Marine Biology (2006) 148: 853–866
DOI 10.1007/s00227-005-0095-1

RESEARCH ARTICLE

S. Reuschel · C. D. Schubart

Phylogeny and geographic differentiation of Atlanto–Mediterranean species of the genus *Xantho* (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses

Received: 27 January 2005 / Accepted: 20 July 2005 / Published online: 18 November 2005

Abstract The crab genus *Xantho* Leach, 1814 is restricted to the northeastern 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*.

Communicated by O. Kinne, Oldendorf/Luhe

S. Reuschel (✉) · C. D. Schubart
Biologie I, Universität Regensburg,
93040 Regensburg, Germany
E-mail: silke.reuschel@biologie.uni-regensburg.de
E-mail: cd_schubart@yahoo.com

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 and 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 northeastern 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 northeastern 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 and 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 snorkeling 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°/1 min 48–50°/1 min 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

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

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

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 Chi-square 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 and Ronquist 2001). The model of DNA substitution that fitted our data best was chosen using the software Modeltetst 3.6 (Posada and 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 tree-bisection-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.

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 F_{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 Schefe 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.

Results

Genetics

The genetic dataset consisted of an alignment of 1,167 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 ($\chi^2 = 34.236$, d$f = 57$, $P = 0.993$). 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 Fig. 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 well-supported 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 (Fig. 1).

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 (Fig. 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* (Fig. 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 Protugal), 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 (Fig. 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 (Fig. 2, Table 3). 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

