

Comparative phylogeography of two symbiotic dorvilleid polychaetes (*Iphitime cuenoti* and *Ophryotrocha mediterranea*) with contrasting host and bathymetric patterns

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Running title: Phylogeography of two crab-associated Dorvilleidae

Abstract

Two symbiotic polychaetes living in brachyuran crabs in the western Mediterranean and nearby Atlantic were analysed to determine their phylogeographical patterns and the possible effects of known oceanographic barriers in the study area. The analysed species live in hosts inhabiting well-differentiated depths, a factor which may be crucial for understanding the different patterns observed in each species. *Iphitime cuenoti* was found in four different host crabs between 100 and 600 m depth and showed some level of genetic homogeneity, reflected in a star-like haplotype network. Furthermore, barrier effects were not observed. In contrast, *Ophryotrocha mediterranea* was exclusively found in a single host crab species living between 600 and 1200 m depth. Phylogeographical analyses showed two lineages that predate the existence of current barriers. The geological history of the study area, including the most recent glaciation events, likely led to a secondary contact between the lineages, thus forming a unique metapopulation. The phylogeographic pattern found in each species may be explained by differences in dispersal ability, habitat, and host crab specificity that has led them to be differentially affected by historical events. This study represents the first phylogeographic approach on symbiotic polychaetes.

Introduction

Population genetic studies of differentiation are particularly helpful in establishing links between a species ecology and evolution (Bohonak 1999). The population genetic structure is determined by historical events and contemporary factors, such as dispersal ability and oceanographic currents that can act as gene barriers. Altogether, these factors may help infer the historical patterns of populations and their current geographic distribution.

In marine species, dispersal is a key factor for explaining genetic diversity. Populations of benthic species with long-living planktonic larvae are hypothesized to be weakly structured, while brooding species or those with short-living planktonic larvae are hypothesized to have limited gene flow and, consequently, high genetic diversity (Palumbi 1994). Similarly, species affected by permeable barriers to gene flow, such as those with strong swimming abilities, tend to have large, weakly structured populations, acting as panmictic units (Palumbi 1994). Nevertheless, in the ocean, there are well-known gene flow barriers, generating different levels of population structure in marine species (Jolly et al. 2005; Pérez-Portela et al. 2010).

The Mediterranean Sea is a semi-enclosed sea, connected to the Atlantic Ocean by the Gibraltar Strait (GS) Figure 1. Through GS, surface Atlantic waters enter the Mediterranean, while deep Mediterranean waters flow into the Atlantic (Tintore et al. 1998; Millot 1999; Bianchi & Morri 2000).

Consequently, GS acts as a strong barrier to gene flow for some Western Mediterranean invertebrate and fish species (Domingues et al. 2007; Palero et al. 2008; Pérez-Portela & Turon 2008; García-Merchán et al. 2012). A second barrier is the Almería-Oran Front (AOF), a semi-permanent dynamic oceanographic front formed by the convergence of two distinct water masses, which is often considered as the main boundary between Atlantic and Mediterranean surface waters (Tintore et al. 1998). Finally, the current flowing southwestwardly along the continental slope of the NE Iberian Peninsula towards the Ibiza Channel (IC) (Millot 1999) generates a disruptive effect on the circulation in the NW Mediterranean basin (Astraldi et al. 1999), which may restrict the movement of some species (Mokhtar-Jamaï et al. 2011). In contrast to AOF and GS, this current is generally not known to have strong effects on population dynamics. However, IC has been implicated in the high degree of genetic structure observed in some species (García-Merchán et al. 2012), including one of the host species of this work, the crab *Liocarcinus depurator*.

Nevertheless, it is unclear whether or how these three oceanographic fronts affect deep-water species. GS sill is approximately 300 m deep (Astraldi et al. 1999) and AOF is limited to the upper 300 m (Tintore et al. 1998). However, both fronts could affect deep-sea species with larval phases that develop in epipelagic waters.

In addition to the different fronts occurring in present times, recurrent events following the Messinian salinity crisis, including sea level fluctuations and climate changes during the Pleistocene (Emig & Geistdoerfer 2004) have had significant contributions in shaping the present distribution of Mediterranean species, including deep-sea ones.

Brachyuran crabs are common inhabitants of the Atlantic-Mediterranean continental shelf and slope, becoming scarcer in deep-sea basins. Therefore, we expect similar factors to be driving the populations associated with these organisms. Well-known brachyuran crab symbionts include polychaete species of the genus *Iphitime* and two species (out of the 60 known to date) of the genus *Ophryotrocha* (Martin & Britayev 1998). Most studies investigating the relationship of these species with their host crabs have focused on describing the patterns of polychaete infestation (Mori & Belloni 1985; Abelló & Masales 1988; Comely & Ansell 1989; Martin et al. 1991; Høisaeter & Samuelsen 2006). However, nothing is known about the genetic diversity within their distributional areas, the evolutionary relationships with host crabs, and in general, the biotic and abiotic factors that play a role in the life history of these polychaete species.

Although different in many aspects, *Iphitime cuenoti* Fauvel, 1914 and *Ophryotrocha mediterranea* Martin, Abelló and Cartes, 1991 are both symbionts inside the branchial chambers of brachyuran crabs. *Iphitime cuenoti* is found in the North Atlantic Ocean and Mediterranean Sea inside different brachyuran hosts, including *Liocarcinus depurator* (Linnaeus, 1758), *Goneplax rhomboides* (Linnaeus, 1758), *Macropipus tuberculatus* (Roux, 1830) and *Bathynectes maravigna* (Prestandrea, 1839) (Abelló & Masales et al. 1988; Comely & Ansell 1989). In contrast, *O. mediterranea* is known only from the Mediterranean Sea, and only from the host *Geryon longipes* A. Milne-Edwards, 1882 (Mori & Belloni 1985; Martin et al. 1991). *Iphitime cuenoti* presents a wide bathymetric distribution, being commonly found at 100 to 400 m depth, but reaching up to 600 m, while *O. mediterranea* is a strictly deep-sea species found between 600 and 1200 m depth.

The reproductive cycle and larval type of both species is unknown. *Iphitime cuenoti* has a characteristic sexual dimorphism: females are longer than males, and their dorsum is covered by bifurcate branchiae that sometimes contain eggs (Comely & Ansell 1989). Although it has been suggested that females and males of some free-living *Ophryotrocha* species can be distinguished by size (Åkesson 1984), the symbiotic *O. mediterranea* does not have an apparent sexual dimorphism (Martin et al. 1991). *Ophryotrocha geryonicola* (Esmark, 1878), which closely resembles *O. mediterranea*, both in morphology and behaviour, is a gonochoric species in which oocytes float freely in the coelomic cavity (Pfannenstiel et al. 1982). However, nothing is known about its larval morphology and dispersion.

The systematics of *Iphitime* and *Ophryotrocha* has been controversial during the last decade. *Iphitime*, which consists of only seven species, all symbionts of brachyuran crabs, had been placed in different families (Høisaeter & Samuelsen 2006). However, a recent phylogenetic analysis showed that *Ophryotrocha* appears monophyletic only when *Iphitime* is included in the analysis (Wiklund et al. 2009; Wiklund et al. 2012), grouping *Iphitime* with *O. geryonicola* in the same clade. Therefore, currently both genera are included within the family Dorvilleidae (Read & Fauchald 2014).

