Palumbi 2003). The larval transport may be affected strongly by local eddies and current reversals (Shanks 1995; Lee et al. 1994). Therefore, the populations might be restricted in their dispersal capability and increasing geographical distance is expected to enhance isolation by distance among them (Palumbi 1994; Roberts 1997). Within the tripelfin fish subspecies *Tripterygion delaisi xanthosoma*, IBD was revealed along the Spanish Mediterranean coast (Carreras-Carbonell et al. 2006).

Dispersal

Most of the marine species pass through a complex life history comprising a planktonic (larval) and a benthic (juvenile-adult) phase (Anger 2001). The pelagic phase is considered to be a prerequisite for a wide geographic range and to ensure genetic exchange between separate populations of the benthic organisms. Molecular studies of many species have shown that high dispersal potential due to planktonic larvae is often associated with only

mild genetic differentiation over large scales e.g. the Norway lobster *Nephrops norvegicus* (see Stamatis et al. 2004), the sponge *Crambe crambe* (see Duran et al. 2004a) and the stone crabs *Menippe mercenaria* and *Menippe adina* (see Schneider-Broussard et al. 1998; Williams & Felder 1986). The lack of a population structure in many marine species over large areas becomes evident when related freshwater species are compared (Avise 2004). The barriers to gene flow in freshwater systems appear clearer because of the contrasting physical structures of freshwater versus marine environments and the diverse life-cycles. For example, freshwater crabs developed different patterns of life-history. The larval development is often abbreviated or direct and the offspring tends to remain in the parental habitat (Schubart & Koller 2005). In addition, geographic barriers permit allopatric differentiation in freshwater systems, because each stream or river system may harbour an isolated population, separated by land from adjacent rivers. A large number of studies have shown that freshwater species with low dispersal tend to have a significant genetic structure (Palumbi 1992; Daniels et al. 2001, 2003; Schubart & Koller 2005; Shih et al. 2006). An intermediate between typical marine and freshwater species is the amphidromous life cycle, which occurs in many limnic species. The adults inhabit freshwater systems and release larvae in the upper reaches of rivers from where they drift passively to coastal environments, where they develop and metamorphose into post-larvae, which subsequently migrate back upstream to the adult habitats (Cook et al. 2006; McDowall 2007). The dispersal capabilities of amphidromous freshwater species and its influence on the population structure is little studied, because the life-history of amphidromous species is less clear than of marine species or fully freshwater species with abbreviated or direct development. The period larvae can survive in the sea, the location of larval development, how they are able to locate and return to the mouths of freshwater streams and the cues of settlement are still unknown (Myers et al. 2000). However, the presence of several larval stages of different amphidromous species in estuaries has been shown (Benstead et al. 2000; Chace & Hobbs 1969; Fiévet et al. 2001). Therefore, dispersal via the ocean is most likely to happen between adjacent or nearby estuaries, only allowing short-range gene flow. The comparison of the population structure of amphidromous species with marine species may reveal differences in the population structure which might result from different dispersal capabilities. The comparison of various marine species which differ in the length of larval development should shed light on the influence of the dispersal capability on the population structure within marine systems.

Nowadays, the dispersal of marine species is affected by anthropogenic transport of small marine organisms, often worldwide, via ballast water in ships. The transported species often

colonize the habitats successfully by endangering the natural fauna. Good examples for such non-indigenous/invasive species are *Carcinus maeans* (see Yamada et al. 2005) outside of Europe and *Eriocheir sinensis* (see Hänfling et al. 2002) in Europe. For these species, new ecological opportunities arose and the potential survival increases in sibling species, due to often similar ecological demands. Selection could take place via predators, habitat condition, and competition.

Not only invasive species have to adapt to different environmental conditions. As environmental parameters are different between the Mediterranean Sea and the Atlantic Ocean, there is an opportunity to estimate the gene flow associated with the adaptation of the marine fauna to their environment. The Adria, Ionian Sea, eastern Mediterranean, Black Sea and Spanish Atlantic Ocean are characterized by different temperature and salinity regimes as well as by different tidal influences (Dimitrov & Dimitrov 2004; Hofrichter 2002). These are differences at wide geographic ranges but there are also differences in the habitat condition at a smaller scale due to anthropogenically disturbed habitats (jetties, eutrophication and anoxic sediments) and differences in the shape of the coastline (sandy bottom or rocky shores). These different ecological conditions can play an important role promoting adaptive radiation and speciation in marine species (Schluter 2001). For example, cryptic species in *Clavelina* were detected in response to different habitats: the “interior” form of *C. lepadiformis* adapted to harbour environment and the “exterior” to rocky littoral habitat (Tarjuelo et al. 2001).

To study the influence of species-specific dispersal capabilities, physical or geographic isolation, dispersal and vicariant events and ecological traits, different decapod species were chosen: the marine crab genus *Xantho* Leach, 1814 (Brachyura, Xanthidae) which has a relatively short larval development with four zoeal stages (Ingle 1983), the marine caridean shrimp *Palaemon elegans* (Rathke, 1837) (Palaemonidae) with nine zoeal stages (Fincham 1977) and the Caribbean amphidromous shrimp *Xiphocaris elongata* (Guérin-Méneville, 1856) for which the number of larval stages is unknown.

***The genus* Xantho**

The genus *Xantho* has an exclusive Mediterranean-Atlantic distribution and consists of four species, *Xantho hydrophilus* (Herbst, 1790), *X. poressa* (Olivi, 1792), *X. pilipes* A. Milne-Edwards, 1867, and *X. sexdentatus* (Miers, 1881). In the literature, these species have often been confused, due to their morphological similarity and complex taxonomic history (Holthuis 1954; Garcia-Raso 1987). *X. hydrophilus* occurs from the North Sea southward to Morocco, including the Azores, Madeira, Canary Islands and Cape Verde Islands (Manning & Holthuis 1981) (Figure 3 and 4b). For the Mediterranean populations of *X. hydrophilus*, Forest (in Drach & Forest 1953) described a variety called "*granulicarpus*", which is often considered as a subspecies. For *X. h. granulicarpus* the following pattern of differentiation is suggested: *X. h. granulicarpus* has probably evolved from the East Atlantic *X. hydrophilus* (see Almaça 1985). At the beginning of the Pliocene, Atlantic water flooded the Mediterranean Basin again (Hsü 1983), allowing Atlantic species to re-colonize the Mediterranean. These species had to adapt to different conditions because the North Atlantic is considerably colder and more nutrient-rich than the Mediterranean (Hofrichter 2002). While *X. hydrophilus* is more common in the Atlantic, *X. poressa* is one of the most frequently found species in the Mediterranean and the Black Sea and its geographical range extends to the Canary Islands and Portugal (Zariquiey-Alvarez 1968) (Figure 3 and 4a). The stone crab shows variability in size and coloration throughout its range of occurrence. The distribution of *X. pilipes* ranges from Norway and the Shetland Islands southward to Angola and into the Mediterranean Sea (d´Udekem d`Acoz 1999). *X. sexdentatus* is only found in the East Atlantic and tropical Atlantic, including the Azores and the Canary Islands (d´Udekem d`Acoz 1999).

***The caridean prawn* Palaemon elegans**

In comparison to the genus *Xantho*, the dispersal capacities of the caridean species *Palaemon elegans* (Figure 4c) are presumably higher, since the complete larval development has nine zoeal stages (Fincham 1977). *P. elegans* is distributed in the Atlantic from Scotland and Norway to Mauritania including Azores, Madeira and the Canary Island, Mediterranean Sea, Baltic Sea, Black Sea, Caspian Sea and Lake Aral (d'Udekem d'Acoz 1999). Besides the wide geographical range, the shrimp has also adapted to different habitat types: it tolerates a wide range of salinities, temperature and oxygen (Berglund 1980; Berlund & Bengston 1981). In the past, morphological variations have been suggested by de Man (1915), and the subgenus *Paleander* Holthuis, 1950 was reintroduced by Chace and Bruce (1993) for *P.*

elegans to separate it from other species of the genus *Palaemon*. Here we wanted to determine the degree of genetic differentiation along the corresponding Atlanto-Mediterranean coastline.

***The amphidromous shrimp* Xiphocaris**

To study the genetic differentiation of an amphidromous species in comparison to marine species we compared different populations of *Xiphocaris elongata* (Guérin-Méneville, 1856) (Figure 4d). The genus *Xiphocaris* occurs only on the West Indian Islands. It was long considered as a member of the familiy Atyidae, although a primitive, aberrant species (Chace & Hobbs 1969). Chace (1992) allocate *Xiphocaris* in a separate family, the Xiphocarididae, which is more closely related to the marine nematocarcinids than to the atyids (Martin & Davis 2001). A characteristic of the species *Xiphocaris elongata* is the extreme variability in the relative length of the rostrum. Taking this into consideration, Pocock (1889) subdivided *Xiphocaris elongata* in three distinct species and one variety: *Xiphocaris brevirostris* Pocock, 1889, *Xiphocaris gladiator* Pocock, 1889, *Xiphocaris gladiator* var. *intermedia* Pocock, 1889 and *Xiphocaris elongata* (Guérin-Méneville, 1856). These morphological forms occur in many diverse types of habitats. Therefore, it appears possible that the length of the rostrum is influenced by biotic and/or abiotic factors. Morphological and genetic comparisons should help to estimate the degree of genetic differentiation and the role of life history and ecological traits.

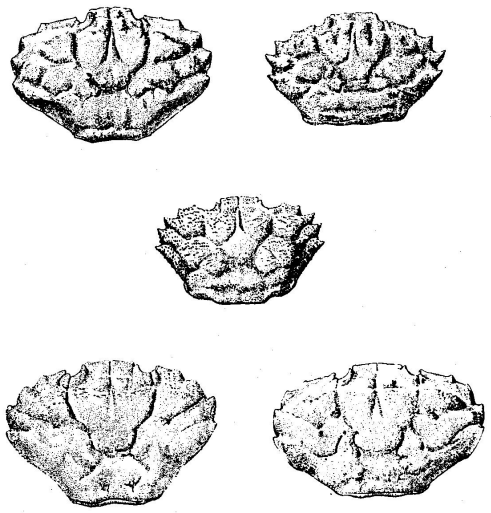
Figure 4. The studied species: (a) *Xantho poressa*; (b) *Xantho hydrophilus*; (c) *Palaemon elegans*; (d) *Xiphocaris elongata*.

Aim of this thesis

The aim of this thesis was to detect mechanisms of generation and maintenance of genetic diversity in marine and freshwater decapods with special emphasis on the geographic structure. We wanted to determine whether Atlantic and Mediterranean populations of *Xantho hydrophilus*, *Xantho poressa* and *Palaemon elegans* can be separated by morphometric and genetic methods (publication one to four). For all these species, we address the question whether the Strait of Gibraltar, the Almería-Oran-Front or the biogeographic history has a measurable influence on gene flow between the Atlantic Ocean and the Mediterranean Sea populations. In addition, the studies were carried out to search for a possible isolation-by-distance pattern. For the amphidromous or adult freshwater shrimp *Xiphocaris elongata,* we addressed the question whether its life cycle has an influence on the population structure and whether the different morphs could be separated genetically (publication five). The ecology of *X. poressa* was studied to document habitat preferences, variety of colour patterns, size variability, population density and to determine if the different colour patterns and size variability can be separated genetically (publication two). Morphometric methods were used to study patterns of relative growth for determining the onset of maturity and allometric growth (publication one, two and five). In addition, the morphometric and genetic results should provide answers if there exist cryptic species within the studied taxa. With focus on a population-level comparison, we used DNA-sequences of the two mitochondrial genes 16S rRNA and COI (publication one to five). Mainly COI has been proved to be a genetic marker exhibiting levels of sequence variation suitable for population analysis of other marine invertebrates (Papadopoulos et al. 2005; Nikula &Väinölä 2003; Tarjuelo et al 2004; Roman & Palumbi 2004; Stamatis et al. 2004).

PUBLICATION 1

PHYLOGENY AND GEOGRAPHIC DIFFERENTIATION OF ATLANTO–MEDITERRANEAN SPECIES OF THE GENUS *XANTHO* (CRUSTACEA: BRACHYURA: XANTHIDAE) BASED ON GENETIC AND MORPHOMETRIC ANALYSES



Silke Reuschel and Christoph D. Schubart

Manuscript published by *Marine Biology*

ABSTRACT

The crab genus *Xantho* Leach, 1814 is restricted to the north-eastern Atlantic Ocean and the Mediterranean Sea. It consists of four species, *Xantho hydrophilus* (Herbst, 1790), *X. poressa* (Olivi, 1792), *X. pilipes* A. Milne-Edwards, 1867, and *X. sexdentatus* (Miers, 1881). *X. hydrophilus* has been divided into two geographic forms, of which one, *X. h. granulicarpus* (Forest, 1953), is postulated to be endemic to the Mediterranean Sea. In this study, we reconstruct phylogenetic relationships of the genus *Xantho* and related genera from the Atlantic Ocean or Mediterranean Sea and compare different geographic populations of *Xantho hydrophilus* and, to a lesser extent, of *X. poressa* by means of population genetic and morphometric analyses. The molecular phylogeny is based on two mitochondrial genes (large subunit rRNA and cytochrome oxidase I) and indicates that *X. poressa*, *X. hydrophilus* and *X. sexdentatus* form a monophyletic group, the latter two species sharing identical haplotypes. On the other hand, *X. pilipes* shows affinities to *Xanthodius denticulatus*. Population genetics based on the COI gene reveal genetic differentiation within *X. hydrophilus*. Morphometric results also give evidence for distinct geographic forms in *X. hydrophilus* with a clear discrimination. In comparison, morphometric discrimination between different geographic populations of *X. poressa* is less clear, but still significant. We therefore suggest a recent/ongoing morphological and genetic differentiation within *Xantho hydrophilus*, restricted gene flow between its Atlantic and Mediterranean populations (not allowing subspecific differentiation) and possible mtDNA introgression between the species *X. hydrophilus* and *X. sexdentatus*.

INTRODUCTION

Population genetic studies of marine invertebrate species have shown that high-dispersal potential due to planktonic larvae is often associated with only mild genetic differentiation over large scales as for example in the stone crabs *Menippe mercenaria* and *Menippe adina* from the Gulf of Mexico and western Atlantic (Williams & Felder 1986; Schneider-Broussard et al. 1998), the batillarid snail *Batillaria multiformis* occurring along the Japanese coast (Kojima et al. 2003) and the calyptraeid snail *Crepidula depressa* from the east coast of North America (Collin 2001). This implies high levels of gene flow within marine coastal megapopulations. However, despite the high-dispersal potential of most marine invertebrates, a variety of mechanisms can prevent gene flow between populations. These mechanisms may act at different levels, even among closely related species. While some species show sufficiently high rates of gene flow to reproduce panmictically, the genetic exchange becomes so remarkably low in other species, that natural selection and genetic drift may occur more or less independently in each deme (Slatkin 1981). Thereby, population structure depends strongly on the dispersal potential of the corresponding larval stages. Within the genus *Littorina*, Kyle and Boulding (2000) found examples for high as well as low population genetic structure in accordance to the duration of larval development. Furthermore, nonobvious barriers, isolation by distance, local genetic drift, introgression due to hybridization and incomplete lineage sorting are additional factors to be taken into account when studying dispersal of marine species (Palumbi 1994; Avise 1994; Zane et al. 2000).

The genus *Xantho* Leach, 1814 has an exclusive Mediterranean–Atlantic distribution and shows a great interspecific as well as intraspecific morphological variability (d'Udekem d'Acoz 1999). All four species of the genus as currently defined (Guinot 1967), i.e. *Xantho hydrophilus* (Herbst, 1790) (= *Xantho incisus* Leach, 1814, see Sakai 1999), *X. poressa* (Olivi 1792) *X. pilipes* A. Milne-Edwards, 1867 and *X. sexdentatus* (Miers 1881) are restricted to the north-eastern Atlantic Ocean and the Mediterranean Sea. While *X. sexdentatus* is only found in the tropical and subtropical Atlantic, the other three species are distributed in the north-eastern Atlantic as well as in the Mediterranean Sea. In the literature, these species have often been confused, due to their morphological similarity and complex taxonomic history (Holthuis 1954; García Raso et al. 1987). For the Mediterranean populations of *X. hydrophilus*, Forest (in Drach & Forest 1953) described a variety called *granulicarpus*, which subsequently was often used as a subspecies name. *X. h. granulicarpus* is characterized by more acute lateral carapace spines, stronger granulated carapace and pereiopods, and a dark pigmentation on the chelar dactyls of the adult males extending onto

the palm region. Transitional forms have been reported from the western Mediterranean Sea and therefore the exact geographic boundaries of the two subspecies of *X. hydrophilus* always remained unclear and the taxonomic status of *X. h. granulicarpus* doubtful (Almaça 1985; d'Udekem d'Acoz 1999).

The western Mediterranean is connected to the Atlantic Ocean through the Strait of Gibraltar. This narrow oceanic strait and the Almería-Oran front have been shown to represent natural gene flow barriers between Atlantic and Mediterranean populations in different marine species and therefore to cause and maintain allopatric separation. Several studies have revealed a restricted gene flow between Atlantic and Mediterranean populations in different marine invertebrate and vertebrate species, e.g. in the barnacle genus *Chthamalus* (see Pannacciulli et al. 1997), the cuttlefish *Sepia officinalis* (see Pérez-Losada et al. 2002), the sea bass *Dicentrachus labrax* (see Naciri et al. 1999) and the mussel *Mytilus galloprovincialis* (see Quesada et al. 1995). In the history of the Mediterranean Sea, there have been numerous instances in which its waters have been isolated from the Atlantic Ocean during extended periods. In the late Miocene, for example, a sea level regression isolated the Mediterranean Sea from the Atlantic, leading to almost complete desiccation of the Mediterranean (Messinian Crisis, e.g. Hsü 1983). Also, during the Quaternary glacial periods, sea level regressions limited the biotic exchange through the Strait of Gibraltar (Vermeij 1978). These historic separations with complete isolation as well as the continuing potential gene flow barrier of the Strait of Gibraltar might have shaped the genetic structure of the Mediterranean fauna. An endemic crab fauna could have originated by isolation from Atlantic populations, repeated recolonizations with adaptation to specialized habitats, or adaptive radiation (Almaça 1985). The allopatric speciation between the morphologically similar Atlantic *Carcinus maenas* and Mediterranean *Carcinus aestuarii* as suggested by Demeusy (1958), and later confirmed with genetic analyses of the 16SrRNA gene by Geller et al. (1997) and with the COI gene by Roman and Palumbi (2004), represents such an Atlanto–Mediterranean separation event.

The present study is designed to reconstruct phylogenetic relationships within the genus *Xantho* and to determine whether Atlantic and Mediterranean populations of *X. hydrophilus* and *X. poressa* can be separated by morphometric and genetic methods. Thereby, we address the question whether the Strait of Gibraltar has a measurable influence on gene flow of xanthid crabs between the Atlantic Ocean and the Mediterranean Sea, or if other separating mechanisms may be involved. The results may provide an answer to the question of the

validity of the subspecies *X. h. granulicarpus* and if this taxon can be considered a Mediterranean endemic.

MATERIALS AND METHODS

Samples for this study were obtained from field trips to Giglio (Italy, 2001), Ibiza (Spain, 2001 and 2003), Parga (Greece, 2003), Corsica (France, 2004), Istra (Croatia, 2001 and 2004) (all Mediterranean), Cádiz (Spain, 2004) (Atlantic), and from colleague donations and museum collections of the Senckenberg Museum Frankfurt (SMF) and Naturalis Museum Leiden (RMNH) (see Table 1).

For the morphometric comparisons, 436 specimens of *Xantho* were included in this study. The sample size per population ranged from 22 to 101 individuals. Material from various geographic areas from the Mediterranean Sea and the Atlantic Ocean including both sexes was examined. Specimens were taken from the intertidal zone to a depth of 10 meters by snorkelling and occasionally by scuba-diving. The following populations were used: from the Atlantic Ocean, Portugal and Bretagne (France) for *X. hydrophilus*, Cádiz (Spain) for *X. poressa*; from the western Mediterranean, Ibiza for both species, Corsica for *X. poressa*; from the central Mediterranean, Greece for both species, and from the Adriatic Sea, Croatia for *X. poressa* only (see Table 1).

For the genetic analyses, genomic DNA was extracted from the muscle tissue of the walking legs using the Puregene kit (Gentra Systems). A total number of 82 specimens of the genus *Xantho* and additional seven species of the family Xanthidae were thereby genetically examined (Table 1). The selective amplification of an approximately 520 basepair fragment from the large subunit rRNA (16S) and a 640 basepair fragment from the cytochrome oxidase subunit I (COI) (in both cases excluding primers) genes was carried out by polymerase chain reaction (PCR) (40 cycles; 45 s 94°/1min 48-50°/1min 72° denaturing/annealing/extension temperatures) with the primers listed in Table 2. In the case of COI, new internal primers to COIf and COIa were designed to allow amplification of *X. poressa* and older museum specimens (see Table 2). The PCR products were purified with Millipore Montage PCR Centrifugal Filter Devices (Millipore, Corp) and both strands were used for cycle sequencing. The products were precipitated with ethanol, resuspended in water and sequenced with the ABI BigDye terminator mix (Big Dye Terminator v 1.1 Cycle Sequencing Kit; Applied Biosystems) in an ABI Prism automated sequencer (ABI Prism 310 Genetic Analyzer; Applied Biosystems). The sequences were analyzed with the program ABI

Sequencing Analysis 3.4 (Applied Biosystems) and manually aligned with BioEdit (Hall 1999).

The two mitochondrial genes 16S rRNA and COI were combined for the phylogeny. *Panopeus herbstii* H. Milne Edwards, 1834 sequences were obtained from the molecular database and used as outgroup (16S: AJ130815; COI: AJ274699). A Chisquare test of homogeneity of base frequencies across taxa was carried out as implemented in PAUP* (Swofford 1998). Test for homogeneity among partitioned datasets was also performed using PAUP* (Swofford 1998), with COI and 16S as predefined partitions. Three methods of phylogenetic inference were applied to our data set: maximum parsimony (MP) and minimum evolution (ME) using the software package PAUP* (Swofford 1998), and Bayesian analysis (BI) as implemented in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). The model of DNA substitution that fitted our data best was chosen using the software Modeltetst 3.6 (Posada & Crandall 1998). This approach consists in successive pairwise comparisons of alternative substitution models by hierarchical likelihood ratio tests. The ME and BI trees were obtained with the suggested model of evolution and the corresponding parameters. MP trees were obtained by a heuristic search with 100 replicates of random sequences addition and tree bisection-reconnection as branch swapping options. Gaps were treated as fifth state. Subsequently, confidence values for the proposed groups within the inferred trees were calculated with the bootstrap method (2,000 pseudoreplicates) with 10 replicates of stepwise random sequence addition and the treebisection-reconnection (TBR) branch swapping algorithm, keeping multiple trees (MulTrees). Otherwise, the default options of PAUP* were used. Only minimal trees were retained and zero length branches were collapsed. A second MP bootstrap analysis was carried out, this time giving transversions five times more weight than transitions and treating gaps as missing, since MP otherwise does not account for different substitution rates. The Bayesian analysis was run with four MCMC chains for 2,000,000 generations, saving a tree every 500 generations (with a corresponding output of 4,000 trees). The –lnL converged on a stable value between 5,000 and 7,500 generations (‘‘burn in phase’’). The first 10,000 generations were therefore not included in the analysis to avoid the possibility of including random and suboptimal trees. The posterior probabilities of the phylogeny were determined for the remaining trees. Consensus trees were constructed using the ‘‘sumpt’’ option in MrBayes.

Table 1 Localities, dates of collection, number of specimens used for genetic (N_g) and morphometric (N_m) comparisons and genetic database accession number of the specimens of the family Xanthidae

Species	Collection site	N_g	N_m	Catalouge No.	EMBL No.16S	EMBL No. COI
Actaeinae Alcock, 1898						
Paractea monodi Guinot, 1969	Greece: Crete, 2001	1		SMF 30114	AM076773	AM076801
Euxanthinae Alcock, 1898						
Monodaeus couchii (Couch, 1851)	France: Roscoff W 345, 43°54.2′N 02°10.9′W, 1970	1		MNHN B29631	AM076771	AM076799
Monodaeus guinotae Forest, 1976	Ibero-Moroccan Gulf, 38°41′N 06°30′W, 1984	1		MNHN B24145	AM076772	AM076800
Xanthinae MacLeay, 1838						
Xantho hydrophilus (Herbst, 1790)	Portugal: Azores: Fayal, 1991			SMF 20457	AM076788	AM076916-27
	Spain: Canary Island, Tenerife, 1975			SMF 6721		
	France: Bretagne, 1999			SMF 27535		
				SMF 10235		
	Italy: Giglio Campese, 2001			SMF 27525-27		
	Spain: Ibiza, 2001	13	24	SMF 27531-32	AM076787	AM076805
						AM076903-15
	Greece: Parga, 2003	20	53	SMF 30115	AM076786	AM076804
						AM076928-36
	Portugal: Setubal, 2004	20	31	SMF 30116-17		AM076803
	Portugal: Praia das Avencas, 2001			SMF 30118		
Xantho pilipes A. Milne-Edwards, 1867	Portugal: Praia das Avencas, 2001	1		SMF 30119	AM076775	AM706806
	France: Corsica, Calvi Area, 1988	1		SMF 30121	AM076776	AM706807
	Western Greece: Parga, 2003	1		SMF 30120	AM076777	AM076938
Xantho poressa (Olivi, 1792)	Croatia: Istra, 2001	1		SMF 27533	AM076937	AM076808
	Spain: Canary Islands	1		RMNH 38643	AM076779	AM076810
	Spain: Ibiza, 2001	1		SMF 27529-30	AM076778	AM076809
	Croatia: Istra, 2004		75			
	Western Greece: Parga, 2003		101			
	Spain: Cádiz, 2004		61	SMF 30122		
	Ibiza (Spain)		27	SMF 30123		
	France: Corsica, 2002		22	SMF30124		
Xantho sexdentatus (Miers, 1881)	Mauritania, 1978	15	42	RMNH 38635	AM076780-81	AM076811-12
						AM076928-36
Xanthodius denticulatus (White, 1848)	Belize: Carrie Bow Bay, 2002	1		ULLZ 5519	AM076782	AM076813
	Puerto Rico: Maunabo, 2004	1		SMF 30125	AM076783	AM076939
Xanthodius inaequalis (Dana, 1852)	Principe and São Tomé: Bom Bom, 2004	1		RMNH 51206	AM076784	AM076814
Xanthodius sternberghii Stimpson, 1859	Mexico: Mulegé, 2001	1		ULLZ 3936	AM076785	AM076815
Zosiminae Alcock, 1898						
Platypodiella picta (Milne Edwards,1869)	Principe and São Tomé: Bom Bom, 2004	1		RMNH D51198	AM076774	AM076802

Abbreviations of museums: RMNH Naturalis Museum Leiden; SMF Senckenberg Museum und Forschungsinstitut, Frankfurt a. M. ULLZ University of Louisiana at Lafayette Zoological Collection, Lafayette; MNHN Muséum national d'Histoire naturelle, Paris

For genetic comparisons of populations, we used a 622 bp fragment of the COI gene. Parsimony networks were built with TCS (estimating gene genealogies version 1.13; Templeton et al. 1992). The Φ_{ST} value (the genetic differentiation between any two subpopulations) was calculated by means of an AMOVA (Excoffier et al. 1992) to determine the degree of genetic differentiation amongst the populations of *X. hydrophilus* (software Arlequin 2.0; Schneider et al. 1999). First, genetic differentiation was tested between the Mediterranean Sea (Greece and Ibiza; N=33) and the Atlantic (Portugal; N=20). Subsequently, the Mediterranean Sea was subdivided into two populations, central Mediterranean (Greece; N=20) and West Mediterranean (Ibiza; N=13) and the genetic differentiation was tested between the three populations and between the two species *X. hydrophilus* (N=53) and *X. sexdentatus* (N=15). Genetic heterogeneity within populations was estimated as haplotype diversity (h=1-$\sum f_i^2$; where f_i is the frequency of the ith haplotype).

For the morphometric analyses the following morphological measurements were used: (1) carapace width (cw); (2) carapace length (cl); (3) body height (bh); (4) frontal width (fw); (5) ventral leg length of the fourth leg. Measurements of the chelae and abdomen were not included in the analyses because of sexual dimorphism. The data were tested for normal distribution by the Kolmogorov–Smirnov-test (software Statistica 6.0; StatSoft). Patterns of morphometric relationships can be influenced by the effect of allometric growth and size in species of undetermined age. To reduce the influence of allometry, all measurements were log-transformed, and ratios were arcsine-transformed. The comparison of morphometric ratios of different populations was carried out with a 1-Factor-ANOVA and a post hoc Schefé test for the comparison within species. We also included a Levene test to test the homogeneity of the data. The equality of variance–covariance matrices were tested with a Box's *M*-test (Box 1953). In addition, discriminant analysis was used for a more accurate differentiation between populations using log-transformed morphometric variables.

Table 2. Primers used for PCR amplification and sequencing of the 16S rRNA and the mitochondrial COI genes.

Name	Primer sequence 5´-3´	References
COIf	CCT GCA GGA GGA GGA GAY CC	Palumbi et al. 1991
COL3	ATR ATT TAY GCT ATR HTW GCM ATT GG	New internal
COIa	AGT ATA AGC GTC TGG GTA GTC	Palumbi et al. 1991
COH3	AAT CAR TGD GCA ATW CCR SCR AAA AT	New internal
16L2	TGC CTG TTT ATC AAA AAC AT	Schubart et al. 2002
16H3	CCG GTT TGA ACT CAA ATC ATG T	New

RESULTS

Genetics

The genetic dataset consisted of an alignment of 1167 basepairs after removal of the primer regions (640 of COI and 527 of 16S). Out of these, 424 positions were variable and 351 parsimony-informative. The test for homogeneity of base frequencies composition indicated homogeneity within the combined COI and 16S dataset (P=0.249) as well as across taxa ($\chi 2$=34.236, df=57, P=0.993). Application of the likelihood ratio tests revealed that the selected model of DNA substitution by Akaike for the combined data was the general time reversible model GTR+I+G (Rodríguez et al. 1990) with an assumed proportion of invariable sites of 0.5445 and the rates following a gamma distribution with a shape parameter of 1.0464. The heuristic search of MP resulted in three most parsimonious trees with a length of 1,013 (CI=0.596, RI=0.731, RC=0.436). Bayesian inference, maximum parsimony (with weighted transversions), and minimum evolution analyses resulted in a similar topology without conflicting branching patterns and are therefore presented together in Figure 1. The resulting 16S-COI gene tree suggests that the genus *Xantho* Leach, 1814 is not necessarily a monophyletic group, since *Xanthodius denticulatus* possibly represents the sister species of *Xantho pilipes* (low confidence values) and is not closely related to other members of the genus *Xanthodius* Stimpson, 1859, including the type species *Xanthodius sternberghii* Stimpson, 1859. The other three species of the genus *Xantho* do form a well supported monophyletic clade (1.0/100/85 posterior probability or bootstrap values in BI/MP/ME). Surprisingly, *X. sexdentatus* cannot be separated from *X. hydrophilus*. Also, the two postulated subspecies of *X. hydrophilus* (*X. h. hydrophilus* and *X. h. granulicarpus*) cannot be distinguished with this approach. Consequently, *X. hydrophilus* from the Atlantic and Mediterranean Sea and *X. sexdentatus* form a genetically wellsupported clade (1.0/100/100). The sister species to this clade is *Xantho poressa*. Similar as in *X. hydrophilus*, also in *X. pilipes* and *X. poressa* all Mediterranean and Atlantic specimens are very closely related and grouped together (1.0/100/100 in both cases). *Xanthodius inaequalis* (Olivier 1791) from Africa and *Xanthodius sternberghii* Stimpson, 1859 from the eastern Pacific form another monophyletic group (1.0/100/100), representing the genus *Xanthodius* and the two species of Monodaeus are also placed in a common clade with strong support (1.0/100/100), supporting the validity of the genus. *Platypodiella picta* and *Paractaea monodi* are characterized by long branches and cannot be placed in phylogenetic vicinity of any other species included in this phylogeny. *Panopeus herbstii* represents the designated outgroup (Figure 1).

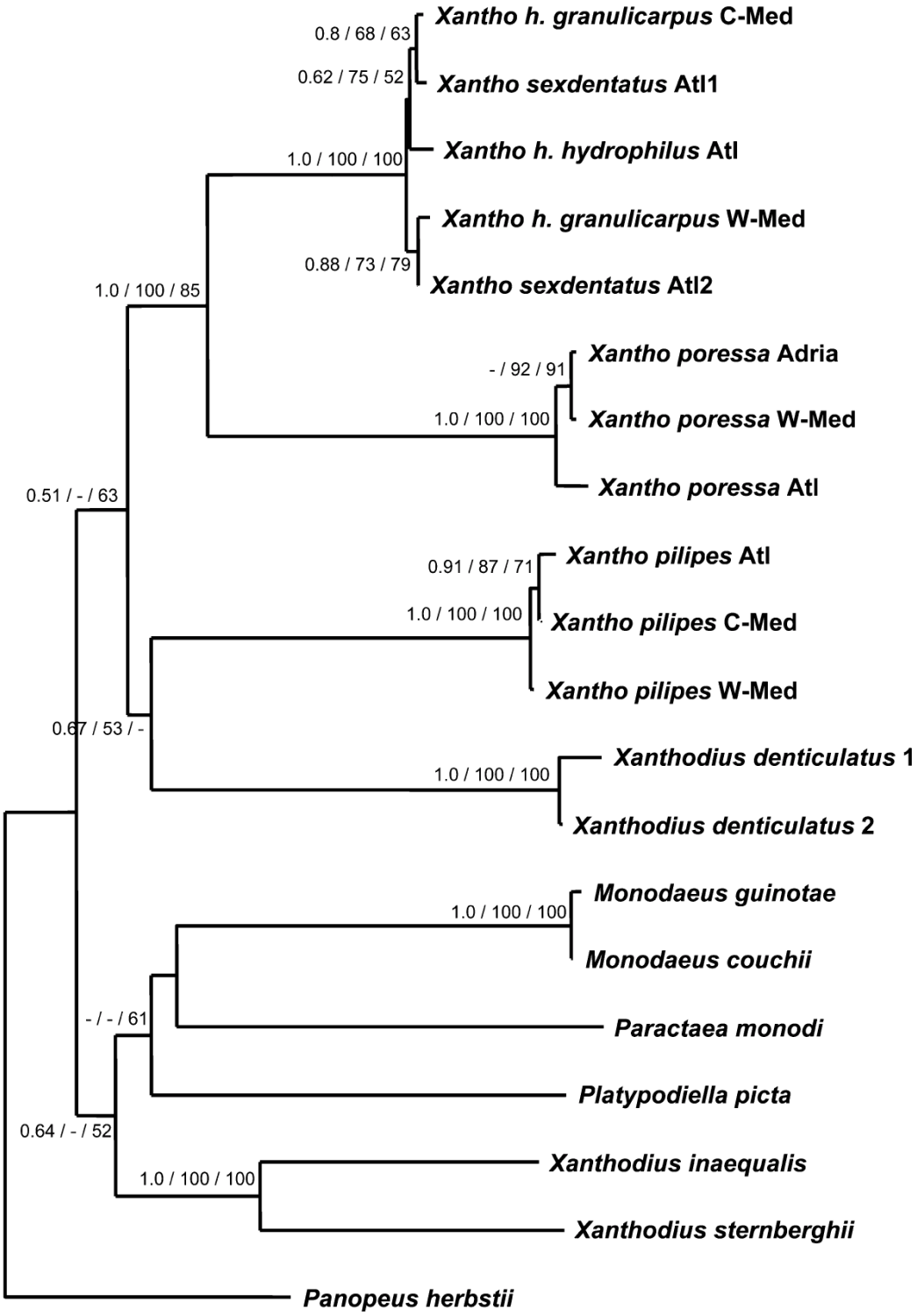


Figure 1. Bootstrap 50% majority-rule consensus tree of phylogenetic relationships within the genus *Xantho* and other Xanthidae with *Panopeus herbstii* as outgroup. Bayesian inference, maximum parsimony and minimum evolution (with GTR+I+G model of evolution) topologies. Confidence values from 2,000 bootstrap replicates (BI/MP/ME confidence values) based on 1,167 basepairs of the 16S and COI mitochondrial genes; only bootstrap values above 50 are shown. C-Med: Central Mediterranean; W-Med: West Mediterranean; ATL: Atlantic.

The comparison of multiple sequences of 520 basepairs of 16S mtDNA (16S) and 622 basepairs of COI in *X. hydrophilus* revealed the existence of most common haplotypes for both genes. Because of the close relationship between *X. sexdentatus* and *X. hydrophilus* in the phylogenetic tree (Figure 1), we included *X. sexdentatus* in the subsequent network construction. For the 16S gene, 12 out of 19 specimens share a common haplotype (seven *X. hydrophilus* from the Mediterranean, four from the Atlantic and one *X. sexdentatus*). Five additional haplotypes of *X. hydrophilus* were found (four being separated by one transition and one from Ibiza by two transitions), but occurred only once in our analysis. Two specimens of *X. sexdentatus* are separated by different transitions. In contrast, *X.poressa* is separated from this complex by at least 20 transitions and one transversion. Within *X. poressa*, five out of six specimens share a common haplotype and one additional haplotype from Italy occurs with one transition. Due to the higher variability and thus separating potential of the COI gene, we included many more specimens of the *X. hydrophilus* complex (N=53) in the intraspecific comparisons together with 15 specimens of *X. sexdentatus*. The minimum spanning tree shows a star-like shape, with most haplotypes being connected by very few mutation steps. The populations from Greece, Ibiza and Portugal of *X. hydrophilus* and *X. sexdentatus* share one most frequent haplotype (HT1) which was present in 18 specimens, six from Greece, three from Ibiza, six from Portugal and three representing *X. sexdentatus* (Figure 2). A large number of rare haplotypes have diverged from the common haplotype. They are generally present in not more than one individual per population: 14 haplotypes with one mutation, 11 with two mutations and 8 with three mutations were found. Eight other haplotypes form a more differentiated group: one haplotype with four (HT20 from Ibiza), one *X. sexdentatus* with five (HT39), one haplotype with six (HT12 from Portugal), one with seven (HT11 from Portugal), two with eight (HT30 from Greece and HT40 representing one *X. sexdentatus*), one haplotype with nine (HT41 representing one *X. sexdentatus*) and one with 11 substitutions (HT13 from Portugal) relative to HT1. Eight of the 15 sequenced specimens of *X. sexdentatus* share one position, always distinguishing them from HT1, the corresponding haplotype is termed HT42. Relative to HT42 one *X. sexdentatus* diverged with one (HT43) and two with three (HT45, 44) transitions. However, also some of the Mediterranean and Atlantic haplotypes of *X. hydrophilus* are derived from HT42 (Figure 2). These results therefore demonstrate the lack of obvious diagnostic differences in the 16S rRNA and COI genes between the species *Xantho hydrophilus* and *X. sexdentatus* and give evidence that Atlantic and Mediterranean populations of *X. hydrophilus* share a most common haplotype (Figure 2, Table 3).