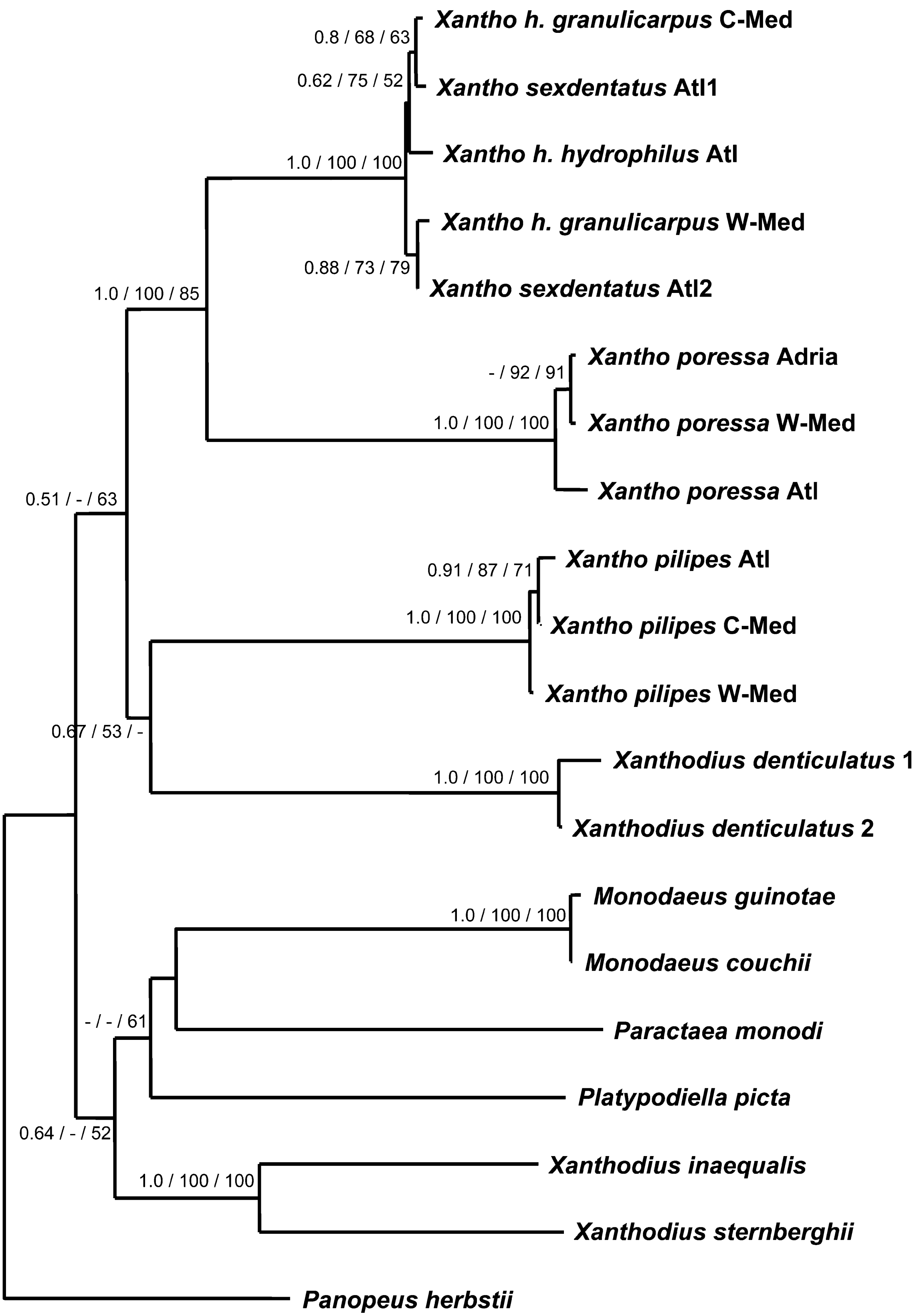

Fig. 1 Bootstrap 50% majority-rule consensus tree of phylogenetic relationships within the genus *Xantho* and other *Xanthidae* with *Panopeus herbstii* as outgroup. Bayesian inference, maximum parsimony and minimum evolution (with GTR + I + G model of evolution) topologies. Confidence values from 2,000 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

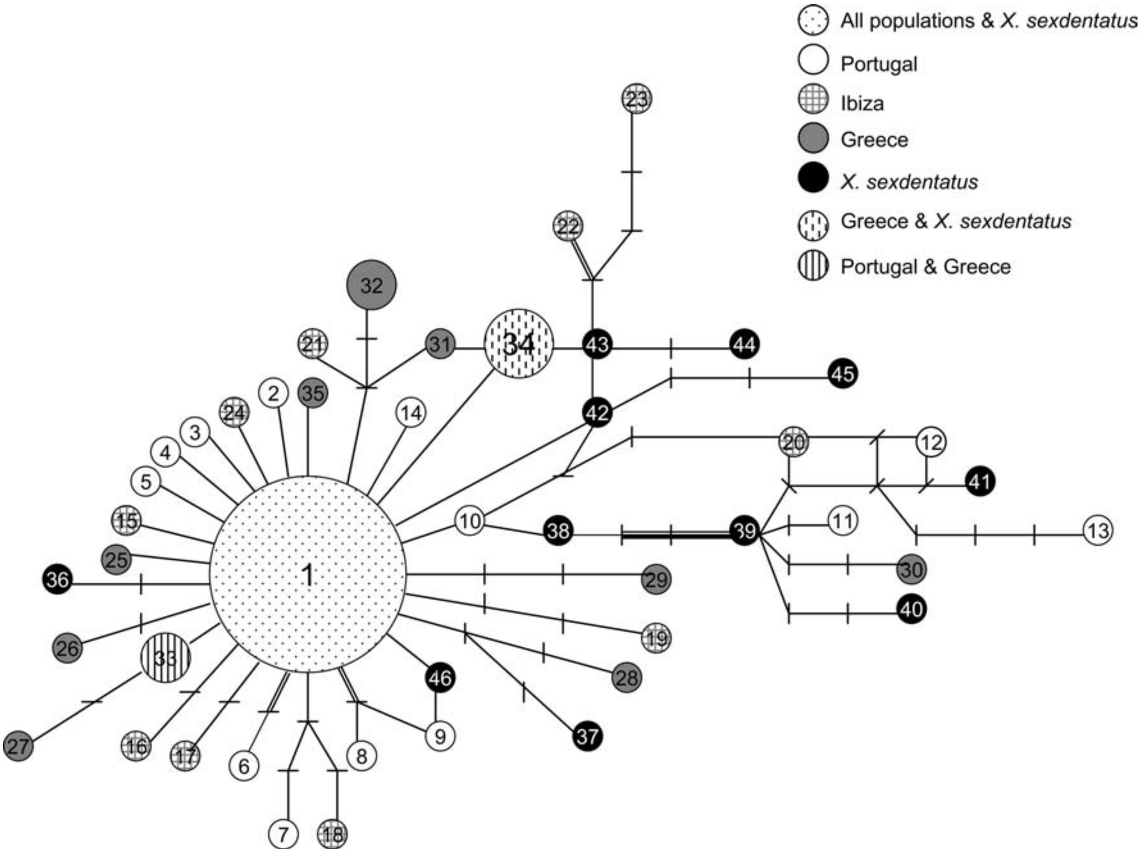

Fig. 2 Minimum parsimonious spanning network constructed with TCS of *X. hydrophilus* ($N = 53$) and *X. sexdentatus* ($N = 15$) of a 622-basepair fragment from the COI gene. Each line represents one substitution; cross lines on lines indicate additional substitutions separating two haplotypes, a double 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