Specimens of symbiotic *Iphitime* and *Ophryotrocha* are difficult to obtain given the deep-sea habitat of the host crabs, which must first be collected. However, by analysing the population structure of these species, we expect to contribute significantly to the understanding of the recent evolution of species with different bathymetric patterns and the relationships between symbionts and hosts. The close phylogenetic relationship (see figure 5 in Wiklund et al. 2009) and common characteristics of these polychaete species, such as living in similar hosts in nearly the same regions, but at different depths, may

help separate factors that have influenced their evolutionary histories. Consequently, in the present study, we compare the population genetic structure of *I. cuenoti* and *O. mediterranea* in the Western Mediterranean Sea and nearby Atlantic waters, analysing potential contemporary genetic breaks and historical events, as determined by current genetic diversity. For these analyses, we use the mitochondrial cytochrome oxidase c subunit I (COI) gene, which has proved useful for assessing relationships among conspecific populations (Avisé et al. 1984) and has been extensively used to barcode marine invertebrates (Bucklin et al. 2011).

Material and methods

Study area and sample collection

Mediterranean *Iphitime cuenoti* (Figure 1A, 2B) and *Ophryotrocha mediterranea* (Figure 1C) specimens were obtained during the MEDITS_ES surveys performed by the Spanish Oceanographic Institute (IEO) on board the R/V Cornide Saavedra in May 2012 (Bertrand et al. 2002) along the Mediterranean coasts of the Iberian Peninsula. The stations were based on a sampling scheme randomly stratified by bathymetric limits (50, 100, 200, 500 and 800 m depth) and geographical sectors. The bottom trawl gear used had a cod-end mesh size of 20 mm, allowing the capture of demersal species.

The Atlantic specimens of *Iphitime cuenoti* were obtained during the ARSA annual bottom trawls surveys also performed by the IEO on board R/V Cornide Saavedra in March 2013 in the Gulf of Cadiz (Silva et al. 2011). The sampled depths in this cruise did not reach the deeper habitat of *Geryon longipes*. These Atlantic samples correspond to the continental shelf and upper-middle slope and were obtained by a random stratified design with five depth strata (15-30, 31-100, 101-200, 201-500 and 500-700 m depth).

Samples from geographically close locations were grouped together into nine populations according to the geomorphology of the area, and previous biogeographic studies (Abelló et al. 2002; Rufino et al. 2005): Gulf of Cádiz (CADI); Western Alborán (WALB) from Gibraltar to Cape Sacratif; Eastern Alborán (EALB) from Cape Sacratif to Cape Gata; Alborán Island (ALBI); Alacant (ALAC) from Cape Palos to Cape La Nao; Valencia (VALE) from Cape La Nao to the Castelló/Columbretes Islands; Ebro Delta region (DELTA) from the Castelló/Columbretes Islands to Cape Salou/Tarragona; Central Catalonia (CCAT) from Cape Salou/Tarragona to Barcelona and North Catalonia (NCAT), from Barcelona to Cape Creus (Figure 2; Table 1). For genetic analyses, only five and one *Iphitime* samples could be obtained from the DELTA and CCAT regions, respectively.

The polychaetes were found inside the branchial chambers of their host brachyuran crabs. *Iphitime cuenoti* was found inside *Liocarcinus depurator* (Figure 1D), *Goneplax rhomboides* (Figure 1E), *Macropipus tuberculatus* (Figure 1F) and *Bathynectes maravigna* (Figure 1G), while *Ophryotrocha mediterranea* occurred only inside *Geryon longipes* (Figure 1H). For all crabs caught, sex and size were measured as in Martin et al. (1991). The carapace of the crab was separated from the body to check for the presence of the polychaetes, which were preserved in absolute ethanol for molecular studies. The prevalence, defined as the ratio between the total number of hosts and those infested with the polychaetes, was examined for *L. depurator*, *M. tuberculatus* and *G. longipes* as a function of its geographic area. Given the low number of polychaetes found inside *G. rhomboides* and *B. maravigna*, prevalence could not be estimated. Significance of infestation prevalence between sexes was assessed with a heterogeneity G-test (Sokal & Rohlf 1981).

DNA extraction, PCR amplification and sequencing

DNA extraction was performed using the QIAGEN Biosprint 15 DNA Blood Kit (Qiagen Iberia S.L., Madrid), following the manufacturer's instructions, with an extended period of proteinase K lysis (about 15 hours incubation at 55°C). Partial fragments for mitochondrial cytochrome oxidase c subunit I (COI) were amplified using the primers LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' (Folmer et al. 1994) and COI-H 5'-TCAGGGTGACCAAAAAATCA-3' (Machordom et al. 2003). The following thermal cycle conditions were used: an initial denaturation step at 94°C (4 min), followed by 40 cycles of 94°C (45 s), 46-48°C (1 min), 72°C (1 min) and a final extension at 72°C (10 min). The PCR mix contained 2 µl of DNA template, 0.16 µM of both primers, 0.2 mM of each dNTP, 5 µl of buffer with final concentration of 2 mM MgCl₂, 1.5 U of *Taq* DNA Polymerase (Biotools, Madrid, Spain) and ddH₂O for a total volume of 50 µl. The amplified fragments (658 base pairs) were cleaned by ethanol precipitation prior to sequencing both strands using the same PCR primers and Big Dye Terminator (Applied Biosystems). Sequences were run in an ABI 3730 Genetic Analyzer (Applied Biosystems).

The DNA sequences obtained were verified, cleaned at the primer ends and edited using the Sequencher program (Gene Code Corporation). Indels were not present for the alignment of the obtained

sequences. The obtained sequences have been deposited in GenBank (reference numbers KR004611 - KR004790) and respective voucher specimens at the Museo Nacional de Ciencias Naturales of Madrid (reference numbers (MNCN 16.01/17001 – MNCN 16.01/17180).

Diversity estimates and genetic differentiation

Number of haplotypes (Nh), haplotype diversity (h) and nucleotide diversity (π) were calculated for both species with the Arlequin v3.5 software (Excoffier et al. 2005). Given the low number of *Iphitime cuenoti* individuals collected from DELT, CCAT and NCAT, and the geographical proximity of these populations coupled with the absence of any putative oceanographic barrier, individuals were grouped as a single population to obtain diversity estimates. The analysis of molecular variance (AMOVA) was also performed with Arlequin to investigate the hierarchical population structure among and within populations, based on haplotype frequencies.

Two approaches were performed for *Iphitime cuenoti*. First, we defined four geographical groups according to possible oceanographic barriers: 1) CADI: west GS Atlantic waters; 2) West AOF: including WALB, EALB and ALBI (which are located between the GS and AOF); 3) ALAC (region from north AOF to south IC) and 4) North of the IC (VALE, DELT, CCAT and NCAT). Second, we defined two groups separated by the AOF: CADI, WALB, EALB and ALBI to the west, and all remaining locations north of the AOF.

Two approaches were also performed for *Ophryotrocha mediterranea*. First, we compared the populations from the Alborán sectors (EALB, WALB, and ALBI) with the Western Mediterranean ones. Second, we compared EALB with all other populations together.

For further analyses of population genetic structure, the pairwise F_{st} values between populations based on haplotype frequencies were assessed by performing 10,000 permutations using Arlequin.

Haplotype networks were constructed for each species using the Median Joining Network algorithm (Bandelt et al. 1999) implemented in Network v4.6.1.2 (Fluxus Technology). The obtained networks allowed examining the geographical distribution of mtDNA haplotypes, and the distribution by depth and brachyuran host (for *Iphitime cuenoti* only).

Due to the divergence and patterns found between two *Ophryotrocha mediterranea* lineages, species differentiation was addressed by adding sequences from GenBank for closely related *Ophryotrocha* species: *O. geryoncola* (GQ415476), *O. adherens* Paavo, Bailey-Brock and Akesson, 2000 (JQ310756) and *O. puerilis* Claparède & Metschnikow, 1869 (GQ415486).