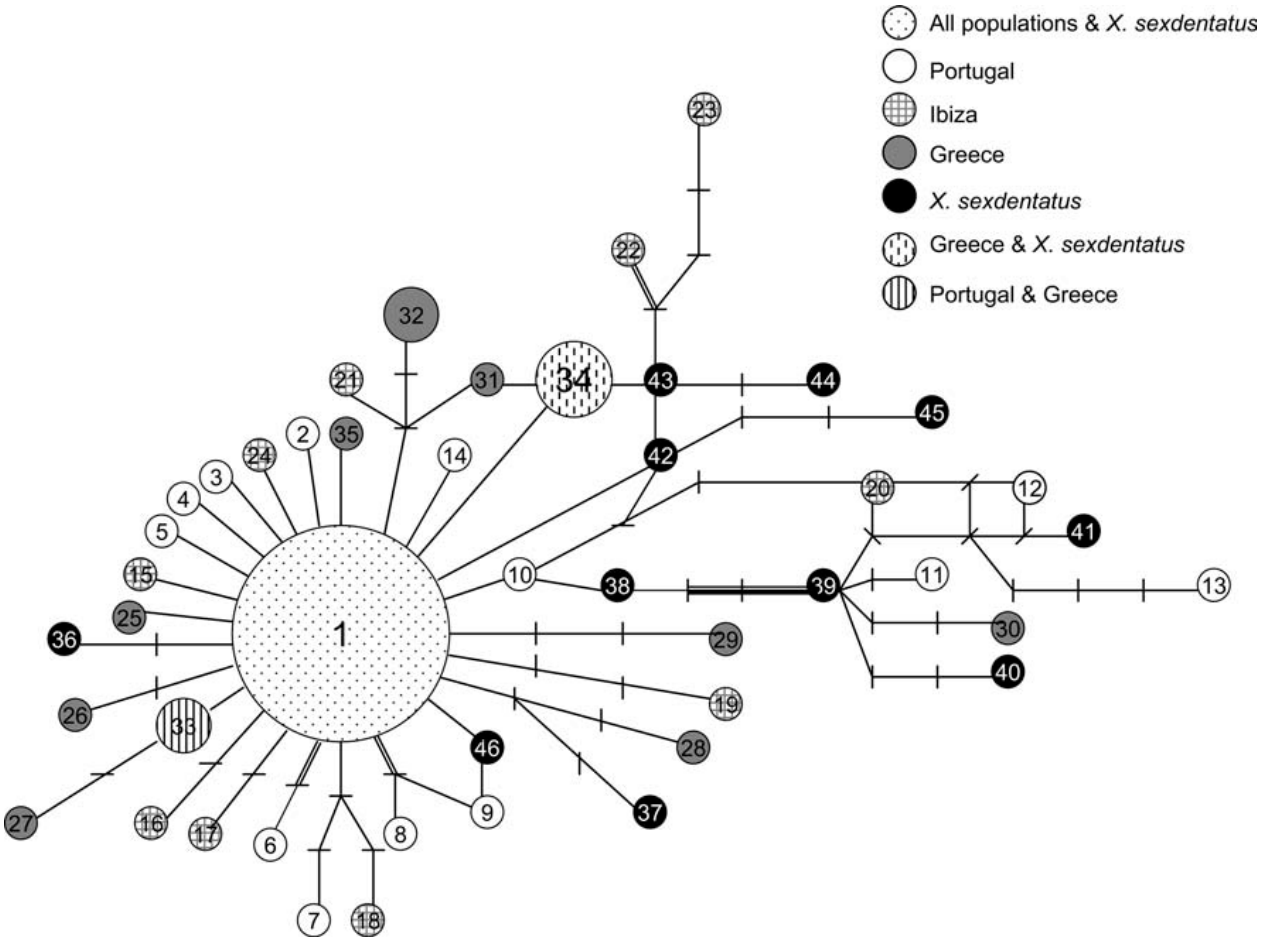


Figure 2. Minimum parsimonious spanning network constructed with TCS of *X. hydrophilus* (N=53) and *X. sexdentatus* (N=15) of a 622-basepair fragment from the COI gene. Each line represents one substitution; cross lines on lines indicate additional substitutions separating two haplotypes, a double line stands for transversions. The size of the circle is representative for the frequency of the haplotypes (small: N=1; medium: N=2–3, large: N>10). Shading corresponds to geographic origin.

Nevertheless, the existence of genetic structure correlated with geography could be shown by F-statistics. The analysis of variance of 622 basepairs of COI between all Atlantic and Mediterranean representatives of *X. hydrophilus* revealed a highly significant genetic differentiation and a Φ_{ST} -value of 0.07 (P<0.001). Moderate genetic differentiation is indicated by values between 0.05 and 0.15 (Wright 1978). To study the degree of homogeneity within the Mediterranean Sea, we further divided the Mediterranean samples into the two corresponding subpopulations; representing Greece and Ibiza (see Material and methods). The Φ_{ST}-values were lower (and less significant) between the populations of Portugal and Ibiza (Φ_{ST}: 0.05, P=0.006), the populations of Greece and Ibiza (Φ_{ST}: 0.05; P=0.005) than between the populations of Portugal and Greece with a highly significant Φ_{ST}-value of 0.08 (P<0.001). A Φ_{ST}-value of 0.04 was estimated between *X. hydrophilus* and *X. sexdentatus*, but with relatively low significance (P=0.004). The haplotype diversity (h) of

Table 3 *Xantho hydrophilus* haplotype distribution within the Mediterranean Sea: Greece (*G*) and Ibiza (*I*); and the Atlantic Ocean: Portugal (*P*); also including *X. sexdentatus* (*X. s.*). n HT is the number of different haplotypes found in each population and *h* is the haplotype diversity according to Nei and Tajima, 1981. The number of substitutions (*N* Sub), transitions (*S*) and transversions (*V*) are defined in comparison to the most common haplotype 1

Locality	Haplotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	n	nHT	*h*
P		6	1	1	1	1	1	1	1	1	1	1	1	1	1																			1															20	14	0.72
I		3															1	1	1	1	1	1	1	1																								13	10	0.81
G		6																							1	1	1	1	1	1	1	2	1	3	1														20	14	0.48
X. s.		3																																1		1	1	1	1	1	1	1	1	1	1	1	15	12	0.81
Total		18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	4	1	1	1	1	1	1	1	1	1	1	1	1	1	68	40	0.99
S			1	1	1	1	3	1	1	1	5	6	9	1	1	2	2	3	3	3	4	2	3	6	1	1	2	3	3	3	6	2	3	1	1	1	2	3	2	3	6	7	1	2	4	4	1				
V								1		1	1	2		2						1																														
N Sub			1	1	1	2	3	2	2	1	7	6	11	1	1	2	2	3	3	4	2	3	6	1	1	2	3	3	3	6	2	3	1	1	1	2	3	2	3	6	7	1	2	4	4	1					

the COI gene within Atlantic and Mediterranean populations is relatively high (0.72 vs. 0.54) (see Table 3).

Morphometrics

There are significant morphometric differences between the four populations of *X. hydrophilus* and *X. sexdentatus* (here included because of its genetic similarity) in single character ratios as well as in overall discriminant analyses. Two geographic forms and transitional forms of *Xantho hydrophilus* could be distinguished, in addition to the separation of *X. sexdentatus*. Most distant are the populations from the Bretagne and Greece while the populations of Ibiza and Portugal represent transitional forms. The 1 Factor-ANOVA analyses of the ratios of *X. hydrophilus* and *X. sexdentatus* revealed significant differences between all the populations of the Mediterranean Sea and the Atlantic Ocean of *X. hydrophilus* and *X. sexdentatus* in carapace length to carapace width (d*f* 4; F=26.469; *P*<0.001), body height to carapace width (d*f* 4; F=39.364; *P*<0.001) and frontal width to carapace width (d*f* 4; 26.407; *P*<0.001). The post hoc Schefé test for the ratio carapace length to carapace width showed significant differences for the population of the Bretagne and *X. sexdentatus* in comparison to all the other populations (*P*<0.001), except between *X. sexdentatus* and the population of Ibiza (*P*>0.1). Furthermore, significant differences (*P*<0.001) were detected in the ratio body height to carapace width between Greece and all other populations. For this ratio, there were no significant differences between the population of Ibiza and *X. sexdentatus* (*P*>0.1), between

X. sexdentatus and the population of the Bretagne (P>0.1) and between the population of the Bretagne and Portugal (P>0.1). Only the population of the Bretagne showed significant differences (P<0.001) in the ratio frontal width to carapace width to Greece, Ibiza, Portugal and *X. sexdentatus*.

In order to test the overall differentiation of the different populations of *X. hydrophilus* and the population of *X. sexdentatus*, a discriminant analysis was carried out using the five log-transformed variables carapace length, carapace width, body height, frontal width and leg length. The dataset were subjected to canonical analyses shown in Figure 3. The group dispersions were not homogeneous (Box's M-test: M=188, $F_{45, 8208}$ = 3.908, P<0.0001) and the discrimination between the groups was highly significant (Wilks' Lambda: 0.11, F (20.538) = 25.271, P<0.00001; 80.11% correct classification). The population of Greece is correctly classified with a likelihood of 90.7%, the population of the Bretagne with 90%, the population of Portugal with 77.42% and *X. sexdentatus* with 92.8% likelihood. In addition, the classification matrix showed that individuals belonging to Ibiza were only correctly classified in less than 50%, (Table 4). The Mahalanobis distances (D^2) of the population of Greece revealed the shortest distance to the population of Ibiza (D^2 2.2) followed by Portugal (D^2 6.9), *X. sexdentatus* (D^2 11.5) and the most distant Bretagne (D^2 19.9). The population of Ibiza has a close relationship to its neighbouring populations of Greece (D^2 2.2) and Portugal (D^2 2.6). *X. sexdentatus* has the smallest distance to the population of Portugal (D^2 5.5), followed by the population of Ibiza (D^2 8.4), Bretagne (D^2 9.6) and at last Greece (D^2 11.5). The population of the Bretagne shows high distances to the population of Ibiza (D^2 20.6), Greece (D^2 19.9) and Portugal (D^2 14.1) (Table 4). The first canonical function (root1) accounted for 64.57% of the explained variance. The first and the second canonical function explained 87.15% of the total variance.

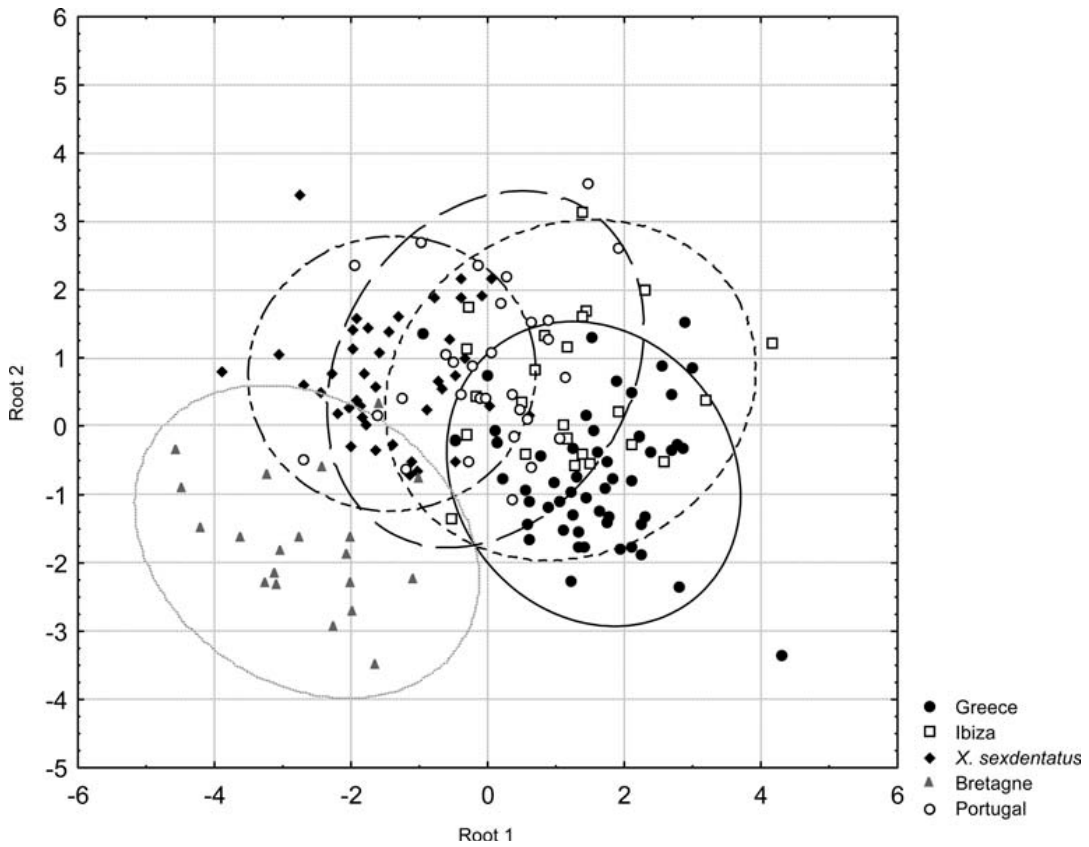


Figure 3. Canonical analysis depicting discrimination by morphometric measurements between *X. sexdentatus* and *X. hydrophilus* from Bretagne (France), Portugal, Ibiza (Spain), and Parga (Greece); plot of the first discriminant function (root 1) against the second (root 2).

The four geographic groups of *Xantho poressa* are not as clearly separable, despite significant differences in two character ratios and overall differences in discriminant analysis. Interestingly, most distant is the Adriatic population from two other Mediterranean populations, while it is morphologically closer to the Atlantic population. The 1-Factor-ANOVA analysis of the ratios revealed no significant difference in the ratio body height to carapace width (d*f* 3; F=1.433; *P*=0.223), but significant differences in the ratios carapace length to carapace width (d*f* 3; F=10.835; *P*<0.001) and frontal width to carapace width (d*f* 3; F=15.890; *P*<0.001).

Table 4 Numbers and percentage correct classification to the different populations (in bold letters) and Mahalanobis distances (D^2) based on the morphometric classification function for the four populations of *X. hydrophilus* and *X. sexdentatus*, and five populations of *X.poresssa*

Populations of *X. hydrophilus*	Bretagne	Portugal	Ibiza	Greece	*X. sexdentatus*	Correct classification (%)
Bretagne	**18**	**1**	**0**	**0**	**1**	**90**
Portugal	**1** D^2 14.1	**24**	**2**	**2**	**2**	**77.4**
Ibiza	**0** D^2 20.6	**5** D^2 2.6	**7**	**10**	**2**	**29.2**
Greece	**0** D^2 19.9	**2** D^2 6.9	**1** D^2 2.2	**49**	**0**	**90.7**
X. sexdentatus	**0** D^2 9.6	**1** D^2 5.5	**1** D^2 8.4	**1** D^2 11.5	**39**	**92.8**

Populations of *X. poressa*	Atlantic	Corsica	Ibiza	Greece	Adria	Correct classification (%)
Atlanitc	**23**	**0**	**0**	**17**	**17**	**40.35**
Corsica	**1** D^2 3.6	**1**	**2**	**17**	**1**	**4.5**
Ibiza	**4** D^2 2.5	**0** D^2 0.5	**1**	**20**	**2**	**3.7**
Greece	**9** D^2 2.9	**0** D^2 0.3	**0** D^2 0.7	**86**	**6**	**85.1**
Adria	**9** D^2 0.8	**0** D^2 4.8	**0** D^2 3.9	**18** D^2 3.1	**48**	**64**

The post hoc Schefé-test was always nonsignificant between the populations of Ibiza, Corsica and Greece and between the populations of the Adria and the Atlantic. However, the analysis revealed significant differences (P<0.001) in the ratios frontal width to carapace width and carapace length to carapace width for both the Atlantic and the Adriatic populations versus the population of Greece, Ibiza and Corsica.

For the discriminant analysis four log transformed variables were used: carapace length, carapace width, body height and frontal width. Leg length was excluded from this analysis, because it was not normally distributed. The four groups are not as clearly separated as the populations of *X. hydrophilus* but also show that the group dispersion was not homogenous (Box's M-test: M=292, $F_{30, 1236}$ = 9,117, P<0.001) and highly significant differences (Wilks' Lambda: 0.49, F (16.837) = 13.414, P<0.00001; 56.38% correct classification) (Figure 4).

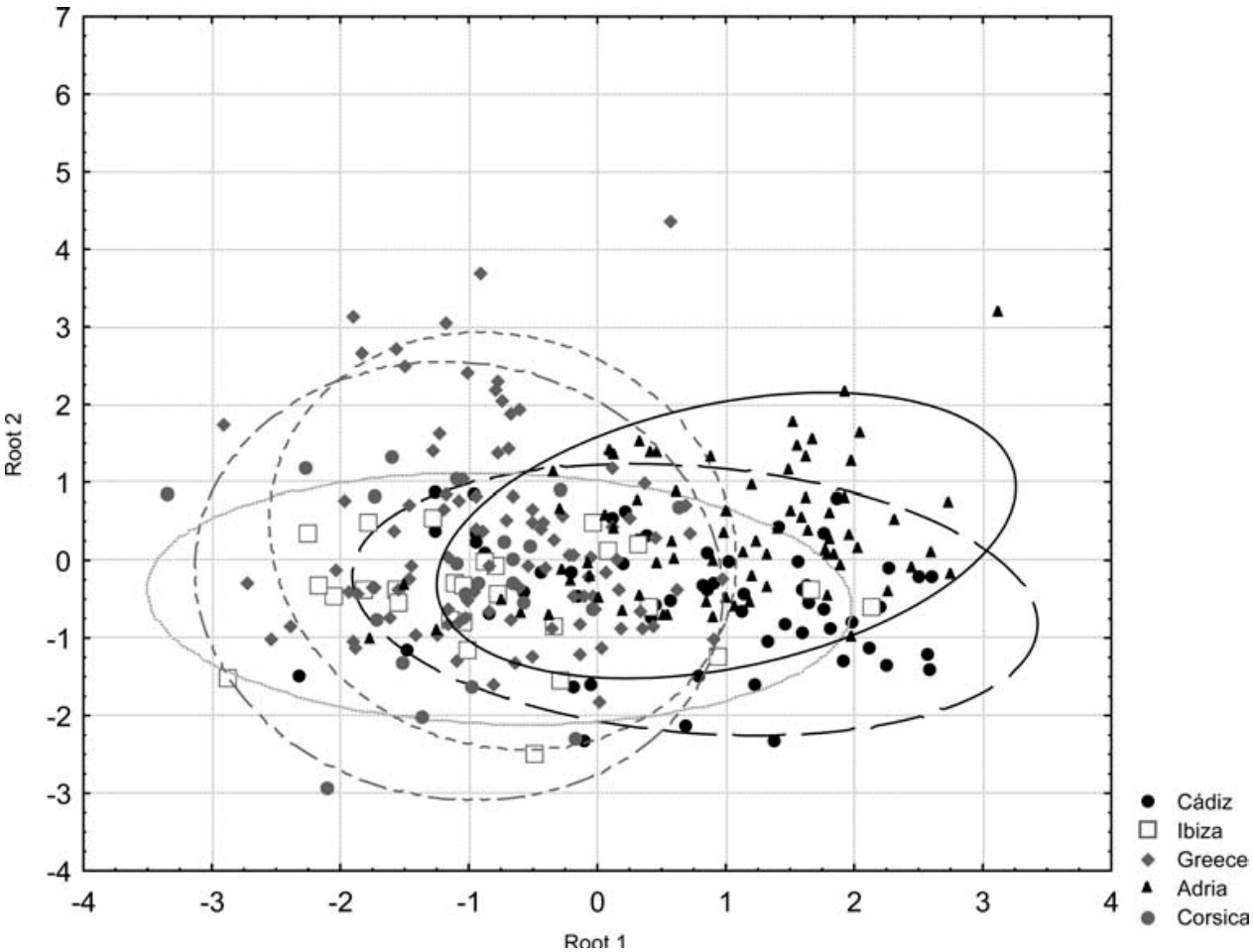


Figure 4. Canonical analysis depicting discrimination by morphometric measurements of *X. poressa* from Cádiz (Spain), Ibiza (Spain), Croatia (Adria) and Parga (Greece); a plot of the first discriminant function (root 1) against the second (root 2).

The canonical analysis showed that the significance was mostly due to the following differentiation: the population of Ibiza and Corsica grouped together with the population of Greece and the Adria population with the Atlantic one. In addition, the classification matrix showed that individuals belonging to Ibiza and Corsica were only correctly classified in less than 10%. Instead, they correspond more often to the population from Greece (Table 4). The Mahalanobis distances (D^2) reveal that populations of Ibiza and Greece (D^2 0.7) and the populations of Corsica and Greece (D^2 0.3) are closer to each other than the Atlantic population to Ibiza (D^2 2.5), Corsica (D^2 3.6) and Greece (D^2 2.9). On the other side, the population from the Adria is very similar to the Atlantic (D^2 0.8) and shows higher distances to Greece (D^2 3.1), Ibiza (D^2 3.9) and Corsica (D^2 4.8) (Table 4). The first canonical function (root1) accounted for 82% and the first and second (root2) for 98% of the explained variance.

DISCUSSION

Population structure, as estimated by neutral molecular markers, is determined by the interactions between gene flow and genetic drift (Wright 1943; Kimura & Weiss 1964; Slatkin 1985). Interpretation of the level of structure can be difficult, because historical events and a variety of nonobvious mechanisms can be involved in the separation processes that eventually lead to future speciation.

The 16S rRNA and the more variable COI mitochondrial genes have been shown to be variable enough for population studies in marine crabs (Schubart et al. 2000a; Fratini & Vannini 2002). In this study, no single nucleotide position along 1167 basepairs of mtDNA corresponding to the 16S rRNA or COI genes, could be used to consistently distinguish different populations within *X. hydrophilus* or *X. poressa* nor to separate *X. sexdentatus* from *X. hydrophilus*. However, the analyses of frequencies of haplotype distributions in the *X. hydrophilus–X. sexdentatus* complex and morphometric comparisons separate *X. sexdentatus* and allows distinction of geographic forms in *X. hydrophilus*, with the geographically most distant populations from the Atlantic (Bretagne) and the Mediterranean (Greece) also being most distinct and the other two populations representing transitional forms. This could be the result of recent separation followed by incomplete lineage sorting and occasional gene flow in neighbouring populations (e.g. Ibiza and Portugal). The geographic separation may have been caused or maintained by the Strait of Gibraltar acting as a gene flow barrier. On the other hand, this study shows that there is also restricted gene flow within the Mediterranean Basin, for which the barriers and exact patterns still need to be determined.

Triantafyllidis et al. (2005) showed that the Aegean population of the European lobster *Homarus gammarus* differ significantly from the Atlantic samples, as well as from the ones from the Adriatic and West Mediterranean based on haplotype frequencies and Φ_{ST} of a 3-kb mitochondrial DNA segment. Duran et al. (2004b) using 644 basepairs of the COI gene, detected a slight but significant pattern of genetic differentiation between the Atlantic and Mediterranean populations of the sea urchin *Paracentrotus lividus*. Zane et al. (2000) also observed a genetic cline between both sides of the Gibraltar Strait for the pelagic crustacean *Meganyctiphanes norvegica* based on a 200 basepair fragment of the mitochondrial NADH subunit I. Borsa et al. (1997) carried out analyses of allozyme variation showing fixed allele differences among populations from the Atlantic, the western Mediterranean, the Adriatic Sea and the Aegean Sea for the fish species *Platichthys flesus* and *P. stellatus*. Furthermore, comparing samples with enzyme electrophoresis, Monteiro et al. (1997) revealed extensive genetic divergence between populations of the common intertidal sea anemone *Actinia*

equina from Britain and the Mediterranean. Additional examples for Atlanto–Mediterranean differentiation were already enumerated in the Introduction (Quesada et al. 1995; Geller et al. 1997; Pannacciulli et al. 1997; Naciri et al. 1999; Pérez- Losada et al. 2002). On the other hand, in the literature we also find examples of closely related pairs of brachyuran crabs, that are treated as distinct taxa, but for which genetic separation has not been demonstrated so far. For example, two Mediterranean species of the varunid genus *Brachynotus*, *B. sexdentatus* and *Brachynotus gemmellari*, (see Froglia & Manning 1978), the stone crabs *Menippe mercenaria* and *Menippe adina* (Menippidae) from the Gulf of Mexico and western Atlantic (Williams & Felder 1986), the panopeid crabs *Panopeus herbstii* and *P. stimpsoni* from the north-western Atlantic (Schubart et al. 2000b) and the varunid crabs *Cyrtograpsus altimanus* and *C. affinis* from the Argentinian coast (Spivak & Schubart 2003) lack consistent differences in the 16S mtDNA and can only be separated on the basis of colour, morphometry or bathymetry, indicating recent separation or phenotypic variability (Schneider-Broussard et al. 1998; Schubart et al. 2001; Spivak & Schubart 2003). These could represent additional examples for the recent insight that morphological differences between regional populations may be independent from the genetic discontinuities between lineages (see also Flowers & Foltz 2001; Wilding et al. 2000). On the other hand, none of these examples have been addressed with population genetic methods. Comparison of a few individuals with the 16S rRNA gene would also have been insufficient in our case study to reveal genetic structure within *X. hydrophilus* and differences in haplotype frequencies between *X. sexdentatus* and *X. hydrophilus*.

The stepping stone model of Kimura and Weiss (1964) assumes a negative correlation between genetic relatedness and geographic distance. In our case, it is possible that gene flow occurs only among adjacent populations of *X. hydrophilus* and therefore the Φ_{ST} is largest between the most distant populations and transitional stages of *X. hydrophilus* exist in the vicinity of the Strait of Gibraltar, all this reflecting isolation by distance. This could be favoured by the relatively short larval development of *X. hydrophilus*, because the genetic structure of populations of marine animals is often correlated with different potential of dispersal in their larval stages (Kyle & Boulding 2000). The larval development of *Xantho* consists of four zoeal stages (Ingle 1983), which is relatively short in comparison to for example the larval development of *Pachygrapsus marmoratus*, another Mediterranean littoral species, with eight zoeal stages (Cuesta & Rodríguez 1994). Pogson et al. (2001) recognized isolation by distance in the Atlantic cod *Gadus morhua*, Planes et al. (1996) in the surgeonfish *Acanthurus triostegus* and Ketmaier (2002) in the central Italian *Porasellus*

coxalis isopod group. Palma and Andrade (2002) found a clear geographic gradient within the fish genus *Diplodus* between Atlantic and Mediterranean samples using morphometric comparsions.

The discriminant analysis of our morphometric data revealed geographic differences for *Xantho hydrophilus* and less pronounced also for *X. poressa*. The classification matrix of four populations of *X. hydrophilus* shows that the population from Greece and the population from the Bretagne can be classified correctly with a high likelihood and thus form morphometrically well separable groups. In contrast, the populations from Ibiza and Portugal do not represent such distinct groups. For the population from Ibiza the Mahalanobis distances indicate a high similarity to the neighbouring populations from Greece and Portugal. Furthermore, the Φ_{ST} values also show that the population from Greece has a larger distance to the population from Portugal than to the intermediate population from Ibiza. In conclusion, also in morphometry the population from Ibiza represents a transitional form between the Atlantic and the Mediterranean Sea. This is exactly what has been postulated in the literature concerning the separating morphological characters of the two subspecies of *X. hydrophilus*: transitional forms in the western Mediterranean Sea were recognized by Almaça (1985) and d'Udekem d'Acoz (1999). In our case, also *X. hydrophilus* from Portugal forms a transitional form in morphometry between the Bretagne and the Mediterranean Sea. Therefore, it remains impossible to define the exact boundaries of the two variations of *X. hydrophilus*, especially when trying to define the ranges of the possible subspecies.

We therefore consider *Xantho hydrophilus* from the Atlantic and the Mediterranean one single species and suggest not to use *X. hydrophilus granulicarpus* as a distinct subspecies. It possibly represents a morphological variant (forma granulicarpus) which seems to be more common in the Mediterranean, but with no taxonomic value. The other taxonomic problem turns out to be the status of *Xantho sexdentatus*. With the current lack of results from nuclear DNA, we suggest that *X. sexdentatus* and *X. hydrophilus* should still be considered as two different species (due to their consistent remarkably different morphologies), between which mitochondrial introgression may occur. Introgression refers to gene movement between species or genetic populations mediated by hybridization or backcrossing (Avise 2004). Thus, introgression of mtDNA between taxa can cause two species that were monophyletic to become para- or even polyphyletic with respect to mtDNA. Rawson and Hilbish (1998) have shown that *Mytilus edulis* mtDNA haplotypes appear in mussel populations from the Baltic Sea, which have predominantly *M. trossulus* nuclear genotypes, indicating that introgressive hybridization is prevalent among European mussel populations. Alternatively, a lack of

concordance between species delineation and mitochondrial gene genealogies can be the result of incomplete lineage sorting (Avise et al. 1984).

It has been suggested that in the marine environment many species may be organized into large panmictic populations (Palumbi 1992, 1994). Our genetic and morphometric data show that *X. hydrophilus* cannot be classified as panmictic. In the case of *X. poressa*, there was only low variability in the morphometric data, but so far population genetic data are lacking. The morphometrically distinct population of the Adria is similar to the Atlantic population and shows larger distances to the western and central Mediterranean populations. The Adria holds an exceptional position within the Mediterranean Sea, because of its different temperature and salinity regimes as well as for its unusual tidal influence. However, the effect of the Adriatic Sea on the morphometry of xanthid crabs is not consistent. A single individual of *X. hydrophilus* obtained from the northern Adria clustered morphometrically with the central Mediterranean and not Atlantic. It has to be considered, however, that in the Mediterranean the two species distribute at different depths: *X. poressa* lives in the shallow subtidal zone (0–2 m) and thus under direct tidal influence, whereas *X. hydrophilus* is more common in deeper rocky areas (1.5–10 m), and less influenced by tides. We therefore propose that the similar morphometry of *X. poressa* from the Adria and the Atlantic Ocean may reflect phenotypic plasticity or convergence and not genetic similarity. This, however, remains to be tested genetically. Similarly, in the study of Schubart et al. (2001), it was suggested that *Brachynotus gemmellari* and *Brachynotus sexdentatus* possibly represent different ecophenotypes of a single species at different depths. Also in *Cyrtograpsus affinis* and *C. altimanus*, molecular and morphometric comparisons revealed no genetic structure, but two different morphs that were always associated with subtidal versus intertidal habitats (Spivak & Schubart 2003). Phenotypic plasticity influenced through the tides was also found in the snail *Littorina saxatilis*, in which a Venice sample and a Swedish sample, both with weak tidal influence, show morphological similarity but are distinct from a British sample and another Swedish with strong tidal influence (Janson 1985). Besides allopatric separation, clinal variation thus represents an alternative explanation for local mechanisms of adaptation (Quesada et al. 1995).

In order to determine (1) how consistently Mediterranean and Atlantic forms can be separated, (2) if the closely related species *X. sexdentatus* hybridises with *X. hydrophilus*, (3) how many genetic and morphometric subunits of *X. hydrophilus* can be recognized and (4) where their exact boundaries are in the Mediterranean Sea, many more specimens of the genus *Xantho* from other areas of its distribution, i.e. Canary Islands, eastern Mediterranean

Sea, and Black Sea need to be included in the analysis. More variable markers (e.g. microsatellites or AFLP) would help to resolve questions concerning local gene flow. In addition, population genetic studies of species with similar distributions and life histories (*X. poressa*, *Brachynotus sexdentatus* complex) shall be carried out for comparative purposes.

ACKNOWLEDGEMENTS

Our special thanks go to Senckenberg Museum Frankfurt, Naturalis Museum Leiden, Muséum National d'Histoire Naturelle Paris, Dirk Brandis, Darryl Felder, Gustavo Flores, Joana Garcia, Danièle Guinot, Jürgen and Maike Heinze, Fernando Mantelatto, Carsten Müller, Tobias Santl, Rafael Robles, Reiner Rubner, José Cuesta, Henrik and Sophia Schubart, Cédric d'Udekem d'Acoz, Peter Wirtz, and Petra Zillner, for helping to collect or sending xanthoid crabs. Thanks are further due to Estelle Roux for helpful discussions of statistical tests, Katja Pusch for support with Arlequin and Anne Hartmann for her comments.

PUBLICATION 2

CONTRASTING GENETIC DIVERSITY WITH PHENOTYPIC DIVERSITY IN COLORATION AND SIZE IN *XANTHO PORESSA* (BRACHYURA: XANTHIDAE), WITH NEW RESULTS ON ITS ECOLOGY

Silke Reuschel and Christoph D. Schubart

Manuscript published by *Marine Ecology*

ABSTRACT

The ecology of *Xantho poressa* (Olivi, 1792) (Brachyura) was studied during field trips to the Mediterranean Sea, the Black Sea and the Spanish Atlantic Ocean. Our results reveal that *X. poressa* lives from the intertidal to the shallow subtidal zone, and inhabits relatively protected rocky shores, often with pebble underground, from juvenile to adult stages. A mark–recapture experiment revealed a high population density in this habitat. All stages, but predominantly juveniles, show a variability of colour patterns, which allow the crabs to blend in with the rocky substratum, thereby hiding from predators as passive defence. Adulthood can be reached with a carapace length smaller than 6 mm. The morphometric analysis of the species revealed allometric growth in carapace shape. Variability in overall size could be observed at different collecting sites. Neither the colour morphs nor the size differences could be attributed to differences of Cytochrome Oxidase subunit I mitochondrial DNA sequences, suggesting that ecological rather than genetic patterns are responsible for the different phenotypes.

PROBLEM

Recently, phenotypic plasticity has become an important concept in evolutionary thinking (Pigliucci 2005). Phenotypic plasticity can be inclusively defined as the production of multiple phenotypes from a single genotype, depending on environmental conditions (Miner et al. 2005). In many marine crabs, phenotypic plasticity in carapace coloration patterns has been observed. This variation could be related to habitat and may involve some advantage against visual predators through crypsis and carapace polymorphism by making it more difficult to create a search image (Todd et al. 2005). In the case of disruptive coloration, the characteristic outline of an individual is broken by bands, stripes and spots. The individual elements of the colour patterns imitate common environmental objects (e.g., in marine habitats, bits of rock, shell or algae) to a visual predator (Cott 1957). The shrimps *Heptacarpus pictus* and *H. paludicola* are under predation pressure by fish, and it is suggested that the colour patterns are camouflage against such visually-hunting predators (Bauer 1981). Moreover, the body size is already known to be important for the evolution of crypsis (Forsman & Appelqvist 1999). Crypsis is predominantly found among several decapods that are fish-bite sized. Small crabs remain cryptic through all developmental stages. The phenomenon of colour polymorphic early settlers and monochromatic large crabs has been reported in environments dominated by small predatory fish, where large crabs are less threatened (Palma & Steneck 2001). In two species of marine crabs it has been suggested that the variability in carapace colour pattern disappears with increasing size (Bedini 2002). In contrast, the importance of the use of colour patterns to differentiate among cryptic and sibling species in decapods was discussed, e.g. for the alpheid shrimp (Knowlton & Mills 1992) and for the grapsid genus *Goniopsis* (von Sternberg 1994).

The so-called European “stone crab” *Xantho poressa* (Olivi, 1792), previously also known as *Xantho rivolosus* (Risso, 1816), is a very common crab in the Mediterranean Sea as well as in parts of the north-eastern Atlantic Ocean. The species is characterized by a wide oval carapace, short legs, unequal chelipeds, a notch in the upper buccal field and keel-like carapace teeth (Forest in Drach & Forest 1953). Its distribution ranges from the Canary Islands to Portugal and it is present in the entire Mediterranean Sea, including the Black Sea. Bedini (2002) suggests that juvenile stone crabs live in *Posidonia oceanica* seagrass meadows until the puberty moult, after which they abandon the prairies as adults for the nearest rocky substrate. He defines as juveniles the size class of up to 8 mm carapace length, and suggests that the adult stage is reached at 12-13 mm. Furthermore, he postulates that *X.*

poressa exhibits a seagrass-specific colour pattern for cryptic mimicry while living as juveniles among the *Posidonia* leaves.

For ecological and morphological studies, it is often important to know when crabs mature, e.g. in our case to be able to determine a possible loss in colour polymorphism as adults. Results from morphometry can be applied to determine patterns of relative growth, establish sexual dimorphism and the approximate size at onset of sexual maturity (Hartnoll 1982). For the latter purpose, allometric relationships of pleon width in females and chelar propodus size in males are related to carapace length or width. An abrupt change of the growth patterns of pleon or chelae characterizes the puberty moult (Hartnoll 1974).

We studied the ecology of *Xantho poressa* at different collection sites along the Adria, Ionian Sea, eastern Mediterranean, Black Sea and Spanish Atlantic Ocean. These sites are characterized by different temperature and salinity regimes as well as by different tidal influences (Hofrichter 2002; Dimitrov & Dimitrov 2004).

The targets of our study were (1) to determine habitat preferences of *Xantho poressa* as juveniles and as adults, (2) to document variety of colour patterns and their importance for camouflage in different habitats, (3) to determine if different colour patterns and geographically distant populations can be separated genetically using DNA sequences of the mitochondrial gene Cytochrome Oxidase subunit I (COI), (4) to study patterns of relative growth for determining the onset of maturity, (5) to test if the morphometry of *X. poressa* is constant throughout its distributionary range and (6) to estimate local population density.

MATERIALS AND METHODS

Habitat preferences were documented in the field and the variability of colour patterns were photo-documented (Figure 1). A large variety of different colour morphs, different geographical regions and different sizes (small, medium, large; see below) were included in the genetic analyses (Table 1).

Figure 1. Examples for different colour morphs of *Xantho poressa*: A: purple colour morph, cl: 16.4 mm; B: white transverse stripes on the walking legs and carapace, cl: 10.77 mm; C: white band on the margin of the frontal carapace, cl: 8.22 mm; D: white transverse stripes on the walking legs and carapace, cl: 7.71 mm; E: orange colour morph, cl: 4.62 mm; F: camouflage: four crabs hidden among colourful pebbles. The colour variability encountered in nature was much higher.

Table 1. Localities, dates of collection (month-year), number of specimens used for genetic (N_g) and morphometric (N_m) comparisons and genetic accession number of all haplotypes of *X. poressa*. Abbreviation of museum: SMF: Senckenberg Museum und Forschungsinstitut, Frankfurt am Main.

collection site	N_g	N_m males	N_m females	catalogue no.	EMBL no.
Spain: Cádiz, 4-2004	10	41	20	SMF 30122	AM418522–23
Spain: Ibiza, 3-2001 & 3-2003	9	14	13	SMF 27529/30123	AM418525–27
Spain: Alicante, 5-2005	4	17	14	SMF 31198–99	AM418524
Spain: Tarragona, 9-2006	0	20	10	SMF 31200–01	n/a
Croatia: Istra, 8-2001, 9-2004 & 9-2005	8	47	28	SMF 27533	AM418516–21
Greece: Parga, 9-2003	4	57	44	SMF 31190	AM418531
Greece: Corfú, 9-2002	2	11	6	SMF 31189	AM418530
France: Corsica, 6-2004, 6-2006	2	18	9	SMF 30124	AM418528–29
Bulgaria: Varna & Sozopol, 6-2005	3	44	25	SMF 31192–93	AM418532
Morocco: Nador, 11-2005	4	4	5	SMF 31195	–

Genomic DNA was extracted from the muscle tissue of a walking leg using the Puregene kit (Gentra Systems). A selective amplification of an approximately 660 bp fragment from the COI (excluding primers) of a total number of 50 specimens of *X. poressa* was carried out by polymerase chain reaction (PCR) (40 cycles; 45 s 94°/1min 48-50°/1min 72° denaturing/annealing/extension temperatures). New primers in the COIf–COIa region (see Palumbi et al. 1991) were designed to allow amplification of *X. poressa*. The following primer combinations were used: COIf (5´-CCT GCA GGA GGA GGA GAY CC-3´) and COIa (5´-AGT ATA AGC GTC TGG GTA GTC-3´) (Palumbi et al. 1991); COIf and the new CO H18 (5´-CTA TGG AAG ATA CGA TGT TTC-3´) and the internal primers COL3 (5´-ATR ATT TAY GCT ATR HTW GCM ATT GG-3´) and COH3 (5´-AAT CAR TGD GCA ATW CCR SCR AAA AT-3´) (Reuschel & Schubart 2006). The PCR products were purified with Millipore Montage PCR Centrifugal Filter Devices (Millipore, Corp.) or with 'Quick/Sure Clean' (Bioline). The products were precipitated with ethanol, resuspended in water and sequenced with the ABI Big Dye terminator mix (Big Dye Terminator® v 1.1 Cycle Sequencing Kit; Applied Biosystems) in an ABI Prism automated sequencer (ABI Prism™ 310 Genetic Analyzer; Applied Biosystems). The sequences were proofread with the program ABI Sequencing Analysis® 3.4 (Applied Biosystems) and manually aligned with BioEdit (Hall 1999). Parsimony networks were built with TCS (estimating gene genealogies version 1.13; Templeton et al. 1992). Genetic heterogeneity within populations was estimated as haplotype diversity (h) (Nei & Tajima 1981) and nucleotide diversity (π) (Nei 1987) with DnaSP 4.00 (Rozas et al. 2003).

For the morphometric comparisons, 414 specimens of *X. poressa* were included in this study. Material from Ibiza (Spain, 2001 & 2003), Alicante and Tarragona (Spain, 2005 & 2006), Corfú and Parga (Greece, 2002 & 2003), Corsica (France, 2004), Istra (Croatia, 2001 & 2004)

(all Mediterranean), Cádiz (Spain, 2004) (Atlantic), Morocco (2005), Sozopol and Varna (Bulgaria, 2005) (Black Sea) including both sexes, was examined (Table 1). Most crabs from Croatia, Greece and Bulgaria were released after taking measurements. Specimens were taken from the intertidal zone to a depth of 10 m by snorkelling and occasionally by SCUBA diving. Visual and manual sampling methods were constant, including all sizes of crabs and carried out by the same collectors and only in clear water conditions, so that no bias could have resulted.

The sample size per population ranged from 9 to 102 individuals. The population was initially defined according to geographical regions. For the morphometric analyses the following morphological measurements were taken: (1) carapace width (cw); (2) carapace length (cl); (3) body height; (4) frontal width; (5) ventral leg length of the fourth leg; (6) length of chelar dactylus; (7) length of chelar propodus; (8) height of chelar propodus; (9) pleon width. The data were tested for normal distribution with the Kolmogorov–Smirnow test (software Statistica 6.0; StatSoft). Growth of female pleon width and male chelar size were plotted against carapace width to test for abrupt changes in growth which may be interpreted as the onset of maturity (Hartnoll 1974, 1982). Patterns of morphometric relationships can be influenced by the effect of allometric growth and size in species of undetermined age. To reduce the influence of allometry, all measurements were transformed to ratios. Morphometric ratios were calculated by relating measurements to carapace width. To enable testing of the remaining effects of allometry, the specimens had to be grouped. We chose the following categories: small (cl: 3–15.50 mm), medium (cl: 15.51–20 mm) and large (<20 mm) in a way that large populations with smaller individuals (e.g., Greece) were entirely represented in the smallest category. To subdivide larger animals further, the 20 mm category was used to maintain approximately 5 mm differences between classes of adult animals. The comparison of morphometric ratios of different groups and geographical regions was carried out with a one-factor ANOVA and a post-hoc Schefé test. We also included a Levene test to determine the homogeneity of the data. In addition, discriminant analysis was carried out for a more accurate differentiation between the groups using ratios. To test possible effects of size of individual crabs on the used ratios, a regression-analysis was used to compare regressions of the ratios carapace length to carapace width against carapace width, carapace height to carapace width against carapace width and frontal width to carapace width against carapace width.