an F_{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 F_{ST}-values were lower (and less significant) between the populations of Portugal and Ibiza (F_{ST} : 0.05, $P = 0.006$), the populations of Greece and Ibiza (F_{ST} : 0.05; $P = 0.005$) than between the populations of Portugal and Greece with a highly significant F_{ST}-value of 0.08 ($P < 0.001$). An F_{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 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. sexdentataus*. 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 Schefe 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

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	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	1	2	2	4	1	1	1	1	1	1	1	1	1	1	1	1	68	40	0.99
S		1	1	1	1	1	3	1	1	1	5	6	9	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			
V						1		1	1		2		2									1																											
N Sub		1	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			

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 Fig. 3. The group dispersions were not homogeneous (Box‘s *M*-test: M = 188, $F_{45,\ 8,208}$ = 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.

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; $F = 15.890$; $P < 0.001$). The post hoc Schefe-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,

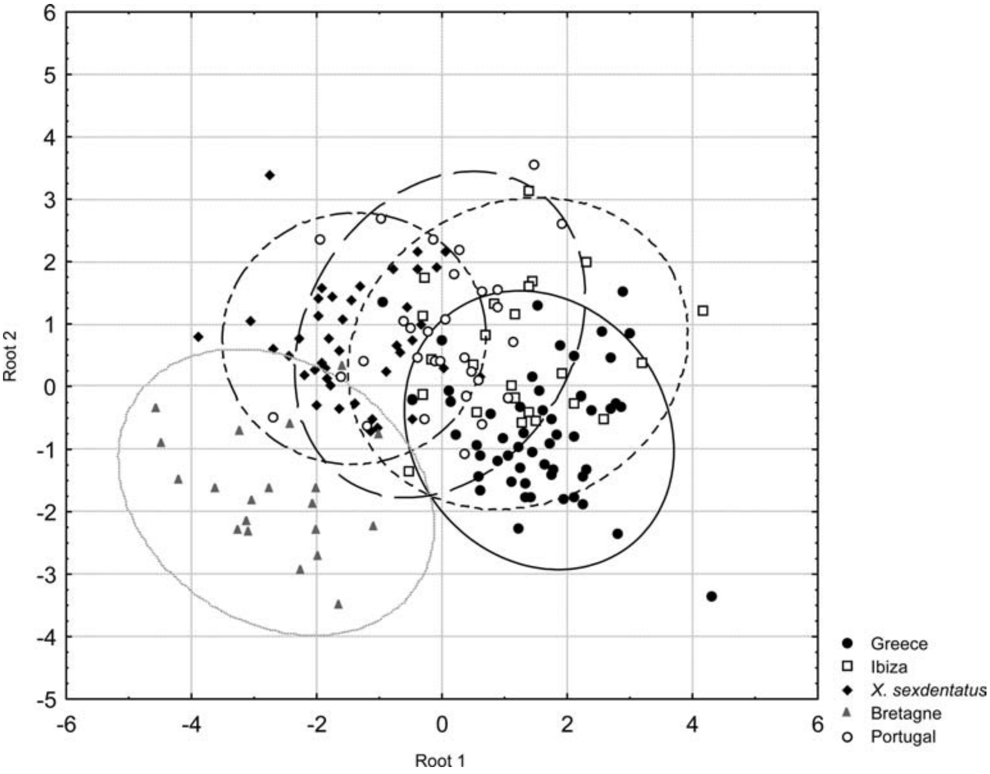

Fig. 3 Canonical analysis depicting discrimination by morphometric measurements between *X. sexdentatus* and *X. hydrophilus* from Bretagne (France), Portugal, Ibiza (Spain), and Parga (Greece); plot of the first discriminant function (root 1) against the second (root 2)