Historical demography

Historical population demography patterns were tested by mismatch distribution, analysing pairwise differences among haplotypes using the model of sudden expansion (Slatkin & Hudson 1991; Rogers & Harpending 1992) and testing the null hypothesis of population growth in Arlequin (Excoffier et al. 2005). Patterns were also assessed by Tajima's D test and Fu's F_s test (Fu 1997), implemented in DnaSP (Librado & Rozas 2009).

The time since the most recent expansion (t) was calculated from mismatch distribution analysis of parameter τ based on the equation $\tau = 2\mu kt$ (Rogers & Harpending 1992), where μ is 1.9% per million years (the nucleotide mutation rate (Hickerson et al. 2003) and k is the sequence length of the analysed fragment (658 bp for COI in both species).

Results

Infestation rates

Of the 1023 *Liocarcinus depurator* and 830 *Macropipus tuberculatus* crabs collected, 67 and 96, respectively, contained the polychaete *I. cuenoti* (Table 2). Thus, the overall prevalence rate was 6.55% in *L. depurator* and 11.57% in *M. tuberculatus*. In *L. depurator*, females showed a significantly ($p < 0.001$) higher prevalence (9.80%) than males (3.44%); in *M. tuberculatus*, there were no significant differences between the sexes (12.04% for males; 10.88% for females; $p > 0.05$). In relation to geographic area, infestation in *L. depurator* was higher in the Alborán Sea sectors (WALB, EALB and ALBI) and in Valencia (VALE), while *M. tuberculatus* showed the highest infestation in the Alborán Island samples, followed by CADI, VALE and ALAC. Infestation was low in the Catalan sectors (DELT, CCAT and NCAT).

The occurrence of *Ophryotrocha mediterranea* was detected in 38 of the 130 male *Geryon longipes* individuals collected. It was not detected in any of the 26 female crabs examined. Thus, the overall prevalence was 24.39%. The highest rates of infestation in *G. longipes* were detected in the

Alborán Sea region (WALB, EALB and ALBI). Rates were much lower in the other geographic sectors sampled (Table 2).

Genetic diversity

A total of 59 and 22 COI haplotypes were detected among 108 and 72 individuals of *Iphitime cuenoti* and *Ophryotrocha mediterranea*, respectively. Polymorphisms were observed at 21 of the 658 bp sequenced in both species. Haplotype diversity was high in both species, but higher in *I. cuenoti* than in *O. mediterranea*, while nucleotide diversity was low in both species (Table 3). Similarly, haplotype and nucleotide diversity of *I. cuenoti* in relation to three of the brachyuran hosts were high and low, respectively (Table 3).

Population differentiation

The AMOVA approaches for *Iphitime cuenoti* and *Ophryotrocha mediterranea* revealed a low, non-significant ($p > 0.05$) percentage of variance among groups and among populations within groups (Tables 4 and 5). In contrast, the percentages of variance within populations were high (94.06 and 91.60% in *I. cuenoti*, and 88.82 and 82.02% in *O. mediterranea* for each approach, respectively) with a significant ($p < 0.05$) amount of explained variance.

In the case of *Iphitime cuenoti*, however, the percentage of variance tended to be slightly higher when comparing the two groups separated by the AOF (7.67%). For *Ophryotrocha mediterranea*, this trend was slightly more marked (8.52%). Interestingly, this variance was much more evident when comparing EALB with all other populations (16.23%).

The pairwise F_{st} for *Iphitime cuenoti* (Table 6) were mainly low and non-significant, except when comparing CADI and WALB with ALAC, VALE and NORTH (DELTA+CCAT+NCAT), and ALBI with ALAC and VALE. When grouping VALE and NORTH (to obtain a more representative population), significant values were found with respect to CADI, WALB and ALBI.

In the case of *Ophryotrocha mediterranea*, the only significant, high pairwise F_{st} values observed were those comparing EALB with VALE and NCAT (0.21667 and 0.32788, respectively). Although the F_{st} obtained when comparing EALB with WALB and ALAC were relatively high (0.17066 and 0.15315, respectively), they were not significant (Table 6).

The network of *Iphitime cuenoti* showed a dominant haplotype, common in all localities, together with several derived ones with low frequencies (Figure 3). Most derived haplotypes occurred in one specimen, and differed from the dominant one in just one or two mutational steps. Nevertheless, some singleton-derived haplotypes from the AOF (CADI and populations around the Alborán Island) were separated up to 13-14 mutational steps from the dominant one. In addition, the haplotype network did not show any evident structure related with the brachyuran hosts (Figure 4).

The haplotype network of *Ophryotrocha mediterranea* showed two main groups of haplotypes (L1 and L2) separated by at least four mutational steps (Figure 5). Each group has two common haplotypes occurring in all localities and several haplotypes derived from each common haplotype, mostly occurring in single individuals.

The genetic divergences found between *Ophryotrocha mediterranea* individuals belonging to L1 and L2, and those between *O. geryonicola* (the Atlantic species) and L2, were close to 1%, while the divergence between *O. geryonicola* and L1 was less than 1%. In contrast, the genetic divergence between *O. geryonicola* and *O. adherens* and *O. puerilis* were 23 and 31%, respectively.

Historical demography

The sums of squared deviations of mismatch distribution for *Iphitime cuenoti* and *Ophryotrocha mediterranea* were not significantly different, indicating a fit to the demographic expansion model tested (Figure 6). Nevertheless, the entire sample of *O. mediterranea* displayed a distinctive bimodal pattern with distant peaks, implying a significant deviation from the distribution expected under the sudden expansion model (as supported by the lack of significance of the Tajima's D test, Table 7). The separate analysis of L1 and L2 did not allow rejecting the hypothesis of sudden expansion (Figure 6).

Both Tajima's D and Fu's F_s tests were high, negative and significant for *Iphitime cuenoti*, in agreement with the sudden expansion model. In *Ophryotrocha mediterranea* (with L1 and L2 either pooled together or separated), only the Fu's F_s tests were negative and significant, but lower than in *I. cuenoti* (Table 7).

Estimates of the approximate time since the most recent expansion suggest that demographic expansion events occurred 85.746 and 250.115 years ago for *Iphitime cuenoti* and *Ophryotrocha mediterranea*, respectively. In the case of the two lineages of *O. mediterranea*, expansion events were estimated to have occurred 72.488 and 88.733 years ago for L1 and L2, respectively (Figure 6).

Discussion

The present study is the first in comparing the phylogeography and genetic structure of two symbiotic polychaete species, *Iphitime cuenoti* and *Ophryotrocha mediterranea*, which lives in association with brachyuran crabs. We used the mitochondrial COI gene, a marker that allows the detection of both historical processes and contemporary gene flow barriers (Avise, et al. 1987) to study patterns of species genetic structure. Our results show that both polychaetes present a weak genetic structure, though higher in *O. mediterranea*. AOF was identified as a weak barrier to gene flow for *I. cuenoti*, in agreement with the AMOVA that revealed a higher among-group variation percentage when comparing the Atlantic and the Mediterranean populations. In contrast, *O. mediterranea* showed a higher among-group genetic structure, especially when the Eastern Alborán population was considered. More interestingly, *O. mediterranea* showed two distinctive haplotype groups in all studied geographic areas. The different patterns may have been driven by both historical and contemporary factors, including behavioural, oceanographic, and climatic processes, which are discussed below.