For the mark–recapture experiment, we used the Schnabel method (Krebs 1999). This method depends on repeated capture and recaptures sessions. The first day, 40 individuals were

marked over a short time and released. The marking was done by clipping the dactyls of one to four walking legs in different combinations. The following days, 40 individuals were captured again, checked for marks, then marked and released again. A series of four independent sample sessions were performed in a confined cove near Parga (western Greece). The cove was 13 m broad, 15 m long and delimited by a steep coast. The population density was estimated after the formula of Schumacher and Eschmeyer (see Krebs 1999).

RESULTS

The study of habitat preference revealed that *Xantho poressa* is a common crab in the intertidal (especially Atlantic) and the shallow subtidal zone, to a depth of 3 m. In *Posidonia oceanica*, the stone crab was very rarely found. Juveniles and adults preferred to stay under larger boulders with a rocky underground consisting of small pebbles of different coloration in shallow areas with low wave exposure, i.e. little rock displacement. All stages, but especially smaller animals, revealed a high variability of colour patterns. In some cases, the crabs show disruptive coloration in the form of white transverse stripes on the walking legs or a white band on the margin of the frontal carapace (Figure 1).

The comparison of 50 sequences of 614 basepairs of COI revealed the existence of one common and probably ancestral haplotype within *X. poressa*. The network shows a star-like shape, with most haplotypes being connected by few mutation steps (Figure 2). Sixteen rare haplotypes diverge from the common haplotype (HT1). They were generally present in not more than one sampled individual. HT1 includes most specimens (n=32) with representatives of different colour morphs and from all geographical regions (Croatia: Pula; France: Corsica; Spain: Cádiz, Alicante, Ibiza; Greece: Corfú, Parga; Bulgaria: Varna, Sozopol; Morroco: Nador). Eight specimens with the colour morph “white front” belong to four different haplotypes (five times HT1, once HT2, HT8 and HT15). Also the “white stripe” morph is not attributable to a specific haplotype (HT1 and HT16). Specimens from the same collecting point (Spain: Alicante) with many different colour patterns shared HT1 except for one individual (HT9). More derived haplotypes do not share a specific coloration or geographic pattern. There is also no separation by size: HT1 includes all three categories, HT 5 is present in a medium and a large crab and HT 10 in a small and a medium one. There is low genetic differentiation within *X. poressa*: haplotype diversity *h* of the COI gene is moderate (0.593) and the nucleotide diversity π is relatively low (0.004).

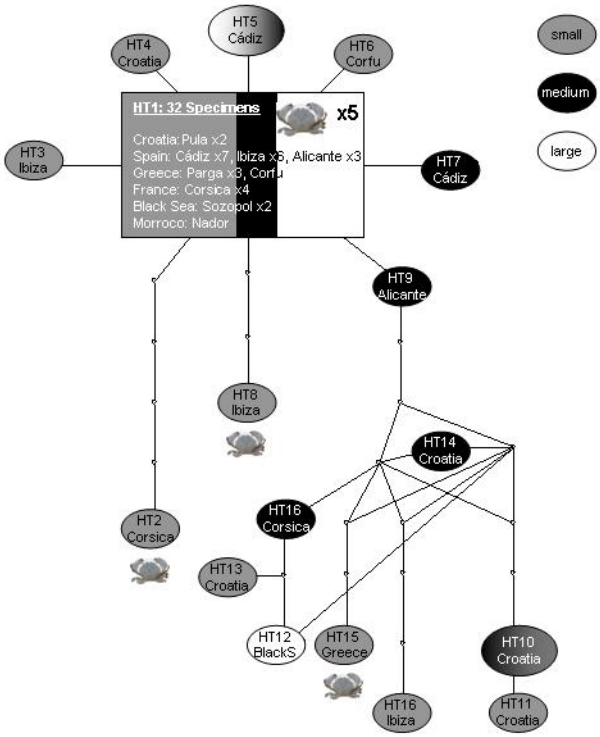


Figure 2. Minimum parsimonious spanning network constructed with TCS of a 614-bp fragment from the COI gene of *X. poressa* (n=50). Each line represents one substitution; black spots on lines indicate additional substitutions separating two haplotypes. The size of the circle is representative for the frequency of the haplotypes (small circle: N=1; medium circle: N=2; the rectangular box stands for the suggested ancestral haplotype). The shading corresponds to the different size categories; the illustrated crabs represent the white front colour morph.

The size of the cove used to estimate population density was approximately 170 m². The capture-recapture technique allowed calculating a density of at least 1.62 specimens / m². The captures and recaptures are listed in Appendix 1. During all our field trips, we always had the impression that *X. poressa* is a species with high population density. The collecting was done by turning stones in a depth from 0.3 to 2 m. Under almost every suitable stone one to four (rarely 10) specimens were found with different sizes and colour patterns.

In Parga (Greece), we collected 274 crab-specimens ranging from 3 to 16 mm in carapace length during three days in the rocky shallow subtidal and in Croatia during one day 60 specimens ranging from 7 to 23 mm. The males are the larger specimens, reaching a carapace length of 29 mm, the females 22 mm (Figure 3A). The most frequent size classes in males and females are shown in Table 2. From 69 specimens collected during two days in Bulgaria, females (n=25) ranged from 6 to 22 mm and males (n=44) from 7 to 29 mm carapace length, which makes the specimens from the Black Sea the largest *Xantho poressa* collected by us. The data of the Black Sea show a lack of medium-sized crabs, which must be the consequence from an unwanted bias during collecting, resulting in higher numbers of large

and small individuals. Only a few and only large crabs were found in the shallow parts of Nador Lagoon (Sebkha Bou Areq) in Morocco. The females (n=5) ranged from 20 to 22 mm carapace length and the males (n=6) from 23 to 27 mm. The Morocco field trip was in the end of November so that the sample size and the collection bias during the short immersion could be influenced by the cold temperature. Alternatively, smaller crabs may have migrated to deeper waters. In general, the specimens from the northern Adria, the Spanish Atlantic, Morocco and the Black Sea were larger animals in comparison to the smaller specimens from the Ionian Sea and the western Mediterranean. To test for the role of habitat on size distribution we sampled in September 2006 two different collection sites along the Spanish province of Tarragona in a distance of approximately 56 kilometres coastline during the same day. In L'Ampolla (Tarragona I in Table 3) we could find all size categories in an anthropogenically disturbed habitat (jetty and anoxic sediments) and at Cap de Salou (Tarragona II in Table 3) only small crabs were found in a relatively undisturbed natural cove (Table 3). Females carrying eggs from Greece and Corsica ranged from 5-12 mm (Figure 3B). In none of the populations, morphometric changes in the measured relationships allowed to determine onset of maturity.

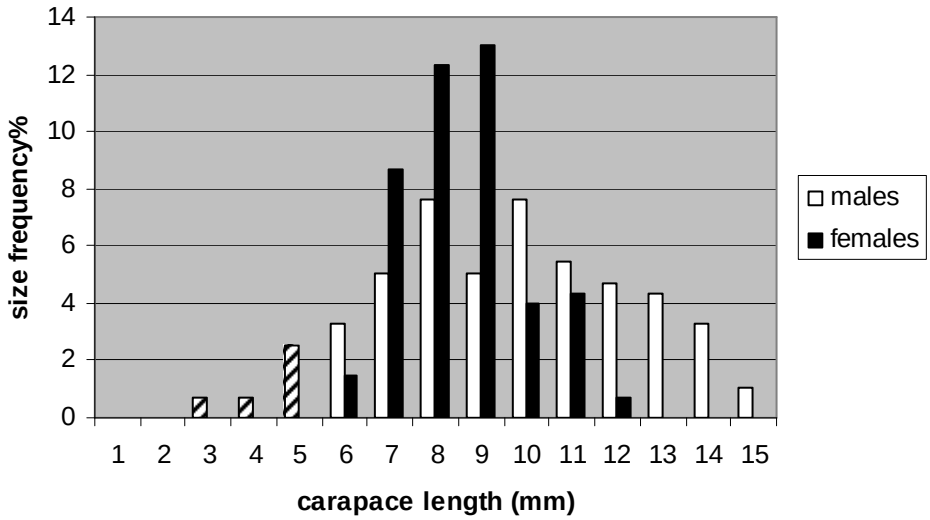
Table 2. Most frequent size classes of males and females of *Xantho poressa* at different collection sites (n = number of sampled specimens; CL = carapace length).

	males (n)	CL (mm)	females (n)	CL (mm)
Greece	22	10–11	36	8–9
Croatia	17	14–15	5	11–12
Ibiza & Corsica	5	9–10	6	8–9
Cádiz	5	17–18	4	12–13

Table 3. Percentages of the various size classes of *Xantho poressa* among the different collecting sites.

geographical region	$\%_{Small}$	$\%_{Medium}$	$\%_{Large}$
Spain: Cádiz	56.4	30.9	12.7
Morocco: Nador	0	0	100
Spain: Alicante	90	10	0
Spain: Ibiza	92.6	7.4	0
Spain: Tarragona I	55.6	38.9	5.5
Spain: Tarragona II	100	0	0
Corsica	84.6	11.5	3.9
Croatia: Pula	51.8	42.9	5.3
Greece: Parga	100	0	0
Bulgaria	47.6	15.8	36.6

A



B

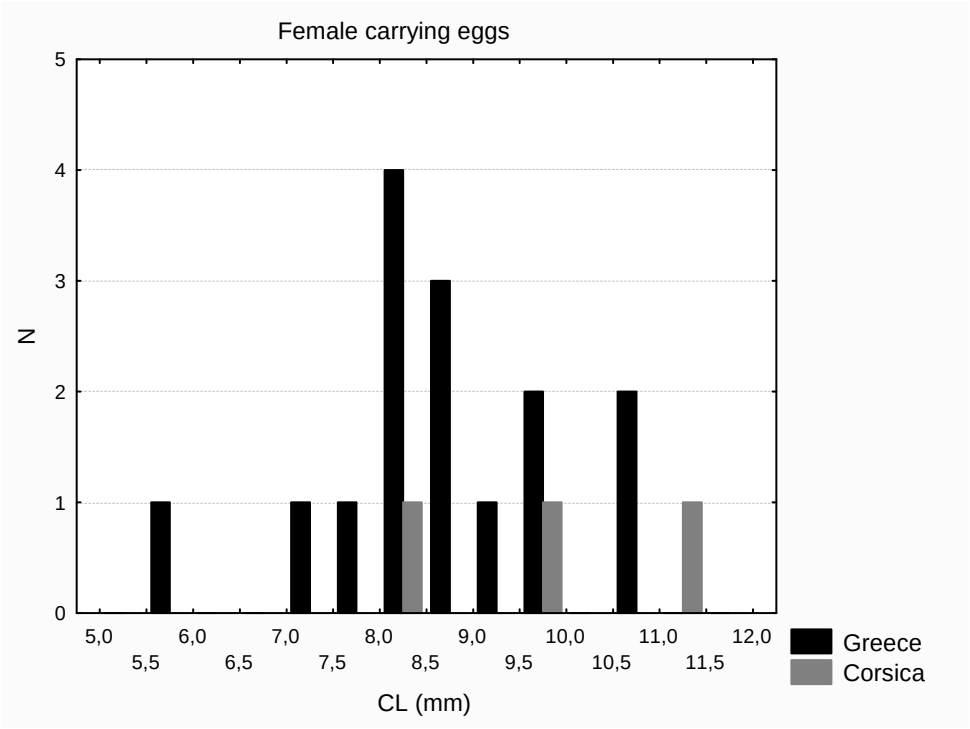


Figure 3. A) Size-frequency distribution of females and males of *Xantho poressa* from Greece, B) carapace length (CL) of females carrying eggs (N=number of animals)

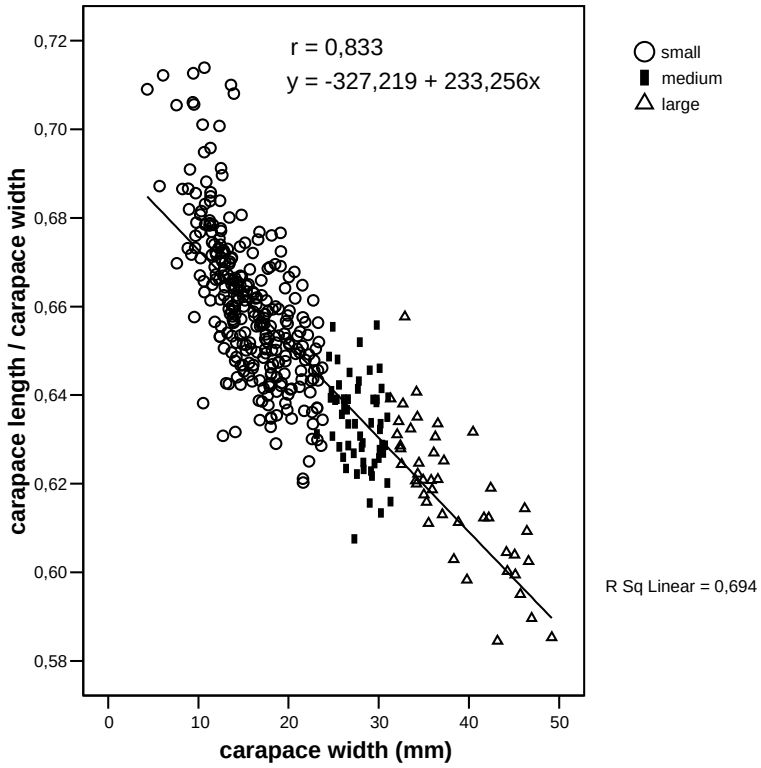
The test of normal distribution revealed a slight left shift for the measurements of carapace width, carapace length, body height and frontal width. Therefore an ln-transformation of the raw data was done previous to statistic analyses. A transformation for the not-normal distributed ventral leg length of the fourth leg was not possible, so that it was excluded from

the subsequent analyses. When using morphometric data for population comparisons, it is also necessary to test the influence of allometry. This became especially important after realizing that different geographic populations consist of animals of different size classes (Table 3). A one-factor ANOVA analysis revealed significant differences between the regression coefficients for carapace length to carapace width against carapace width (df 1; $F=865.501$; $p<0.001$) and frontal width to carapace width against carapace width (df 1; $F=1179.862$; $p<0.001$) but not for carapace height to carapace width against carapace width (df 1; $F=0.481$; $p=0.488$) (Figure 4). Therefore, the specimens were grouped in categories of small (cl: 3-15.5 mm), medium (cl: 15.51-20 mm) and large (>20 mm) animals to test for morphometric differences between sizes.

The morphometric comparisons of *Xantho poressa* revealed only significant differences in two ratios between the size groups and the specimens show a clear separation in the discriminant analysis. The 1-Factor-ANOVA analysis of the ratios revealed no significant difference in the ratio body height to carapace width (df 2; $F=0.844$; $p=0.431$), but significant differences in the ratios carapace length to carapace width (df 2; $F=168.93$; $p<0.001$) and frontal width to carapace width (df 2; $F=186.12$; $p<0.001$). The post-hoc Schefé-test revealed significant differences ($p<0.001$) in the ratios frontal width to carapace width and carapace length to carapace width for all three groups.

For the discriminant analysis, the three ratios were used. The three groups show highly significant differences (Wilks' Lambda: 0.47, $F(6.756) = 58.57$, $p<0.00001$; 86.17% correct classification) (Figure 5). The canonical analysis confirmed that there is a separation among the size classes. The first canonical function (root1) accounted for 99% and the first and second (root2) for 100% of the explained variance.

A



B

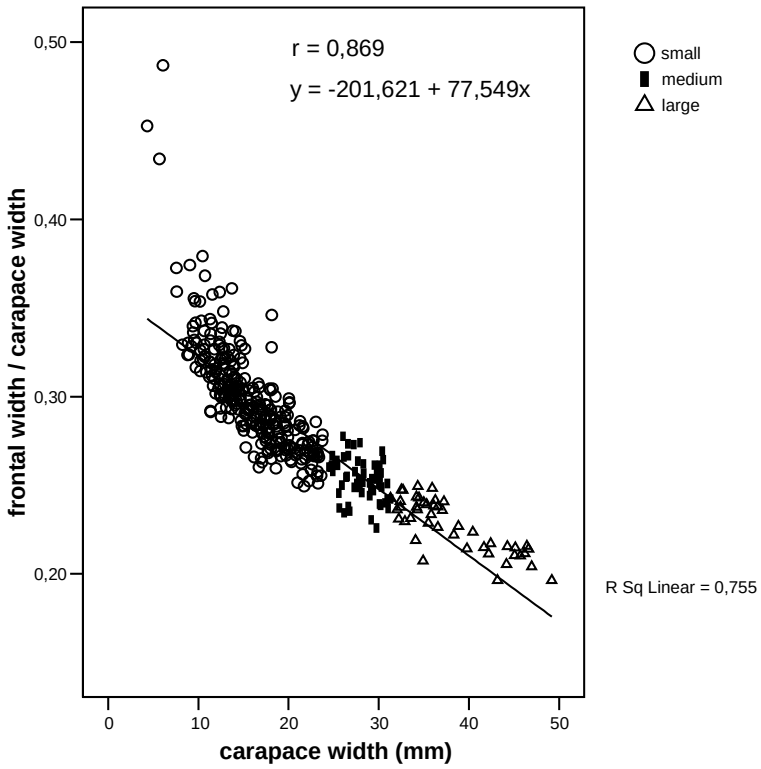


Figure 4. *Xantho poressa*. A) Regression of carapace length to carapace width against carapace width, B) regression of frontal width to carapace width against carapace width.

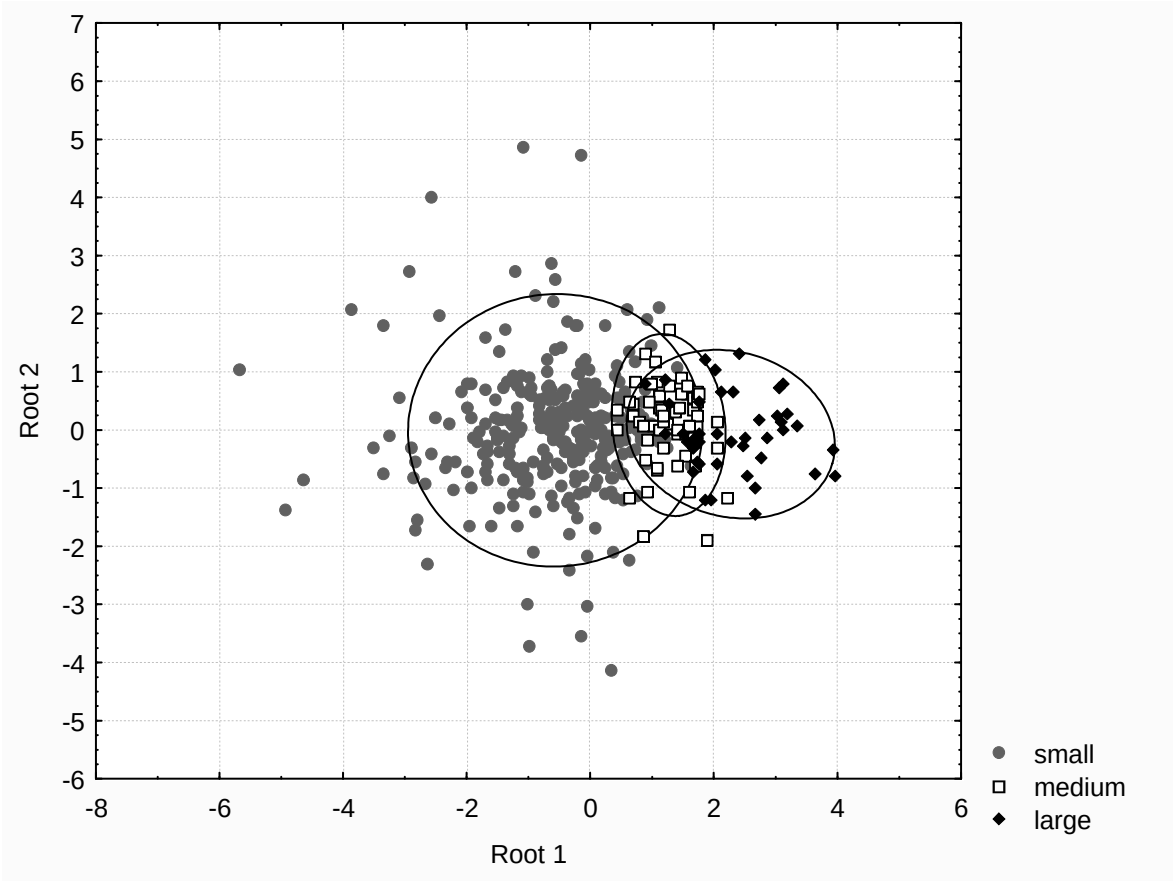


Figure 5. Canonical analysis depicting discrimination by morphometric measurements of *Xantho poressa* of three different size groups: small, medium and large; a plot of the first discriminant function (root 1) against the second (root 2).

To test if there are differences between geographically distant populations we carried out one-factor-ANOVA of the ratios and a discriminant analysis for the specimens of the small group only of the following geographical regions: Cádiz, Ibiza, Corfú, Corsica, Croatia, Parga and the Black Sea. The 1-Factor-ANOVA analysis of the ratios revealed significant differences in the ratios carapace length to carapace width (df 7; $F=2.599$; $p<0.013$) and frontal width to carapace width (df 7; $F=3.562$; $p<0.001$). The *post-hoc* Schefé-test showed that there were no more significant differences between the geographic populations. In the ratio body height to carapace width, there were no significant differences at all (df 7; $F=0.669$; $p=0.699$). In the discriminant analysis the geographic regions show significant differences, but there is no separation or a reliable correct classification among the regions (Wilks' Lambda: 0.81, F (24.870) = 2.8779, $p<0.000$; 34.08% correct classification).

DISCUSSION

The field observations revealed that *X. poressa* is a very common crab at all our collection sites at rocky shores of the shallow subtidal zone in the Mediterranean Sea (intertidal in the Atlantic). The crabs were almost never found in *P. oceanica* meadows or on sandy-dominated shores. In seagrass *P. oceanica* meadows, Bedini (2002) collected 23 crabs (*Carcinus maenas* and *X. poressa*) during 2 years by SCUBA diving at a depth of 20–30 m and suggested that this represents the juvenile habitat of *X. poressa*. According to our results and the small sample size of Bedini, the collected specimens of Bedini (2002) must represent a very small fraction of the overall size class and have settled in the sea grass meadows in absence of more suitable habitat. Concerning the adult specimens, also Riedl (1983) described *X. poressa* as a common species in shallow harbours and shores, mostly occurring under stones. For the three species *X. poressa*, *X. hydrophilus* and *X. pilipes*, a bathymetric separation in the Mediterranean becomes apparent. *Xantho poressa* occurs mostly in the shallow subtidal; *X. hydrophilus* prefers depths below 2 m. *Xantho pilipes* was most often found by SCUBA diving at depth of 15 m. This was already described by Almaça (1985). Also in the literature it is commonplace that adults of *X. poressa* are inhabitants of the shore and *X. hydrophilus* of the littoral down to 40 m depth (Pesta 1918; Zariquiey 1968; Riedl 1983).

In the case of the observed colour patterns of *X. poressa*, all stages – juveniles as well as adults – were found with highly variable colours and colour patterns (Fig. 1). In the literature, *X. poressa* is also described as variable, brown to olive-green, with red dots and white stripes (Pesta 1918; Riedl 1983). Bedini (2002) suggests that the juveniles of *X. poressa* have colour patterns that conform to cryptic mimicry in sea grass, as the coloration provides an excellent camouflage among the *Posidonia* leaves and that the adults change their colour at their final moult to adulthood completely and move to rocky shores. We propose that *X. poressa* juveniles mostly live among small colourful pebbles and larger rocks and reduce the likelihood of a predator spotting them by matching their underground with variability in colour, also using white transverse stripes on the legs and frontal carapace to disrupt their outline (Fig. 1F). Disruptive cryptic coloration as passive defence (camouflage) is also found in the colour pattern of *Heptocarpus pictus* and *H. paludicola* (see Bauer 1981). More examples of camouflage are found in seagrass meadows. The old leaves of *Posidonia* are often epiphytized by pink to brown *Fosiella pneophyllum*, and *Hippolyte* spp. is mimicking these thalli with the epiphytes (d'Udekem d'Acoz 1999). Spider crabs (Majidae) are often overgrown by algae to match the underground (Carmona-Suárez 2002). In the case of *Cancer irroratus*, crabs display a spectrum of non-adult colours that disappear as they grow (Palma &

Steneck 2001). *Xantho poressa* is smaller and more vulnerable than *Cancer irroratus*. Therefore, it is important for them to keep the polymorphism also as adults, like the chip crab *Heterocrypta granulata* (see Gosner 1978). In the Mediterranean Sea, we could observe repeatedly the fish species *Coris julis* and *Thalassoma pavo* preying on adults of *X. poressa*. Our mtDNA data are not linked to colour morphs and thus provide no evidence for colour heritability. Breeding experiments have to be carried out to obtain final evidence that colour morphs are the result of phenotypic plasticity. It would be interesting to compare juveniles from the same hatch exposed to differently coloured sediment throughout several moults.

With our morphometric data, it was not possible to show an abrupt change of growth in *X. poressa* and thus to determine the approximate size of sexual maturity. Egg-carrying females ranged from 5.5 to 11 mm, in the Black Sea up to 16 mm (Fig. 3B). Ovigerous females were found from mid May to mid September. No ovigerous females were found in the northern Adriatic Sea in August. In Greece, egg-carrying females ranged from 6 to 11 mm. So, only crabs with CL < 6 mm may be considered juveniles and the adulthood may already be reached at a size of 6 mm carapace length, at least in some of the studied populations. This differs from Bedini (2002), who suggested a carapace length of 11–12 mm to reach the adult stage. Therefore, Bedini's definition of the juvenile stage and the postulated seagrass-specific colour pattern are herewith put to question.

A pattern of allometric growth could be recognized in the carapace shape of *X. poressa*: the smaller the crabs are, the rounder is the carapace shape, and the larger the crabs are, the wider and more oval the carapace becomes. Likewise, the front is relatively more narrow in larger animals. It could be an advantage against predators to grow fast to outgrow predation size. This is possibly also the case in the other species of the genus *Xantho*. For *X. hydrophilus* it was suggested that there exists a clear geographical morphometric variation in concordance with a genetic gradient (see Reuschel & Schubart 2006). However, the morphometric results are in part also influenced by allometric growth (own unpublished data).

In this study, we document variability in size among our different collecting sites (Table 3). When the examined organism spans a broad size range, it can be difficult to extract the relationship between size and shape from a set of measurements (Zelditch et al. 2004). In our previous study, the discriminant analysis of the data revealed geographical differences for *X. hydrophilus* and less for *X. poressa* (see Reuschel & Schubart 2006). In the present study, new analyses of *X. poressa*, in which the data set was split in three size categories, show no remaining geographical separation. Therefore, in *X. poressa* and *X. hydrophilus*, we should expect size to be the dominant explanation for morphometric variance and not geographical

separation. The observed similarity between Adriatic and Atlantic specimens of *X. poressa* in Reuschel & Schubart (2006) can now be fully attributed to similar body sizes. The first canonical function (root 1) of the discriminant function of size classes in *X. poressa* accounted for 99% of the variance. The first function is interpreted as a measure of size, and all the others are interpreted as measures of shape (Zelditch et al. 2004). It is unlikely that the remaining 1% explain anything but noise. Rincón (2000) showed in two sturgeon species that the two composite variables used in a previous study were badly affected by ontogenetic allometry, thereby leading to the ascription of large and small specimens to different groups.

We could not relate the differences in size of *X. poressa* to a gradual geographical pattern or a special season of the year. Instead, it may be related to a different spectrum of predators, temperature, salinity regimes and unusual tidal influence at the collecting points. In L'Ampolla we found specimens in all size classes but with no big variability in colour pattern. There, the habitat consisted of large boulders in front of a jetty overgrown with algae and partly muddy anoxic sediments. At the same day, 56 km further north at Cap de Salou, only small crabs with a high variability in colour morphs were found. Here, the bottom consisted of small colourful pebbles and the crabs were found under medium-sized stones. We therefore propose that the size variation of *X. poressa* may reflect phenotypic plasticity, but without fully understanding the factors favouring different sizes. In the case of the shore crab, *Carcinus maenas*, Brian et al. (2006) hint that patterns of morphological variability in this species are largely determined by local environmental conditions and the species may exhibit phenotypic plasticity in UK populations. Schubart et al. (2001) suggested that *Brachynotus gemmellari* and *Brachynotus sexdentatus* possibly represent different ecophenotypes of a single species at different depths. Also in *Cyrtograpsus affinis* and *C. altimanus*, molecular and morphometric comparisons revealed no genetic structure, but two different morphs that were always associated with subtidal *versus* intertidal habitats (Spivak & Schubart 2003).

It remains to be tested how geographical size variation is determined. For this purpose, future experiments documenting growth in response to ecological conditions should be carried out in parallel to population genetics. Until now, there is a lack of structured genetic variation in *X. poressa* based on mtDNA (see also Reuschel & Schubart 2006). For further studies on population genetics and colour morphs, variable nuclear markers (microsatellites or AFLPs) would be important to complement our results. According to the estimated high population density and the wide distribution range of the species, *X. poressa* may represent a coastal metapopulation, reproducing panmictically and showing phenotypic plasticity with respect to colouration and size.

SUMMARY

Field studies of habitat preferences of *X. poressa* revealed that the species is abundant in a wide size range in the intertidal and shallow subtidal zone hiding between small and often colourful pebbles under larger rocks. A mark–recapture experiment revealed that the species reaches population densities about 1.62 specimens m^{-2}. Juveniles and adults of *X. poressa* use different colours and disruptive cryptic coloration as passive defence (camouflage). A pattern of allometric growth in the carapace shape and variability in size at our different collecting sites was observed. The different colour patterns and different sized populations seem not be genetically separated. These results suggest phenotypic plasticity in the species. Geographical separation does not seem to play a role for the genetic and morphometric variability. In contrast, the comparison of size classes resulted in highly significant morphometric differences, underscoring the important effect of allometric growth for morphometric analyses.

ACKNOWLEDGEMENTS

Our special thanks go to the staff of the Senckenberg Museum Frankfurt, Jürgen and Maike Heinze, Carsten Müller, Tobias Santl, Reiner Rubner, José Cuesta, Henrik and Sophia Schubart, Cédric d'Udekem d'Acoz and Petra Zillner, for helping to collect and measure specimens and to Benno Darnhofer-Demar for his sceptical comments on an earlier version of this study. Field trips were financially supported by DAAD grant (D/03/40344) funds from the Lehrstuhl für Biologie I and from Freunde der Universität Regensburg.

Appendix 1

A list of the captures and recaptures for calculating the population–density in the field.

S	C	M	R	U
1	30	–	–	30
2	32	30	8	24
3	30	54	6	24
4	40	78	7	33

S: successive days capture, C: number of captured specimens, M: number of marked specimens, R: number of recaptured specimens and U: number of new marked specimens.

PUBLICATION 3

POPULATION GENETIC ANALYSES OF THE PRAWN *PALAEMON ELEGANS* CONFIRM PRESENCE OF MARINE BIOGEOGRAPHIC BARRIERS AND HUMAN INTRODUCTION ALONG THE EUROPEAN COAST

Silke Reuschel, José A. Cuesta and Christoph D. Schubart

Manuscript submitted to *Molecular Ecolgoy*

ABSTRACT

Intraspecific genetic diversity is investigated in the prawn *Palaemon elegans* (Rathke, 1837) (Palaemonidae), a common representative of the intertidal and shallow subtidal of the northeastern Atlantic, including the Baltic Sea, the whole Mediterranean, as well as Black and Caspian Seas. The Mediterranean has been strongly affected by well documented Pleistocene glaciations, possibly acting as gene flow barriers in addition to contemporary oceanographic boundaries. In order to test potential genetic differentiation in dependence of the biogeographic history we carried out a population genetic comparison with two mitochondrial genes (16S rRNA and COI). Our study revealed a surprisingly high population structure. Three main groups of haplotypes can be separated, one from the Atlantic Ocean (Type I) and two from the Mediterranean Sea (Type II and III). While Type II and III occur in sympatry, a clear phylogeographic break was observed for Type I, giving evidence for an ongoing genetic isolation of Atlantic and Mediterranean populations. The borderline lies in the westernmost Mediterranean Sea and seems to correspond to the Almería-Oran-Front. Type III consists of a very distinct group of haplotypes. The high levels of nucleotide divergence in COI and the 16S rRNA suggest a cryptic species within *P. elegans*. Type III might have a Messinan origin, when the nearly desiccated Mediterranean Sea was completely isolated from the Atlantic, while Type II could be the result from posterior introduction events of Atlantic specimens. The colonization of the southern Baltic Sea is most likely due to human introduction. Our results also indicate restriction to gene flow within the Atlantic Ocean.

INTRODUCTION

The common European littoral prawn *Palaemon elegans* is adapted to cope with extremely variable environmental conditions: it tolerates a wide range of salinities, temperature and oxygen (Berglund 1980; Berglund & Bengston 1981). The species can be found from hypersaline lagoons to tidal rock pools, shallow rocky coasts, *Zostera*, *Posidonia* and *Cymodocea* sea grass meadows to partly brackish estuaries. It is also common along man-made rock jetties and within harbours. The native distribution ranges from the Atlantic Ocean (from Scotland and Norway to Mauritania including the Azores, Madeira and Canary Islands) to the entire Mediterranean Sea and Black Sea (d'Udekem d'Acoz 1999). Nowadays, the species can also be found in the Aral and Caspian Sea, but this goes back to unintentional introductions in the 1950s (Zenkevich 1963; Grabowski 2006). Since 2000, it has also colonized the southern coast of the Baltic Sea, where it is already replacing the native *Palaemon adspersus* (Grabowski 2006). It is discussed, if the introduction there is due to natural dispersal or to human transport (Grabowski 2006). The broad ecological niche and the recent range expansion of the distribution of *P. elegans* make the prawn an important player of the European marine littoral fauna. The dispersal capacities are probably high, since the complete larval development takes place in the ocean with nine zoeal stages (Fincham 1977), possibly resulting in high rates of gene flow and panmictic population structure. Nevertheless, Berglund and Lagercrantz (1983) found significant genetic heterogeneity by horizontal starch gel electrophoresis including six sampling sites along the Atlantic coast from Sweden to France. Fortunato and Sbordoni (1998) showed also high genetic variability with allozymes within the Mediterranean Sea. Previously, morphological variations had been suggested by de Man (1915).

The narrow connection between the Atlantic Ocean and the Mediterranean Sea has been proposed to represent an important phylogeographic break in several marine species based on oceanographic models and molecular population genetics (e.g. turtles, Reece et al. 2005; cirripedes, Pannancciulli et al. 1997; bivalves, Quesada et al. 1995; crustaceans, Zane et al. 2000, Triantafyllidis et al. 2005; sea urchin, Duran et al. (2004); fish, Borsa et al. 1997; cuttlefish, Pérez-Losada et al. 2002). The Mediterranean Sea was strongly affected by well documented sea level regressions and Pleistocene glaciations. It was isolated several times from the Atlantic. In the late Miocene (Messinium), a sea level regression separated the Mediterranean Sea from the Atlantic, leading to full or partial desiccation of the Mediterranean Sea. At the beginning of the Pliocene, Atlantic water flooded the Mediterranean Basin again (Hsü et al. 1977), allowing Atlantic species to re-colonize the

Mediterranean. Also during the Quaternary glacial periods, sea level regressions limited the biotic exchange through the Strait of Gibraltar (Vermeij 1978). The opening and closing of the Strait of Gibraltar has made the Mediterranean a region of high endemism and a generator of diversity. This This narrow oceanic street could still represent a barrier to gene flow between Atlantic and Mediterranean populations and be responsible for ongoing allopatric separation. It is possible that dispersal is prevented by oceanographic patterns or by behavioral mechanisms that act to prevent transport of larvae between populations (Palumbi 2003). The Mediterranean crab fauna could thus have originated by repeated or continuous multiple colonization events with adaptation to specialized habitats and adaptive radiation (Almaça 1985). This could have lead to marked genetic differences resulting in cryptic speciation and a high proportion of endemism (28.6 % according to Hofrichter 2002) in the Mediterranean Sea. Cryptic species are defined as morphological indistinct lineages separated by species level genetic differences (Belfiore et al. 2003). Knowlton (1993, 2000) has indicated that the phenomenon of cryptic species is widespread in the oceans. In addition, several studies have shown the great utility of molecular markers in diagnosing endemism and cryptic speciation, even when traditional markers fail or are ambiguous (Avise 2004). Cryptic species of *Clavelina* (Ascidiae) could be identified in the north-western Mediterranean with mtDNA data (Tarjuelo et al. 2001). *Carcinus aestuarii* of the Mediterranean Sea is morphologically very similar to the Atlantic *Carcinus maenas.* Its cryptic species status, however, was confirmed with genetic analyses of the 16SrRNA gene and the COI gene (Geller et al. 1997; Roman & Palumbi 2004).

The purpose of this study is to determine the degree of genetic differentiation of *Palaemon elegans* along the corresponding Atlanto-Mediterranean European coastline, with focus on a population-level comparison using DNA-sequences of the two mitochondrial genes 16S rRNA and COI. Our comparison includes animals from the Atlantic and Mediterranean coast as well as from Norway, the North Sea and Baltic Sea coasts of Germany and Poland, the Black and Caspian Sea. This way, we also address the questions whether the Strait of Gibraltar has a measurable effect on gene flow between Atlantic and Mediterranean populations and if restricted gene-flow is resulting in cryptic-speciation.

MATERIALS AND METHODS

Sampling

Specimens of *P. elegans* were collected from as many different geographic regions as possible, comprising the Baltic Sea (2004-05), Norwegian Sea (2007), North Sea (2004-05), Atlantic coast of Portugal (2006), Spain (2004-05), the Canary Islands (2005), the Mediterranean coast of Spain (2004-06), Croatia (2004-2005), Italy (2006), Greece (2003) Tunisia (2006), Turkey (2007), Ibiza (2003 and 2007), the Black Sea (2005) and the Caspian Sea (see Table 2, Figure 1). The samples were collected with a hand net, most often from rock pools, along man-made rock jetties and within harbours. After collection, the samples were preserved in ethanol (70%).

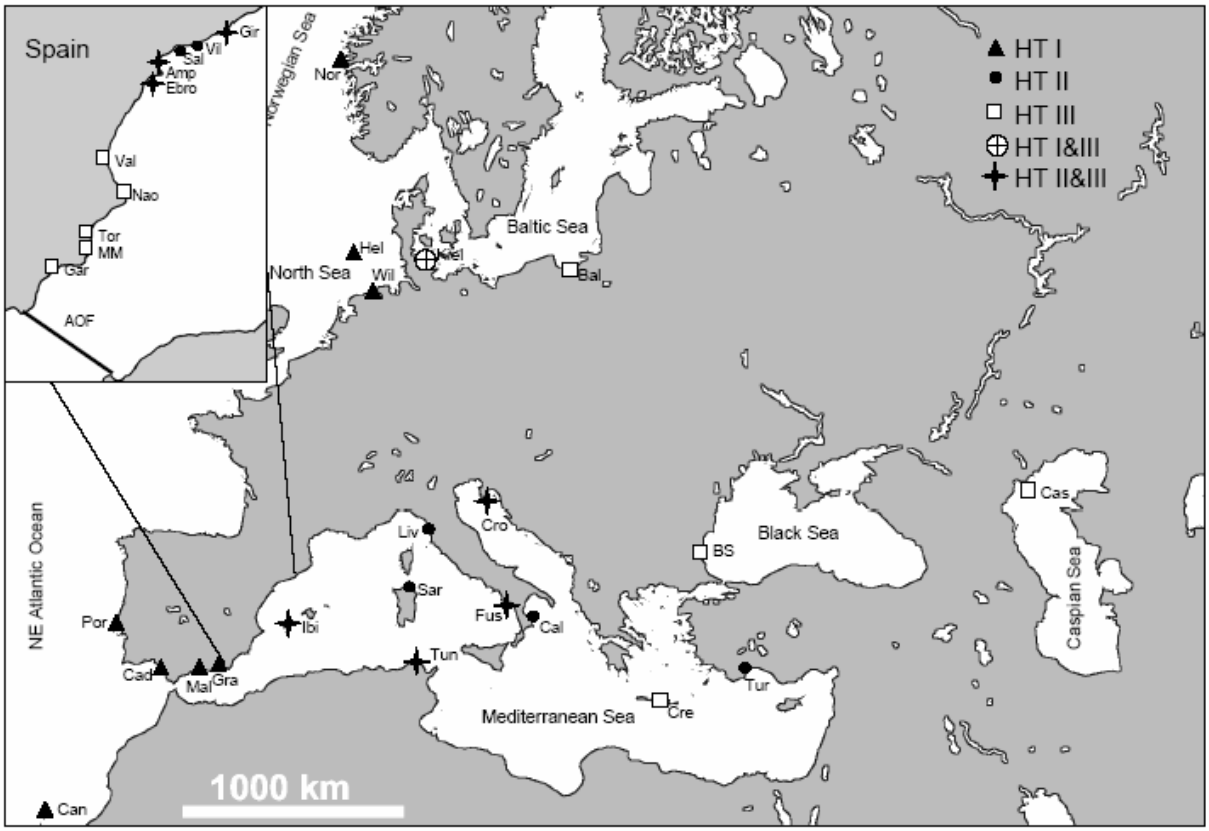


Figure 1. Geographical locations of collection sites of *P. elegans*. The different types are shown in symbols; for the abbreviations of collection sites look also Table 1 (AOF = Almería-Oran-Front).