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: $M = 292$, $F_{30,\ 1{,}236} = 9{,}117$, $P < 0.001$) and highly significant differences (Wilks' Lambda: 0.49, *F* (16.837) = 13.414, $P < 0.00001$; 56.38% correct classification) (Fig. 4). 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

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**	**24**	**2**	**2**	**2**	**77.4**
	D^2 14.1					
Ibiza	**0**	**5**	**7**	**10**	**2**	**29.2**
	D^2 20.6	D^2 2.6				
Greece	**0**	**2**	**1**	**49**	**0**	**90.7**
	D^2 19.9	D^2 6.9	D^2 2.2			
X. sexdentatus	**0**	**1**	**1**	**1**	**39**	**92.8**
	D^2 9.6	D^2 5.5	D^2 8.4	D^2 11.5		

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

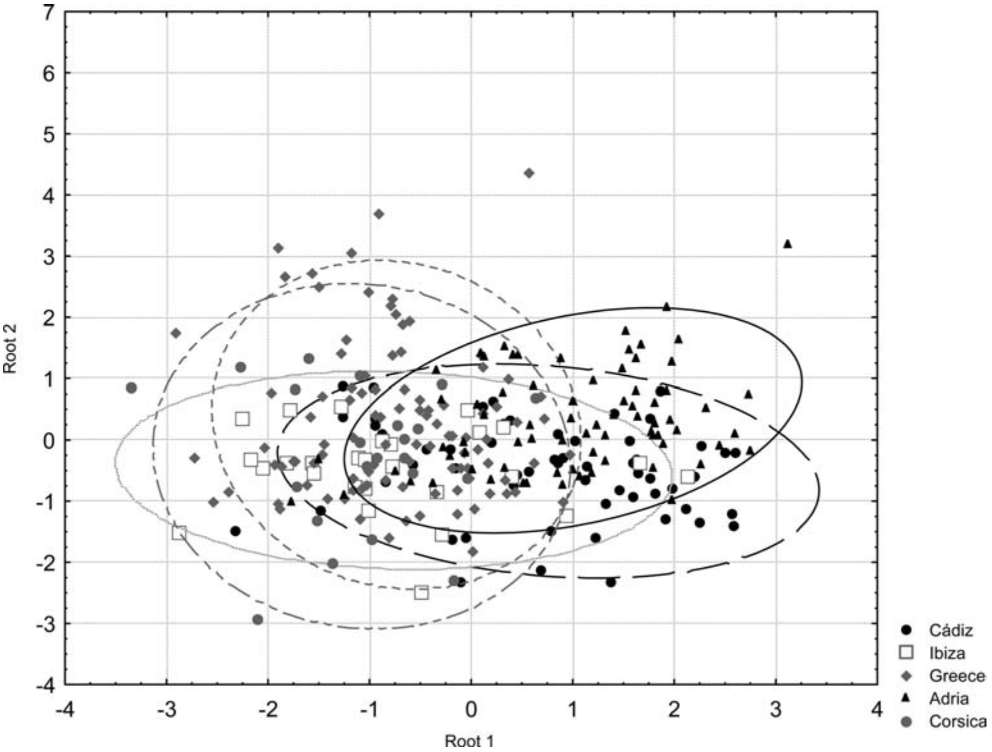

Fig. 4 Canonical analysis depicting discrimination by morphometric measurements of *X. poressa* from Cádiz (Spain), Ibiza (Spain), Croatia (Adria) and Parga (Greece); a plot of the first discriminant function (root 1) against the second (root 2)

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 and 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 and 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 F_{st} of a 3-kb mitochondrial DNA segment. Duran et al. (2004) 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 and Manning 1978), the stone crabs *Menippe mercenaria* and *Menippe adina* (Menippidae) from the Gulf of Mexico and western Atlantic (Williams and Felder 1986), the panopeid crabs *Panopeus herbstii* and *P. stimpsoni* from the northwestern Atlantic (Schubart et al. 2000b) and the varunid crabs *Cyrtograpsus altimanus* and *C. affinis* from the Argentinian coast (Spivak and 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 and 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 and 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 F_{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 and 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 et al. 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 F_{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 predominanty *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 and 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.