1. Population structure affected by oceanographic discontinuities and other contemporary effects

The high haplotype and low nucleotide diversities in *Iphitime cuenoti* and *Ophryotrocha mediterranea* indicate a high degree of genetic homogeneity among the studied populations. Nevertheless, the genetic differentiation differed for each species. *Iphitime cuenoti* showed a star-shaped haplotype network, with some derived haplotypes almost exclusively in individuals from the Atlantic and Mediterranean transition zone and, according to the population structure analysis, a certain geographic structuring. The significant F_{st} of the NW Mediterranean vs. the Atlantic and Alborán region populations could result from an effect of AOF on gene flow. This front is considered a barrier of gene flow for various marine organisms (Zane et al. 2000; Patarnello et al. 2007; Pérez-Losada et al. 2007; Galarza et al. 2009; Mokhtar-Jamaï et al. 2011; Palero et al. 2011), but apparently has no effect for many others (Patarnello et al. 2007; Zulliger et al. 2009; Boissin et al. 2011; Borrero-Pérez et al. 2011). The effect of AOF is therefore species-specific and likely depends on the main dispersion mechanism and coupling behaviour characteristics of each species.

GS and IC do not affect the genetic structure of *Iphitime cuenoti*. The effect of GS as a gene flow barrier for marine species has been controversial due to the discordant results, which includes null or very weak effects (Calderón et al. 2008; Guerra-García et al. 2009; Pérez-Portela et al. 2013), obtained for different Atlantic-Mediterranean species (Bargelloni et al. 2003; Patarnello et al. 2007). GS was reported as a barrier for only seven species among the thirty published papers examined since 2008, which included studies of marine invertebrates and fishes (Table 8). Supporting this finding, a high percentage of species are common to the western Mediterranean and GS (Carballo et al. 1997). The possible influence of GS seems weak in shaping population structure compared with other factors, such as reproductive biology, behaviour, bathymetric distribution or historical events. In particular, the recolonization events after the Messinian salinity crisis (García-Castellanos et al. 2009) and during the Pleistocene interglacial periods (Bianchi et al. 2012) certainly contributed to the Atlantic-Mediterranean faunal similarities and dissimilarities.

The dumbbell-like haplotype network of *Ophryotrocha mediterranea* is typical for species with two distinctive lineages (see below for detailed discussion). This species appears to be genetically more structured than *Iphitime cuenoti*, as indicated by the high and significant F_{st} values of EALB vs. NCAT and VAL, and the AMOVA results. Although non-significant, the percentage of variation between EALB and all other populations was high (> 16%). The percentage of variation of all Alborán populations vs. the NW Mediterranean ones was lower but still higher than that for *I. cuenoti* (> 8%). The bathymetric distribution of *O. mediterranea*, a deep-water species typically found between 600 and 1200 m depth, prevents acting the shallower AOF as a gene flow barrier. Therefore, the results suggest that isolation of the EALB populations of *O. mediterranea* may be related to a particular deep-sea circulation system in the area.

Besides the possible barrier effect of the oceanographic configuration of the area, biology may also play a relevant role in understanding the current population structure. The peculiarities of the symbiotic relationships of *Iphitime cuenoti* and *Ophryotrocha mediterranea* with their host crabs may also affect or determine their distributions. *Iphitime cuenoti* occurs inside various host species (Abelló et al. 1988; Comely & Ansell 1989; Høisaeter & Samuelsen 2006), whereas *O. mediterranea* only occurs inside *Geryon longipes*. *Liocarcinus depurator* and *Macropipus tuberculatus*, the most frequent *I. cuenoti* hosts, are swimming crabs that inhabit muddy bottoms. The former is found more frequently and abundantly over the continental shelf (< 200 m depth), and the latter between 200 and 400 m depth (Abelló et al. 2002; Rufino et al. 2005). In contrast, *G. longipes* inhabits characteristically waters deeper than 500 m (Abelló & Valladares 1988; Cartes 1993). According to our results, *I. cuenoti* did not show a

defined population structure in relation to host species. In other words, there are no haplotypes that are preferentially distributed in one host species. Furthermore, the population genetic structure of *L. depurator* and *M. tuberculatus* did not show any similarity to that of *I. cuenoti*: for both crab species, GS was detected as gene barrier, as well as IC for *L. depurator* (García-Merchán et al. 2012). The low infestation percentage of the crabs, which would likely be higher in the case of a strict species-specific endosymbiotic relationship, supports these findings. Therefore, *I. cuenoti* seems to be an opportunistic symbiont that evolved independently from each host crab. Some *I. cuenoti* hosts are portunid, or swimming crabs (Abelló et al. 1988), at least in their early crab juvenile stages, implying they are able to move vertically in the water column, and thus may account for the wider distribution and higher gene flow of *I. cuenoti*.

Unlike *Iphitime cuenoti*, *Ophryotrocha mediterranea* is considered a Mediterranean, species-specific symbiont, currently known from a single host crab, *Geryon longipes*, whose bathymetric distribution ranges from 450 to almost 2000 m depth (Desportes et al. 1977; Mori & Belloni 1985; Martin et al. 1991; Cartes & Sardà 1992). Conversely, the closely related species, *O. geryonicola*, is known to live inside three North Atlantic crabs, *G. tridens* Krøyer, 1837, *G. quinquedens* Smith, 1897 and *Cancer borealis* Stimpson, 1859 (Gaston & Benner 1981; Pfannenstiel et al. 1982). Two Geryonidae crabs, *Chaceon mediterraneus* Manning and Holthuis, 1989 and *Zariquieyon inflatus* Manning and Holthuis, 1989, are known to occur in the Mediterranean, although very scarcely in waters deeper than 2000 m. However, the presence of *Ophryotrocha* spp. within these species has not been reported to date. *Ophryotrocha mediterranea* is thus more specific in choice of host crab than *O. geryonicola* and *I. cuenoti*, suggesting that the bathymetric distribution of the species and the relatively larger crab size may, in part, explain host specificity. It should be noted that *O. mediterranea* is much larger in size than *I. cuenoti* and is usually present in a higher number of individuals within its host. Unfortunately, the genetic population structure of *G. longipes* remains yet unknown, preventing co-evolutionary inferences on the host/symbiont relationship or on common biogeographic influences (such as barriers to gene flow). *Geryon longipes*, like *O. mediterranea*, is widely distributed in the Mediterranean Sea (Company et al. 2008; Di Camillo et al. 2008; Follesa et al. 2009). However, it is also found in adjacent Atlantic waters, from Morocco to the Bay of Biscay (Orsi-Relini & Mori 1979; Guerao et al. 1996), which does not seem to be the case for *O. mediterranea* (Martin et al. 1991). *Ophryotrocha mediterranea* were found exclusively in male crabs, and with low infestation rates. The low number of total females found and the larger size of the males may account for these observations. Therefore, the symbiotic and evolutionary relationships between this species and its host crab are certainly an interesting topic for future studies.