DNA isolation, PCR amplification and DNA sequencing

A total of 282 specimens of *Palaemon elegans* were used for this study. For the genetic analyses, genomic DNA was extracted from the muscle tissue of the abdomen using the Puregene kit (Gentra Systems). We amplified mitochondrial DNA from the large subunit rRNA (16S) gene, and from the cytochrome oxidase subunit I (COI) gene by means of polymerase chain reactions (PCR) (4min 94°; 40 cycles with 45sec 94° / 1min 48-50° / 1min 72° denaturing/annealing/extension temperatures respectively and 10min final extension at 72°). The following primer combinations were used: 16L2 (5´-TGC CTG TTT ATC AAA AAC AT-3´) (Schubart et al. 2002) and16H3 (5´- CCG GTT TGA ACT CAA ATC ATG T -3´) (Reuschel & Schubart 2006). In the case of COI, the specifically designed new primers COL6Pe (5´- AAG ATA TTG GAA CTC TAT AT-3´) and COH6Pe (5´- GTG SCC AAA GAA YCA AAA TA-3´) were employed. Sequences were compared in DNA alignments of 520 basepairs (bp) 16S and 621 bp COI mtDNA. PCR products were purified with Quick / Sure Clean (Bioline). The products were ethanol-precipitated, resuspended in water and sequenced with the ABI BigDye terminator mix (Big Dye Terminator® v. 1.1 or 3.1 Cycle Sequencing Kit; Applied Biosystems) in ABI Prism automated sequencers (ABI Prism™ 310; Applied Biosystems).

Data analysis

The sequences were analyzed and proofread with the program ABI Sequencing Analysis® 3.4 (Applied Biosystems) and manually aligned with BioEdit (Hall et al. 1999) excluding the primer regions and could be aligned unambiguously. For genetic comparisons of populations, parsimonious networks for both genes were built with TCS (estimating gene genealogies version 1.21; Templeton et al., 1992). Loops in the networks were solved after the three criteria suggested by Crandall and Templeton (1993): (1) geographic criterion, (2) frequency criterion and (3) topological criterion. Genetic heterogeneity within populations was estimated as haplotype diversity (*h*) (Nei & Tajima 1981), nucleotide diversity (π) (Nei 1987) and the mean number of pairwise differences (*k*) computed with DnaSP 4.00 (Rozas et al. 2003). The Φ_{ST} values were calculated with Arlequin 3.1 (Excoffier et al. 2005) to estimate genetic divergences between populations for the more variable COI dataset. The comparisons were performed within the different haplotype-groups (see below). A histogram was done to illustrate the distribution of the different haplotype-groups (Figure 4).

Mismatch analysis

To determine the historical demography of the species and its genetic subunits we analysed the mismatch distributions with the model of Rogers and Harpending (1992) for the COI dataset. The mismatch distributions are used to assess fit of haplotype data to the sudden demographic expansion model (Rogers & Harpending 1992). We tested the null hypothesis of neutrality, which may be rejected when a population has experienced population expansion (Tajima 1989). Therefore, Tajima´s *D*-test (Tajima 1989) and Fu´s (1997) F_s test and their significance levels were estimated using DnaSP 4.00 based on 1000 simulated re-sampling replicates. Mismatch distribution analyses, under the assumption of selective neutrality, were also used to evaluate possible historical events of population growth and decline (Rogers & Harpending 1992). Past demographic parameters, including τ (Li 1977), θ_0 and θ_1 and their probabilities (Rogers & Harpending 1992) were estimated with Arlequin and DnaSP.

Table 1. Localities; locality name of the sampled populations and the number of specimens used for genetic comparisons (*N*) of the 16S gene; the haplotype diversity (*h)* and nucleotide diversity (π) within the examined population and the three types of *P. elegans*.

Collection site of *P. elegans*	**Abbr.**	***N***	**Type**	***h***	**π**
Germany: Kiel: Nord-Ostseekanal	Kiel	2	I	0	0
Germany: Helgoland	Hel	1	I	0	0
Germany: Wilhelmshaven (Niedersachsenbrücke)	Wil	3	I	0	0
Spain: Canary Islands: Gran Canaria (La Isleta)	Can	7	I	0.28	0.00055
Spain: Cádiz: Rota	Cád	6	I	0.6	0.00128
Spain: Málaga: Marbella: Cabo Pino	Mal	2	I	0	0
Spain: Granada: Almunecar	Gra	2	I	0	0
Spain: Almería: Garrucha	Gar	4	III	0.5	0.00096
Spain: Murcia: Mar Menor	MM	1	III	0	0
Spain: Tarragona: Ebro Delta (2006)	Eb06	6	III	0.33	0.00128
Spain: Girona: L´Escala	Gir	2	II	-	-
Spain: Balearic Islands: Ibiza (Cala Llenya)	Ibi	5	III	1	0.01385
Greece: Kalami Beach	Gre	3	II	-	-
Croatia: Pula	Cro	4	II	0.83	0.00224
Black Sea: Bulagari: Sozopol	BS	5	III	0.6	0.00115

Table 2. Localities; locality name of the sampled populations and the number of specimens used for genetic comparisons (*N*) of the COI gene; the haplotype diversity (*h)* and nucleotide diversity (π) within the examined population and the three types of *P. elegans* (type which dominates the population in bold; the number of specimens which belong to the type in brackets).

Collection site of *P. elegans*	**Abbr.**	***N***	**Type**	***h***	***π***
Poland: Baltic Sea: Gulf of Dansk	Bal	10	III	0.2	0.00129
Norway: Bergen: Fantoft	Nor	8	I	0.89	0.00397
Germany: Kiel: Nord-Ostseekanal	Kiel	13	**I (9)** & III (4)	0.9	0.00483
Germany: Helgoland	Hel	11	I	1	0.0065
Germany: Wilhelmshaven (Niedersachsenbrücke)	Wil	4	I	1	0.00483
Portugal: Lisbon : Cabo Raso	Port	10	I	0.93	0.00569
Spain: Canary Islands: Gran Canaria (La Isleta)	Can	10	I	0.87	0.0039
Spain: Cádiz: Rota	Cád	10	I	0.78	0.0029
Spain: Málaga: Marbella: Cabo Pino	Mal	4	I	1	0.00403
Spain: Granada: Almunecar	Gra	10	I	0.87	0.0033
Spain: Almería: Garrucha	Gar	10	III	0.91	0.0034
Spain: Murcia: Mar Menor	MM	2	III	0	0
Spain: Alicante: Torrevieja	Tor	10	III	0.84	0.00272
Spain: Alicante: Cabo Nao	Nao	5	III	0.7	0.00225
Spain: Valencia: Puig	Val	10	III	0.78	0.00186
Spain: Tarragona: Ebro Delta (2006)	Eb06	10	III	0.53	0.00129
Spain: Tarragona: Ebro Delta (2005)	Eb05	9	II	0.91	0.0049
Spain: Tarragona: Ampolla	Amp	10	**II (8)** & III (2)	0.84	0.00408
Spain: Tarragona: Salou	Sal	10	II	0.87	0.00433
Spain: Tarragona: Vilanova di Geltrú	Vil	10	II	0.93	0.00530
Spain: Girona: L´Escala	Gir	10	**II (9)** & III (1)	0.96	0.02344
Spain: Balearic Islands: Ibiza (Cala Llenya)	Ibi	10	II (3) & **III (7)**	0.96	0.04376
Italy: Sardegna: Palau	Sar	5	III	1	0.00386
Italy: Livorno	Liv	9	III	0.92	0.00403
Italy: Fusaro: Lago di Fusaro near Pozzuoli	Fus	10	**II (7)** & III (3)	0.98	0.04409
Italy: Calabria: Torre Melissa	Cal	7	II	0.95	0.00598
Greece: Kalami Beach	Gre	4	II	1	0.00832
Greece: Crete	-	1	III	-	-
Croatia: Pula	Cro	12	**II (11)** & III (1)	0.96	0.02344
Turkey: Phaselis	Tur	10	II	0.98	0.00686
Tunisia	Tun	9	II (2) & **III (7)**	0.86	0.02312
Black Sea: Bulagari: Sozopol	BS	10	III	0.89	0.00573
Caspian Sea	Cas	10	III	0.47	0.00269

Molecular clock

A relative-rate-test was done with MEGA 4.0 (Tamura et al. 2007) to test the constancy of evolutionary rates. We applied the relative rate tests between the common haplotypes of Type I and Type III and the common haplotypes of Type II and Type III using *Palaemon serratus* from Ibiza as an outgroup (EMBL number pending). The snapping shrimp mitochondrial for clock COI of 1.4 % sequence divergence between pairs of lineages per Myr was used (Knowlton & Weigt 1998) to date the timing of population isolation.

RESULTS

Fifty-three individuals over a length of 520 basepairs (bp) fragment were used for the analysis of the 16S rRNA, resulting in 17 haplotypes (hts) and 29 variable sites, with 17 parsimony informative sites. The genetic heterogeneity revealed a relative high haplotype diversity (h = 0.811 +/- 0.04) and nucleotide diversity (π = 0.01438 +/- 0.00056) and k = 7.48 as the overall mean number of pairwise differences.

For the mitochondrial COI gene, 282 individuals were included and a fragment of 621bp was used. From Crete we only had one specimen which was donated by a colleague. 136 different hts were detected resulting in high haplotype (h = 0.950 +/- 0.0009) and nucleotide (π = 0.0472 +/- 0.00060) diversities. The mean number of pairwise differences is k = 29.28. A total of 131 variable sites (21.1 %) were detected with 85 parsimony informative sites. Most substitutions involved transitions, with a transition/transversion ratio amounting to 3.3. Nine mutations resulted in an amino acid substitution and only two of the nine are fixed.

Haplotype networks

Within *Palaemon elegans*, the comparison of multiple sequences of 16S mtDNA and COI revealed the existence of three different haplotype groups (referred to as types) for both of the genes (Figs. 2-3). For the 16S gene (Figure 2), twenty-three out of fifty-three specimens belong to the Atlantic Type I. Out of these twenty-three, nineteen share a common haplotype (ht), which is suggested to represent the ancestral haplotype (HT I). Four additional hts are separated by one transition from HTI. Type II is separated with at least six (p=1.2%) and Type III with at least thirteen (p=2.5%) fixed mutation steps from all Type I haplotypes. Within Type II, there exists a common ht (HT II), consisting of five specimens, and five additional hts, of which four differ by one mutation and one by two mutations with respect to HT II. Type III is separated by nine differences (p=1.7%) from Type II and has a common ht (HT III) found in thirteen specimens and additional four hts separated by one mutation and

one ht by two mutations. The haplotype diversity, nucleotide diversity and the mean number of pairwise differences for each collection site are listed in Table 1.

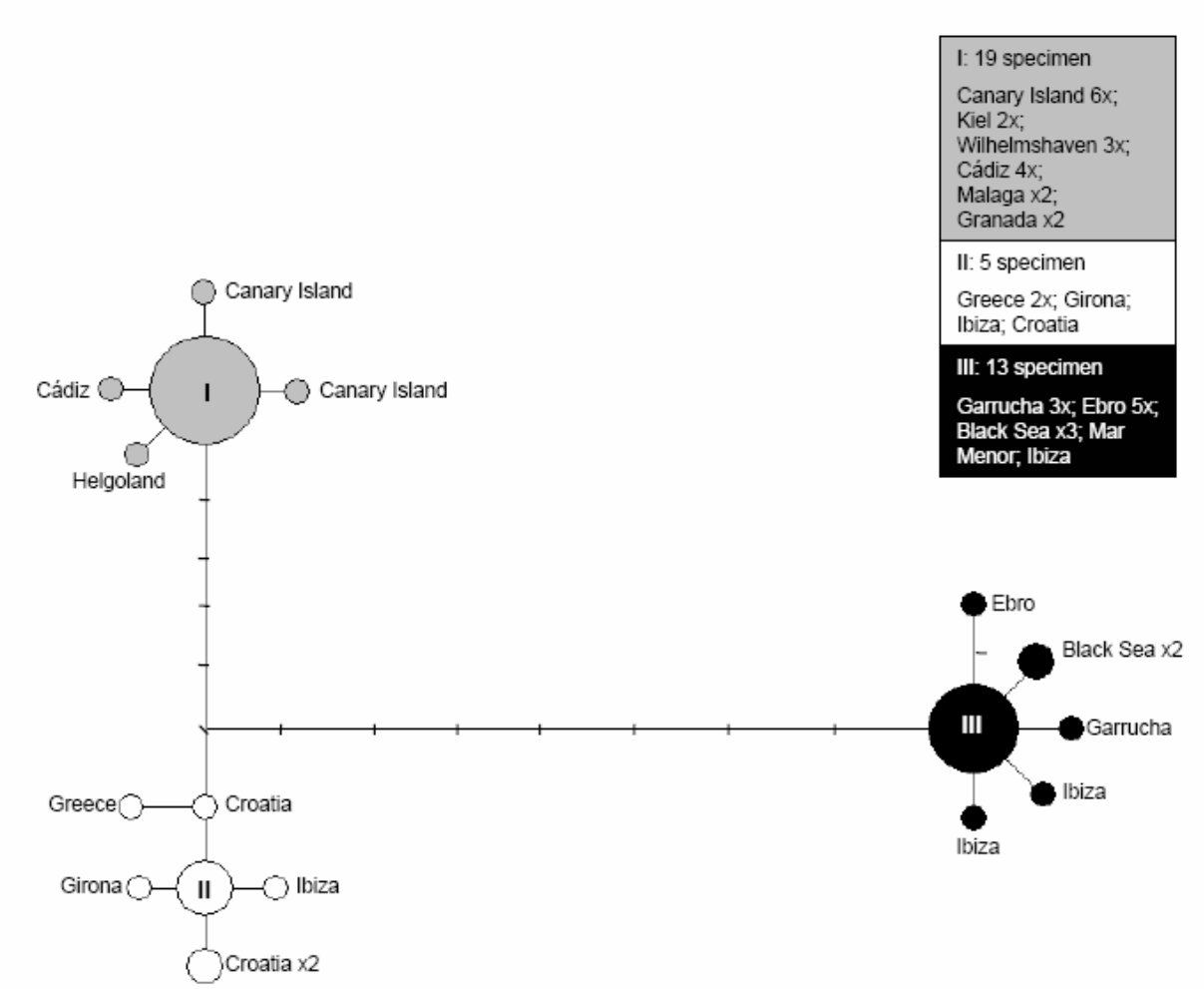


Figure 2. Minimum parsimonious spanning network of 16S RNA constructed with TCS. The network is based on the haplotypes of 53 individual prawns. Each circle represents a single haplotype and its diameter is proportional to the frequency of the haplotype, with the smallest circle representing a single individual. Each line represents one substitution; lines indicate additional substitutions separating two haplotypes. The shading corresponds to the different types.

In case of the COI gene of *P. elegans* (Figure 3), the minimum spanning network shows a higher genetic differentiation in comparison to 16S and results in a multiple star-like minimum spanning tree. Type III hts could not be parsimony-connected anymore to the Type I-II network, because they are separated by over 50 mutation steps from the other two types.
Type I includes 80 specimens from Norway (Bergen), Germany (Eckernförde, Helgoland, Wilhelmshaven, Kiel), Portugal and Spain (Canary Islands, Cádiz, Granada, Málaga). The haplotype-diversity within this type was high (h = 0.956 +/- 0.014; N (ht) = 49) and the nucleotide diversity relative high (π = 0.0013 +/- 0.00035) (see also Table 2). The central ht

of Type I (HT I) includes 14 individuals from different geographic regions. 21 rare haplotypes are directly connected to HT I. Eleven specimens from northern Europe are diverging as the so called haplotype group *b* from HT *a*. HT *a* and its seven minimally diverged hts are from Portugal, Canary Islands, Granada and Norway. Most specimens of the Canary Islands resulted in another haplotype-group around HT *c* with four connected hts. HT *a* is separated by four mutation steps and HT *c* by five mutation steps from the HT I; both lie between HT I and HT II. HT I is separated from HT II by 12 mutation steps (p=1.9%). Four fixed steps separate the two groups. The farthest connections within Type I consists of thirteen mutation steps.

The second haplotype group Type II includes 89 specimens exclusively from the Mediterranean Sea: Spanish coast (Ampolla, Salou, Villanova, Ebro05, Girona, Ibiza), Italy (Fusaro, Calabria), Greece, Croatia, Tunisia and Turkey. We obtain similar results for the haplotype-diversity (h = 0.930 +/- 0.020; N (ht) = 52) and the nucleotide diversity (π = 0.0056 +/- 0.0004) as within Type I (see also Table 2). The postulated ancestral ht of Type II (HT II) includes 21 specimens. 27 hts are directly connected to HT II by only a few mutation steps in a star-like fashion. A haplotype group around HT *d* is separated with three mutation steps and lies between HT I and HT II. 20 rare haplotypes diverge more or less directly from HT d. Within Type II, the most distant connections sum up to fourteen mutation steps.

Type III includes most of our specimens (N=118), but only 36 hts diverged here from the central ht resulting in a lower haplotype-diversity (h = 0.771 +/- 0.041) and nucleotide diversity (π = 0.0029 +/- 0.00032) (see also Table 2). The geographic distribution of Type III includes the Polish and German Baltic Sea and otherwise ranges from Mediterranean Spain (Garrucha, Mar Menor, Torrevieja, Cabo de la Nao, Valencia, Ebro06, Ampolla, Ibiza) to Italy (Fusaro, Livorno), Tunisia, the Black Sea and Caspian Sea. It was also found in one specimen from Croatia, Crete and Girona each. Type III has one common ht (HT III), which was found in fifty-six specimens. HT III is separated from HT I by 54 mutation steps (p=8.7%) and from HT II by 53 mutation steps (8.5%). Out of these mutation steps twenty-six are exclusively for Type III and therefore characteristic for this type (Table 3 and 4). 36 hts or haplotype-groups are separated by only a few mutation steps in a star-like shape. Geographical overlap between Types II and III is so far recorded from the population of Girona, Ampolla, Ebro, Ibiza, Fusaro, Croatia and Tunis (see also Figure 4). For convenience, the haplotype diversity, nucleotide diversity and the mean number of pairwise differences for each collection site was always estimated for the type which dominates the population listed in Table 2 (bold).

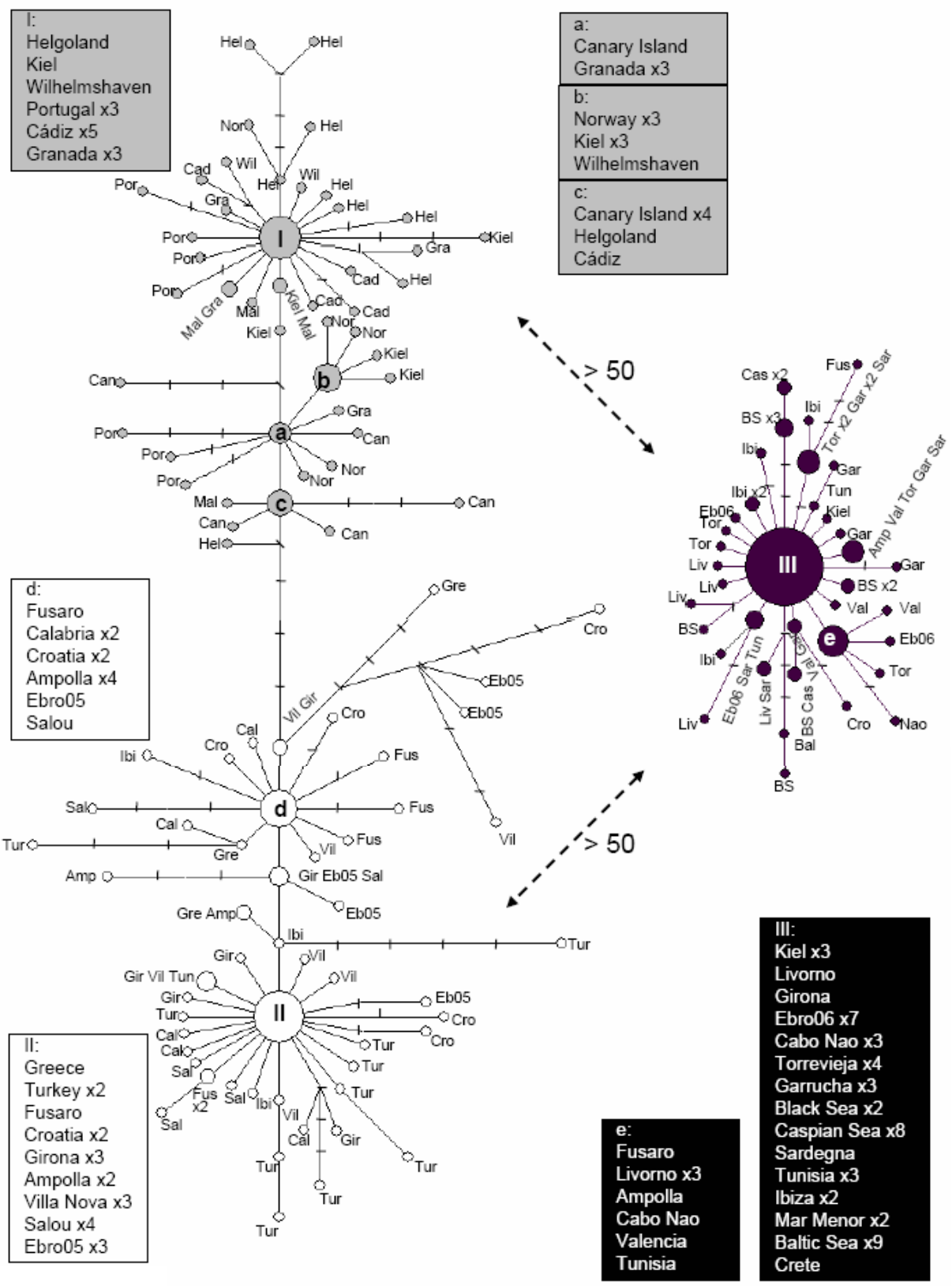


Figure 3. Minimum parsimonious spanning network of Cytochrome Oxidase I constructed with TCS. The network is based on the haplotypes of 282 individual prawns. Each circle represents a single haplotype and its diameter is proportional to the frequency of the haplotype, with the smallest circle representing a single individual. Each line represents one substitution; lines indicate additional substitutions separating two haplotypes. The shading corresponds to the different types. See Table 1 for the abbreviations of the localities.

Table 3. Variable sites of the 520 bp fragment of the 16S gene for Type I, II and III. Asterisks in the lower sequence indicate nucleotides that are identical to those in the upper sequence and the last line shows the diagnostic sites for the Type III specimens. Sequence position after universal primer 16Sar (Palumbi et al. 1991).

Type	148	152	227	229	303	323	344	461
I	G	A	G	C	G	T	T	T
II	.	.	.	.	.	.	.	.
III	A	G	A	T	A	C	C	G

Table 4. Variable sites of the 621 bp fragment of the COI gene for Type I, II and III. Asterisks in the lower sequence indicate nucleotides that are identical to those in the upper sequence and the last line shows the diagnostic sites for the Type III specimens. Sequence position after universal primer CO1472 (Folmer et al. 1994).

Type	68	110	125	140	152	161	221	233	245	281	296	299	341	344
I	G	C	A	G	C	A	C	C	C	A	A	A	C	G
II	.	.	.	.	.	.	.	.	.	.	.	.	.	.
III	A	T	C	T	T	G	T	A	T	C	G	G	T	A

Type	345	348	353	374	479	506	527	557	569	581	599	620
I	T	A	T	C	A	A	C	C	T	A	C	C
II	.	.	.	.	.	.	.	.	.	.	.	T
III	G	G	A	T	T	S	T	R	C	T	T	A

Population genetic parameters

Analysis of molecular variance of the 16S shows that most molecular variance can be attributed to the differences between the three types (93.85%) whereas a very small portion of the variance was due to variation within populations (5.79%) and among populations within types (0.36%). The AMOVA between all subpopulations reveals a significant mean value of overall Φ_{ST} of 0.94 ($p>0.0001$). A strikingly similar result was obtained from the COI dataset, the molecular variance between the three types being 93.68%, within populations 5.98% and among populations within types 0.33%. The AMOVA between all subpopulations reveals a significant mean value of overall Φ_{ST} of 0.94 ($p>0.0001$). Pairwise Φ_{ST} values between populations are listed in Table 3. The comparison was done between all collection sites where at least seven specimens where available. The Φ_{ST} values were analysed combining Type I and II (Table 5) and due to the high genetic differentiation, separately within Type III (not shown). In the case of the few sequences documenting the

geographical overlap between Type II and III (Ampolla, Girona, Ibiza, Fusaro, Croatia and Tunisia) the rare ones had to be excluded from the respective analysis to avoid overly pronounced differentiation. The number of included specimens is shown in Table 1. The analysis revealed significant differences between populations of Types I and II. Within Type I and II almost all possible Φ_{ST} comparisons of the Canary Islands, Norway (except to Kiel), and Helgoland (except to Cádiz) were significant. Within the Mediterranean Sea (Type II) the Φ_{ST} values are low and almost all of them not significant. The same pattern was revealed for all Φ_{ST} values within Type III, being very low (>0.09) and only few of them being significant without a clear geographical pattern. To confirm restricted gene-flow between the northern and the north-east populations of Type I we did an AMOVA. A comparison between the North-Atlantic (Norway, Helgoland, Wilhelmshaven, Kiel) and the North-East-Atlantic (Portugal, Cádiz, Canary Islands) reveals a significant mean value of overall Φ_{ST} of 0.19031 (p>0.0001).

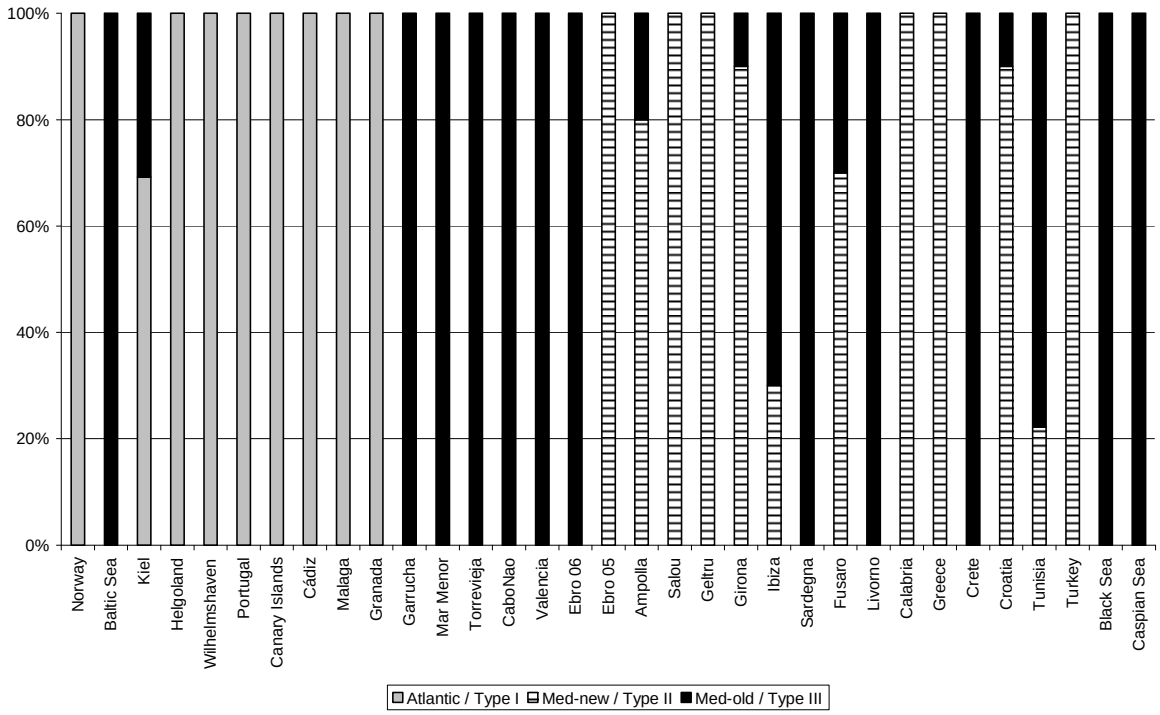


Figure 4. Histogram showing the distribution of the three types and illustrating the geopraphical overlaps. The shading corresponds to the three types.

Table 5. Pairwise Φ_{ST} values between all populations of the Type I and Type II, based on haplotype frequencies. Significant values are presented in bold (significance level 0.05 was calculated from 10000 permutations).

	Canary I	Norway	Kiel	Helgoland	Portugal	Cádiz	Granada	Ebro	Ampolla	Salou	Vilanova	Girona	Fusaro	Calabria	Croatia
Norway (8)	**0.17209**														
Kiel (9)	**0.27355**	0.03415													
Helgoland (11)	**0.27268**	**0.26671**	**0.19290**												
Portugal (10)	**0.32169**	**0.23885**	**0.10244**	**0.07197**											
Cádiz (10)	**0.47745**	**0.41803**	**0.23295**	0.07046	0.00498										
Granada (10)	**0.33987**	**0.25447**	0.10156	**0.09130**	-0.04089	0.05229									
Ebro (9)	**0.70204**	**0.72885**	**0.72288**	**0.66915**	**0.71533**	**0.78745**	**0.76480**								
Ampolla (8)	**0.71651**	**0.74359**	**0.73557**	**0.67276**	**0.72285**	**0.80423**	**0.77947**	0.01669							
Salou (10)	**0.74134**	**0.76526**	**0.75659**	**0.70848**	**0.74870**	**0.81389**	**0.79502**	-0.01300	0.08247						
Vilanova (10)	**0.70316**	**0.72738**	**0.72197**	**0.67116**	**0.71479**	**0.77951**	**0.76048**	-0.02737	0.08579	-0.04507					
Girona (9)	**0.75743**	**0.78253**	**0.77021**	**0.72001**	**0.76071**	**0.82891**	**0.81056**	0.02130	**0.15401**	-0.04220	-0.04715				
Fusaro (7)	**0.67896**	**0.70300**	**0.70007**	**0.63968**	**0.68959**	**0.77175**	**0.74598**	-0.02288	-0.04135	0.03474	0.03525	0.10746			
Calabria (7)	**0.68154**	**0.69962**	**0.69618**	**0.64125**	**0.68608**	**0.76846**	**0.74220**	-0.02708	-0.07055	0.02922	0.03031	0.08436	-0.05686		
Croatia (9)	**0.63850**	**0.66347**	**0.66741**	**0.61565**	**0.66337**	**0.73380**	**0.70874**	-0.04633	-0.02965	0.01227	-0.00724	0.05263	-0.07758	-0.05414	
Turkey (12)	**0.68543**	**0.70469**	**0.70432**	**0.67172**	**0.70779**	**0.76066**	**0.74253**	0.06072	**0.14896**	-0.00813	-0.00615	-0.00526	**0.11147**	0.09474	0.07803

Mismatch analysis

Three separate assemblages of mismatch distributions were constructed: (A) Type I; (B) Type II and (C) Type III (Figure 5). The mismatch distributions for all types were not significantly different from the sudden expansion model of Rogers and Harpending (1992). The smooth unimodal mismatch distributions of the separated types and the statistics of the other neutrality tests, Tajima´s *D* and Fu´s F_s were significant and negative (Table 6) thus suggesting sudden population expansion. Negative values of Tajima´s *D* suggest deviations from mutation-drift equilibrium, possibly caused by populations bottlenecks (Tajima 1989). The diversity indices and the magnitude of the values of Fu´s F_s and of demographic estimates were similar in Type I and Type II. These results suggest that both types have undergone a sudden expansion around the same time. The steep display of the mismatch curve of Type III, the small θ_0 and τ are consistent with a recent sudden expansion from a small initial population (Rogers & Harpending 1992; Avise 2000). The smaller the initial population is the steeper will be the leading face of the curve of the mismatch distribution (Rogers & Harpending, 1992). The diversity indices and the values of demographic estimates of Type III are different from these of Type I and II suggesting a distinct demographic history. The τ estimates appear to support that Type I and Type II have an older history than Type III. The pattern of the multiple starlike networks may suggest otherwise (Bremer et al. 2005), but Type III has the shortest maximum distances (10) between hts, again arguing for a more recent evolution.

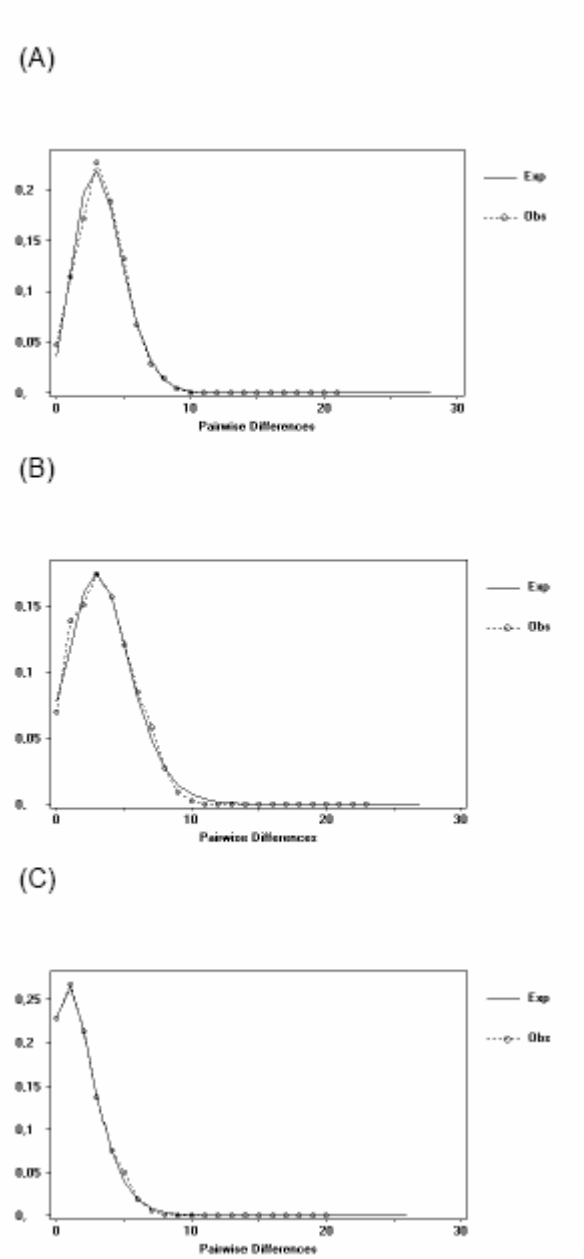


Figure 5. Mismatch distributions and τ values obtained from COI gene sequence data. The dotted line represents the observed pairwise differences. The solid curve is the expected distribution under the sudden expansion model.

Table 6. Summary of statistics of the COI gene; N, number of sequences; M, number of haplotypes; h, haplotypic diversity; n, nucleotide diversity; S, number of segregation (polymorphic) sites; k, mean number of pairwise differences between individuals; SD, standard deviation; values of Tajima´s D and Fu with probability values (P).

Sample	*N*	M	*h*(SD)	n (SD)	*S*	*k* (SD)		*D (P)*	*Fu´s (P)*
TypeI	80	49	0.96 (0.014)	0.0013 (0.0004)	86	8.21 (3.72)	0.082 3.545 33.477	-1.76 (0.01)	-24.77 (0.001)
TypeII	89	52	0.93 (0.020)	0.0056 (0.004)	59	3.47 (1.8)	1.019 2.992 15.566	-2.28 (0.001)	-26.17 (0.001)
TypeIII	117	37	0.77 (0.041)	0.0029 (0.0018)	43	1.83 (1.06)	0.896 1.235 6.401	-2.39 (0.01)	-27.72 (0.001)

DISCUSSION

For most marine species, a high level of gene flow is suggested, due to the preponderance of pelagic larval stages and the absence of obvious distribution barriers for them. Therefore, panmictic species are predicted and marine speciation appears only probable with long term geographical barriers or rare colonization events (Palumbi 1994). However, the extant high level of marine biodiversity suggests that genetic differentiation and speciation must be common in marine systems (Mathews 2006). There are several non-obvious barriers to gene flow: larval behaviour favouring retention, isolation by distance, local genetic drift, or historical events (Avise 1994; Palumbi 1994; Queiroga 1996, 1998; Zane et al. 2000). These barriers have to be taken into consideration in marine systems, when estimating the hidden marine biodiversity. Most species are defined by morphological characters, despite our modern knowledge that morphology is a quite complex marker and could lead to under- or overestimation of biodiversity (Lefébure et al. 2006).

Genetic - geographical structure of populations

In our study, the 16S rRNA and the more variable COI mitochondrial genes revealed a surprisingly high population structure in the marine species *Palaemon elegans* and clearly distinct genetic lineages. Three haplotype-groups can be defined: Type I (Atlantic and Alboran Sea), Type II (entirely Mediterranean) and Type III (Mediterranean plus Baltic, Caspian and Black Seas). The common ht of Type III haplotype-group is separated by 13 differences in the 16S rRNA and by over 50 differences in the COI gene from both common hts of the haplotype groups I and II. The haplotype group III can be distinguished by eight fixed differences of the 16S gene and twenty-six fixed differences of the COI gene to both other types of *P. elegans* (see Table 3 and 4). Since no morphological differences could be found between the different genetic types so far, we suggest a species complex for *P. elegans* with the existence of one cryptic species. Many marine cryptic crustaceans have been detected (Knowlton 1993), e.g. in the snapping shrimp genus *Alpheus* using the 16S and COI mitochondrial genes (Mathews 2006), in the seabob shrimp species *Xiphopenaeus kroyeri* and *Xiphopenaeus riveti* with COI (Gusmao 2006), and among the mysid shrimp *Mesopodopsis slabberi* with 16S and COI mitochondrial genes (Remerie et al. 2006). When considering the existence of a cryptic species within *P. elegans*, the taxanomic status of the species needs to be discussed. Since *P. elegans* was first described by Rathke, 1837 from specimens of the Black Sea, the name *P. elegans* corresponds to our Type III populations and would thus be restricted to the Black Sea, Caspian Sea, eastern Baltic Sea and all of the Type

III specimens in the Mediterranean Sea. De Man (1915) recognized three forms within *P. elegans*, a species which he referred to as *Leander squilla*. The form *L. squilla var. intermedia* can be distinguished from the typical form by a shorter ramus of the outer flagellum and by the second leg – the carpus appears a little shorter than the chelae - and was described from the Dutch province of Zeeland, the English Channel, the Irish Coast, Scotch waters, France and Portugal. Therefore, for typical Atlantic (and the Mediterranean populations of Type II), the name *Palaemon intermedius* (De Man, 1915) would become available. So far the three types could not distinguished by morphological characters. It remains to be tested whether hybridization is taking place.