References

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

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

Avise JC (2004) Molecular markers, natural history and evolution. Sinauer Associates, Inc Publishers, Sunderland (Massachusetts), pp 1–684

Avise JC, Neigel JE, Arnold J (1984) Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J Mol Evol 20:99–105

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

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

Collin R (2001) The effects of mode of development on phylogeography and population structure of North Atlantic *Crepidula* (Gastropoda: Calyptraeidae). Mol Ecol 10:2249–2262

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 laboratary. Scientia Marina 58(4):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

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

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

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

Flowers JM, Foltz DW (2001) Reconciling molecular systematics and traditional taxonomy in a species-rich clade of sea stars (*Leptasterias* subgenus *Hexasterias*). Mar Biol 139(3):475–483

Fratini S, Vannini M (2002) Genetic differentiation in the swimming crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. J Exp Mar Biol Ecol 272:103–116

Froglia C, Manning RB (1978) *Brachynotus gemmellari* (Rizza, 1839), the third Mediterranean species of the genus (Crustacea, Decapoda, Brachyura). Proc Biol Soc Wash 91:691–705

García Raso JE, González Gurriarán E, Sardá F (1987) Estudio comparativo de la fauna de crustáceos decápodos braquiuros de tres áreas de la Península Iberica (Galicia, Málaga y Cataluna). Investigación Pesquera 51 Suplemento 1:43–55

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

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(4):695–727

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

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 (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

Ingle RW (1983) A comparative study of the larval development of *Monodaeus couchi* (Couch), *Xantho incisus* Leach and *Pillumnus hirtellus* (Linnaeus) (Crustacea: Brachyura: Xanthidae). J Nat Hist 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. Biol J Linn Soc 24:51–59

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

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 Japaneses Islands. Zool Sci 20:1423–1433

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

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. Mar Biol 129:425–433

Naciri M, Lemaire C, Borsa P, Bonhomme F (1999) Genetic study of the Atlantic/Mediterranean transition in sea bass (*Dicentrachrus labrax*). Am Genet Assoc 90:591–596

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

Palma J, Andrade JP (2002) Morpholgical study of *Diplodus sargus*, *Diplodus puntazzo*, and *Lithognathus mormyrus* (Sparidae) in the Eastern Atlantic and Mediterranean Sea. Fish Res 57(1):1–8

Palumbi SR (1992) Marine speciation on a small planet. Trends Ecol Evol 7(4):114–117

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. Ann Rev Ecol Syst 25:547–572

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. University of Hawai, Honolulu

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. Mar Biol 128:73–82

Pérez-Losada M, Guerra A, Carvalho GR, Sanjuan A, Shaw PW (2002): Extensive population subdivision of the cuttlefish *Sepia officinalis* (Mollusca: Cephalapoda) around the Iberian Peninsula indicated by microsatellite DNA variation. Heredity 89(6):417–424

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

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(1):131–146

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

Quesada H, Beynon CM, Skibinski DOF (1995) A mitochondrial discontinuity in the mussel *Mytilus galloprovincialis* Lmk: pleistocene vicariance biogeography and secondary intergradation. Mol Biol Evol 12(3):521–524

Rawson PD, Hilbish TJ (1998) Asymmetric introgression of mitochondrial DNA among European populations of blue mussels (*Mytilus* spp.). Evolution 52(1):100–108

Rodríguez F, Oliver JF, Marín A, Medina JR (1990) The general stochastic model of nucleotide substitution. J Theor Biol 142:485–501

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

Sakai K (1999) J.F.W. Herbst-collection of decapod Crustacea of the Berlin Zoological Museum, with remarks on certain species. Naturalists, Tokushima Biol Lab (6):1–45

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(6):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. Mar Biol 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. J Crustacean Biol 22(1):28–44

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

Slatkin M (1985) Gene flow in natural populations. Ann Rev Ecol Syst 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). J Crustacean Biol 23(1):212–222

Swofford DL (1998) PAUP*: Phylogenetic analysis using parsimony and other methods (software). Sinauer Associates, Sunderland, MA

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

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. Mar Biol 146:223–235

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 Histoire naturelle) Paris 40:1–383

Vermeij GJ (1978) Biogeography and adaptation. Patterns of marine life. Harvard University Press, Cambridge, Massachusetts and London, pp 1–332

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

Williams AB, Felder DL (1986) Analysis of stone crabs: *Menippe mercenaria* (Say), restricted, and a previously unrecognized species described (Decapoda: Xanthidae). Proc Biol Soc Wash 99(3):517–543

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

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

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. Mar Biol 136:191–199