Unfortunately, little information is available about the reproductive cycle of these polychaete species. *Iphitime cuenoti* is sexually dimorphic: males and females differ in size and type of branchiae, similar to *I. paguri* Fage and Legendre, 1934 (a similar species from North Atlantic waters, commensal of hermit crabs). Females apparently use branchiae as pouches for the developing gametes (Comely & Ansell 1989; Høisaeter & Samuelsen 2006). We did not observe females with gametes, but juveniles were found in some samples. According to Abelló et al. (1988), Comely & Ansell (1989) and our own observations, there may be from one to four, or rarely five, *I. cuenoti* individuals inside a branchial chamber, some of which were juveniles. Both characteristics (i.e., gamete development close to or on females and the presence of juveniles) suggest larval development inside the branchial chambers, although a more complex mechanism, including a planktonic phase and a chemically mediated host-attraction driving settlement, cannot be fully discarded. In fact, this second possibility agrees well with the finding that the haplotypes from pairs of worms sharing the same host crab were always different. Therefore, it seems evident that *I. cuenoti* is able to leave its host to infest other crabs. However, we cannot assess at which stage (i.e., larvae, juvenile or adult) this may occur as the life cycle of *I. cuenoti* is currently unknown. The moulting and mating behaviour of the host may also be connected to the reproductive cycle of *I. cuenoti*. However, at present, we have no data to support this interesting possibility.

Information on the reproductive cycle of *Ophryotrocha mediterranea* is also scarce. In contrast, it is well known that *O. geryonicola* is gonochoric, has oocytes that float freely in the coelom, and does not form egg masses, like other species of the genus (Åkesson 1973; Pfannenstiel et al. 1982). Most species of *Ophryotrocha* are continuous breeders: fertilization seems to be a kind of pseudocopulation where eggs and sperm are spawned together in a gelatinous matrix, and the free-swimming larval stage is short, with the larvae sometimes laying in the vicinity of the egg case (Åkesson 1984; Paavo et al. 2000; Paxton & Åkesson 2007). However, there are many different reproduction strategies within the genus (Åkesson, 1984), which prevent attributing one to *O. mediterranea*. The presence of juveniles, the number of worms per crab (up to 15) and the fact that all crabs hosted several different haplotypes, including representatives of the two lineages identified in the haplotype network, all strongly suggest the lack of a recent selective process in *O. mediterranea* and the presence of a homogenized stable

metapopulation. A long-lasting planktonic stage contributing to this homogenization could be logically inferred, but further studies are required to define the links between reproduction and the genetic structure of this symbiotic polychaete. Whether the life cycle of the symbiont is connected with peculiarities of the host is also an open question. The fact that all infested hosts were males suggests that such a link may be more than a simple hypothesis.

2. *Demographic history and genetic diversity*

The COI sequences of both symbiotic worms were characterized by high haplotype diversity (higher in *Iphitime cuenoti*) and low nucleotide diversity, like in other Atlantic-Mediterranean invertebrates (Pérez-Portela et al. 2010; Borrero-Pérez et al. 2011; García-Merchán et al. 2012; Pérez-Portela et al. 2013), including polychaetes (Jolly et al. 2006; Iannotta et al. 2007). Interestingly, the demographic history of both species seems to be different. The star-shaped network, the significant Fu's F_s and Tajima's D indexes and the unimodal mismatch distribution of *I. cuenoti*, are characteristic of species that have undergone a recent expansion (since selection must not be considered if COI acts as a neutral marker) (Rogers & Harpending 1992). Conversely, the bimodal mismatch distribution in *Ophryotrocha mediterranea* and the non-significant Tajima's D index indicate a long-term stable population, according to Rogers & Harpending (1992) and Slatkin & Hudson (1991). In addition, the presence of two groups of haplotypes in all localities with no obvious morphologically distinct characters suggests that *O. mediterranea* may currently have a Western Mediterranean panmictic population.

The dumbbell-like haplotype network and the bimodal distribution of *Ophryotrocha mediterranea* follow the same pattern as populations that have experienced a secondary contact between two genetically differentiated populations (Avise 2000; Avise et al. 1992; Tabata & Watanabe 2013). If two long-term isolated populations experienced population expansions, the species should display a dumbbell-shaped gene tree with two star-bursts connected by a longer branch (Avise 2000), as is observed in the case of *O. mediterranea*. Moreover, these potential expansions may have occurred during the same period of expansion of *Iphitime cuenoti*.

According to the estimates for both species, expansion probably happened after the late Pleistocene, when drastic climatic changes occurred in both the Atlantic and Mediterranean basins, and the sea level dropped 100 to 120 m below current levels, limiting the entrance of Atlantic species into the Mediterranean basin (Emig & Geistdoerfer 2004). Following this period, Mediterranean marine conditions were restored with the flow of Atlantic waters through GS, and the Mediterranean Sea was recolonized by species of Atlantic origin or those that found refuge there during such periods (Bianchi et al. 2012). These past events support the hypothesis of a secondary contact between allopatrically divergent lineages of *Ophryotrocha mediterranea*, which probably evolved from an originally Atlantic population, as discussed below. However, these climatic changes seem not to have similarly affected *Iphitime cuenoti*, as it showed a star-like network, which might indicate a reduction of the sample size due to sea level changes but without the isolation and posterior differentiation of two lineages as in *O. mediterranea*. The genetic structure observed for the two analysed species are likely a result of contrasting features (mainly depth range and host specificity), which have led the species to be differentially affected by historical events.

3. *Lineages of Ophryotrocha mediterranea*

Ophryotrocha mediterranea shares many features with the Atlantic species *O. geryoncola*. Their morphology is very similar: both have long bodies and a large number of segments (Martin et al. 1991). Both are also deep-sea species living commensally with Geryonid crabs. However, there are a number of taxonomically robust morphological characters that differentiate both species (Table 9).

The low molecular divergences found between the two Mediterranean lineages and the Atlantic species support the hypothesis of a unique metapopulation, in which the two lineages experienced a secondary contact, and suggest that *Ophryotrocha mediterranea* and *O. geryoncola* may have a common ancestor. The similar behavioural features and low molecular divergence between *O. geryoncola* and *O. mediterranea* may indicate the possible existence of a single species, widely distributed from the NE Atlantic Ocean to the Mediterranean Sea. Accepting this hypothesis means that *O. mediterranea* would be considered a junior synonymy of *O. geryoncola*. However, further research must be addressed to validate this hypothesized synonymy. In particular, it must be determined whether the two lineages occur within Atlantic populations and whether the characteristic morphological features reported in the original description of *O. mediterranea* are insufficient for distinguishing these two species.

Conclusions

This research compares the phylogeography of two symbiotic polychaetes found in brachyuran crabs that show distinctive evolutionary histories, as well as different phylogeographic patterns based on haplotype networks and mismatch distributions.

Iphitime cuenoti has a weak population structure, with highly homogenized gene flow between Mediterranean and near Atlantic waters. Populations from Cádiz and Western Alborán showed the highest and significant F_{st} when compared with the Western Mediterranean ones, indicating that AOF has been a weak gene flow barrier for the species. The wide bathymetric distribution (caused by a low host specificity), together with the vertical swimming capacity of the main host crabs, may have contributed to this gene flow homogenization.

A star-like network and unimodal distribution of haplotypes in *Iphitime cuenoti* likely indicates a recent population expansion, which may have occurred after the Pleistocene glaciations. Further analysis of the genetic structure and phylogeographic patterns of the North Atlantic populations of the species would shed light on its evolutionary history.

Ophryotrocha mediterranea also showed a homogenized gene flow; however, in this case, the Eastern Alborán population can be well distinguished from all the other populations (according to AMOVA and F_{st} results), probably a result of local oceanographic constraints such as a deep-sea current system.

The dumbbell-like haplotype network and bimodal distribution observed for *Ophryotrocha mediterranea* typically indicate the existence of a unique metapopulation with two distinctive lineages that had secondary contact as a result of an expansion after isolation event, likely during the Pleistocene glaciations, and subsequently gave rise to homogenized gene flow.