Causes of dispersal limits

Although Rathke, 1837 described *P. elegans* based on specimens from the Black Sea, Type III animals probably do not have there origin there. The Black Sea was primarily a freshwater basin and was flooded with Mediterranean saline water about only 6800 to 9630 years ago. As a result, the Mediterranean fauna was introduced into the Black Sea (Dimitrov & Dimitrov 2004). The geology of the Mediterranean Basin gives evidence for at least one major isolation event from Atlantic waters followed by massive decrease of the water level (Messinian Crisis in the Pliocene) (Por & Dimentman 1989). Assuming that marine animals survived in the Mediterranean during that time, despite existing evidence of hypersaline conditions, allopatric speciation from the Atlantic forms would have been the logical consequence (e.g. Schubart et al. 2001). The relative rate test was not significant between Type II and the other two types ($p = 0.75$ for Type I and III; $p = 0.76$ for Type II and III). Therefore, an estimate of divergence could be carried out, which was based on the calibration of a molecular clock based on other caridean shrimps, the snapping shrimp of the genus *Alpheus* isolated by the closure of the Isthmus of Panama (Knowlton & Weigt 1998). For the calculation, we used the sequences with the lowest and highest divergence to obtain the mean value and standard deviation of divergence per million years. Our results indicate that the divergence between the ancestors of types I&II and Type III occurred 6.85 ± 0.85 Myr ago. This confirms the possibility that Type III ancestors were isolated from the “Atlantic” populations during the Messinian Crisis, since the beginning of the crisis is dated around 5.96 Myr ago (see Krijgsman et al. 1999). For the European shore crab genus *Carcinus*, genetic differences of 2.5% in the 16S and 11% in the COI between the Atlantic *C. maenas* and Mediterranean *C. aestuarii* were detected (Geller et al. 1997; Roman & Palumbi 2004). This is comparable to *P. elegans* with genetic differences of 2.5% in the 16S and 8.7% in the COI

genes. Demeusy (1958) proposed that the isolation during the Messinian Crisis between the Mediterranean and the Atlantic could have provided the geographic barrier permitting allopatric speciation within *Carcinus.* The same has been suggested for the split in the varunid genus *Brachynotus*, between the Atlantic *B. atlanticus* and the Mediterranean *B. foresti* (see Schubart et al. 2001). There is also strong support that the origin of the snail *Salenthydrobia ferrerii* correlates with the crisis (Wilke 2003). With the flooding of the Mediterranean Basin with Atlantic water after the Messinian Crisis, Atlantic forms were re-introduced into the Mediterranean Sea, possibly without reproducing with the local Type III ancestors, and progressively separated from Atlantic Type I. Thereafter they evolved to the new Mediterranean Type II populations, possibly due to isolation by distance, later gene flow barriers like Pliocene/Pleistocene sea level regressions and ongoing limited exchange due to oceanic currents (e.g. Almería Oran Front). The demographic analyses of Type I and II support a similar sudden expansion around the same time. In the polychaete *Lysidice ninetta* (Audouin & Milne-Edwards, 1833), the presence of intraspecific cryptic lineages was recorded. At some sites this species is sympatric with *L. collaris* Grube, 1870. For *L. ninetta* a re-colonization of the Mediterranean Basin from the Atlantic, after the Messinian Crisis is assumed (Iannotta et al. 2007).

While Type II is found only in the Mediterranean Sea, Type III has a broader distribution. It also occurs in the Baltic, Black and Caspian Seas and seems to dominate the Mediterranean Sea (see also Figure 4). Often the two types were found in sympatry (e.g. northeastern Spain, southwestern Italy, Croatia, and Tunisia).

Invasive species

Grabowski (2006) reported the rapid colonization of the Polish Baltic coast by *Palaemon elegans*. The author discusses the possibility of the species being an introduced invasive as opposed to a natural range expansion. In the last decades, several crustaceans spread by human activity, for example with ballast water (Gollasch et al. 2000; Leppäkoski & Olenin 2000; Tavares & de Melo 2004). Since our study revealed that all souttern Baltic Sea specimens belong to the genetic Type III, which is otherwise confined to the Mediterranean Sea, it is indeed likely according to our model, that *P. elegans* was introduced to the Baltic Sea by humans and therefore represents an invasive species. In support of that is also the finding that the Baltic Sea population shows the lowest haplotype diversity (0.2) of all studied populations. A possible bottleneck can have lead to chance reduction in genetic variation so that the haplotype diversity was reduced compared to the ancestral population

(see also Hedrick 2005). Limited mtDNA haplotype diversity was also detected in the Baltic populations of the cladoceran *Cercopagis pengoi* (see Cristescu et al. 2001). The authors suggest that the population was founded by a small number of colonizers from the Black Sea and have undergone a bottleneck (Cristescu et al. 2001). The cause of this translocation remains unknown. A similar case of a bottleneck can also be assumed for the population of *P. elegans* in the Caspian Sea. Again we have a relatively low haplotype diversity (0.47) compared to the population in the Black Sea (0.89). Several geographically isolated lineages from different species, that currently inhabit fragmented habitats in the Ponto-Caspian region, show a limited genetic divergence, which might be attributed to genetic bottlenecks (Grigorovich et al. 2004).

Physical barriers to gene flow

Our haplotype networks of *Palaemon elegans* show a geographic distinction between Atlantic (Type I) and Mediterranean populations, but with an extension of the Atlantic population into the Mediterranean Basin (Alborán Sea). For a long time, the relatively shallow Strait of Gibraltar, representing the geographic boundary between the western Mediterranean and the Atlantic Ocean, was assumed to act as a potential barrier of gene flow. However, more recently it has been suggested that the Almería-Oran front between Cabo de Gata (province of Almería in south-eastern Spain) and Oran in Algeria represents the more important hydrographic boundary between Atlantic and Mediterranean surface waters. Circular jetties predominate between this front and the Strait of Gibraltar (Tintoré et al. 1988). It has been confirmed for different marine species, e.g. the cuttlefish *Sepia officinalis* (see Pérez-Losada et al. 2002, 2007), the pelagic euphausiid crustacean *Meganyctiphanes norvegica* (see Zane et al. 2000) and the scallops *Pecten jacobeus* and *P. maximus* (see Ríos et al. 2002) that the Almería-Oran front represents a barrier to gene flow between Atlantic and Mediterranean populations, which might be also the case for *Palaemon elegans*.

Another European physical marine geographic boundary confirmed by our data is the English Channel. The English Channel is an arm of the Atlantic Ocean that separates the island of Great Britain from northern France and connects the North Sea and the Atlantic. The hydrodynamic features of the English Channel and the northern Atlantic coasts of France may also shape gene flow and separate water masses on both sides from the English Channel (Salomon & Breton 1993; Jolly et al. 2005). For the Atlantic populations of *Carcinus maenas*, a slight but significant break between western Europe and northern Europe along the

English Channel was found (Roman & Palumbi 2004). A genetic break was also observed for the common goby *Pomatoschistus microps* around the British Isles, with distinct haplotypes dominating at either side of the English Channel (Gysels et al. 2004). In this study, we revealed a significant differentiation between the populations of the North Sea (east of the English Channel) and the open northeastern Atlantic, which must be assigned to the effect of the English Channel. The Φ_{ST}-values showed evidence of a strong correlation between genetic differentiation and geographical distance (from Norway to Cádiz). An isolation-by-distance pattern within the Atlantic is thus plausible.

Conclusions

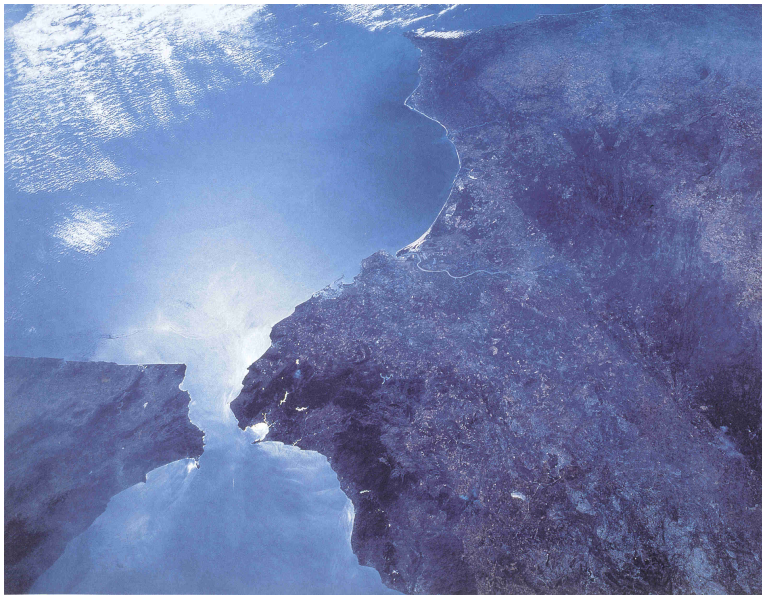
The results of this study give clear evidence for three haplotype groups within *Palaemon elegans*. Due to the high genetic differentiation of one of the three haplotype groups (2.5% in 16S and 8.7% in COI), we propose the occurrence of a cryptic species within this complex (Type III) resulting from an isolation event during the Messinian Crisis based on a molecular clock calibrating with the COI gene. The genetic diversity of the different types is furthermore influenced by physical barriers like the Almería-Oran Front and the English Channel. In order to determine, if members of the three types hybridise and to confirm the presence of a cryptic species a nuclear marker and breeding experiments should be performed. In addition, a fine scale sampling around Cabo de Gata would be important to determine the exact population structures along this biogeographic boundary. Further sampling is also needed in populations originating from the Atlantic coast, Irish Sea and North Sea to investigate more closely the phylogeographic separation within *P. elegans* in this region. This is of particular importance, because the Φ_{ST} values among the Atlantic populations show a higher genetic differentiation than those within the Mediterranean Sea and thus give evidence for a higher potential of local endemisms.

ACKNOWLEDGEMENTS

Our special thanks go to Carsten Müller, José Paula, Ines C. Silva, Urszula Janas and Sammy de Grave, Lapo Ragionieri, Enrique González-Ortegón, Ruth Jesse, Sara Fratini, Sonya Uzunova, Henrik and Sophia Schubart, Florian Gmeiner, Andrea Schott, Veronika Ebe and students of the Universtiy of Regensburg for helping to collect and sending specimens of *P.elegans*. Thanks are further due to Graciela Sotelo for helpful discussions of statistical tests.

PUBLICATION 4

GEOGRAPHIC BREAK AND GENE FLOW AMONG ATLANTIC AND MEDITERRANEAN POPULATIONS OF THE EUROPEAN PRAWN *PALAEMON ELEGANS* ALONG THE ALMERÍA-ORAN-FRONT



Silke Reuschel, José A. Cuesta and Christoph D. Schubart

Manuscript to be submitted to *Journal of Biogeography*

ABSTRACT

Surprisingly high levels of genetic differentiation have been recorded for the European prawn *Palaemon elegans* and three main haplotype groups can be distinguished: one from the Atlantic Ocean and two from the Mediterranean Sea. The geographic separation of the two haplotype groups of the Mediterranean Sea from the Atlantic one lies within the Alboran-Gyre and is proposed to be due to the Almería-Oran-Front (AOF). A number of intraspecific phylogeographic studies have already suggested that the AOF is an important barrier to gene flow, but without determining the barrier more specifically or with absolute certainty. A fine-scale sampling around Cabo de Gata (Spain: Almería) was performed to study the distribution of the three haplotype groups of *Palaemon elegans* and to determine more precisely the geographic barrier to gene flow caused by the AOF. The mitochondrial gene COI was used for a phylogeographic population comparison,. A geographical overlap of the three haplotype groups in this area was revealed and spatial structuring of genetic diversity was shown in an isolation-by-distance pattern along the Mediterranean Spanish coastline. The AOF is hereby confirmed as an important phylogeographic break with restricted gene flow.

INTRODUCTION

Ocean currents are a major factor for the dispersal of many marine life forms as they may increase the spread of larvae as well as the distance travelled (Palumbi 2003). Approximately 70% of the extant marine organisms have a life history comprising a planktonic larval (juvenile or adult) and a benthic phase (Thorson 1950). The planktonic larval stages allow many marine species a large dispersal before settlement (Palumbi 2003). It has been suggested that the dispersal capability, and therefore genetic differentiation, depends on the duration of the larval development (Kyle & Boulding 2000): high dispersal is coupled with low genetic differentiation and low dispersal with high genetic differentiation. But a number of studies have revealed that species with long larval development and high dispersal potential still may show genetic structuring and restricted gene flow for example within the marine bivalve *Macoma balthica* with a planktonic larval development up to five weeks (Luttikhuizen et al. 2003). This phenomenon is often explained by different oceanographic mechanisms like wind drift or tidal currents, density-driven flow, coastal boundary layer and eddies (Shanks 1995). Therefore, in some cases, ocean currents can also prevent gene flow between populations.

Oceanographically, the western Mediterranean is an interesting geographical area (Carreras-Carbonell 2006). The Almería-Oran-Front (AOF) is an effective boundary between Atlantic and Mediterranean surface waters. The cold and less saline Atlantic surface waters enter the Strait of Gibraltar and induces a jet of surface water toward North Africa (see Figure 1). A part of the Atlantic water returns westwards in form of two gyres (West and East Alborán Gyre) and most of the remaining surface water flows eastward into the Mediterranean Basin along the coast of North Africa. In contrast, thermohaline Mediterranean water sinks below the constantly incoming Atlantic waters to form bottom water, called the Mediterranean outflow (Tintoré et al. 1988; Hofrichter 2002).

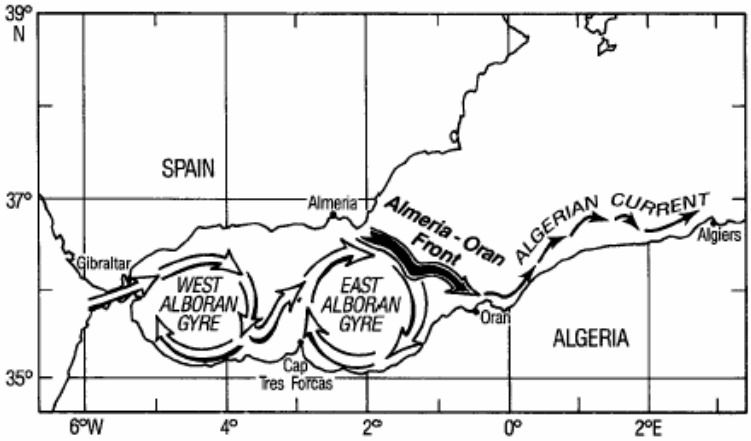


Figure 1. Major surface currents in the Iberian-Moroccan Bay and Alboran Sea from Tintore et al. (1988).

Therefore, the AOF is a potential physical barrier for migration of larval stages and for gene flow of populations between the Atlantic Ocean and Alborán Sea on one hand and the Mediterranean Sea on the other (Naciri et al. 1999; Lemaire et al. 2005). Estrada et al. (1985) have found different planktonic communities on each side. Furthermore, a number of studies have suggested that the AOF is the responsible phylogeographic break in the described population genetic structuring (Pannacciulli 1997; Naciri 1999; Zane et al. 2000; Rios et al. 2002; Cimmaruta et al. 2005; Saavedra & Viñas 2005; Gonzáles-Wangüemert et al. 2006; Pérez-Losada et al. 2007). But in most of these studies the sampling design was not specific enough to confirm with reasonable certainty the AOF as a barrier to gene flow (see Table 1). Therefore, performing a fine-scale sampling for the comparison of populations around Cabo de Gata would be important in order to determine, if the AOF actc as a phylogeographic break between the Atlantic-Alborán and the Mediterranean Sea and on what scale it is functioning.

Within the European prawn *Palaemon elegans* (Rathke, 1837), Reuschel et al. (submitted) gave evidence for a surprisingly high population structure using the mitochondrial genes 16S and COI. Three main groups of haplotypes could be clearly separated with such a high genetic differentiation that a species complex is suggested with one cryptic species. The haplotype groups were defined as Type I, II and III. Type I presents the Atlantic haplotype-group, Type II the New Mediterranean haplotype group and Type III the Old Mediterranean haplotype group. The Old Mediterranean Type is separated from Type I and II with over 50 mutation steps in the mitochondrial gene COI. Within the Mediterranean Sea, Type II and III can occur in sympatry. The Atlantic and New Mediterranean haplotype groups also revealed a clear genetic differentiation along the Atlanto-Mediterranean border (Reuschel et al. submitted). Within the Mediterranean Sea, the separation was so far established between Almuñecar (Granada) and Garrucha (Almería) and was interpreted to probably be caused by the AOF. In this study, a short-range sampling is carried out around Cabo de Gata to determine the distribution of the three previously described haplotype groups, to define more precisely the borderline between the Atlantic and the Mediterranean types and to confirm the AOF as a barrier to gene flow using population genetic comparisons with the mitochondrial gene COI.

Table 1. Overview of phylogeographic studies of marine taxa and coastal plants which suggested the Almería-Oran-Front (AOF) as barrier to gene flow; if populations are included from the Spanish coast they are listed under sampling design (Al = Alboran Sea, Sp = Spanish coast north of Cabo de Gata), otherwise the rough sampling design is shown (Atl = Atlantic; Med = Mediterranean Sea).

AUTHOR	*MOL. MARKER*	*TAXON*	*SAMPLING DESIGN*	*RESULT*
Baus et al. (2005)	AFLP	*Asterina gibbosa*	5 in Atl, 3in Med	"high levels of genetic differentiation between the Med and Atl basin"
Carreras-Carbonell et al. (2006)	mtDNA	*Trypterygion delaisi xanthosoma* *Trypterygion d. delaisi*	2 in Atl, respectively 1 in Tarifa (Al), Cabo de Gata, Cabo de Palos (Sp), Blanes (Sp), Tossa (Sp), Cap de Creus (Sp), 2 in western Med	"constant genetic differentiation between Tarifa and all other Med populations"
Cimmaruta et al. (2005)	allozymes	*Merluccius merluccius*	4 in Atl, Malaga (Al), Alicante (Sp), 9 in western and eastern Med	"Atl and Med are separated by the AOF"
Domingues et al. (2007)	mt/nDNA	*Coryphoblennius galerita*	7 in Atl , Cabo de Gata, 3 in western and eastern Med	"major barrier between Med and Atl populations"
Kadereit et al. (2005)	AFLP	*Eryngium maritimum* *Halimione portulacoides* *Cakile maritime* *Salsola kali*	every 100 to 200 km along the coast from Turkey to Sweden	"a distinct gap was found"
Lemaire et al. (2005) Naciri et al. (1999)	mtDNA microsatellites	*Dicentrarchus labrax*	7 in Atl, 3 in Alboran Sea, 8 in western Med	"effective gene flow is limited between the Med and Atl basin"
Pannacciulli et al. (1997)	Protein electrophoresis	*Chtalamus montagui* *Chtalamus stellatus*	8-12 in Atl, 1 in Gibraltar, 8-9 in western and eastern Med	"AOF major barrier between Med and Atl forms of the two species"
Perez-Losada et al. (2002, 2007)	mtDNA	*Sepia officinales*	9 in Atl, respectively 1 in Roquetas del Mar (Al), Alicante (Sp), Torre de Sal (Sp) and Vilanova (Sp), 10 in western and eastern Med	"restricted gene flow between southern and eastern Spanish samples due to the AOF"
Rios et al. (2005) Saavedra & Peña (2005)	allozymes mtDNA	*Pecten maximus* *Pecten jacobaeus*	1 in Atl, Fuengirola (Al), Valencia, Moixó, Carreró, 1 in Adriatic Sea	"species divison is due to the AOF"
Zane et al. (2000)	mtDNA	*Meganyctiphanes norvegica*	4 in Atl, respectively 1 in Cádiz, Alboran Sea (in the near of Cabo de Gata), Ligurian Sea	"restricted gene flow between the NE Atl and the Ligurian Sea, the Alboran Sea is an intermediate"

MATERIALS AND METHODS

A total of 369 shrimps (including 282 samples from Reuschel et al. submitted) were available to assess the genetic variability within *P. elegans*. Four new sequences from Corsica and 83 specimens collected along the Spanish coast to document the faunal change in this area were included in this study. The major focus of this study lies on the 83 prawns which were obtained during collecting trips to the Spanish Mediterranean coast by the second author (Figure 2 and Table 2). The collected material was preserved in 70% ethanol.

For the extraction of genomic DNA, muscle tissue was removed from the abdomen of the shrimp and DNA isolated using the Puregene kit (Gentra Systems). Selective amplification of a 640 basepair product from the mitochondrial gene cytochrome oxidase subunit I (COI) was carried out by a polymerase-chain-reaction (PCR) (40 cycles: 45sec 94°/1min 48-50°/1min 72° denaturing/annealing/extension temperatures). The following primer combination was used: COL6Pe (5´- AAG ATA TTG GAA CTC TAT AT-3´) and COH6Pe (5´- GTG SCC AAA GAA YCA AAA TA-3´) (from Reuschel et al. submitted). The PCR products were purified with "Quick / Sure Clean" (Bioline). The products were precipitated with ethanol, resuspended in water and sequenced with the ABI BigDye terminator mix (Big Dye Terminator® v 1.1 Cycle Sequencing Kit; Applied Biosystems) in an ABI Prism automated sequencer (ABI Prism™ 310 Genetic Analyzer; Applied Biosystems). The sequences were proofread with the program ABI Sequencing Analysis® 3.4 (Applied Biosystems) and manually aligned with BioEdit (Hall 1999) excluding primer regions. Genetic heterogeneity within populations was estimated as haplotype diversity (*h*) (Nei & Tajima, 1981), nucleotide diversity (π) (Nei 1987) and the mean number of pairwise differences (*k*) computed with DnaSP 4.00 (Rozas et al., 2003). The AMOVA and Φ_{ST} values were calculated with Arlequin 3.1 (Excoffier et al. 2005) for the whole data set. For population comparison analyses, sequences from fourteen populations from the Spanish coastline from Reuschel et al. (submitted) and the nine populations from this study were used (Table 2) to show isolation-by-distance effects and the haplotype-distribution along the Spanish Mediterranean coastline (Figure 4 and Table 3). The comparison was done with Cádiz (southern Spain, Atlantic side) as reference point. The same populations were included in a histogram to illustrate the haplotype-distribution in the area of Cabo de Gata (Figure 3). The geographical overlaps are also shown with pie charts in Figure 2.

Table 2. Localities; locality abbreviations of the sampled populations, number of specimens used for genetic comparisons (*N*), haplotype diversity (*h)* and nucleotide diversity (π) within the examined population and the three types of *P. elegans* (type which dominates the population in bold; number of specimens of respectively type in brackets).

Collection site of *P. elegans*	**Abbr.**	***N***	**Type**	***h***	***π***
Spain: Almería: Almería	Alm	10	**I (6)** / II (4)	0.91	0.01014
Spain: Almería: Los Genoveses	Gen	10	I (4) / **II (6)**	0.91	0.01095
Spain: Almería: San José	Jos	9	**I (6)** / II (3)	0.89	0.01102
Spain: Almería: Cala de las Toros	Tor	10	I (5) / II (5)	0.93	0.01216
Spain: Almería: Las Salinicas	Sal	8	I (1) / **II (4)** / III (3)	1	0.00926
Spain: Almería: Carboneras	Car	10	I (1) / **II (6)** / III (3)	1	0.01196
Spain: Almería: Macenas	Mac	9	I (3) / II (2) / **III (4)**	1	0.01488
Spain: Almería: Mojacar	Moj	7	**II (6)** / III (1)	1	0.00771
Spain: Castellón	Cas	10	I (1) / **II (6)** / III (3)	1	0.01236
France: Corsica: St. Florent	Cor	4	I (1) / **III (3)**	0.67	0.0011

RESULTS

Together with results from our previous study (Reuschel et al. submitted), sequences of the COI gene consisting of 605 basepairs were available from 369 specimens of *Palaemon elegans*. The molecular analysis of the whole dataset revealed 144 different haplotypes, 90 parsimony-informative sites and 10 non-synonymous mutations. The AMOVA between all subpopulations, including results from Reuschel et al. (submitted), reveals a significant mean value of overall Φ_{ST} of 0.91 ($p<0.0001$), the molecular variance between the three types being 81.24%, among populations within types 9.94% and within populations 8.82%. The AMOVA between the Atlantic Type and the New Mediterranean Type reveals a significant mean value of overall Φ_{ST} of 0.58641 ($p<0.0001$). In this case, the molecular variance can be attributed to the differences between the two types (48.87%) and within populations (41.36%) whereas a very small portion of the variance was due to variation among populations within types (9.77%).

The 83 sequences from southwestern Spain (Alm-Cast in Fig. 2) could be assigned as follows to the three previously designed haplotype groups. 27 individuals belong to haplotype group I (Atlantic Type); they are from Almería, Los Genoveses, San José, Toros, Macenas and one specimen from Las Salinicas, Carboneras and Castellón respectively. Haplotype group II (New Mediterranean Type) includes 42 specimens from Almería, Los Genoveses, San José, Toros, Las Salinicas, Carboneras, Macenas, Mojacar and Castellón. These two types show

similar haplotype and nucleotide diversities (Table 3). Type III (Old Mediterranean Type) haplotypes (hts) are separated by over 50 mutation steps from the other two types. Type III includes 14 specimens, but with a lower haplotype-diversity and nucleotide diversity. This third haplotype group includes specimens from Las Salinicas, Carboneras, Macenas, Mojacar, Castellón and is the only type present in Garrucha, Mar Menor, Torrevieja, Cabo del Nao and Valencia. The geographical overlap between all types is shown in Figure 2 and 3. One of the four sequences from Corsica belongs to the Atlantic Type the other three to the Old Mediterranean Type (not shown).

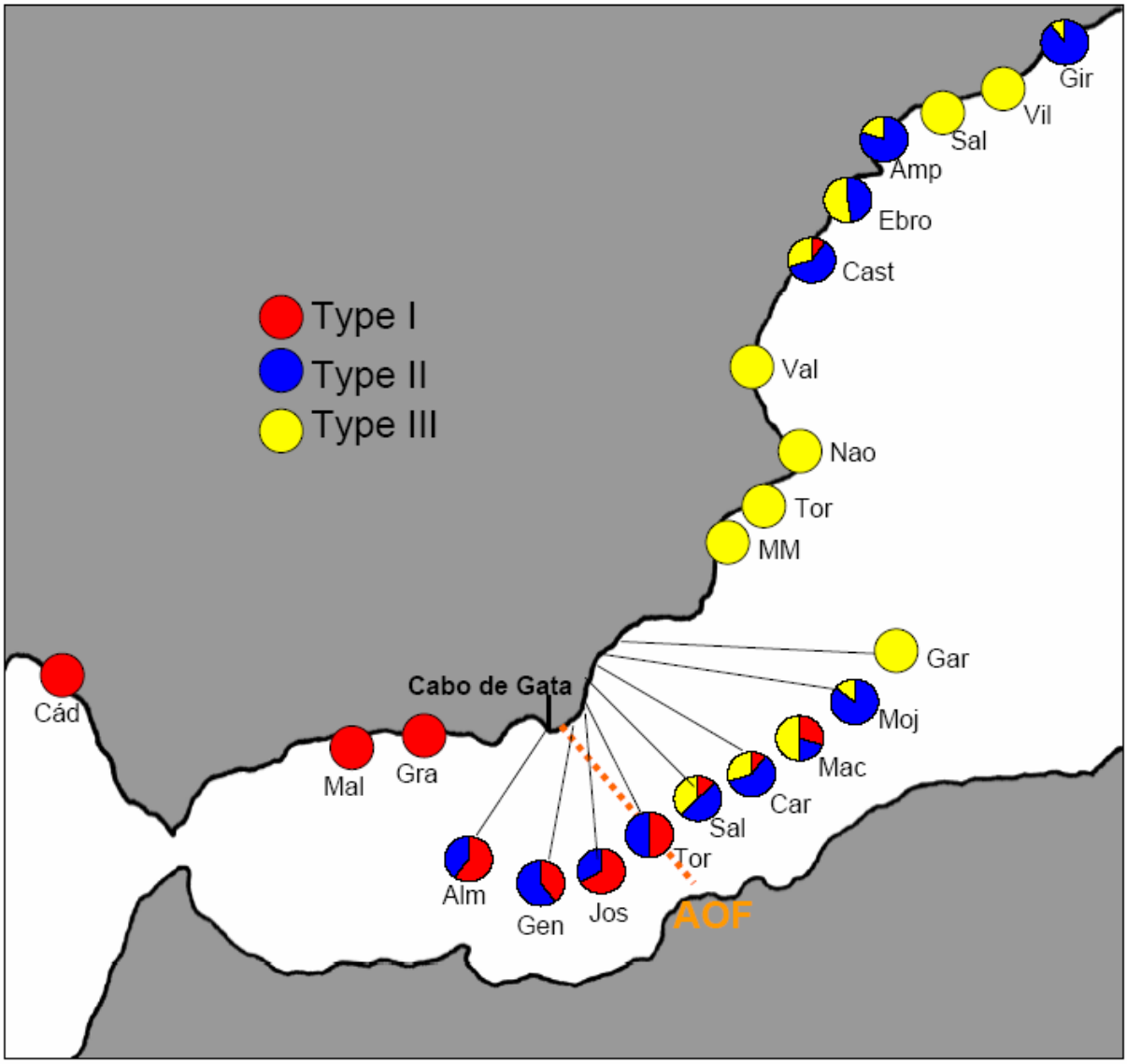


Figure 2. Geographical locations of collection sites of *P. elegans*. The shading corresponds to the different types and is proportional to the number of specimens of the types which are found at the site. For the abbreviations of collection sites see also Table 1 (Cád = Cádiz; Mal = Malaga; Gra = Granada; Gar = Garrucha; MM = Mar Menor; Tor = Torrevieja; Nao = Cabo Nao; Val = Valencia; Cast = Castellon; Ebro = Ebro Delta; Amp = Ampolla; Sal = Salou; Vil = Vilanova; Gir = Girona; AOF = Almería-Oran-Front).

Table 3. Summary of statistics of the COI gene; *N*, number of sequences; M, number of haplotypes; *h*, haplotypic diversity; π, nucleotide diversity; S, number of segregation (polymorphic) sites; k, mean number of pairwise differences between individuals; SD, standard deviation.

Gen	Sample	*N*	M	*h*(SD)	π (SD)	*S*	*k* (SD)
COI	TypeI	27	16	0.89 (0.054)	0.0047 (0.001)	27	2.84 (3.72)
	TypeII	42	32	0.97 (0.014)	0.0069 (0.0008)	44	4.21 (1.8)
	TypeIII	14	7	0.8 (0.09)	0.0031 (0.0008)	10	1.87 (1.06)

In our previous study, we only had evidence that two types can occur in sympatry. However, the present study reveals cases where all types occur together (see Figure 3). Type I decreases more or less gradually from Almería to Macenas. Type III dominates the central Spanish Mediterranean coast: from Garrucha to Valencia only the Old Mediterranean Type is found. This is also the case in the eastern Baltic, Black and Caspian Seas (Reuschel et al., submitted). The distribution of Type III in the Baltic and Caspian Sea probably goes back to unintentional human introductions (see Grabowski 200?; Reuschel et al. submitted). The New Mediterranean Type is only found in the Mediterranean Sea. Within the Mediterranean Sea, one specimen of the Atlantic haplotype group was found in Castellón and Corsica, respectively.

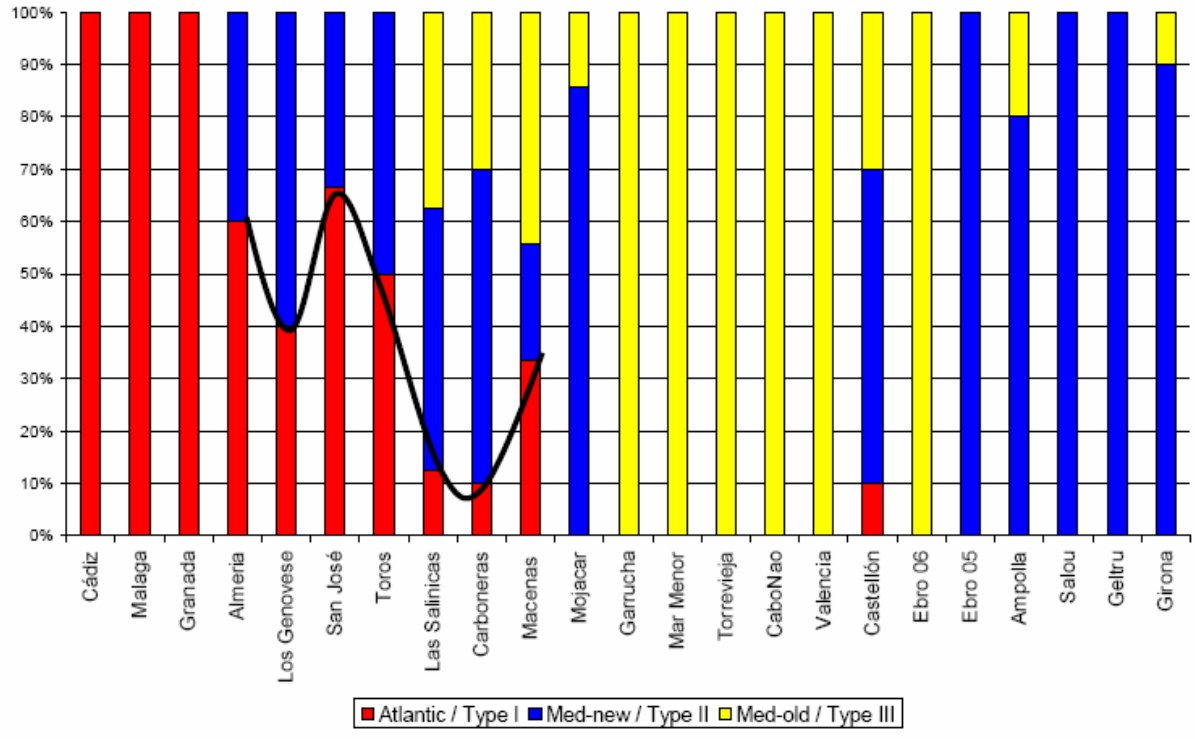


Figure 3. Histogram showing the distribution of the three types and illustrating the geographical overlaps. The shading corresponds to the three types and the line shows the decline of the Atlantic Type.

In order to determine, if there is an isolation-by-distance pattern the pairwise Φ_{ST} values – with Cádiz as reference population - are presented in Table 4 and Figure 4. The comparison was done between all collection sites along the Spanish coast and only with the type which dominates the population. Type I and II was thereby considered a single gene pool and Type III as a second one, due to its high differentiation. The few sequences causing the geographical overlap of Type I&II and Type III were excluded from the respective analysis. The populations of Type I&II around Cabo de Gata show a tendency of isolation-by-distance along the Spanish coast from Granada (Φ_{ST}: 0.05498) to Girona (Φ_{ST}: 0.83631). From Almería to Girona the analysis reveals significant Φ_{ST} values. Only the population of San José is not significant. The values increase in a non-linear way and stagnate around 0.8. All Type III populations showed significant Φ_{ST} values close to 1.

Table 4. Pairwise Φ_{ST} values between Cádiz and populations of Type I, II and III. Significant values are in bold.

	Cádiz
Granada	0.05498
Almería	**0.2328**
Los Genoveses	**0.40932**
San José	0.1281
Toros	**0.29595**
Las Salinicas	**0.68**
Carboneras	**0.62754**
Mojacar	**0.78214**
Garrucha	**0.96538**
Torrevieja	**0.96915**
Cabo Nao	**0.97093**
Valencia	**0.97405**
Castellón	**0.58557**
Ebro06	**0.97753**
Ebro05	**0.79495**
Ampolla	**0.81271**
Salou	**0.8207**
Vilanova	**0.78632**
Girona	**0.83631**

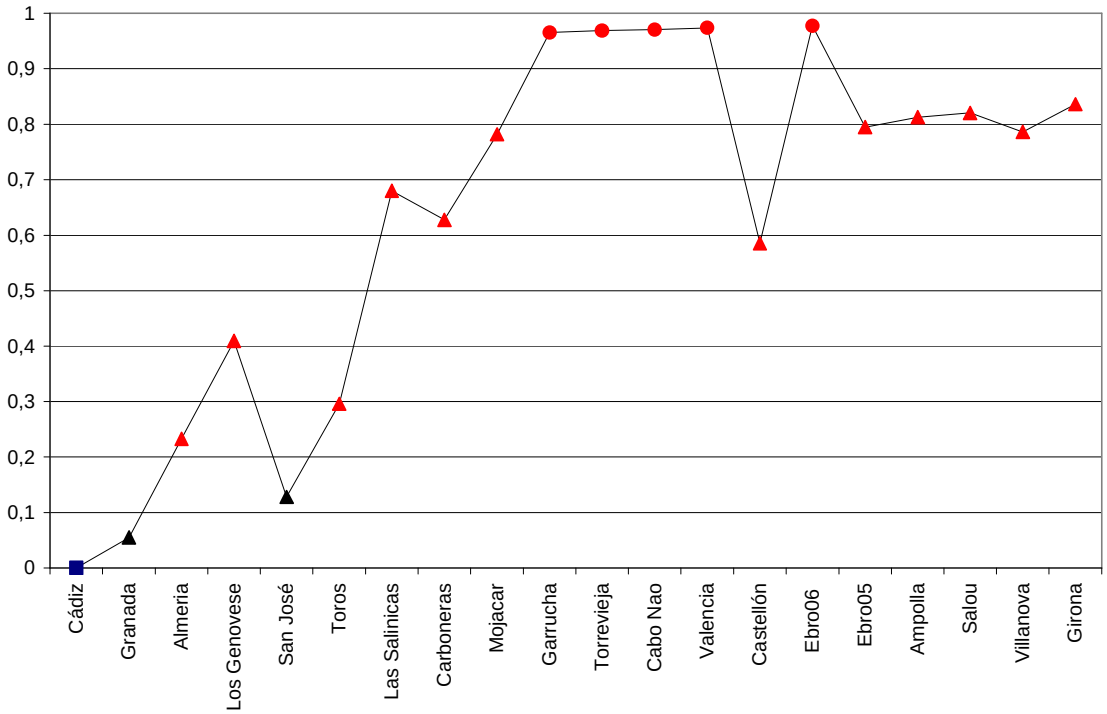


Figure 4. The graph demonstrate an isolation-by-distance pattern shown by the pairwise Φ_{ST} values - with Cádiz (in blue) as reference population; triangles belong to Type I and II; red triangles are significant; dots belong to Type III and are all significant.

DISCUSSION

Marine organisms often show biogeographical patterns that can be associated with historical changes in the relative positions of land and sea masses. Variations in the hydrographic conditions may influence speciation and the isolation of taxa (Naranjo et al. 1998; Schubart et al. 2006). For *P. elegans*, we revealed an isolation of populations due to historical changes and ocean currents. The Old Mediterranean Type consists of a very distinct group of haplotypes and its isolation might go back to the Messinian Crisis while the New Mediterranean Type (Type II) could be the result from a reintroduction of Altantic specimens after the Crisis without total isolation (see Reuschel et al. submitted). For the Atlantic Type (Type I) we here describe a phylogeographic break. The barrier to gene flow lies in the western Mediterranean Sea and corresponds to the Almería-Oran-Front. It separates the populations of the Old Mediterranean Type, which can probably be considered as a different species, and the New Mediterranean Type form the Atlantic Type. Therefore, the AOF might play a central role in maintaining previously acquired allopatric separation which has been suggested for several marine species and plants, for example for the cuttlefish *Sepia officinalis* (see Pérez-Losada et al., 2002, 2007), for the sea star *Asterina gibbosa* (see Baus et al. 2005), for the scallops *Pecten jacobeus* and *P. maximus* (see Rios et al., 2002) and for four coastal flowering plant species (see Kadereit et al. 2005).

In comparison to these studies (see also Table 1), we did a finer scaled geoghraphic sampling to confirm the influence of the AOF on the population structuring. All the three types can occur in sympatry in this region. In the area of Cabo de Gata, we revealed a geographic break with a narrow zone of overlap: specimens of the populations from Almería to Toros belong to the Atlantic Type I as well as to Type II, while specimens of the populations from Las Salinicas to Macenas belong to all three types (see Figure 2 and 3). The Atlantic Type decreases gradually along the Spanish coast from Almería to Macenas. This geographical overlap could be explained due to the instability of ocean currents. They are often not stable during a year and vary due to the season which is also shown for the Almería-Oran front. The AOF is variable in its shape and position, a fact that could influence the distribution of zooplankton and its dispersal at the front (Fielding et al. 2001). A zone of overlap between the populations from the Alboran-Gyre and the western Mediterranean is the effect. Therefore, the AOF is a phylogeographic break with a transition zone where mixing between populations of the Atlantic (Type I) and the Mediterranean Sea (Type II) is still possible. For example Zane et al. (2000) revealed that the population of the Alboran-Gyre is an intermediate between the Atlantic and western Mediterranean. Our results also show that

there is occasional transport of Atlantic haplotypes into the Mediterranean Sea (Castellón and Corsica). This is most likely due to ocean currents occasionally transporting Atlantic propagules into the western Mediterranean. However, it is interesting to note that there is no transport of Mediterranean haplotypes into the western Alboran Gyre or the Atlantic Ocean. Most of the zooplankton is restricted to the surface water where its food source, the phytoplankton, is located (Hofrichter 2002). Since the Mediterranean waters enter the Atlantic as bottom water, the dispersal of pelagic larvae into the Atlantic seems to be impossible. In contrast, due to the Atlantic surface water the dispersal of larvae from the Atlantic into the Alborán and Mediterranean Seas is more likely. In future studies, a finer scaled sampling between Cádiz and Almería will be carried out in order to confirm this hypothesis.