The low molecular divergence between the Atlantic and Mediterranean species of *Ophryotrocha* associated with brachyuran hosts, together with the historical demography of *O. mediterranea*, suggest that this species may be a junior synonymy of *O. geryoncola*, which would then have a wide Atlantic-Mediterranean distribution similar to that of *Iphitime cuenoti*. To confirm this synonymy, however, it is necessary to perform a more extensive population study along the entire distribution area of both species, to determine if a barrier exists to separate the species or if a cline exists in which only the extremes have been studied.

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Figure 1. Map of Western Mediterranean and Cadiz showing sampled localities of *I. cuenoti* and *O. mediterranea*. The major oceanographic fronts showed in black lines (GS: Gibraltar Strait, AOF: Almería-Oran front; IC: Ibiza Channel). Studied populations: CADI: Gulf of Cádiz, WALB: Western Alborán, EALB: Eastern Alborán, ALBI: Alborán Island, ALAC: Alacant; VALE: Valencia; DELT: Ebro delta region, CCAT: Central Catalonia and NCAT: Northern Catalonia.

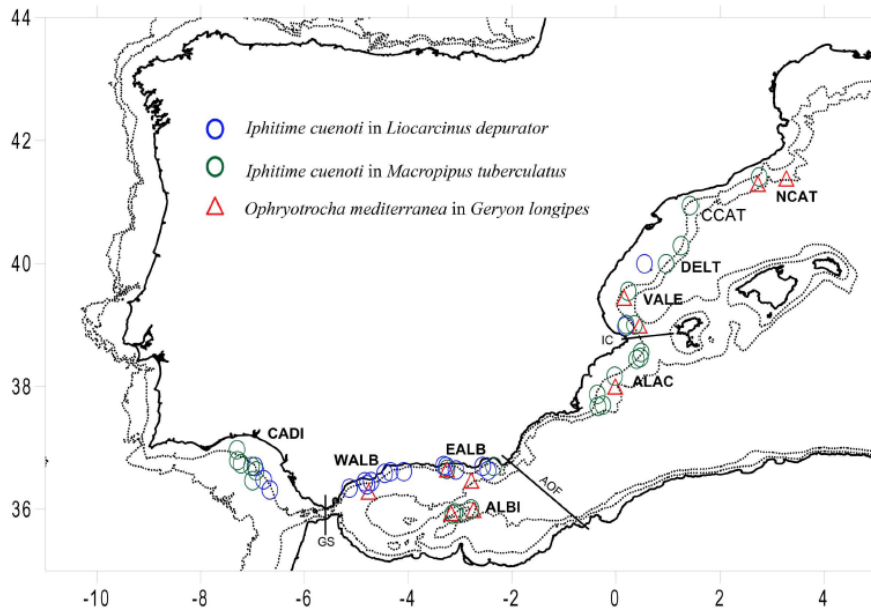


Figure 2. A. *Iphitime cuenoti*, male. B. *Iphitime cuenoti*, female. C. *Ophryotrocha mediterranea*. D. *Liocarcinus depurator*. E. *Goneplax rhomboides*. F. *Macropipus tuberculatus*. G. *Bathynectes maravigna*. H. *Geryon longipes*.

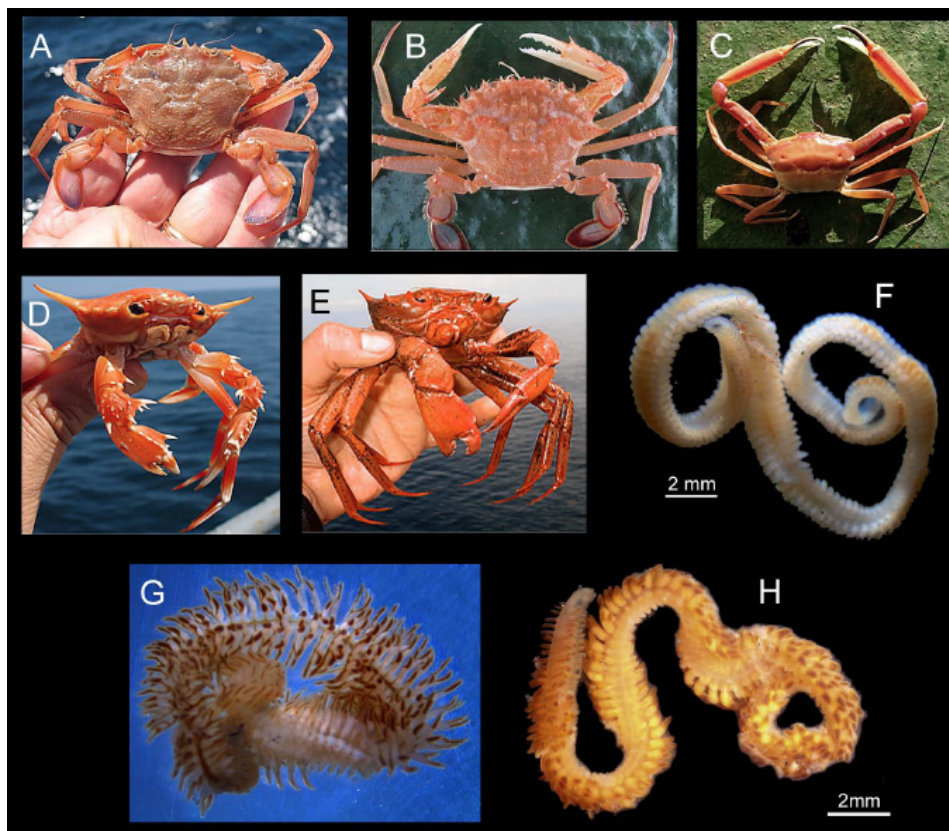


Figure 3. Median Joinin haplotype networks of mtDNA COI sequences for *Iphitime cuenoti*. Empty circles represent missing haplotypes. Black points represent mutational steps. Circle size and pie size are proportional to the haplotype frequency. Alborán includes WALB, EALB and ALBI. WALB: Western Alborán, EALB: Eastern Alborán, ALBI: Alborán Island, CADI: Gulf of Cádiz (Atlantic), VALE: Valencia, DELT: Ebro Delta region, CCAT: Central Catalonia, NCAT: Northern Catalonia, ALAC: Alicante.

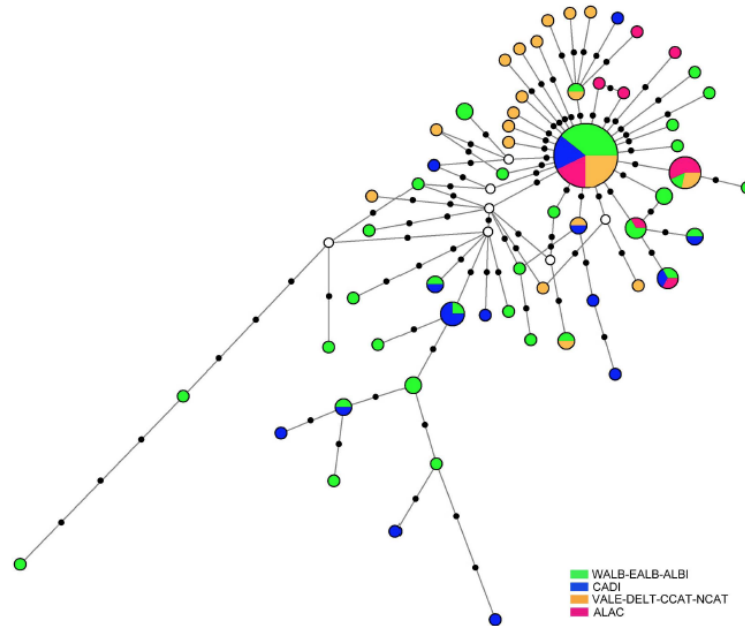


Figure 4. Median Joining haplotype networks of mtDNA COI sequences for *Iphitime cuenoti* in relation to its host crab. Empty circles represent missing haplotypes. Black points represent mutational steps. Circle size and pie size are proportional to the haplotype frequency.