In addition to restricted gene flow due to marine currents occurring in the Gibraltar area, other factors might explain the Atlantic-Mediterranean division of Type I and II such as historical events. Mediterranean and Atlantic populations have been isolated several times during sea level regressions in the Plio-/Pleistocene, with subsequent genetic divergence and secondary contact. Such biogeographic scenarios have been suggested for other marine species as the bonito *Sarda sarda* (see Viñas et al. 2004), the mussel *Mytilus galloprovincalis* (see Quesada et al. 1995) and the scallops *Pecten jacobeaus* and *Pecten maximus* (see Ríos et al. 2002). In addition, the different conditions of the North Atlantic, which is considerably colder and more nutrient-rich than the Mediterranean Sea, could facilitate a phylogeographic break due to differences in adaptation (Hofrichter 2002). Cimmaruta et al. (2005) suggested that the genetic differentiation within the European hake (*Merluccius merluccius*) is due to differences in water temperatures and salinity in the two basins. In the case of the Ebro Delta, we collected two populations in different years, 2005 outside the harbour of St. Carles de la Rapita and 2006 inside the harbour. The population of 2005 belongs to Type II and the populations of 2006 to Type III (see Reuschel et al. submitted). It is possible, that different types adapted to different environmental conditions. Genetic differentiation in response to different habitats is shown for the ascidian genus *Clavelina*: the “interior” form of *C. lepadiformis* adapted to harbour environment and the “exterior” to rocky littoral habitat (Tarjuelo et al. 2001).

There is no abrupt or strict linear genetic differentiation of the closely related Type I and Type II along the Mediterranean Spanish coastline (see Figure 4). Within the Mediterranean Sea, the complexity of the coastline and the numerous islands create many small eddies and other local currents (Send et al. 1999). This and the low amplitude of tidal currents in the

Mediterranean Sea could reduce the dispersal capability of marine species (Shanks 1995). Therefore, the gene flow between populations and the spreading of the different types could be different along the coastline. This might also explain that in some places the different types occur in sympatry and in other places or broader regions only one type is found (e.g. between Garrucha and Valencia).

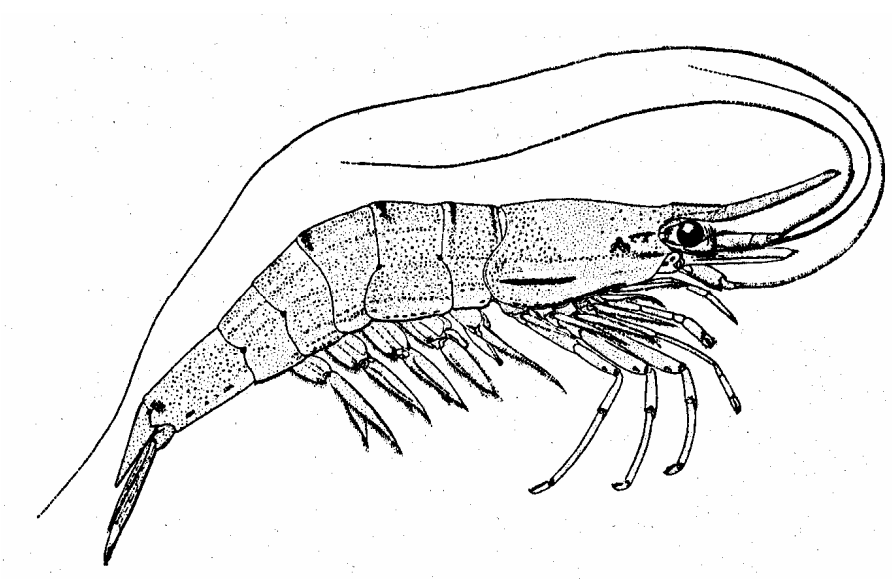
This is the first study that clearly shows in detail the effect of the AOF as barrier to gene flow for the marine fauna. We suggest the AOF to be one of the main reasons for maintaining cryptic and endemic species in the Mediterranean Sea. A high genetic differentiation due to the AOF was revealed between Type I and II and additionally it acts as a barrier for dispersal of Type III into the Atlantic Ocean. However, in the area of Cabo de Gata restricted haplotype mixing is still possible between the Atlantic and New Mediterranean Type populations. Therefore, this narrow zone is a transition zone for marine species. Another important result is the evidence of transport of Atlantic propagules into the Mediterranean Basin, while no Mediterranean propagules were found in the Atlantic.

ACKNOWLEDGEMENTS

Our special thanks go to Carsten Müller, Lapo Ragionieri, Enrique González-Ortegón, Ruth Jesse, Sara Fratini, Henrik Schubart, Florian Gmeiner and students of the Universtiy of Regensburg for helping to collect and sending specimens of *P.elegans*. Thanks are further due to Graciela Sotelo for helpful discussions of statistical tests.

PUBLICATION 5

GENETIC VARIABILITY IN THE CARIBBEAN FRESHWATER SHRIMP *XIPHOCARIS ELONGATA* (CRUSTACEA: CARIDEA) DOES NOT REFLECT MORPHOLOGICAL NOR GEOGRAPHICAL PATTERNS



Silke Reuschel and Christoph D. Schubart

Hydrobiologia (accepted)

ABSTRACT

Genetic divergence in the West Indian freshwater shrimp *Xiphocaris elongata* (Guérin-Méneville, 1856) is here described as DNA-sequence variation in fragments of the mitochondrial genes COI (48 individuals) and 16S rRNA (13 individuals) from five different river systems of Puerto Rico and comparative material from Cuba, the Dominican Republic and Jamaica. The study revealed high genetic differentiation within *X. elongata,* but without an apparent geographic pattern. The demographic analyses of the species suggest a small initial population that experienced a sudden expansion. The previously described variability in the relative length of the rostrum resulting in three to four morphs (in part considered as species) was tested with genetic and morphometric methods. While the morphometric analysis confirmed significant differences between three distinct morphological forms, molecular results gave no evidence for a mitochondrial differentiation of these phenotypes. We therefore propose that the variable length of the rostrum represents phenotypic variability, only partly depending on carapace size.

INTRODUCTION

Species with different life history strategies are likely to display different genetic structuring, because population structure, genetic divergence and larval dispersal are strongly coupled (Slatkin 1981; Palumbi 1992). In marine species, high rates of gene flow are assumed to maintain panmictic reproduction. Indeed, population genetic studies of many species have shown that high dispersal potential due to planktonic larvae is often associated with only mild genetic differentiation over large scales (see Schneider-Broussard et al. 1998; Kojima et al. 2003; Spivak & Schubart 2003; Reuschel & Schubart 2007). In freshwater systems, barriers to gene flow promoting speciation are more prominent than in marine systems, because each stream and its tributaries harbours a population potentially separated by land from adjacent ones. Therefore, species that inhabit fresh water often display higher population genetic structure, which has been proposed as a general rule for species of freshwater fish (Avise 2004). For example, recent analyses of mtDNA in the marine and riverine populations of *Atherina*, showed that the marine *A. presbyter* presents a pattern of high level of gene flow and low degree of genetic differentiation, whereas the riverine species *A. boyeri* shows differentiation between the populations (Francisco et al. 2006). In freshwater species, the potential for larval dispersal is furthermore limited, because larval development is often abbreviated or reduced and the offspring tends to remain in the parental habitat like in sesarmid crabs of Jamaica (Schubart & Koller 2005). Therefore, levels of gene flow are often so low that natural selection and genetic drift may occur more or less independently in each deme. For example, Hughes et al. (1996) revealed for the freshwater shrimp *Caridina zebra*, a species with a highly abbreviated larval development with no planktonic stage, a high genetic differentiation among nine isolated populations.

The Caribbean endemic freshwater shrimp *Xiphocaris elongata* has an amphidromous life cycle and thus represents an intermediate between typical marine and freshwater species. Females release larvae in the upper reaches of rivers from where they drift passively to coastal environments to develop and metamorphose into postlarvae that subsequently migrate back upstream to adult (freshwater) habitats (see also Myers et al. 2000; Cook et al. 2006 and McDowall 2007 for other amphidromous species). Therefore, the question arises, whether the population structure shows a pattern of typical freshwater or marine species. For *X. elongata*, the length of the larval development, the habitat of larval development (in the open ocean or in estuaries) and the mode of localization and return to the mouths of freshwater streams should determine the degree of geographic structure. Currently, this important life history information is unknown. Benstead et al. (2000) discovered the presence of several larval

stages of *X. elongata* in two estuaries and suggested that at least a proportion of larvae develop to post-larval stage in estuarine habitats. In general, Chace and Hobbs (1969) and Fiévet et al. (2001) suggest for Caribbean shrimp and freshwater species the presence of their larvae in estuaries, close to the coast or upriver. If we predict a larval retention life history strategy, as opposed to larval dispersal, then dispersal may be limited to adjacent or nearby estuaries and restricted gene flow would be the logical consequence.

Morphologically, the most evident characteristic of *X. elongata* is the extreme variability in the relative length of the rostrum. Based on differences in rostral length, Pocock (1889) subdivided *X. elongata* into three distinct species and one variety. The proposed taxa, *Xiphocaris brevirostris* Pocock, 1889, *Xiphocaris gladiator* Pocock, 1889, *Xiphocaris gladiator* var. *intermedia* Pocock, 1889 and *Xiphocaris elongata* in correspondence to the length of their rostra, are here used for morphological subdivison only. Ortmann (1894), Bouvier (1925) and Hart (1961) argued that only one species should be recognised and that Pocock´s species are, if anything, only subspecies or varieties. The reason for the variability in the relative length of the rostrum could not be explained so far. Chace and Hobbs (1969: 87) pointed out the following pattern for the phenomenon: “the rostrum increases rapidly in relative length in the youngest juveniles, and then gradually decreases in proportion as the body lengthens and broadens”.

The genus *Xiphocaris* occurs only on the West Indian Islands and no close relatives are known from the mainland. Most of the islands are not older than the late Oligocene or early Miocene. For the colonisation of the Greater Antilles, two hypotheses are being discussed: the dispersal and the vicariance hypothesis (Williams 1989; Page et al. in press). Both result in geographical isolation (allopatry), which in time allows separated populations to evolve independently (Humphries & Ebach 2004). The dispersal model postulates that either four centres of origin contributed through massive long or short term dispersal to a Caribbean fauna, or that one Caribbean centre contributed to an eastern Pacific, eastern Atlantic, North and South American fauna (Hedges et al. 1992). The vicariance model, on the other hand, considers the Caribbean biota a result of fragmented ancestral biota that occupied a “proto Antillean archipelago”, colonised from North and South America during times of direct contact in the Mesozoic. Portions of Pacific seafloor moved eastward carrying the archipelago with it. Islands and their biota moved to their present location during late Mesozoic & Cenozoic (Rosen 1976). Phylogenetic studies are supporting vicariance (Chakrabarty 2006) and dispersal (Perdices et al. 2005), highlighting the unsolved history of Caribbean freshwater fauna. For *Xiphocaris*, Chace and Hobbs (1969) pointed out that the

species may represent the remnant of an old stock that has disappeared from its original distribution and has found a congenial habitat in the West Indies.

In this study, we used a molecular phylogeographic approach to investigate the population structure of *X. elongata* at two different scales: between different river-systems of Puerto Rico and between the islands Puerto Rico, Dominican Republic, Cuba and Guadeloupe, with single individuals from the latter two localities. We used mitochondrial DNA sequences of the 16S rRNA and the COI gene. The morphological varieties *"X. brevirostris"*, *"X. gladiator"* and *X. elongata* were compared with genetic and morphometric methods in order to investigate whether the species can be split in separable distinct morphs or whether there is a gradient of rostral lengths. With the whole dataset, we also investigated the demographic history of the species.

MATERIALS AND METHODS

Genetics

For the intra-island comparison we analyzed 22 specimens from five different river systems of Puerto Rico. For the comparison among islands, we included twelve additional individuals from different river systems of the Dominican Republic and twelve from different river systems of Jamaica (see Table 1 and Figure 1). One specimen each from Cuba and from Guadeloupe was available for this study.

For the genetic analyses, genomic DNA was extracted from the muscle tissue of the abdomen using the Puregene kit (Gentra Systems). A total number of 48 specimens of the genus *Xiphocaris* was examined (Table 1 and 2). The selective amplification of an approximate 540 basepair fragment from the large subunit rRNA gene (16S) for 13 specimens and a 640 basepair fragment of the cytochrome oxidase subunit I gene (COI) for all specimens was carried out by polymerase chain reaction (PCR) (40 cycles; 45sec 94°/1min 48-50°/1min 72° denaturing/annealing/extension temperatures). The following primer combinations were used: for the COI, the new primers COL15 (5´ - CCT GCT GGD GGW GGW GAC CC - 3´) and COH19 (5´ - TAT ATA AGC ATC GGG GTA ATC – 3`) were designed; for 16S 16L2 (5´ - TGC CTG TTT ATC AAA AAC AT - 3´) (Schubart, et al. 2002) and 1472 (5´ - AGA TAG AAA CCA ACC TGG - 3´) (Crandall & Fitzpatrick 1996). The PCR products were purified with Quick Clean (Bioline), precipitated with ethanol, resuspended in water and sequenced with the ABI BigDye terminator mix (Big Dye Terminator® v 1.1 Cycle

Sequencing Kit; Applied Biosystems) in an ABI Prism automated sequencer (ABI Prism™ 310 Genetic Analyzer; Applied Biosystems). The sequences were proofread with the program ABI Sequencing Analysis® 3.4 (Applied Biosystems). Alignments were carried out by hand with BioEdit (Hall 1999), excluding the primer regions.

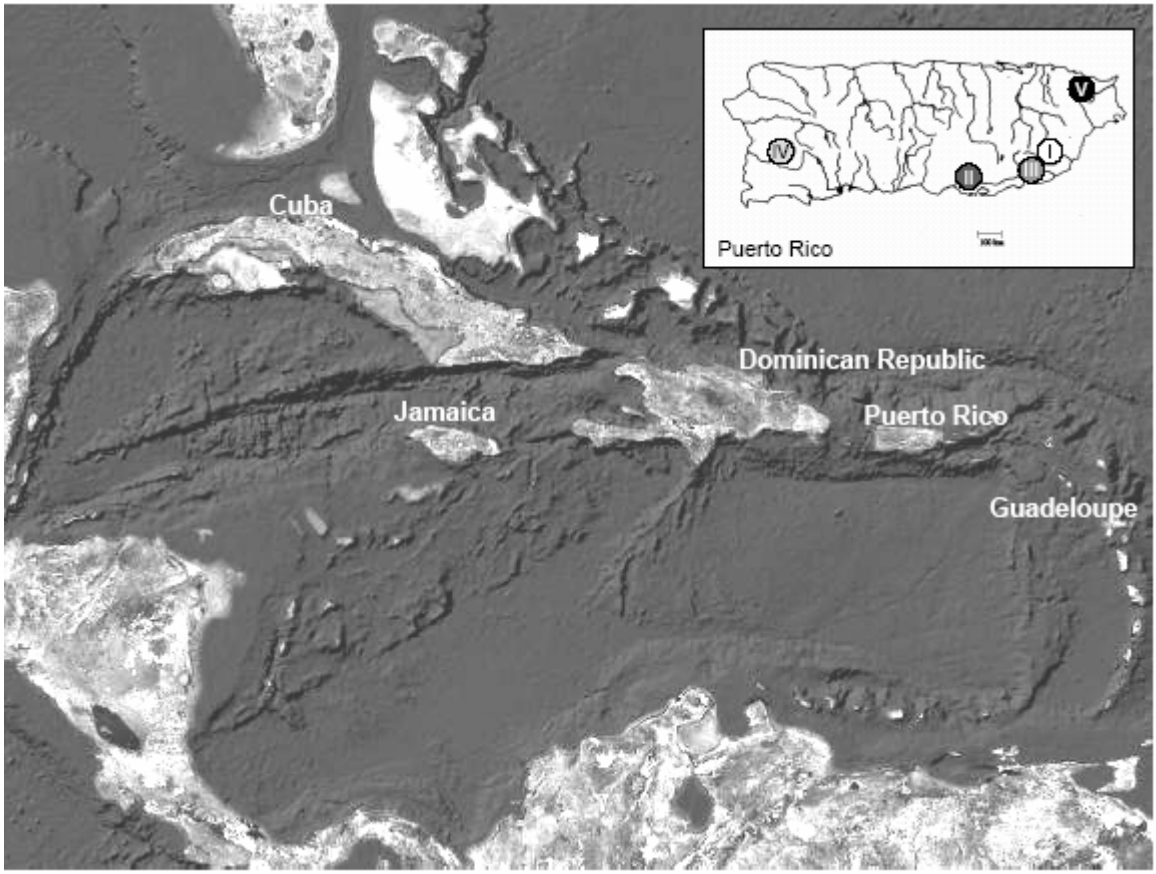


Figure 1. Map of the Caribbean Islands and of Puerto Rico including collection sites of the different river systems of Puerto Rico (I = Río Guayanés, II = Río Guamaní, III = Río Jacaboa, IV = Río Nueve Pasos, V = Río Fajardo; shading corresponds to collection site).

Table 1. Localities (PR=Puerto Rico, DR=Dominican Republic), number of specimens used for genetic (*N*) comparisons of the 16S gene.

Species	Collection site	*N*
Xiphocaris"brevirostris"		
Xiphocaris"brevirostris"	Jamaica: Morgan River	1
Xiphocaris"brevirostirs"	DR: Río Yásica	2
Xiphocaris"brevirostirs"	PR: Río Guayanés N18°03,883 W65°59,727	1
Xiphocaris"brevirostirs"	PR: Río Jacaboa N18°01,149 W65°59,727	1
Xiphocaris "gladiator"		
Xiphocaris "gladiator"	PR: Río Fajardo N18°16,904 W65°43,896	1
Xiphocaris elongata		
Xiphocaris elongata	Jamaica: Great River: Marchmount	2
Xiphocaris elongata	Jamaica: Rio Bueno	1
Xiphocaris elongata	PR: Río Guamaní N18°02,031 W66°06,110	1
Xiphocaris elongata	PR: Río Fajardo N18°16,904 W65°43,896	1
Xiphocaris elongata	PR: Río Nueve Pasos N18°09,467 W67°04,534	1
Xiphocaris elongata	Cuba: Río Canas,Wof Trinidad N21°50,23 W80°01,47	1

Table 2. Localities (PR=Puerto Rico, DR=Dominican Republic), number of specimens used for genetic (N_g) comparisons of the COI gene, number of individuals used for the morphometric (N_m) comparisons.

Species	Collection site	N_g	N_m
Xiphocaris"brevirostris"			
Xiphocaris"brevirostris"	Jamaica: Upper Cabarita River	5	-
Xiphocaris"brevirostris"	Jamaica: Rio Negro	4	11
Xiphocaris"brevirostirs"	DR: Río Yásica	4	-
Xiphocaris"brevirostirs"	PR: Río Guayanés N18°03,883 W65°59,727	5	9
Xiphocaris"brevirostirs"	PR: Río Jacaboa N18°01,149 W65°59,727	5	12
Xiphocaris "gladiator"			
Xiphocaris "gladiator"	Jamaica: Green Island River	-	4
Xiphocaris "gladiator"	DR: Los Patos	3	3
Xiphocaris "gladiator"	PR: Río Fajardo N18°16,904 W65°43,896	1	2
Xiphocaris elongata			
Xiphocaris elongata	Jamaica: Rio Bueno	1	-
Xiphocaris elongata	Jamaica: Great River: Marchmount	2	1
Xiphocaris elongata	Jamaica: Morgan River	-	3
Xiphocaris elongata	Jamaica: Bluefields	-	4
Xiphocaris elongata	DR: La Escalareta	5	5
Xiphocaris elongata	PR: Río Guamaní N18°02,031 W66°06,110	3	3
Xiphocaris elongata	PR: Río Fajardo N18°16,904 W65°43,896	3	4
Xiphocaris elongata	PR: Río Nueve Pasos N18°09,467 W67°04,534	5	6
Xiphocaris elongata	Guadeloupe	1	-
Xiphocaris elongata	Cuba: Río Canas,WofTrinidad N21°50,23 W80°01,47	1	-

To determine the historical demography of the population we analysed the mismatch distributions with the model of Rogers & Harpending (1992). The mismatch distributions are used to assess fit of haplotype data to the sudden demographic expansion model (Rogers & Harpending 1992). We tested the null hypothesis of neutrality, which may be rejected when a population has experienced population expansion (Tajima 1989). Therefore, Tajima´s *D*-test (Tajima 1989) and Fu´s (1997) F_s test and their significance levels were estimated using DnaSP based on 1000 simulated re-sampling replicates. Mismatch distribution analyses, under the assumption of selective neutrality, were also used to evaluate possible historical events of population growth and decline (Rogers & Harpending 1992). Past demographic parameters, including τ (Li 1977), θ_0 and θ_1 and their probabilities (Rogers & Harpending 1992) were estimated with Arlequin and DnaSP.

Morphometrics

For the morphometric comparisons, 21 *"X. brevirostris"*, 13 *X. elongata* and 2 *"X. gladiator"* from Puerto Rico, 11 *"X. brevirostris"*, 8 *X. elongata* and 4 *"X. gladiator"* from Jamaica and 5 *X. elongata* and 3 *"X. gladiator"* from the Dominican Republic were included in the analysis. The following morphological measurements were taken: 1) carapace length with rostrum and 2) carapace length up to the orbit (cl). The rostrum length (rl) was then calculated as the difference from these two measurements. The data were tested independently for normal distribution by the Kolmogorov-Smirnow-test (software Statistica 6.0; StatSoft). To reduce the influence of allometric growth, the rostrum-carapace ratios were used for subsequent analyses. The comparison of the morphometric ratio of *"X. brevirostris"*, *"X. gladiator"* and *X. elongata* and a geographical comparison of *X. brevirostris* and *X. elongata* from the different islands were carried out with a 1-Factor-ANOVA and a post-hoc Schefé test. In order to test possible effects of size of individual shrimps on the used ratios, a regression-analysis was used to compare regression of the ratio of rostrum length to carapace length against carapace length. A scatterplot was constructed to show the relationship of rostrum length to carapace length of both proposed species.

RESULTS

Genetics

For genetic comparisons of populations, we used a 540 basepair alignment of 16S mtDNA and a 620 basepair fragment of the COI gene. The TCS network of the 16S showed a high number of haplotypes with thirteen specimens resulting in twelve different haplotypes and a high haplotype diversity (*h*) of 0.98, whereas the nucleotide diversity (π) was comparatively low (0.00589). The maximum pairwise difference between two haplotypes was six mutation steps. The network revealed no geographical pattern (Figure 2).

Due to the higher variability and thus separating potential of the COI gene, we included many more specimens in this comparison (N=48). We first built a minimum spanning network out of 22 specimens from the five different river systems of Puerto Rico. The network showed a star-like shape, with no frequent haplotype. The haplotype with most connected haplotypes was suggested as central haplotype. 18 rare haplotypes have diverged from the central haplotype. Haplotypes were generally present in not more than one individual per population and most haplotypes were connected by only few mutational steps, again resulting in high haplotype diversity and low nucleotide diversity (*h*: 0.987; π: 0.0082). The most distant connection of 11 mutational steps was between a haplotype from the river Guamaní and a haplotype of the river Guayanés, both river systems being in the southeast, but draining independently from each other (Figure 3).

The second network includes all specimens (N=48) and showed a similar pattern: it revealed a star-like shape with high haplotypic diversity (0.99) but low nucleotide diversity (0.00832) (Figure 4). The haplotype diversity, nucleotide diversity and the mean number of pairwise differences for Jamaica, the Dominican Republic and Puerto Rico is listed in Table 4. Thrity-three of the 40 haplotypes were not present in more than one individual; six were represented in two haplotypes and the central haplotype was found in three individuals. The most distant connections sum up to 15 mutation steps between individuals from Guadeloupe and Puerto Rico and between two individuals from Puerto Rico.

There was no clear evidence for a correlation between genetic structure and geography or morphology as shown by the minimum spanning tree and by AMOVA and F-statistics: the analysis of variance of 620 basepairs of COI between all subpopulations revealed a mean value of overall Φ_{ST} of 0.0052 (p=0.48). The highest percentage of variation was within populations of single river-systems (99.48%).

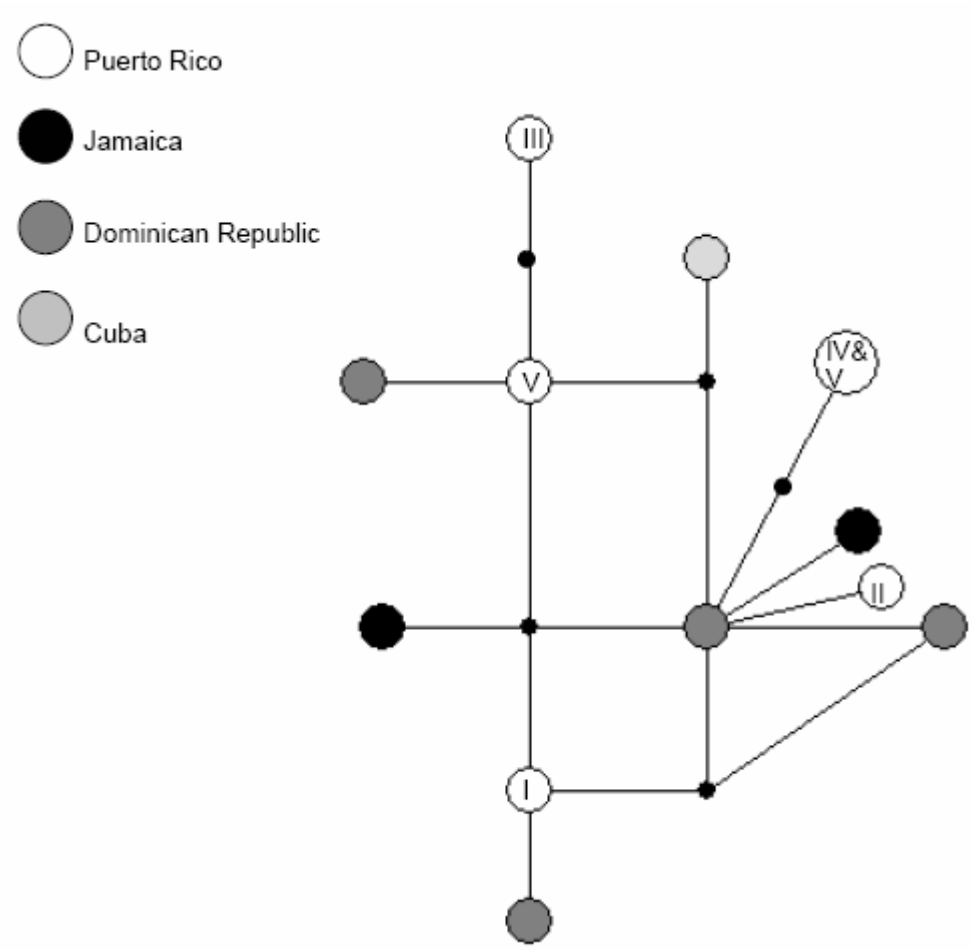


Figure 2. Minimum parsimonious spanning network of *X. elongata* (N=13) constructed with TCS corresponding to a 540-basepair fragment from the 16S rRNA gene. Each line represents one substitution; circles indicate additional substitutions separating two haplotypes. The size of the circle is representative for the frequency of the haplotypes (small: N=1; large: N=2). The shading corresponds to geographic origin and numbers corresponds to the different river systems of Puerto Rico (black = Dominican Republic, white = Puerto Rico, gray = Jamaica; I = Río Guayanés, II = Río Guamaní, III = Río Jacaboa, IV = Río Nueve Pasos, V = Río Fajardo).

To study the exact degree of differentiation between the different populations, we further subdivided the samples into the three islands.The pairwise Φ_{ST} –values were low, not significant and within the range of 0.00084 to 0.0151 ($p>0.1$). The percentage of variation among the three groups was 0.40%. The results show clearly that there is no genetic differentiation among populations.

The morphological varieties *X. elongata* and *"X. brevirostris"* revealed no genetic differentiation (Φ_{ST} – value of 0.00239; p=0.33). The high haplotype diversity and low nucleotide diversity of both genes suggest rapid population growth from an ancestral population with small size, provided the time was sufficient for detection of haplotype

variation via mutation, yet too short for an accumulation of large sequence differences (Avise 2000).

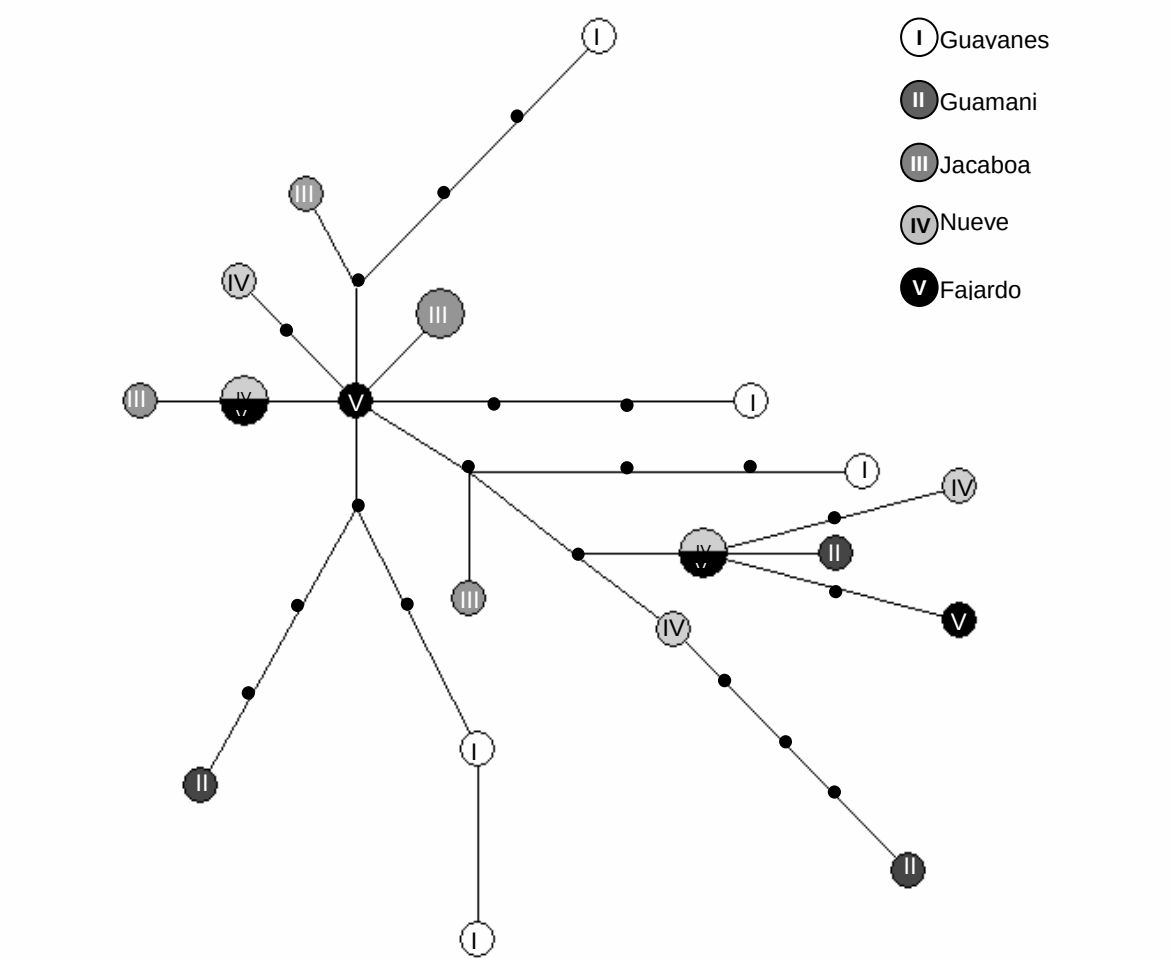


Figure 3. Minimum parsimonious spanning network of *X. elongata* from Puerto Rico (N=22) constructed with TCS corresponding to a 620-basepair fragment from the COI gene. Each line represents one substitution; circles indicate additional substitutions separating two haplotypes. The rectangular box stands for the suggested ancestral haplotype, circle size corresponds to haplotype frequency (small: N=1; large: N=2). Shading and numbers correspond to the different river systems (I = Río Guayanés, II = Río Guamaní, III = Río Jacaboa, IV = Río Nueve Pasos, V = Río Fajardo).

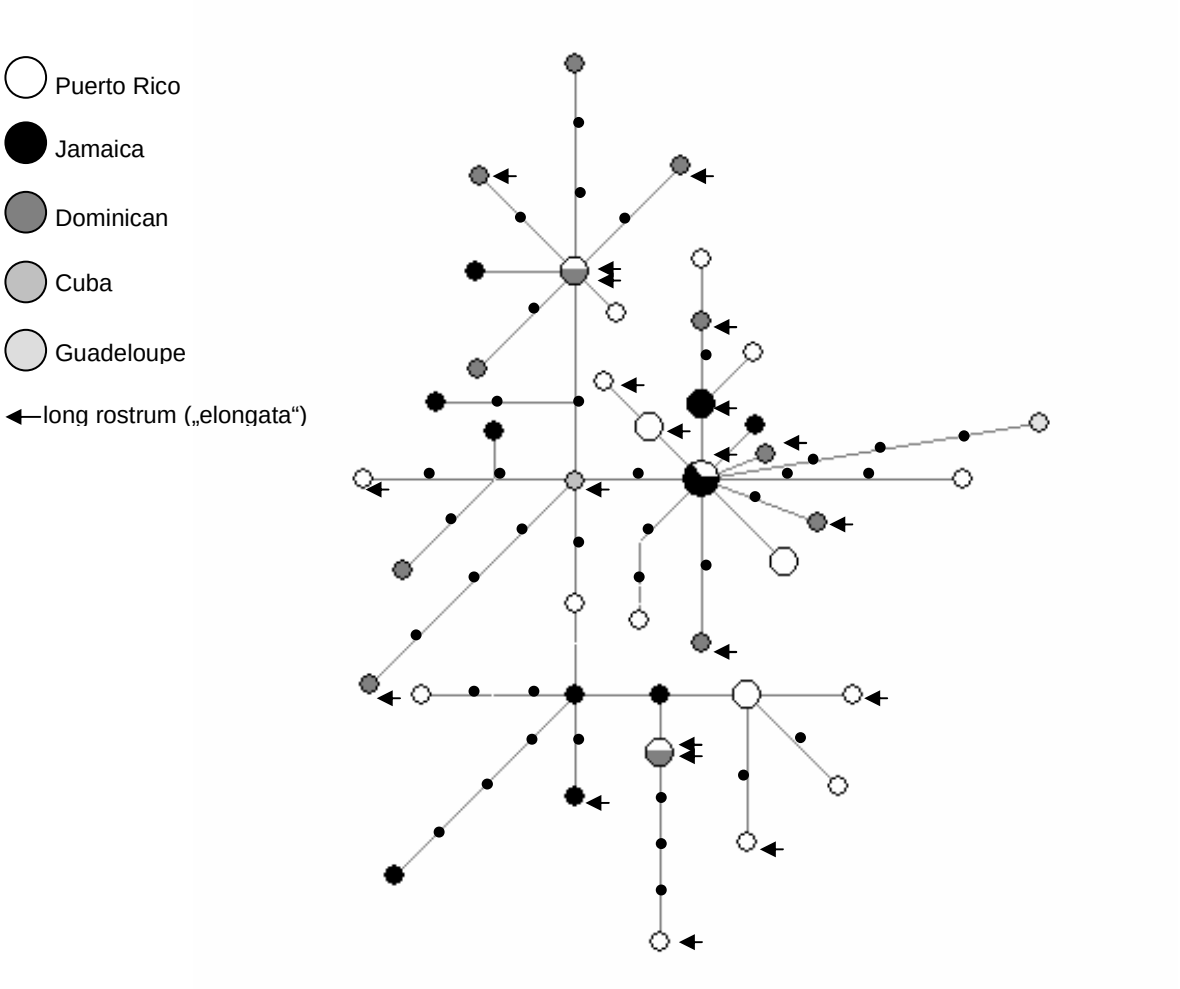


Figure 4. Minimum parsimonious spanning network of *X. elongata* (N=48) constructed with TCS corresponding to a 620-basepair fragment from the COI gene. Each line represents one substitution; circles indicate additional substitutions separating two haplotypes. The circle size corresponds to haplotype frequency (small: N=1; medium: N=2; large: N=3). The arrow indicates the morph with the long rostrum and the shading corresponds to geographic origin (black = Dominican Republic, white = Puerto Rico, gray = Jamaica).

Table 4. Summary of statistics of the COI gene separated by islands; N, number of sequences; M, number of haplotypes; *h*, haplotypic diversity; *n*, nucleotide diversity; S, number of segregation (polymorphic) sites; k, mean number of pairwise differences between individuals.

Sample	**N**	**M**	***h***	***n***	***S***	***K***
Puerto Rico	22	19	0.98	0.0082	31	5.08
Jamaica	12	10	0.96	0.0064	18	3.98
Dominican Republic	12	12	1	0.0094	26	5.83

The pairwise mismatch distributions within the whole population was analysed and the observed distribution of these data corresponds closely to the expected (Figure 5), which means that a sudden expansion occurred. The mismatch distributions were not significantly different from the sudden expansion model of Rogers & Harpending (1992). The statistics of the other neutrality tests, Tajima´s *D* and Fu´s F_s were significant (Table 3). These results and the small τ of 5.393 suggest a relatively recent and sudden expansion of the population.

Table 3. Summary of overall statitstics for the COI gene of *X. elongata*; *N*, number of sequences; *M*, number of haplotypes; *h*, haplotypic diversity; π nucleotide diversity; *S*, number of segregating (polymorphic) sites; *k*, mean pairwise differences between individuals; SD, standard deviation; values of Tajima´s *D*, Fu´s F_s and Harpending´s Raggedness index with probability values (*P*).

X. elongata	N	*M*	h *(SD)*	*π (SD)*	S	k *(SD)*
	48	40	0.99 (0.006)	0.00832 (0.00058)	51	5.16 (4.79)

X. elongata	θ_0, τ, θ_1	D (P), F_s (P), *Harp.*(P)
	0.003, 5.393, 235.034	- 1.9146 (<0.05), -25.4954 (<0.05), 0.0121 (0.51)

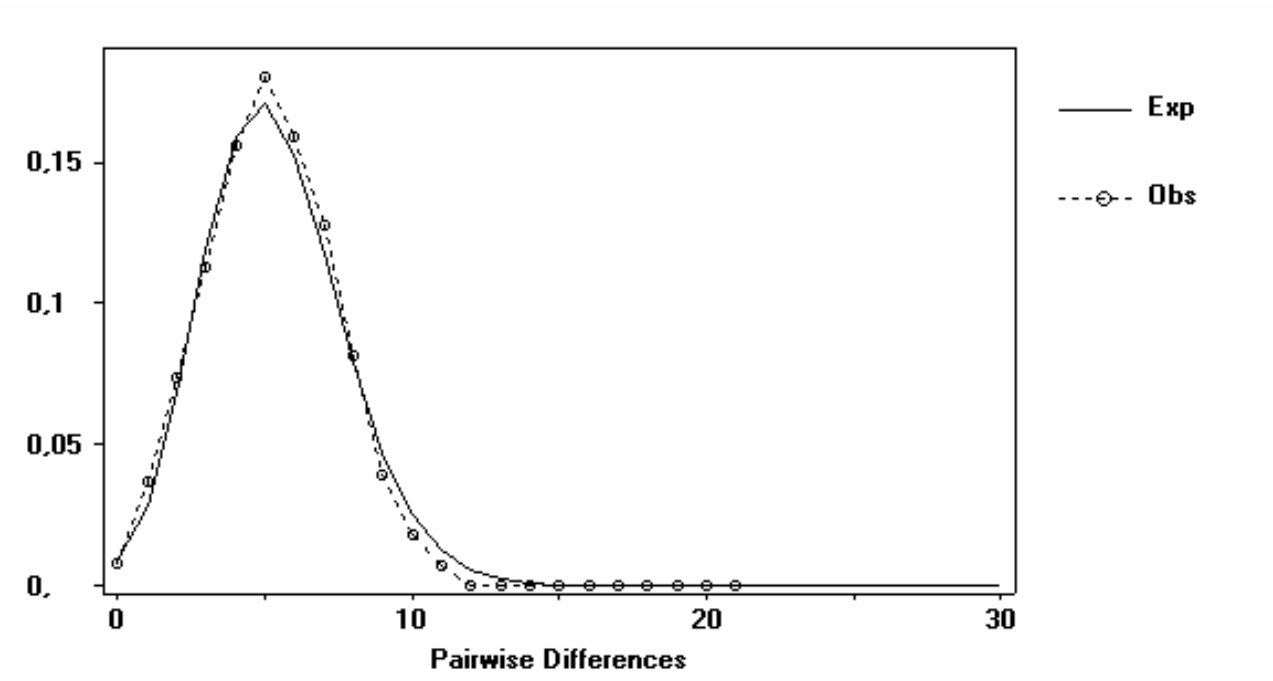


Figure 5. Mismatch distributions and τ values obtained from COI gene sequence data. The dotted line represents the observed pairwise differences. The solid curve is the expected distribution under the sudden expanison model.

Morphometrics

The 1-Factor-ANOVA and post-hoc Schefé test revealed significant morphometric differences between *"X. brevirostris", "X. gladiator"* and typical *X. elongata* (df 2; F=141.95; p<0.0001). The post-hoc Schefé test of the geographical comparison between *X. brevirostris* of Puerto Rico and Jamaica and between *X. elongata* of Puerto Rico, Jamaica and Dominican Republic revealed no significant differences. "*X. gladiator*" was here excluded due to the small sample size in Puerto Rico. The scatterplot has shown a weak correlation of the carapace lengths and the rostrum lengths (R=0.38) (Figure 6). The corresponding ANOVA revealed a significant correlation between the ratio of rostrum length to carapace length and overall carapace length (df 1; F=10.7; p<0.002). Some specimens of the Dominican Republic have an extremely long rostrum so that it seems that there is also variability in the length within the morph *X. elongata*.