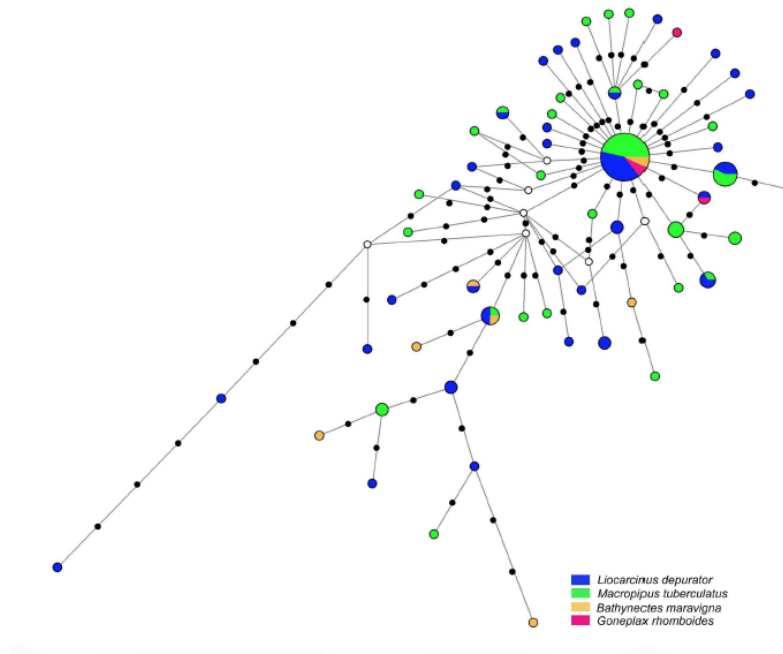


Figure 5. A: Median Joining haplotype networks of mtDNA COI sequences for *Ophryotrocha mediterranea*. Empty circles represent missing haplotypes. Black points represent mutational steps. Circle size and pie size are proportional to the haplotype frequency. B. Haplotype distribution in the studied populations of both lineages 1 and 2. WALB: Western Alborán, EALB: Eastern Alborán, ALBI: Alborán Island, VALE: Valencia, NCAT: Northern Catalonia, ALAC: Alicante.

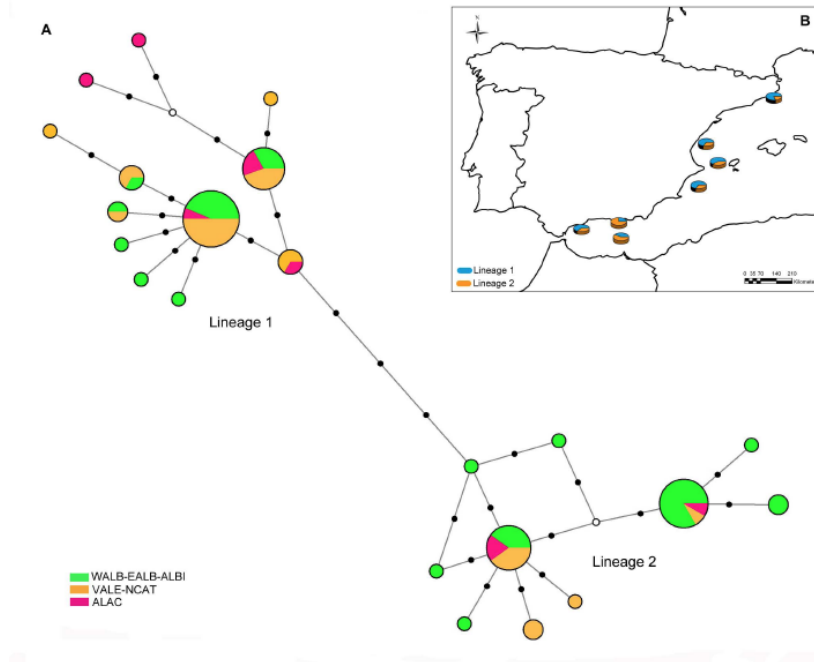


Figure 6. Mismatch distributions. Graph for *Iphitime cuenoti* represent the mismatch distribution for W. Mediterranean basin and Atlantic waters. Graphs for *Ophryotrocha mediterranea* include the whole population from W. Mediterranean and lineages 1 and 2.

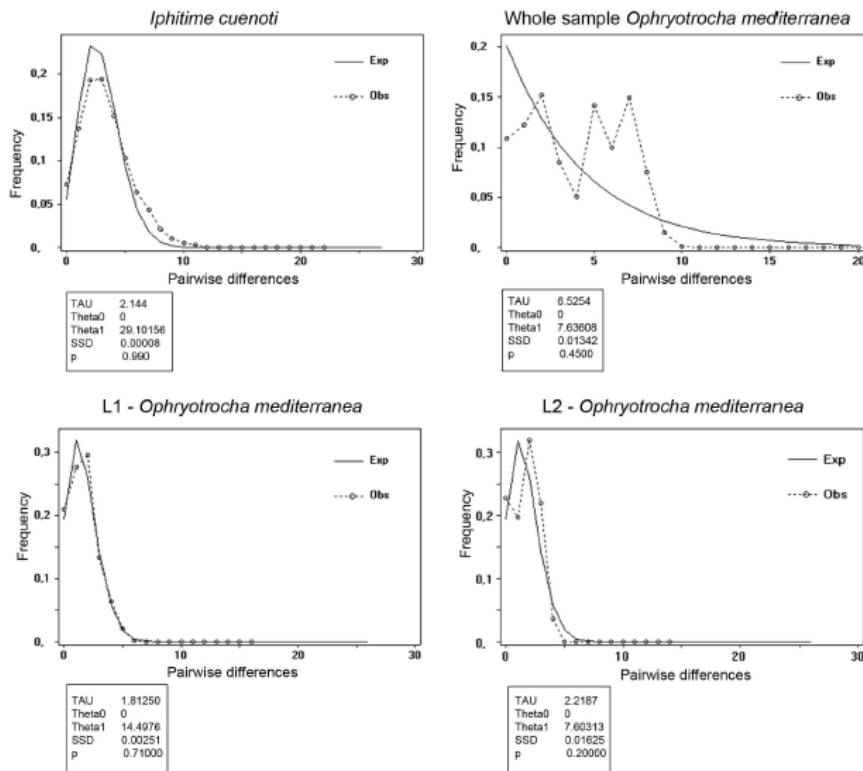


Table 1. Sequenced samples of *Iphitime cuenoti* (found inside *Liocarcinus depurator*, *Macropipus tuberculatus*, *Bathynectes maravigna* and *Goneplax rhomboides*) from Western Mediterranean and Gulf of Cádiz, and *Ophryotrocha mediterranea* (found inside *Geryon longipes*) from Western Mediterranean. In COI column, the number of individuals sequenced. CADÍ: Gulf of Cádiz (Atlantic); WALB: Western Alborán; EALB: Eastern Alborán; ALBI; Alborán Island; ALAC: Alicante; VALE: VALENCIA; DELT: Ebro Delta region; CCAT: Central Catalonia; NCAT: Northern Catalonia. GenBank sequence numbers: KR004611-KR004790. Voucher specimens at MNCN, Madrid: MNCN 16.01/17001 to 16.01/17180.