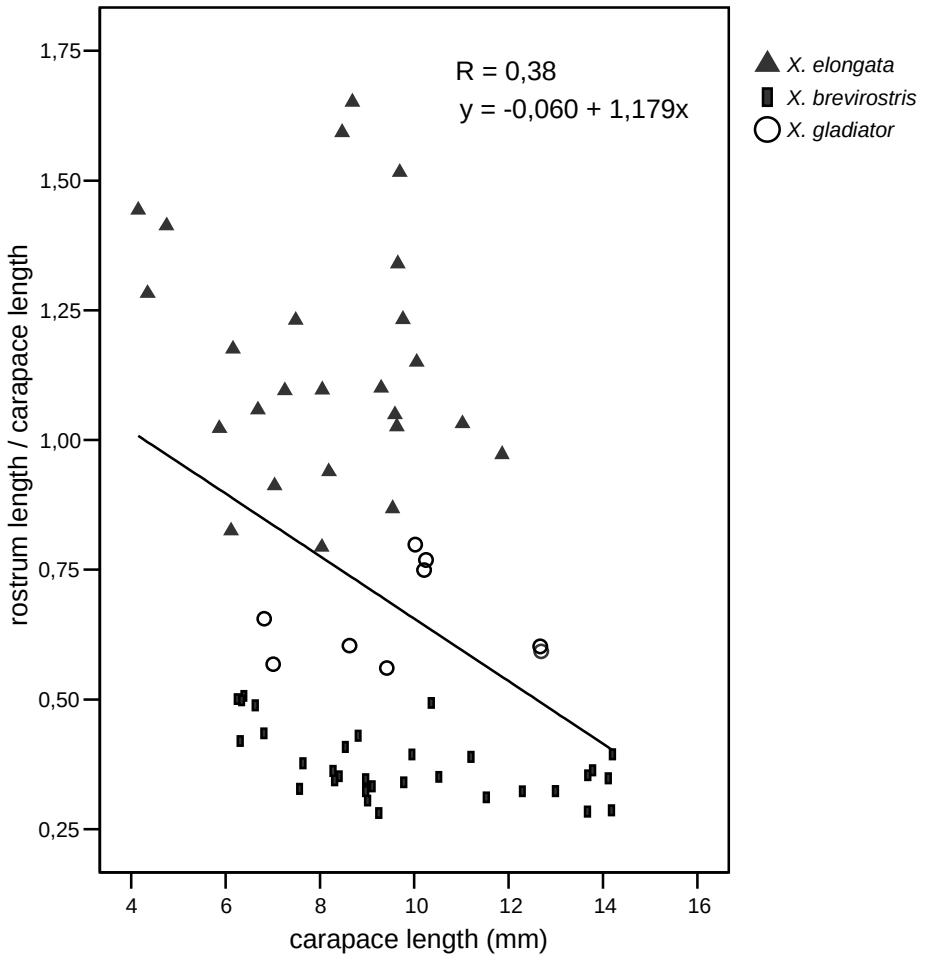


Figure 6. Scatter plot showing the relationship of rostrum length to carapace length of the three proposed species. A 1-Factor ANOVA revealed significant differences (p<0.0001) between the three morphs *X. elongata*, *X. gladiator* and *X. brevirostris*; shading corresponds to morphological variation (black = *X. elongata*, white = *X. brevirostris*, gray = *X. gladiator*).

DISCUSSION

Some freshwater decapods evolved an abbreviated or reduced larval development to complete their life cycle on land or in fresh-water, thereby leading to restricted gene-flow and differentiation processes like in potamid crabs from Taiwan (Shih et al. 2006) or in the freshwater genus *Macrobrachium* (see Murphy & Austin 2004). The diversification potential of a freshwater species with a marine planktonic development is profoundly influenced by the effectiveness of the larval dispersal. With larval retention in estuaries, the gene flow is most likely restricted to adjacent or nearby populations, like in *Rhithropanopeus harrisii* (personal communication). In contrast, a marine planktonic development would result in a widespread distribution via ocean currents with a high level of gene flow for example in freshwater fish on islands throughout the Indo-West Pacific (McDowall 2003). There is little published evidence for genetic differentiation in freshwater shrimps with an amphidromous life cycle, but recently, Cook et al. (2006) found for the Australian atyid shrimp, *Paratya australiensis*, nine highly divergent mtDNA lineages. In contrast, our study revealed no geographic differentiation within *X. elongata* based on population genetics of the COI gene. This suggests that there is no retention of the larvae in estuaries, that part of the larval development takes place in the open ocean, or that distribution through the ocean currents seems to occur regularly. Especially in the Caribbean Sea, hurricanes may enhance the dispersal of freshwater fauna (Calsebeek & Smith 2003). In conclusion, the amphidromy life history strategy may facilitate a continuing oceanic dispersal (McDowall 2007). But considering this postulated dispersal potential, it remains unclear, why there are no established populations of this species along the South and Central American mainland. Similarly, for the amphidromous snail *Clithon spinosus* from French Polynesia, Myers et al. (2000) could not detect genetic population structure between streams or islands with mtDNA data.

The steep display of the mismatch curve, the small θ_0 and τ, the high *h* and low π revealed in this study for *X. elongata* are consistent with a model for recent sudden expansion from a small initial population (Rogers & Harpending 1992; Avise 2000). The smaller the initial population, the steeper will be the leading face of the curve of the mismatch distribution (Rogers & Harpending 1992). In addition, a starlike haplotype network (Figure 4) is an expected signature for an abundant species that has expanded its range rather recently from a small or modest number of founders (Avise 2000). The recent expansion of the species could be an alternative explanation for the low genetic separation within the island and between the islands. McGlashan & Hughes (2001) revealed similar results in the freshwater fish

Hypseleotris compressa. Assuming that we are dealing with a young species, the separation processes may be still ongoing, and it is difficult to predict whether this would lead to future geographic and genetic differentiation.

We here demonstrate lack of genetic differentiation among the different morphological varieties of *X. elongata*. Morphometric results give evidence for at least three distinct morphological forms in *X. elongata* described as species by Pocock (1889). With the exception of the rostra, Hart (1961) was unable to find any significant differences between these varieties. Our results support that the rostrum tends to show high relative length in younger juveniles, and gradually decreases in proportion to the carapace length (Figure 6), but not as rapidly and consistently as proposed by Chace & Hobbs (1969). *X. elongata* occurs in many diverse types of habitat, but it is not abundant everywhere. The factors determining its absence or presence are not understood (Chace & Hobbs 1969). Usually, we found them in swift currents as well as in pools of streams at altitudes of 85 to 515m on Puerto Rico. On Jamaica, we observed juveniles at lower altitudes which may have been migrating upstream to their adult habitats. Females carrying eggs were mostly found in streams at higher altitudes. In the Dominican Republic, large adult specimens, including one female with eggs, were also found in a river mouth at about sea level. During our collecting, the different morphological varieties were only found once in the same locality: two specimens of *X. gladiator* and four of *X. elongata* in Río Fajardo in Puerto Rico. Otherwise, rostrum shape was consistent within localities. In some of the pools fishes occur. March et al. (2002) recognised a different behaviour (not specified) of *X. elongata* in presence of fishes. It appears possible, that the length of the rostrum is influenced by the presence of fish (similar to cyclomorphis in water fleas) or by other biotic or abiotic characteristics of the habitat, and thus reflect adaptive plasticity. The modern view of plasticity can be generalised with the statement that phenotypic plasticity evolves to maximise fitness in variable environments (the adaptive plasticity hypothesis) (Agrawal 2001). This, however, remains to be tested by trying to induce change of rostral length as a consequence of modified habitat or presence/absence of predators.

Rosen (1976) has championed the view that the present distribution of Caribbean biota is most simply explained by the movement of the islands between North and South America, rather than by dispersal from the continents to the islands in their present geographical arrangement (vicariance model hypothesis). It appears possible that the genus *Xiphocaris* is the remnant of an old stock belonging to the proto Antillean archipelago and thus to the former Pacific seafloor. This theory has also been provided for land snails (Bishop 1979;

Goodfriend 1989). But dispersal from the American mainland could be an alternative explanation for the history of the genus *Xiphocaris*. This study does not provide conclusive evidence for any of the two models due to the lack of knowledge concerning the founder population. But our results of a recent expansion favour the hypothesis that *Xiphocaris* reached the West Indies via dispersal. The dispersal model is also suggested for other freshwater shrimps like *Atya* (see Page et al. in press) and *Typhlatya* (see Hunter et al. in press) based on molecular data. In future studies, variable nuclear markers should be applied to complement our results and to confirm or refute the lack of structured genetic variation.

ACKNOWLEDGEMENTS

Our special thanks go to Owen McMillan, Jürgen Heinze and the Deutsche Forschungsgemeinschaft (Schu 1460/3-2) for supporting this project. We thank Andreas Karge, Tobias Santl, Luise Heine, Nicole Rivera, Richard Landstorfer and Martin Huber for helping with the collection of *Xiphocaris* or for helpful discussions.

GENERAL DISCUSSION

Phylogeography of marine "high-dispersal species"

Population structure, as estimated by neutral molecular markers, is determined by the interactions between gene flow and genetic drift (Wright 1943; Kimura & Weiss 1964; Slatkin 1985). In recent years, more and more studies revealed that habitat, dispersal capabilities, ocean conditions and biogeographic history are limitations to the dispersal of marine species (Peijnenburg et al. 2004; Triantafyllidis et al. 2005; Casu & Curini-Galletti 2004; Zardoya et al. 2004; Pérez-Losada et al. 2007). These limits could create absolute barriers or restriction to gene flow and could support speciation events in the marine fauna (Palumbi 1994). This thesis was designed to clarify mechanisms of generation and maintenance of genetic diversity in order to detect geographic patterns and to identify endemic or cryptic species in marine and freshwater decapods.

Dispersal capabilities

It was shown that the genetic structure of marine animal species with planktonic larvae is often correlated with different levels of dispersal in their larval stages (Kyle & Boulding 2000). Species that live as larvae in the plankton for a short period show a more pronounced population structuring (Kirkendale & Meyer 2004 for *Patelloida profunda*; Todd et al. 1998 for *Adalaria proxima*) than species with larvae that are in the plankton for a longer period (Uthicke & Benzie 2003 for *Holothuria nobilis*; Todd et al. 1998 for *Goniodoris nodosa*). We revealed a less pronounced genetic differentiation for the crab genus *Xantho* with four zoeal stages than for the prawn *P. elegans* with nine zoeal stages. The lack of structured genetic variation in *X. poressa* suggests a marine coastal megapopulation, which reproduces panmictically. The analyses of frequencies of haplotype distributions within *X. hydrophilus* suggest low but significant differentiation with three distinct geographic forms in *X. hydrophilus*, which can be interpreted as one Atlantic form, one central Mediterranean form and a transitional western Mediterranean form. Though *P. elegans* has potentially the higher dispersal capability, we revealed a more pronounced genetic differentiation within this species (publication three and four). Three main groups of haplotypes can be separated, one from the Atlantic Ocean and two from the Mediterranean Sea. This becomes even more surprising when we compare the results with the amphidromous shrimp *X. elongata*. For the latter, we expected a higher genetic differentiation than for the marine species. Although we

revealed high genetic variability within *X. elongata,* we could not detect a population subdivision across the islands or between the different river systems, presumably because the marine larvae mediate high inter-island gene flow. It has been found in shrimp studies from the Pacific that the amphidromous life cycle facilitates dispersal between isolated freshwater systems through oceanic currents counteracting otherwise restricted gene flow between the systems (Page et al. 2005; Fiévet 1998). Therefore, amphidromous taxa tend to display patterns more similar to those of marine forms than to exclusively freshwater species. Some points should thus be emphasized about the phylogeographic structure of the examined species. First, a short larval development does not necessarily imply a more pronounced population structure than a longer development. Second, historical and/or biological factors may influence interpretations of phylogeographic population structure more distinctly than dispersal capability.

Dispersal and vicariance

Dispersal and vicariant events are often discussed as opposing models for spatial isolation and consequently as a barrier to gene flow. The Greater Antilles have been prime example for this debate. For the majority of the Antillean freshwater fauna, neither the time of arrival of their ancestors to the islands nor the routes taken by them can be postulated with any degree of certainty (Chace & Hobbs 1969; Avise 2000). The colonisation of the Greater Antilles could have taken place either through massive long or short term overwater dispersal (Hedges et al. 1992). According to the vicariance scenario many extant species are descendants of early colonizers who remained after the island separated from continental landmasses in the late Cretaceous, about 80 Mya (Rosen 1976). Publication five does not provide conclusive evidence for any of the two models, due to the lack of knowledge concerning the founder population. However the revealed recent expansion favours the hypothesis that *Xiphocaris* reached the West Indies via dispersal. Dispersal events are suggested to be younger, and only older divergences could be consistent with vicariant theories for the Caribbean fauna (Humphries & Ebach 2004). Colonisation via dispersal is also suggested to be the dominant process for the atyid fauna of the Caribbean Islands, because there are only very shallow genetic differences within each atyid species, implying that these divergences occurred relatively recently in geological terms (Page et al. submitted). Other important geographical/ecological factors as islands age, like ocean currents, number of freshwater habitats, freshwater flow rate, as well as many others can influence present day distribution and degree of isolation (Bass 2003; Darlington 1938; Fryer 1977, Covich 2006).

Historical geographical factors seem to play a major role within the Mediterranean Sea. Around one third of the Mediterranean Brachyura seems to have speciated in the Mediterranean Sea, as their present geographic ranges are exclusively or almost exclusively restricted to the Mediterranean (Almaça 1985). Most of the autochthonous Mediterranean species seem to be derived from East-Atlantic species in post-Messinian times. The littoral and sublittoral biotopes have provided the most suitable ecological niches for the speciation of the Mediterranean autochthonous crab fauna (Almaça 1985). *X. poressa* seems to be originally Mediterranean but extends to the Atlantic Ocean (Forest 1972 in Almaça 1985). New results (not shown) have revealed restricted gene flow between West Mediterranean and central and East Mediterranean populations of *X. poressa* (Φ_{ST} – value: 0.2; $p < 0.0001$). If the species is originally Mediterranean and has extended its distribution later, then it is more likely to result in genetic differentiation within the Mediterranean Sea than between the Mediterranean and the Atlantic Ocean. For different marine species, genetically discrete populations have been revealed in the eastern basin of the Mediterranean Sea: for the Mediterranean poor cod *Trisopterus minutus capelanus* (see Mattiangeli et al. 2003); for the lagoon cockle *Cerastoderma glaucum* (see Nikula & Väinölä 2003); for the bluefin tuna *Thunnus thynnus thynnus* (see Carlsson et al. 2004). For *X. hydrophilus* we suggest a recent genetic differentiation, with a low but significant genetic differentiation between the Atlantic and Mediterranean populations (Φ_{ST} – value: 0.07; $p < 0.0001$). The geographic separation may have been caused or maintained by the Strait of Gibraltar during sea-level regressions in the Plio-Pleistocene. For the western Mediterranean Sea, we revealed a transitional form, which is also reported in morphological comparisons (Almaça 1985; d'Udekem d'Acoz 1999). These results suggest ongoing gene flow between the western Mediterranean and the Atlantic population which resulted in an isolation-by-distance (IBD) pattern. A comparable historical demography and biogeographic scenario is suggested for the bonito *Sarda sarda*: allopatric isolation during the Pleistocene, secondary contact with the Atlantic population, which resulted in an IBD pattern (Viñas et al. 2004). Since we could not discover constant genetic or morphometric differences that would allow a subspecies status of *X. h. granulicarpus*, it should be treated as a variety and not as endemic subspecies. For the two species *X. hydrophilus* and *X. poressa* it is possible that a habitat and geographic exclusion has been established. While *X. hydrophilus* is the more dominant species in the Atlantic, in the Mediterranean Sea it is *X. poressa*. In the Mediterranean Sea *X. hydrophilus* is found in deeper regions and *X. poressa* in the littoral.

In publication three, we reveal a surprisingly high population structure for *P. elegans*. Three types of haplotype groups can be defined, the Atlantic Type (Type I), the New Mediterranean Type (Type II) and the Old Mediterranean Type (Type III). The Old Mediterranean Type might have its origin during the Messinan Crisis, when the nearly desiccated Mediterranean Sea was completely isolated from the Atlantic. Assuming that crabs survived in the Mediterranean during that time, despite possible hypersaline conditions, allopatric speciation from the Atlantic forms would have been the logical consequence (Schubart et al. 2001). Based on the marked genetic differences which may be translated into separation times of 6.85 ± 0.85 million years according to the molecular clock by Knowlton and Weigt (1998) (1.4 % sequence divergence between pairs of lineages per Myr), we suggest that Type III was isolated from Atlantic populations during the Messinian Crisis (5.5-6 mya, see Hsü 1983). With the flooding of the Mediterranean Basin with Atlantic waters after the Messinian Crisis, Atlantic forms were re-introduced into the Mediterranean Sea without reproducing with the local Type III ancestors and progressively separated into Atlantic (Type I) and New Mediterranean (Type II) populations due to gene flow barriers like Pliocene/Pleistocene sea level regressions or oceanic currents. These results provide evidence that the Mediterranean fauna is strongly influenced by its geological evolution and that the recent populations may be affected by oceanographic patterns.

Oceanography

For a long time, the relatively shallow Strait of Gibraltar, representing the geographic boundary between the western Mediterranean and the Atlantic Ocean, was assumed to act as a potential barrier of gene flow. However, more recently it has been suggested that the Almería-Oran front between Cabo de Gata (Almería Province in south-eastern Spain) and Oran in Algeria represents an alternative hydrographic boundary between Atlantic and Mediterranean surface waters. Circular jetties dominate between this front and the Strait of Gibraltar (Tintoré et al. 1988). This barrier to gene flow between Atlantic and Mediterranean populations has been suggested for different marine species, for example the cuttlefish *Sepia officinalis* (see Pérez-Losada et al. 2002, 2007), the pelagic euphausiid crustacean *Meganyctiphanes norvegica* (see Zane et al. 2000), the Atlantic *Pecten maximus* versus the Mediterranean *Pecten jacobaeus* scallops (see Saavedra & Viñas 2005), and the European hake *Merluccius merluccius* (Cimmaruta et al. 2005). In publication three and four we have shown that the Almería-Oran-front is acting as the barrier to geneflow and disperal between

the exclusively Atlantic Type I and the Mediterranean Type haplotype groups of *P. elegans*. Fine scale geographic analyses revealed no clear break between the types at the front. In the area of Cabo de Gata there is a zone of overlap: specimens of the populations from Almería to Toros belong to the Atlantic Type I as well as to Mediterranean Type II and specimens of the populations from Las Salinicas to Macenas to all three types. Current flows could vary spatially and temporally throughout the year. Also the Almería-Oran front is variable in its shape and position which could influence the distribution of zooplankton at the front (Fielding et al. 2001). Due to this inconsistency, it could be impossible to determine the exact break between the Atlantic Type I and the Mediterranean haplotype groups in a range below 50 kilometres. We detected a gradual decline of the Atlantic Type along the Spanish coast while the Mediterranean Type II increased in an isolation-by-distance pattern along the coast: from Granada to Girona. Although restricted gene flow is possible the AOF is confirmed as a strong barrier to gene flow and therefore plays an important role in maintaining allopatric separation between the Mediterranean Sea and the Atlantic Ocean. We also revealed a clear isolation-by-distance pattern within the Atlantic Type I of *P. elegans*: the Φ_{ST}-values are increasing in a northern direction from Portugal to Norway. Further the results are allowing to separate the Canary Island population. The significant differentiation of the Northern Atlantic and the North-East Atlantic populations could be assigned to the effect of the English Channel. The English Channel apparently restricted gene flow between Western Europe and northern Europe as in the Atlantic populations of *Carcinus maenas* (see Roman & Palumbi 2004) and for the common goby *Pomatoschistus microps* around the British Isles (Gysels et al. 2004).

In addition, very recent results revealed (not shown) that there could be a barrier to gene flow between the central-western and eastern Mediterranean populations of *X. poressa*. Therefore, oceanographic patterns are a generator in maintaining genetic differentiation. These are consequences over large geographical scales. But ecological traits could influence the population structure on a smaller scale.

Ecological traits

During our field observations, we could find size differences in two populations of *X. poressa* in a distance of approximately 56 kilometres coastline. In an anthropogenically disturbed habitat (jetty and anoxic sediments) crabs in all sizes were found and in a relatively undisturbed natural cove only small crabs occurred. In publication two we could not relate the differences in size of *X. poressa* to a genetic pattern. We therefore propose that size

variation of *X. poressa* may reflect phenotypic plasticity, which may be related to a different spectrum of predators, temperature, salinity regimes and unusual tidal influence at the collecting points. The high variability of colour patterns of *X. poressa* was also to be concluded phenotypic. We observed that *X. poressa* lives among small colourful pebbles and larger rocks. They reduce the probability of a predator spotting them by matching their underground with variability in colour, also using white transverse stripes on the legs and frontal carapace to disrupt their outline. We therefore propose that the variable coloration is the outcome of passive defence (camouflage).

We tested if morphometric differences in size could be attributed to a geographic pattern. In publication one, we revealed geographic differences for *X. hydrophilus* and less for *X. poressa*. In publication two we could show that *X. poressa* is affected by allometric growth in the carapace shape. Therefore, the dataset was split in three size categories and the size classes were analysed separately. The same was done for *X. hydrophilus* (data not shown). The analyses revealed no remaining geographic separation and for both species size should be the dominant explanation for morphometric variance and not geographic separation.

Also for *X. elongata* a pattern of allometric growth is suggested: the rostrum gradually decreases in proportion as the body lengthens and broadens (Chace & Hobbs 1969). In publication five, the morphological varieties *"X. brevirostris"*, *"X. gladiator"* and *X. elongata* were compared with genetic and morphometric methods in order to investigate whether the species could be split in separable distinct morphs or whether there is a gradient of rostral lengths. The morphometric analyses showed only a weak correlation of the carapace lengths and the rostrum lengths, and therefore rostrum length is partly depending on carapace size. While the morphometric analysis confirmed significant differences between three distinct morphological forms, molecular results gave no evidence for a mitochondrial differentiation of these phenotypes. Thus, adaptive plasticity could be an explanation for the variability in the rostrum length of *X. elongata*. Consequently, March et al. (2002) recognised a different behaviour of *X. elongata* in presence of fishes.

Examinations of my own methodology and of other studies (Garrido-Ramos et al. 1997; Rincón 2000) lead me to the conclusion that the problem of allometric growth received less careful attention than it deserved. Morphometric data have first to be tested for this phenomenon before carrying out with intra- or interspecific analyses. Pairwise comparisons of the genetic data revealed no correlation between the degree of morphological and genetic similarity of crabs and shrimps. Therefore, the patterns of morphological and colour variability are largely determined by environmental conditions and in part by allometric

growth and heterogeneous distribution of size classes.

The importance of phylogeographic studies

The discovery of endemic and cryptic species is getting more and more important for evolutionary theory, biogeography and conservation planning (Bickford et al. 2007). For detecting and differentiating such morphologically similar species, DNA sequencing and population genetics have given biologists useful methods (Pfenninger & Schwenk 2007). In crustaceans a lot of cryptic species could be detected e.g. in the snapping shrimp genera *Alpheus* using the 16S and COI mitochondrial genes (Mathews 2006), in the seabob shrimp species *Xiphopenaeus kroyeri* and *Xiphopenaeus riveti* based on COI (Gusmao 2006), among the mysid crab *Mesopodopsis slabberi* carried out with 16S and COI mitochondrial genes (Remerie et al. 2006). With the same markers we detected a high genetic differentiation within *P. elegans* which suggest at least a cryptic species for the haplotype group III with no morphological differences between the different types. At the same time, we could confirm that *P. elegans* is an invasive species in the Baltic Sea, where it is already replacing the native *Palaemon adspersus* (see Grabowski 2006). With both results we have demonstrated the utility of phylogeographic studies: on one side to discover the biodiversity of the marine fauna, which is important to protect areas where cryptic or endemic species occur. On the other hand it is useful to uncover invasive species which could become a problem when they endanger the native fauna.

Phylogeographic studies using mtDNA

Approximately 70 percent of phylogeographic studies are based on analyses of mtDNA either primarily or exclusively (Avise 2000). The phylogenetically favourable properties of maternal transmission, extensive intraspecific variation, and absence of intermolecular genetic recombination make the marker very useful for phylogeographic investigations.

One particular problem is the possibility of introgression of mtDNA from one species into another. Introgression can result in significantly different gene genealogies for mtDNA than for most other genes in the species (Ballard & Whitlock 2004). Furthermore, introgressive hybridization can be so extensive that populations merge into one panmictic gene pool (Avise 2004). In publication one, the molecular phylogeny and population genetics indicate that *X. sexdentatus* is very closely related to *X. hydrophilus.* This could be the result either of introgression or of a recent separation event followed by incomplete lineage sorting, with or without subsequent hybridization. Introgression is particularly important for closely related

sympatric taxa – successful hybridization is more likely with closely related taxa and is only possible with some sympatry (Ballard & Whitlock 2004). *X. sexdentatus* and *X. hydrophilus* occur only partly in sympatry which favours hybridization. The suggested types of *P. elegans* - Atlantic, New and Old Mediterranean Type - occur in sympatry, for which the following phylogenetic pattern could be expected: allopatric differentiation followed by secondary contact and hybridization. Natural hybridization may occur sporadically between broadly sympatric species (Avise 2004). Until now we have no results whether hybridization occurs within *Palaemon*. Therefore, hybridization has to be tested for *Palaemon* and *Xantho* with a nuclear marker and breeding experiments.

There was no evidence for pseudogenes in my data: both genes are always consistent in their results and revealed equal geographical patterns; specific primer combinations were used and the sequences are without double peaks, indels or inserts. Thus, mtDNA is still a useful approach in phylogeographic studies as a quick and effective step to detect geographical patterns, cryptic species, oceanographic patterns or invasive species.

Conclusion

The relationship between dispersal capability and population structure plays a minor role for the studied species than biogeographic history and oceanography. A more distinct geographic structure for *Palaemon elegans* with a longer larval development than *Xantho* was detected. The amphidromous shrimp *Xiphocaris* has no geographic pattern which means that its life history is similar to marine species and increases the dispersal capability. This study confirms that the history of the Mediterranean Sea has played a role in speciation processes. A cryptic species was detected within *Palaemon elegans* and it is possible that during the Messinian crisis allopatric speciation has taken place. The Strait of Gibraltar in the past and the Almería-Oran-Front at the present time is an important phylogeographic break between the Mediterranean basin and the Atlantic Ocean (e. g. within *Xantho* and *P. elegans*). It was confirmed that the AOF is a barrier to gene flow between populations of the Atlantic/Alboran Gyre and the western Mediterranean Sea, where restricted gene flow is possible nevertheless (within *P. elegans*). For the studied species ecological traits support no speciation processes, instead, they could be interpreted as phenotypic plasticity.

SUMMARY

In the last decades a number of phylogeographic studies arose to determine the genetic differentiation between Atlantic and Mediterranean populations of a species or sister-species. The Strait of Gibraltar is an important phylogeographic barrier. Sea-level regressions prevented gene flow several times between the two basins during the Messinian crisis and the Plio-/Pleistocene. These events could have influenced allopatric separation and speciation. In addition ocean currents and the phenomenon isolation-by-distance are important factors in maintaining gene flow barriers. Especially the Almería-Oran-Front restricted the gene flow between Atlantic and western Mediterranean populations.

The aim of this study was to determine the influence of biogeography, oceanography and ecology on the population structure of different crustacean species with emphasis on their larval development. It is predicted that the duration of larval development influences the dispersal capability and therefore the level of genetic differentiation. In addition, the results should provide answers if there are cryptic species. The two atlanto-mediterranean crab species *Xantho hydrophilus* (Herbst, 1790) and *Xantho poressa* (Olivi, 1792) and the European prawn *Palaemon elegans* (Rathke, 1837) were included as marine species. *Xantho* has a shorter larval development with four zoeal stages than *Palaemon* with nine larval stages. Therefore, a higher gene flow within *Palaemon* was expected. We used also the amphidromous species *Xiphocaris elongata* (Guérin-Méneville, 1856) which occurs in freshwater systems of the Caribbean islands. The life cycle of amphidromous *X. elongata* is intermediate between freshwater and marine species and thus, high genetic differentiation was predicted. A comparative population genetics analyses was conducted to reveal differences in the population structure due to the distinct larval development and the amphidromous life-cycle.

In the case of *X. hydrophilus,* results show a restricted gene flow between the populations of the Atlantic and the Mediterranean Sea. However, the taxonomic status of the Mediterranean subspecies *X. h. granulicarpus* is not valid as long as no single mutation step was detected to distinguish constantly between the two subspecies *X. hydrophilus* and *X. h. granulicarpus*. In addition, morphological transitional forms of *X. hydrophilus* are found within the Western Mediterranean Sea. *X. poressa* is suggested to reproduce panmictically because no differences are found between the populations of the Mediterranean Sea and the Atlantic Ocean and a mark recapture experiment showed that the species reaches high population densities. This species is well adapted to its habitat due to its variability in colouration and size which is the result of passive defence (camouflage). A pattern of allometric growth in

the carapace shape was observed. Phylogenetic analyses revealed that *X. sexdentatus* is very closely related to *X. hydrophilus*. This could be either due to hybridization or introgression.

Despite the fact that *Palaemon elegans* has nine larval stages, there is surprisingly high genetic differentiation. Normally such strong differences are only found between species and thus a cryptic species within the *P. elegans* complex is suggested. Three types of haplotype groups are found: Type I from the Atlantic and the Alboran Sea, Type II only within the Mediterranean Sea and Type III within the Mediterranean, Black, Caspian, Baltic and East Sea. Type III could be a relict of the Messinian crisis due to its high genetic differentiation, while Type II has recolonized the Mediterranean Sea after the crisis and ongoing separation mechanisms have established restricted gene flow between Type I and II. The barrier to gene flow between the Atlantic Type and the two Mediterranean Types is the Almería-Oran-Front. The Almería-Oran-Front is an important phylogeographic break, where restricted gene flow is possible. An isolation-by-distance pattern could be detected within the Atlantic Ocean. Furthermore, there is significant genetic differentiation between the Northern Atlantic and the North-East Atlantic due to the English Channel. These results provide evidence that dispersal play a minor role in determining the genetic structure of a species, compared to the biogeographic history and physical factors.

This becomes even more obvious by comparison with the shrimp *Xiphocaris elongata,* for which a higher genetic differentiation due to the isolated freshwater systems and the amphidromous life cycle was expected. Although there is a high genetic differentiation, no geographic pattern emerges. The amphidromous life cycle enhance the dispersal capability and gene flow. It displays rather patterns of a marine species than of an exclusively freshwater species. The species is characterized by its variability in the rostrum length, but the results give no evidence for a genetic differentiation of the phenotypes. Thus, adaptive plasticity due to predators and in part allometric growth could be an explanation for the variability.

ZUSAMMENFASSUNG

Die genetische Differenzierung zwischen atlantischen und mediterranen Vertretern einer Art oder von Schwesterarten ist Schwerpunkt vieler phylogeographischer Arbeiten. Die Meerenge von Gibraltar stellt dabei eine wichtige Barriere dar, da sie den freien Genfluss zwischen Atlantik und Mittelmeer während der Messinischen Salinitätskrise und durch Meeresspiegelschwankungen des Plio-/Pleistozäns mehrfach unterbunden hat. Weiterhin zeigte sich, dass sowohl konstante Meeresströmungen als auch Phänomene wie „isolation-by-distance“ Genflussbarrieren darstellen können. Besonders die so genannte Almería-Oran-Front unterbindet den freien Genaustausch zwischen atlantischen und mediterranen Populationen. Diese historischen und physikalischen Faktoren könnten zu allopatrischen Separationen und Speziationen beigetragen haben.

In dieser Arbeit wurde die Populationsstruktur verschiedener Crustaceenarten untersucht, um den Einfluss der Biogeographie, Ozeanographie und Ökologie auf deren genetische Differenzierung zu bestimmen und kryptische bzw. endemische Arten zu erfassen. Dabei wurde auch die Larvalentwicklung der jeweiligen Arten berücksichtigt, da diese die Verbreitungsmöglichkeit und somit den Grad an genetischer Differenzierung beeinflussen kann. Die beiden atlanto-mediterranen Krabben *Xantho hydrophilus* (Herbst, 1790) und *Xantho poressa* (Olivi, 1792) unterscheiden sich von der europäischen Felsengarnele *Palaemon elegans* (Rathke, 1837) in der Dauer ihrer Larvalentwicklung. Während *Xantho* vier Zoeastadien durchläuft, weißt *Palaemon elegans* neun Stadien auf, so dass bei letzterer ein höherer Genfluss zu erwarten ist als bei *Xantho*. Ferner ist der amphidrome Lebenszyklus der Garnele *Xiphocaris elongata* (Guérin-Méneville, 1856), deren Verbreitungsgebiet sich auf die Süßwassersysteme der Karibik erstreckt, eine Zwischenform zwischen im Meer und im Süßwasser lebenden Tieren. Vergleichende Studien sollten zeigen, ob die unterschiedlich langen Larvalphasen und der amphidrome Lebenszyklus einen Einfluss auf die genetische Differenzierung der verschiedenen Arten haben.

Im Fall von *X. hydrophilus* konnte ein eingeschränkter Genfluss auf Grund von „isolation-by-distance“ zwischen Populationen des Atlantiks und des Mittelmeeres festgestellt werden. Die beschriebene Unterart des Mittelmeeres *X. h. granulicarpus* konnte nicht bestätigt werden, da es sowohl morphologische, wie auch genetische Indizien für Übergangsformen im westlichen Mittelmeer gibt und keine genetisch konstanten Unterschiede vorliegen. Bei *X. poressa* handelt es sich wahrscheinlich um eine panmiktische Einheit, da bisher keine genetisch signifikanten Unterschiede zwischen Atlantik und Mittelmeer festgestellt wurden.

Ein Fang-Wiederfang-Experiment zeigte, dass die Krabbe eine hohe Populationsdichte aufweist. Die Art hat sich durch phänotypische Variabilität in Farbe und Größe an ihr Habitat angepasst und ist dadurch gut getarnt und vor Räubern geschützt (camouflage). Außerdem konnte allometrisches Wachstum festgestellt werden. Durch die phylogenetische Analyse der 16S rRNA und des COI Gens stellte sich heraus, dass *X. sexdentatus* mit der Art *X. hydrophilus* sehr nah verwandt ist. Dies lässt sich entweder durch mögliche Hybridisierung oder Introgression erklären.

Obwohl *P. elegans* eine längere Larvalentwicklung als *Xantho* durchläuft, zeigten die genetischen Studien, dass sich die Tiere aus dem Mittelmeer von den Tieren aus dem Altantik in ihrer mtDNA auf einem so hohem Differenzierungsniveau unterscheiden, wie es sonst nur zwischen Arten zu finden ist. Deshalb wird hier eine kryptische Art angenommen. Es konnten drei Typen von Haplotypgruppen gefunden werden: Typ I beschränkt sich auf den Atlantik und das Alboranmeer, Typ II ist nur innerhalb des Mittelmeeres zu finden und Typ III innerhalb des Mittelmeeres, aber auch in der Ostsee, im Schwarzen, Kaspischen und Baltischen Meer. Typ III unterscheidet sich von Typ I und II so weit, dass es sich hier um ein Relikt der Messinischen Salinitätskrise handeln könnte, während Typ II eine Wiederbesiedlung der Atlantikform nach der Krise darstellt und sich auf Grund anderer Separationsmechanismen von Typ I zu differenzieren beginnt. Die Almería-Oran-Front zeigt sich hier als Genflussbarriere zwischen dem Atlantiktyp und den beiden Mittelmeertypen und ist eine wichtige phylogeographische Barriere für marine Arten, die eine geringe Menge an Genfluss zulässt. Innerhalb des Atlantiks konnte „isolation-by-distance“ gezeigt werden. Außerdem steht die genetische Differenzierung zwischen Nordatlantik und Nordostatlantik mit dem Englischen Kanal in Verbindung. Mit diesen Ergebnissen konnte gezeigt werden, dass die Biogeographie und Ozeanographie einen größeren Einfluss auf die genetische Differenzierung der untersuchten Arten haben, als die Dauer der Larvalentwicklung.

Dies wird besonders deutlich, wenn man zusätzlich die Ergebnisse der Garnele *Xiphocaris elongata* gegenüberstellt. Auf Grund der voneinander abgegrenzten Fluss-Systeme und des amphidromen Lebenszyklus wurde ein stark eingeschränkter Genfluss erwartet. Es ergab sich zwar eine hohe genetische Differenzierung, jedoch zeigen Genetik und Geographie keine zusammenhängende Strukturierung. Der amphidrome Lebenszyklus scheint daher der Verbreitung zu dienen und trägt zu freiem Genfluss bei, was jedoch eher einer marinen Art entspricht. Zusätzlich weist die Garnele eine Variabilität innerhalb ihrer Rostrumlänge auf, die zum Teil durch allometrisches Wachstum, aber auch durch Räuberdruck (phenotypische Plastizität) beeinflusst werden könnte.

ACKNOWLEDGEMENTS

I have heard that these last pages of a PhD thesis are the most widely read pages of the entire publication because it is here where you think that you will find out whether you have meant something in the life of the PhD candidate. So I will try to do my very best.

First of all I am grateful to Jürgen Heinze for giving me the possibility to do this thesis, the working space and facilities at the University of Regensburg, although I am working on ten legged and not on six legged animals.

Special thanks go to my supervisor Christoph Schubart who was continuously helping me with my smaller and bigger “Besenstriche” (this goes back to a wisdom of the street-cleaner Beppo, from Michael Endes book “Momo”); for his ideas and his open ear for questions, problems and discussions. He gave me the opportunity to expand my horizon by travelling and to encourage my scientific self-confidence by visiting conferences.

To José Cuesta I would like to say “muchas gracias” for the two visits in his lab in Cádiz where I could learn more about larval biology, AFLPs and “el mundo de español”. He and the students there made me feel very comfy during my stays. I am also grateful to Klaus Anger who gave me the opportunity to work in his lab in Helgoland to get more insights in the larval world and for fruitful evening discussions during my stay on a really small but impressive island. Thank goes to Sonya Uzunova for her logistic support in Bulgaria.

Further I want to thank Tobias Santl for his support in many ways; him and Luise Heine, but also the turtles and cockroaches as great officemates. For good times in the field and supporting me in my field work I want to mention my Caribbean fellows: Tobias Santl and Luise Heine; and my Mediterranean fellows: Reiner Rubner, Carsten Müller, Lapo Raginonieri, Nicole Rivera and Petra Zillner. Thanks to our foreign crab-colleagues and visitors Joana Garcia, Graciela Ostelo and especially to Lapo Ragionieri who brought the southern flair to our lab; to Sebastian Klaus and Nicole Rivera for their contractible laugh; to Ruth Jesse, Martin Huber, Richard Landstorfer, Peter Koller and all before mentioned crab-people and our crab supervisor Christoph Schubart for the cheerful working atmosphere.

Many thanks go to Andreas Trindl, Tina Wanke and Maria Schiwek for always helping me in the lab. I would also like to thank you all others for the nice, funny and sometimes crowded team play in the lab.

Appreciation is extended to all those persons who spent their time for helpful corrections of this thesis or helping me with figures: Wolfgang Göttler, Sebastian Klaus, Anne Hartmann, Alexandra Schrempf and Christoph Schubart; Graciela Ostelo for her very useful discussions about population genetics. I have separately mentioned people who supported different parts of this work at the end of the respective publication. I thank all of them.

I would like also to thank friends of former years in Regensburg: Andrea, Vroni, Ruth, Markus, Hansi, Estelle and Katja; and people of former days and nowadays: Anne, Tina, Alex and Anna. We had a lot of good, funny and chatty times together and I hope that we will be able to stay in touch despite the distances between us.

Finally special thanks go to Wolfgang for his patience in listening to crab stories and the thesis about them and his decision to do his PhD in Regensburg. I am also grateful to all my friends and my family in particular to my parents who supported me to go on. All of them I want to say thank you for being there when I needed them.

Last but not least I want to thank my crabs and shrimps, this PhD thesis would not have been written without them, and my laptop MEDION that it stayed the course till the end.

REFERENCES

Agrawal AA (2001) Phenotypic Plasticity in the Interaction and Evolution of Species. *Science* **294**: 321-326.

Almaça C (1985) Evolutionary and zoogeographical remarks on the Mediterranean Fauna of the brachyuran crabs. In: M. Moraitou-Apostolopoulou and V, Kirotsis (ed.). *Mediterranean Marine Ecosystems*: 347-366.

Anger K (2001) *The Biology of decapod crustacean larvae*. Zoological Museum, University of Amsterdam. Crustacean Issues 14.

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. *Molecular Ecology* **7**: 1053–1064.

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution* **20**: 99–105.

Avise JC (1994) *Molecular markers, natural history and evolution*. Chapman & Hall, New York.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press. Cambridge (Massachusetts); London (England).

Avise JC (2004) *Molecular markers, natural history and evolution*. Sinauer Associates, Sunderland, (Massachusetts).

Bahri-Sfar L, Lemaire C, Hassine OKB, Bonhomme F (2000) Fragmentation of sea bass populations in the western and eastern Mediterranean as revealed by microsatellite polymorphism. *Proceedings of the Royal Society B: Biological Sciences* **267**: 929-935.

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology* **13**: 729-744.

Bass D (2003) A comparison of freshwater macroinvertebrate communities on small Caribbean islands. *Bioscience* **53**: 1094-1100.

Bargelloni L, Alarcon JA, Alvarez MC, Penzo E, Magoulas A, Reis C, Patarnello T (2003). Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic-Mediterranean divide. *Journal of Evolutionary Biology* **16**: 1149-1158.

Bauer RT (1981) Color patterns of the shrimps *Heptacarpus pictus* and *Heptacarpus paludicola* (Caridea: Hippolytidae). *Marine Biology* **64**: 141-152.

Baus E, Darrock DJ, Bruford MW (2005) Gene-flow patterns in Atlantic and Mediterranean populations of the Lusitanian sea star *Asterina gibbosa*. Molecular *Ecology* **14**: 3373-3382.

Bedini R (2002) Colour change and mimicry from juvenile to adult: *Xantho poressa* (Olivi, 1792) (Brachyura, Xanthidae) and *Carcinus maenas* (Linnaeus, 1758) (Brachyura, Portunidae). *Crustaceana* **75**: 703-710.

Belfiore NM, Hoffmann FG, Baker RJ, Dewoody JA (2003) The use of nuclear and mitochondrial single nucleotide polymorphisms to identify cryptic species. *Molecular Ecology* **12**: 2011-2017.

Benstead JP, March JG, Pringle CM (2000) Estuarine larval development and upstream post-larval migration of freshwater shrimps in two tropical rivers of Puerto Rico. *Biotropica* **32**: 545-548.

Berglund A (1980) Niche differentiation between two littoral prawns in Gullmar Fjord, Sweden: *Palaemon adspersus* and *P. squilla*. *Holarctic Ecology* **3**: 111-115.

Berglund A, Bengston J (1981) Biotic and abiotic factors determining the distribution of two prawn species: *Palaemon adspersus* and *P. squilla*. *Oecologia* **49**: 300-304.

Berglund A, Lagercrantz U (1983) Genetic differentiation in populations of two Palaemon prawn species at the Atlantic east coast: does gene flow prevent local adaptation? *Marine Biology* **77**: 49-57.

Bickford D, Lohman DJ, Sodhi NS, Ng PKL, Meier R, Winker K, Ingram KK, Indraneil D (2007) Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* **22**: 148-155.

Billot C, Engel CR, Rousvoal S, Kloareg B (2003) Current patterns, habitat discontinuities and population genetic structure: the case of the kelp *Laminaria digitata* in the English Channel. *Marine Ecology Progress Series* **253**: 111–121.

Bishop MJ (1979) A new species of *Caracolus* (Pulmonata: Camaenidae) from the Oligocene of Nebraska and the biotic history of the American camaenid land snails. *Zoological Journal of the Linnean Society* **67**: 269-284.

Borsa P, Blanquer A, Berrebi P (1997) Genetic structure of the flounders *Platichtys flesus* and *P. stellatus* at different geographic scales. *Marine Biology* **129**: 233-246.

Bouvier EL (1925) *Recherches sur la morphologie, les variations, la distribution géographique des crevettes de la famille des Atyidae*. Encyclopédie Entomologique, Série A, Volume 4, Paul Lechevalier, Paris.

Box GEP (1953) Non-normality and tests on variances. *Biometrika* **40**: 318-335.

Bremer JRA, Viñas J, Mejuto J, Ely B, Pla C (2005) Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular Phylogenetics and Evolution* **36**: 169-187.

Brian JV, Fernandes T, Ladle RJ, Todd PA (2006) Patterns of morphological and genetic variability in UK populations of the shore crab, *Carcinus maenas* Linnaeus, 1758 (Crustacea: Decapoda: Brachyura). *Journal of Experimental Marine Biology and Ecology* **329**: 47-54.

Briggs JC (1974) *Marine Zoogeography*. New York: McGraw-Hill.

Calsbeek R, Smith TB (2003). Ocean currents mediate evolution in island lizards. *Nature* **246**: 552-555.

Carlsson J, McDowell JR, Díaz-Jaimes P, Carlsson JEL, Boles SB, Gold JR, Graves JE (2004) Microsatellite and mitochondrial DNA analyses of Atlantic bluefin tuna (*Thunnus thynnus thynnus*) population structure in the Mediterranean Sea. *Molecular Ecology* **13**: 3345-3356.

Carreras-Carbonell J, MacPherson E, Pascual M (2006) Population structure within and between subspecies of the Mediterranean triplefin fish *Tripterygion delaisi* revealed by highly polymorphic microsatellite loci. *Molecular Ecology* **15**: 3527–3539.

Casu M, Curini-Galletti M (2004) Sibling species in interstitial flatworms: a case study using *Monocelis lineata* (Proseriata: Monocelididae). *Marine Biology* **145**: 669-679.

Chace FA Jr., Bruce AJ (1993) The Caridean Shrimps (Crustacea: Decapoda) of the Albatros Philippine Expedition 1907-1910, Part6: Superfamily Palaemonoidea. Smiths. *Contribution to Zoology* **543**: i-vii + 1-152.

Chace FA Jr. (1992) On the classification of the Caridea (Decapoda). *Crustaceana* **63**: 70-80.

Chace FA Jr., Hobbs HH Jr. (1969) Decapod crustaceans of the West Indies. U.S. *National Museum*

Bulletin **292**: 1-258.

Chakrabarty P (2006). Systematics and historical biogeography of Greater Antillean Cichlidae. *Molecular Phylogenetics and Evolution* **39**: 619-627.

Cimmaruta R, Bondanelli P, Nascetti G (2005) Genetic structure and environmental heterogeneity in the European hake (*Merluccius merluccius*). *Molecular Ecology* **14**: 2577–2591.

Collin R (2001) The effects of mode of development on phyleography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). *Molecular Ecology* **10**: 2249-2262.

Cook BD, Baker AW, Page TJ, Grant SC, Fawcett JH, Hurwood DA, Hughes JM (2006) Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): the role life history transition in phylogeograpic diversification. *Molecular Ecology* **15**: 1083-1093.

Cott HB (1957) *Adaptive coloration in animals*. Methuen, London.

Covich AP (2006) Disperal-limited biodiversity of tropical insular streams. *Polish Journal of Ecology* **54**: 523-547.

Crandall KA, Tempelton AR (1993) Empirical tests of some predictions from coalescent theory with applications to intraspecific phylogeny reconstruction. *Genetics* **134**: 959-969.

Crandall KA, Fitzpatrick Jr, JE (1996) Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic of Biology* **45**: 1-26.

Cristescu MEA, Hebert PDN, Witt JDS, MacIsaac HJ, Grigorovich IA (2001) An invasion history for *Cercopagis pengoi* based on mitochondrial gene sequences. *Limnology and Oceanography* **46**: 224-229.

Cuesta JA, Rodríguez A (1994) Early zoeal stages of *Pachygrapsus marmoratus* (Fabricius), *P. transversus* (Gibbes), and *P. maurus* (Lucas) (Decapoda: Brachyura: Grapsidae) reared in the laboratory. *Scientia Marina* **58**: 323-327.

Demeusy N (1958) Recherches sur la mue de puberté du décapoda brachyoure Carcinus maenas. *Archives de Zoologie Expérimentale et Générale* **95**: 253-492.

Daniels SR, Stewart BA, Burmeister L (2001) Geographic patterns of genetic and morphological divergence amongst populations of a river crab (Decapoda: Potamonautidae) with the description of a new species from mountain streams in the Western Cape. *Zoologica Scripta* **30**: 181-197.

Daniels SR, Gouws G, Stewart BA, Coke M (2003) Molecular and morphological data demonstrates the presence of cryptic lineages among freshwater crabs (Decapoda: Potamonautidae: *Potamonautes*) from the Drakensberg Mountains, South Africa. *Biological Journal of the Linnean Society London* **78**: 129-147.

Darlington PJ (1938) The origin of the fauna of the Greater Antilles, with discussion of dispersal of animals over water and through the air. *Quarterly Review of Biology* **13**: 274-300.

De Man (1915) On some European species of the genus Leander desm., also a contribution to the fauna of Dutch waters. 1-179.

Dimitrov P, Dimitrov D (2004) *The Black Sea*. Slavena, Varna, Bulgaria.

Domingues VS, Santos RS, Brito A, Alexandrou M, Almada VC (2007) Mitochondrial and nuclear markers reveal isolation by distance and effects of Pleistocene glaciations in the northeastern Atlantic and Mediterranean populations of the white seabream (*Diplodus sargus*, L.). *Journal of Experimental Marine Biology and Ecology* **346**: 102-113.

Drach P, Forest J (1953) Description et répartition des *Xantho* des mers d`Europe. *Archives de Zoologie Expérimentale et Générale* **90**:1-35.

Duran S, Giribet G, Turon X (2004a) Phylogeographical history of the sponge *Crambe crambe* (Porifera, Poecilosclerida) : range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. *Molecular Ecology* **13**: 109-122.

Duran S, Palacín C, Beccerro A, Turon X, Giribet G (2004b) Genetic diversity and population structure of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). *Molecular Ecology* **13**: 3317–3328.

Estrada M (1985) Deep phytoplankton and chlorophyll maxima in the Western Mediterranean. *CSA Illumina* (online).

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 491-497.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.1: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1**: 47 50.

Fielding S, Crisp N, Allen JT, Hartman MC, Rabe B, Roe HSJ (2001) Mesoscale subduction at the Almeria–Oran front. Part 2. Biophysical interactions. *Journal of Marine Systems* **30**: 287-304.

Fièvet E (1998) Distribution et capacités d´expansion des crevettes d´eau douce de la région caraibe: exemple des genres *Marcrobrachium* et *Atya* (Crustacea: Caridea). *Biogeographica* **74**: 1-22.

Fièvet E, Dolédec S, Lim P (2001) Distribution of migratory fishes and shrimps along multivariate gradients in tropical island streams. *Journal of Fish Biology* **59**: 390-402.

Fièvet V, Touzet P, Arnaud JF, Cuguen J (2007) Spatial analysis of nuclear and cytoplasmic DNA diversity in wild sea beet (*Beta vulgaris* ssp. *maritima*) populations: do marine currents shape the genetic structure? *Molecular Ecology* **16**: 1847-1864.

Fincham AA (1977) Larval development of Bristish prawns and shrimps (Crustacea: Decapoda: Natantia). 1. Laboratory methods and a review of *Palaemon* (*Palaeander*) *elegans* Rathke, 1837. Bulletin of the British Museum (Natural History), *Zoology* **31**: 1-19.

Flowers JM, Foltz DW (2001) Reconciling molecular systematics and traditional taxonomy in a species-rich clade of sea stars (*Leptasterias* subgenus *Hexasterias*). *Marine Biology* **139**: 475-483.

Folmer O, Black M, Hoeh W, Lutz R,Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Technology* **3**: 294-299.

Fortunato C, Sbordoni V (1998) Allozyme variation in the Mediterranean rockpool prawn (*Palaemon elegans*): environmental vs. historical determinants. *Proceeding and Abstracts of 4th international Crustacean congress.* Amsterdam.

Forsman A, Appelqvist S (1999) Experimental manipulation reveals differential effects of colour pattern on survival in male and female pygmy grasshoppers. *Journal of Evolutionary Biology* **12**: 391-401.

Francisco SM, Cabral H, Vierira MN, Almada VC (2006) Contrasts in genetic structure and historical demography of marine and riverine populations of *Atherina* at similar geographical scales. *Estuarine, Coastal and Shelf Science* **69**: 655-661.

Fratini S, Vannini M (2002) Genetic differentiation in the swimming crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology* **272**: 103-116.

Froglia C, Manning RB (1978) *Brachynotus gemmellari* (Rizza, 1839), the third Mediterranean species of the genus (Crustacea, Decapoda, Brachyura). *Proceeding of the Biological Society of Washington* **91**: 691-705.

Fryer G (1977) Studies on the functional morphology and ecology of the atyid prawns of Dominica. *Philosophical Transactions of the Royal Society of London Series B – Biological Sciences* **277**: 57-129.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915-925.

García-Raso JE, González Gurriarán E, Sardá F (1987) Estudio comparativo de la fauna de crustáceos decápodos braquiuros de tres áreas de la Peninsula Iberica (Galicia, Málaga y Cataluna). *Investigación Pesquera* **51**: 43-55.

Garoia F, Guarniero I, Ramsak A, Ungaro N, Landi M, Piccinetti C, Mannini P, Tinti F (2004) Microsatellite DNA variation reveals high gene flow and panmictic populations in the Adriatic shared stocks of the European squid and cuttlefish (Cephalopoda). *Heredity* **93**: 166-174

Garrido-Ramos MA, Soriguer MC, de la Herrán R, Jamilena M, Rejón CR, Domezain A, Hernando JA, Rejón MR (1997) Morphometric and genetic analysis as proof of the existence of two sturgeon species in the Guadalquivir river. *Marine Biology* **129**: 33-39.

Geller JB, Walton ED, Grosholz ED, Ruiz GM (1997) Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* **6**: 901-906.

Gollasch S, Lenz J, Dammer M, Andres HG (2000) Survival of tropical ballast water organisms during a cruise from the Indian Ocean to the North Sea. *Journal of Plankton Research* **22**: 923-937.

Gonzáles-Wangüemert M, Giménez-Casalduero F, Pérez-Ruzafa A (2006) Genetic differentiation of *Elysia timida* (Risso, 1818) populations in the Southwest Mediterranean and Mar Menor coastal lagoon. *Biochemical Systematics and Ecology* **34**: 514-527.

Goodfriend GA (1989) *Quaternary biogeographical history of land snails in Jamaica. Biogeography of the West Indies: Past, Present and Future*. Sandhill Crane Press, Gainesville, Florida.

Gosner KL (1978) *A field guide to the Atlantic seashore. The Peterson Field Guide Series.* Houghton Mifflin, Boston, Massachusetts, USA.

Grabwoski M (2006) Rapid colonization of the Polish Baltic coast by an Atlantic palaemonid shrimp *Palaemon elegans* Rathke, 1837. *Aquatic invasions* **1**: 116-123.

Grigorovich IA Therriault TW, MacIsaac HJ (2004) History of aquatic invertebrate invasions in the Caspian Sea. *Biological Invasions* **5**: 103-115.

Guinot D (1967) Recherches préliminaires sur les groupements naturels chez les Crustacés Décapodes Brachyoures. IV. Observations sur quelques genres de Xanthidae. *Bulletin du Muséum national D`Histoire naturelle, Paris 2* **39**: 695-727.

Gusmao J, Lazoski C, Monteiro FA, Solé-Cava AM (2006) Cryptic species and population structuring of the Atlantic and Pacific seabob shrimp species, *Xiphopenaeus kroyeri* and *Xiphopenaeus riveti*. *Marine Biology* **149**: 491-502.

Gysels ES, Hellemans B, Pampoulie C, Volckaert FAM (2004) Phylogeography of the common goby, *Pomatoschistus microps*, with particular emphasis on the colonization of the Mediterranean and the North Sea. *Molecular Ecology* **13**: 403–417.

Hall TA (1999) *BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series* **41**: 95-98.

Hänfling B, Carvalho GR, Brandl R (2002) mt-DNA sequences and possible invasion pathways of the Chinese mitten crab. *Marine Eocolgy Progress Series* **238**: 307-310.

Hart CW (1961) The freshwater shrimps (Atyidae and Palaemonidae) of Jamaica, W. I.; with a discussion of their relation to their ancient geography of the western Caribbean area. *Proceedings of the Academy of Natural Sciences, Philadelphia* **113**: 61-80.

Hartnoll RG (1974) Variation in growth pattern between some secondary sexual characters in crabs (Decapoda: Brachyura). *Crustaceana* **27**: 131-136.

Hartnoll RG (1982) *Growth. In: The biology of Crustacea, vol. 2: Embryology, morphology and genetics.* L. G. Abele, ed., pp. 111-196. Academic Press, New York.

Hedges SB, Hass CA, Maxson LR (1992). *Caribbean biogeography: Molecular evidence for dispersal in West Indian terrestrial vertebrates.* Proceedings of the National Academy of Sciences, USA.

Hedrick PW (2005) *Genetics of Population*. Jones and Bartlett Publishers, Sudbury, Massachusetts.

Heyden S, Lipinski MR, Matthee CA (2007) Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* (online).

Hofrichter R (2002) *Das Mittelmeer – Fauna, Flora, Ökologie Band I*. Spektrum Akademischer Verlag, Heidelberg.

Holthuis LB (1954) The names of the European species of the genus *Xantho* Leach, 1814 (Crustacea Decapoda Brachyura). *Proceedings, Koninklijke Nederlandse Akademie van Wetenschappen, Amsterdam, series C* **57**: 103-107.

Hsü KJ (1972) Origin of salinit giants: a critical review after the discovery of the Mediterranean evaporites. *Earth Science Review* **8**: 371-396.

Hsü KJ (1983) *The Mediterranean was a Desert*. Princeton University Press. Princeton New York.

Huelsenbeck, JP, Ronquist, F (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**: 754-755.

Hughes JM, Bunn SE, Hurwood S, Choy S, Pearsons RG (1996) Genetic differentiation among populations of *Caridina zebra* (Decapoda: Atyidae) in tropical rainforest streams, northern Australia. *Freshwater Biology* **36**: 289-296.

Humphries CJ, Ebach MC (2004) Biogeography on a dynamic Earth. *In Fronties of Biogeography.* Sinauer Associates. Sunderland (Massachusetts). Chapter 4: 67-86.

Hunter R, Webb MS, Iliffe TM (in press) Phylogeny and historical biogeography of the cave-adapted shrimp genus *Typhlatya* (Atyidae) in the Caribbean Sea and western Atlantic. *Journal of biogeography*.

Iannotta MA, Patti FP, Ambrosino M, Procaccini G, Gambi MC (2007) Phylogeography of two species of *Lysidice* (Polychaeta, Eunicidae) associated to the seagrass *Posidonia oceanica* in the Mediterranean Sea. *Marine Biology* **150**: 1115-1126.

Ingle RW (1983) A comparative study of the larval development of *Monodaeus couchi* (Couch), *Xantho incisus* Leach and *Pillumnus hirtellus* (Linnaeus) (Crustacea: Brachyura: Xanthidae). *Journal of Natural History* **17**: 951-978.

Janson K (1985) A morphologic and genetic analysis of *Littorina saxatilis* (Prosobranchia) from Venice, and on the problem of *saxatilis – rudis* nomenclature. *Biological Journal of the Linnean Society* **24**: 51-59.

Jolly MT, Jollivet D, Gentil F, Thiébaut E, Viard F (2005) Sharp genetic break between Atlantic and English Channel populations of the polychaete *Pectinaria koreni*, along the North coast of France. *Heredity* **94**: 23-32.

Kadereit JW, Arafeh R, Somogyi G, Westberg E (2005) Terrestrial growth and marine dispersal? Comparative phylogeography of five coastal plant species at a European scale. *Taxon* **54**: 861-876.

Ketmaier V (2002) Isolation by distance, gene flow and phylogeography in the *Proasellus coxalis*-group (Crustacea, Isopoda) in central Italy: allozyme data. *Aquatic Science* **64**: 66-75.

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* **49**: 561-576.

Kirkendale LA, Meyer CP (2004) Phylogeography of the *Patelloida profunda* group (Gastropoda: Lottidae): diversification in a dispersal-driven marine system. *Molecular Ecology* **13**: 2749–2762.

Knowlton N (1993) Sibling species in the sea. *Annual Review of Ecological Systematics* **24**: 189-216.

Knowlton N, Weigt LA (1998) New Dates and New Rates for Divergence across the Isthmus of Panama. *Proceedings of Biological Sciences* **265**: 2257-2263.

Knowlton N (2000) Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* **420**: 73-90.

Kojima S, Kamimura S, Kimura T, Hayashi I, Iijima A, Furota T (2003) Phylogenetic relationships between the tideland snails *Batillaria flectosiphonata* in the Ryukyu Islands and *B. multiformis* in the Japanese Islands. *Zoological Science* **20**: 1423-1433.

Krebs CJ (1999): *Ecological Methodology* (2. edition). Benjamin cummings, Menlo Park.

Krijgsman W, Hilgen FJ, Raffi I, Sierro FJ, Wilsonk DS (1999) Chronology, causes and progression of the Messinian salinity crisis. *Nature* **400**: 652-655.

Kyle CJ, Boulding EG (2000) Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. *Marine Biology* **137**: 835-845.

Lee TN, Clarke ME, Williams E, Szmant AF, Berger T (1994) Evolution of the Tortugas Gyre and its influence on recruitment in the Florida Keys. *Bulletin of the Marine Science* **54**: 621-646.

Lefébure T, Douady CJ, Gouy M, Trontelj P, Briolay J, Gibert J (2006) Phylogeography of a subterranean amphipod reveals cryptic diversity and dynamic evolution in extreme environments. *Molecular Ecology* **15**: 1797–1806.

Lemaire C, Versini JJ, Bonhomme F (2005) Maintenance of genetic differentiation across a transition zone in the sea: discordance between nuclear and cytoplasmic markers. *Journal of Evolutionary Biology* **18**: 70–80.

Leppäkoski E, Olenin S (2000) Non-native Species and Rates of Spread: Lessons from the Brackish Baltic Sea. *Biological Invasions* **2**: 151-163.

Li WH (1977) Distribution of nucleotide differences between two randomly chosen cistrons in a finite

population. *Genetics* **85**: 331-337.

Luttikhuizen PC, Drent J, Van Delden W, Piersma T (2003) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. *Journal of Evolutionary Biology* **16**: 260–272.

Manning RB, Holthuis LB (1981) *West African Brachyuran Crabs (Crustacea: Decapoda)*. Smithsonian Institution Press. City of Washington.

March JG, Pringle CM, Townsend MJ, Wilson AI (2002) Effects of freshwater shrimp assemblages on benthic communities along an altitudinal gradient of a tropical island stream. *Freshwater Biology* **47**: 377-390.

Martin JW, Davis GE (2001) *An updated classification of the recent Crustacea*. Science Series 39. Natural History Museum of Los Angeles County, California.

McDowall RM (2003) Hawaiian biogeography and the islands´ freshwater fish fauna. *Journal of Biogeography* **30**: 703-710.

McDowall RM (2007) On amphidromy, a distinct form of diadromy in aquatic organisms. *Fish and Fisheries* **8**: 1-13.

McGlashan JD, Hughes JM (2001) Low levels of genetic differentiation among populations of the freshwater fish *Hypseleotris compressa* (Gobiidae: Eleotridinae): implications for its biology, population connectivity and history. *Heredity* **86**: 222-223.

McKenzie JA (1999) From desert to deluge in the Mediterranean. *Nature* **400**: 613-614.

Mathews LM (2006) Cryptic biodiversity and phylogeograhic patterns in a snapping shrimp complex. *Molecular Ecology* **15**: 4049-4063.

Mattiangeli V, Ryan AW, Galvin P, Mork J, Cross TF (2003) Eastern and Western Poor Cod (*Trisopterus minutus capelanus*) Populations in the Mediterranean Sea: Evidence from Allozyme and Minisatellite Loci. *Marine Ecology* **24**: 247–258.

Miner BG, Sultan SE, Morgan SG, Padilla DK, Relyea RA (2005) Ecological consequences of phenotypic plasticity. *Trends in Ecology and Evolution* **20**: 685-692.

Monteiro FA, Solé–Cava AM, Thorpe JP (1997) Extensive genetic divergence between populations of the common intertidal sea anemone *Actina equina* from Britain, the Mediterranean and the Cape Verde Islands. *Marine Biology* **129**: 425-433.

Murphy NP, Austin CM (2004) Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxanomy and the convergent evolution of abbreviated larval development. *Zoologica Scripta* **34**: 187-197.

Myers MJ, Meyer CP, Resh VH (2000) Neritid and thiarid gastropods from French Polynesian streams: how reproduction (sexual, parthenogenetic) and dispersal (active, passive) affect population structure. *Freshwater Biology* **44**: 535-545.

Naciri M, Lemaire C, Borsa P, Bonhomme F (1999) Genetic Study of the Atlantic/Mediterranean Transition in Sea Bass (*Dicentrachrus labrax*). *The American Genetic Association* **90**: 591-596.

Naranjo S, Carballo JL, García-Gómez JC (1998) Towards a knowledge of marine boundaries using ascidians as indicators: characterising transition zones for species distribution along Atlantic-Mediterranean shores. *Biological Journal of the Linnean Society* **64**: 151-177.

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. *Genetics* **97**: 145-163.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Néraudeau D, Goubert E (2002) The Messinian event… thirty years after. *Geodiversitas* **24**: 508-510.

Nikula R, Väinölä R (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia: Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology* **143**: 339-350.

Ortmann AE (1894) A study of the systematic and geographical distribution of the decapod family Atyidae Kingsley. *Proceeding of the National Academy of Sciences Philadelphia*: 397-416.

Page TJ, Baker AM, Cook BD, Hughes JM (2005) Historical transoceanic dispersal of a freshwater shrimp: the colonization of the South Pacific by the genus *Paratya* (Atyidae). *Journal of Biogeography* **32**: 581–593.

Page TJ, Cook BD, von Rintelen T, von Rintelen K, Hughes JM (in press). Evolutionary relationships of freshwater atyid shrimp imply both ancient Caribbean radiations and common marine dispersals.

Palma AT, Steneck RS (2001) Does variable coloration in juvenile marine crabs reduce risk of visual predation? *Ecology* **82**: 2961–2967.

Palma J, Andrade JP (2002) Morphological study of *Diplodus sargus*, *Diplodus puntazzo*, and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. *Fisheries Research* **57**: 1-8.

Palumbi SR, Martin A, Romano S, Mcmillan WO, Stice L & Grabowski G (1991) *The simple fool`s guide to PCR*. A collection of PCR Protocols, version 2. Honolulu: University of Hawai.

Palumbi SR (1992) Marine speciation on a small planet. *Trends of Ecology and Evolution* **7**: 114-117.

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* **25**: 547-572.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* **13**: 146-158.

Pannacciulli FG, Bishop JDD, Hawkins SJ (1997) Genetic structure of populations of two species of *Chthamalus* (Crustacea: Cirripedia) in the north-east Atlantic and Mediterranean. *Marine Biology* **128**: 73-82.

Papadopoulos LN, Peijnenburg KTCA, Luttikhuizen PC (2005) Phylogeography of the calanoid copepods *Calanus helgolandicus* and *C. euxinus* suggests Pleistocene divergences between Atlantic, Mediterranean, and Black Sea populations. *Marine Biology* **147**: 1353-1365.

Peijnenburg KTC, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ (2004) Phylogeography of the planktonic chaetogenath *Sagitta setosa* reveals isolation in European seas. *Evolution* **58**: 1472-1487.

Perdices A, Doadrio I, Bermingham E (2005) Evolutionary history of the synbranchid eels (Teleostei: Synbranchidae) in Central America and the Carribean islands inferred from their molecular phylogeny. *Molecular Phylogenetics and Evolution* **37**: 460-473.

Pesta O (1918) *Die Dekapodenfauna der Adria*. Franz Deuticke, Leipzig und Wien.

Pérez-Losada M, Guerra A, Carvalho GR, Sanjuan A, Shaw PW (2002): Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalopoda) around the Iberian Peninsula indicated by microsatellite DNA variation. *Heredity* **89**: 417-424.

Pérez-Losada M, Nolte MJ, Crandall KA, Shaw PW (2007) Testing hypotheses of population structuring in

the Northeast Atlantic Ocean and Mediterranean Sea using the common cuttlefish *Sepia officinalis. Molecular Ecology* **16**: 2667-2679.

Pfenninger M, Schwenk K (2007) Cryptic animal species are homogeneously distributed among taxa and biogeographical region. *Evolutionary Biology* **7**: 1-6.

Pigliucci M (2005) Evolution of phenotypic plasticity: where are we going now? *Trends in Ecology and Evolution* **20:** 481-486.

Planes S, Galzin R, Bonhomme F (1996) A genetic metapopulation model for reef fishes in oceanic islands: the case of the surgeonfish, *Acanthurus trisotegus*. *Journal of Evolutionary Biology* **9**: 103-117.

Pocock RI (1889) Contribution to our knowledge of the Crustacea of Dominica. *Annual Magazine of Natural History* **3**: 6-22.

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by Distance in the Atlantic Cod, *Gadus morhua*, at large and small geographic scales. *Evolution* **55**: 131-146.

Posada D, Crandall, KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Por FD, Dimentman C (1989) The legacy of Tethys: on aquatic biogeography of the Levant (L'héritage de la Téthys: biogéographie aquatique du Levant). *Monographiae biologicae* **63**: 190-214.

Queiroga H (1996) Distribution and drift of the crab *Carcinus maenas* (L.) (Decapoda, Portunidae) larvae over the continental shelf of northern Portugal in April 1991. *Journal of Plankton Research* **18**: 1981-2000.

Queiroga H (1998) Vertical migration and selective tidal stream transport in the megalopa of the crab *Carcinus maenas*. *Hydrobiología* **375/376**: 137-149.

Quesada H, Beynon CM, Skibinski DOF (1995) A Mitochondrial Discontinuity in the Mussel *Mytilus galloprovincialis* Lmk: Pleistocene Vicariance Biogeography and Secondary Intergradation. *Molecular Biology and Evolution* **12**: 521-524.

Rawson PD, Hilbish TJ (1998) Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* spp.). *Evolution* **52**: 100–108.

Reece JS, Castoe TA, Parkinson CL (2005) Historical perspectives on population genetics and conservation of three marine turtle species. *Conservation Genetics* **6**: 235-251.

Remerie T, Bourgois T, Peelaers D, Vierstraete A Vanfleteren J, Vanreusel A (2006) Phylogeographic patterns of the Mysid *Mesopodopsis slabberi* (Crustacea, Mysida) in Western Europe: evidence for high molecular diversity and cryptic speciation. *Marine Biology* **149**: 465-481.

Reuschel S, Schubart CD (2006) Phylogeny and geographic differentiation of Atlanto-Mediterranean species of the genus *Xantho* (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses. *Marine Biology* **148:** 853-866.

Reuschel S, Schubart CD (2007) Contrasting genetic diversity with phenotypic diversity in coloration and size in *Xantho poressa* (Brachyura: Xanthidae), with new results on its ecology. *Marine Ecology* **28**: 1-10.

Reuschel S, Cuesta JA, Schubart CD (submitted) Population genetic analyses of the prawn *Palaemon elegans* confirm presence of marine biogeographic barriers and human introduction along Europe coast. *Molecular Biology*.

Riedel R (1983) *Fauna und Flora des Mittelmeeres*. Paul Parey, Hamburg und Berlin.

Rincón PA (2000) Big fish, small fish: still the same species. Lack of morphometric evidence of the existence of two sturgeon species in the Guadalquivir river. *Marine Biology* **136**: 715-723.

Rios C, Sanz S, Saavedra C, Peña JB (2002) Allozyme variation in populations of scallops, *Pecten jacobaeus* (L.) and *P. maximus* (L.) (Bivalvia: Pectinidae), across the Almeria–Oran front. *Journal of Experimental Marine Biology and Ecology* **267**: 223-244.

Roberts CM (1997) Connectivity and management of Caribbean coral reefs. *Science* **278**: 1454-1457.

Rodríguez, F Oliver JF Marín A, Medina JR (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**: 485-501.

Rogers AR, Harpending H (1992) Population Growth Makes Waves in the Distribution of Pairwise Genetic Differences. *Molecular Biology of Evolution* **9**: 552-569.

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology* **13**: 2891-2898.

Rosen DE (1976) A vicariance model of Caribbean biogeography. *Systematic Zoology* **24**: 431-464.

Rozas J, Sánchez-del Barrio JC, Messeguer X, Roza R (2003) DnaSP, DNA-polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**: 2496-2497.

Saavedra C, Viñas J (2005) Nucleotide diversity and Pleistocene population expansion in Atlantic and Mediterranean scallops (*Pecten maximus* and *P. jacobaeus*) as revealed by the mitochondrial 16S ribosomal RNA gene. *Journal of Experimental Marine Biology and Ecology* **323**: 138-150.

Salomon JC, Breton M (1993) An atlas of long term currents in the Channel. *Oceanological Acta* **16**: 439-448.

Schluter D (2001) Ecology and the origin of species. *Trends in Ecology and Evolution* **16**: 372-380.

Schneider S, Roessli D, Excoffier L (1999) *Arlequin ver. 2.0: software for population genetic data analysis.* Genetics and Biometry Laboratory, University of Geneva, Geneva (Switzerland).

Schneider-Broussard R, Felder DL, Chlan CA, Neigel JE (1998) Tests of Phylogeographic models with nuclear and mitochondrial DNA sequence variation in the stone crabs, *Menippe adina* und *Medina mercenaria*. *Evolution* **52**: 1671-1678.

Schubart CD, Neigel JE, Felder DL (2000a) Use of the mitochondrial 16S rRNA gene for phylogenetic and population studies of Crustacea. *Crustacean Issues* **12**: 817-830.

Schubart CD, Neigel JE, Felder DL (2000b) Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. *Marine Biology* **137**: 11-18.

Schubart CD, Cuesta JA, Rodríguez A (2001) Molecular phylogeny of the crab genus *Brachynotus* (Brachyura: Varunidae) based on the 16S rRNA gene. *Hydrobiologia* **449**: 41-46.

Schubart CD, Cuesta JA, Felder DL (2002) Glyptograpsidae, a new brachyuran family from central america: Larval and adult morphology, and a molecular phylogeny of the Grapsoidea. *Journal of Crustacean Biology* **22**: 28-44.

Schubart CD, Koller P (2005) Genetic diversity of freshwater crabs (Brachyura: Sesarmidae) from central Jamaica with descritption of a new species. *Journal of Natural History* **39**: 469-481.

Send U, Font J, Krahmann G (1999) Recent advances in observing the physical oceanography of the

western Mediterranean Sea. *Progress in Oceanography* **44**: 37-64.

Shanks AL (1995) Mechanisms of cross-shelf dispersal of larval invertebrates and fish. *Marine Science Series* **6**: 323-367.

Shih HT, Hung HC, Schubart CD, Allen C, Chang C, Chang HW (2006) Intraspecific genetic diversity of the endemic freshwater crab *Candidopotamon rathbunae* (Decapoda, Brachyura, Potamidae) reflects five million years of the geological history of Taiwan. *Journal of Biogeography* **33**: 980-989.

Slatkin M (1981): Estimating levels of gene flow in natural populations. *Genetics* **99**: 323-335.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16**: 393-430.

Spivak ED, Schubart CD (2003) Species status in question: A morphometric and molecular comparison of Cyrtograpsus affinis and C. altimanus (Decapoda, Brachyura, Varunidae). *Journal of Crustacean Biology* **23**: 212-222.

Stamatis C, Triantafyllidis A, Moutou KA, Mamuris Z (2004) Mitochondrial DNA variation in Northeast Atlantic and Mediterranean populations of Norway lobster, *Nephrops norvegicus. Molecular Ecology* **13**: 1377–1390.

Swofford DL (1998) *PAUP*: Phylogenetic Analysis Using Parsimony and other method Version 4*. Sinauer Associates, Sunderland (MA).

Tajima F (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596-1599.

Tarjuelo I, Posada D, Crandall KA, Pascual M, Turon X (2001) Cryptic species of *Clavelina* (Ascidiacea) in two different habitats: harbours and rocky littoral zones in the northwestern Mediterranean. *Marine Biology* **139**: 455-462.

Tarjuelo I, Posada D, Crandall KA, Pascual M, Turon X (2004) Phylogeography and speciation of colour morphs in the colonial ascidian *Pseudodistoma crucigaster*. *Molecular Ecology* **13**: 3125–3136.

Tavares M, de Melo GAS (2004) Discovery of the first known benthic invasive species in the Southern Ocean: the North Atlantic spider crab *Hyas araneus* found in the Antarctic Peninsula. *Antartic science* **16**: 129-131.

Templeton AR, Crandall KA, Sing CF (1992) A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogramm estimation. *Genetics* **132**: 619-633.

Thorson, G (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews* **25**: 1-45

Tintoré J, LaViolette P, Blade I, Cruzado A (1988) A study of an intense density front in the eastern Alboran Sea. The Almería-Oran front. *Journal of Oceanography* **18**: 1284-1397.

Todd CD, Lambert WJ, Thorpe JP (1998) The genetic structure of intertidal populations of two species of nudibranch molluscs with planktotrophic and pelagic lecithotrophic larval stages: are pelagic larvae "for" dispersal? *Journal of Experimental Marine Biolgoy and Ecology* **228**: 1-28.

Todd PA, Briers RA, Ladle RJ, Middleton F (2005) Phenotype-environment matching in the shore crab

(*Carcinus maenas*). *Marine Biology* **148:** 1357-1367.

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine Ecology Progress Series* **297**: 225-231.

Triantafyllidis A, Apostolidis AP, Katsares V, Kelly E, Mercer J, Hughes M, Jorstad KE, Tsolou A, Hynes R, Triantaphyllidis C (2005) Mitochondrial DNA variation in the European lobster (*Homarus gammrus*) throughout the range. *Marine Biology* **146**: 223-235.

Triantafyllidis A, Leonardos I, Bista I, Kyriazis ID, Stoumboudi MT, Kappas I, Amat F, Abatzopoulos TJ (2007) Phylogeography and genetic structure of the Mediterranean killifish *Aphanius fasciatus* (Cyprinodontidae). *Marine Biology* **152**: 1159-1167.

Udekem d'Acoz C d' (1999) *Inventaire et distribution des Crustacés Décapodes de l'Atlantique nord-oriental, de la Mediterranée et des eaux continentales adjacentes au nord de 25°N*. Collection Patrimoines Naturels (Muséum national D`Histoire naturelle) Paris, 40.

Uthicke S, Benzie JAH (2003) Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology* **12**: 2635–2648.

Vermeij GJ (1978) *Biogeography and Adaptation. Patterns of Marine Life*. Harvard University Press, Cambridge, Massachusetts and London.

Viñas J, Bremer JA, Pla C (2004) Phylogeography of the Atlantic bonito (*Sarda sarda*) in the northern Mediterranean: the combined effects of historical vicariance, population expansion, secondary invasion, and isolation by distance. *Molecular Phylogenetics and Evolution* **33**: 32-42.

Wilding CS, Grahame J, Mill PJ (2000) Mitochondrial DNA COI haplotype variation in sibling species of rough periwinkles. *Heredity* **85**: 62-74.

Wilke T (2003) *Salenthydrobia* gen. nov. (Rissooidea: Hydrobiidae): a potential relict of the Messinian salinity crisis. *Zoological Journal of the Linnean Society* **137**: 319–336.

Williams AB, Felder DL (1986) Analysis of stone crabs: *Menippe mercenaria* (Say), restricted, and a previously unrecognized species described (Decapoda: Xanthidae). *Proceedings of the Biological Society of Washington* **99**: 517-543.

Williams EE (1989) *Old problems and new opportunities in West Indian Biogeography*. *Biogeography of the West Indies: Past, Present and Future*. Sandhill Crane Press, Gainesville, Florida.

Wright S (1931) Evolution in Mendelian populations. *Genetics* **16**: 97-159.

Wright S (1940) Breeding structure of populations in relation to speciation. *American Naturalist* **74**: 232-248.

Wright S (1943) Isolation by distance. *Genetics* **28**: 114-138.

Wright S (1978) Variability within and among natural populations. Chicago, University of Chicago Press. Evolution and the Genetics of Populations.

Yamada SB, Dumbauld BR, Kalin A, Hunt CE, Figlar R (2005) Growth and persistence of a recent invader *Carcinus maenas* in estuaries of the northeastern Pacific. *Biological Invasions* **7**: 309-321.

Zane L, Ostellari L, Maccatrozzo L, Bargelloni L, Cuzin-Roudy J, Buchholz F, Patarnello T (2000) Genetic differentiation in pelagic crustacean (*Meganycthphanes norvegica*: Euphausiacea) from the North Atlantic and the Mediterranean Sea. *Marine Biology* **136**: 191-199.

Zardoya R, Castilho R, Grande C, Favre-Krey L, Caetano S, Marcato S, Krey G, Patarnello T (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology* **13**: 1785–1798.

Zariquiey AR (1968). Crustáceos decápodos ibéricos. *Investigacíon Pesquera* **32**: 1-510.

Zelditch ML, Swiderski DL, Sheets H.D, Fink WL, (2004) *Geometric morphometrics for Biologists: a primer*. Elsevier, Amsterdam.

Zenkevich L (1963) *Biology of the seas of the U.S.S.R.*. Interscience Publishers, New York.

Regensburg, 14.02.2008