Study Area	COI	Polychaete species	Brachyuran host	Depth (m)
CADIZ	7	<i>I. cuenoti</i>	<i>L. depurator</i>	92-404
	8	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	92-486
	5	<i>I. cuenoti</i>	<i>B. maravigna</i>	530-640
WALB	13	<i>I. cuenoti</i>	<i>L. depurator</i>	85
	1	<i>I. cuenoti</i>	<i>B. maravigna</i>	540
	8	<i>O. mediterranea</i>	<i>G. longipes</i>	759
EALB	11	<i>I. cuenoti</i>	<i>L. depurator</i>	131-340
	2	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	240-551
	2	<i>I. cuenoti</i>	<i>G. rhomboides</i>	68-340
	12	<i>O. mediterranea</i>	<i>G. longipes</i>	551-774
ALBI	4	<i>I. cuenoti</i>	<i>L. depurator</i>	329
	13	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	117-330
	3	<i>I. cuenoti</i>	<i>B. maravigna</i>	545
	16	<i>O. mediterranea</i>	<i>G. longipes</i>	764-791
ALAC	2	<i>I. cuenoti</i>	<i>L. depurator</i>	111
	12	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	175-414
	1	<i>I. cuenoti</i>	<i>G. rhomboides</i>	591
	9	<i>O. mediterranea</i>	<i>G. longipes</i>	591
VAL	7	<i>I. cuenoti</i>	<i>L. depurator</i>	111-345
	10	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	298-367
	17	<i>O. mediterranea</i>	<i>G. longipes</i>	739-581
DELT	4	<i>I. cuenoti</i>	<i>L. depurator</i>	73
	1	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	108
CCAT	1	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	330
NCAT	1	<i>I. cuenoti</i>	<i>M. tuberculatus</i>	274
	10	<i>O. mediterranea</i>	<i>G. longipes</i>	622-657

Table 2. Infestation rates of the polychaetes *Iphitime cuenoti* and *Ophryotrocha Mediterranea* for the crabs *Liocarcinus depurator*, *Macropipus tuberculatus* and *Geryon longipes* in function of their geographic area.

<i>Liocarcinus depurator</i> (+ <i>Iphitime cuenoti</i>)										
GEOGRAPHIC AREA	Males				Females				Overall	
	non-infested	Infested	Total males	% Infested	non-infested	Infested	Total females	% Infested	Total	% Infested
CADI	56	5	61	8,2	63	2	65	3,1	126	5,6
WALB	144	7	151	4,6	167	25	192	13,0	343	9,3
EALB	43	4	47	8,5	44	9	53	17,0	100	13,0
ALBI	4	0	4	0,0	1	2	3	66,7	7	28,6
ALAC	15	0	15	0,0	12	2	14	14,3	29	6,9
VALE	46	2	48	4,2	28	6	34	17,6	82	9,8
DELT	184	0	184	0,0	120	3	123	2,4	307	1,0
CCAT	9	0	9	0,0	4	0	4	0,0	13	0,0
NCAT	4	0	4	0,0	12	0	12	0,0	16	0,0
Total	505	18	523	3,4	451	49	500	9,8	1023	

<i>Macropipus tuberculatus</i> (+ <i>Iphitime cuenoti</i>)										
GEOGRAPHIC AREA	Males				Females				Overall	
	non-infested	infested	Total males	% infested	non-infested	infested	Total females	% infested	Total	% infested
CADI	35	4	39	10,3	15	4	19	21,1	58	13,8
WALB	0	0	0	----	0	0	0	----	0	---
EALB	7	2	9	22,2	2	0	2	0,0	11	18,2
ALBI	10	30	40	75,0	16	14	30	46,7	70	62,9
ALAC	77	11	88	12,5	63	6	69	8,7	157	10,8
VALE	68	10	78	12,8	84	12	96	12,5	174	12,6
DELT	203	0	203	0,0	94	1	95	1,1	298	0,3
CCAT	6	1	7	14,3	5	0	5	0,0	12	8,3
NCAT	25	1	26	3,8	24	0	24	0,0	50	2,0
Total	431	59	490	12,0	303	37	340	10,9	830	

<i>Geryon longipes</i> (+ <i>Ophryotrocha mediterranea</i>)										
GEOGRAPHIC AREA	Males				Females				Overall	
	non-infested	infested	Total males	% infested	non-infested	infested	Total females	% infested	Total	% infested
CADI	0	0	0	---	0	0	0	---	0	---
WALB	0	2	2	100,0	1	0	1	0,0	3	66,7
EALB	3	6	9	66,7	1	0	1	0,0	10	60,0
ALBI	13	14	27	51,9	1	0	1	0,0	28	50,0
ALAC	26	3	29	10,3	11	0	11	0,0	40	7,5
VALE	31	9	40	22,5	4	0	4	0,0	44	20,5
DELT	0	0	0	---	0	0	0	---	0	---
CCAT	0	0	0	---	0	0	0	---	0	---
NCAT	19	4	23	17,4	8	0	8	0,0	31	12,9
Total	92	38	130	29,2	26	0	26	0,0	156	

Table 3: Diversity measures for populations of *Iphitime cuenoti* and *Ophryotrocha mediterranea* in relation to geographical location and brachyuran hosts. N, number of individuals; Nh, number of haplotypes; h, haplotype diversity; π , Nucleotide diversity. LIODEP: *Liocarcinus depurator*; MACTUB: *Macropisus tuberculatus*; BATMAR: *Bathynectes maravigna*. Abbreviations of localities as in Table 1.

Population		N	Nh	H	π
CADI	<i>I. cuenoti</i>	20	14	0.9316 +/- 0.0438	0.0058+- 0.00348
WALB	<i>I. cuenoti</i>	14	11	0.9560 +/- 0.0447	0.0069 +/- 0.0040
	<i>O. mediterranea</i>	8	5	0.8571+/- 0.1083	0.0065 +/- 0.0041
EALB	<i>I. cuenoti</i>	14	11	0.9341 +/- 0.0542	0.0057 +/- 0.0033
	<i>O. mediterranea</i>	12	5	0.8333+/- 0.0691	0.0054 +/- 0.0033
ALBI	<i>I. cuenoti</i>	20	14	0.9158 +/- 0.0546	0.0042 +/- 0.0026
	<i>O. mediterranea</i>	16	9	0.8917+/- 0.0543	0.0062 +/- 0.0036
ALAC	<i>I. cuenoti</i>	15	8	0.8476 +/- 0.0712	0.0024 +/- 0.0017
	<i>O. mediterranea</i>	9	7	0.9444+/- 0.0702	0.0059 +/- 0.0037
VALE	<i>I. cuenoti</i>	17	13	0.9265 +/- 0.0579	0.0036 +/- 0.0023
	<i>O. mediterranea</i>	17	10	0.8676+/- 0.0543	0.0054 +/- 0.0036
NCAT+DELT +CCAT	<i>I. cuenoti</i>	7	6	0.9524 +/- 0.0955	0.0051 +/- 0.0034
NCAT	<i>O. mediterranea</i>	10	6	0.8889+/- 0.0754	0.0045 +/- 0.0029
TOTAL	<i>I. cuenoti</i>	108	59	0.9273 +/- 0.0207	0.0049 +/- 0.0028
	<i>O. mediterranea</i>	72	22	0.8920+/- 0.0192	0.0061 +/- 0.0034
LIODEP	<i>I. cuenoti</i>	50	33	0.9486 +/- 0.0236	0.0055 +/- 0.0031
MACTUB	<i>I. cuenoti</i>	46	27	0.9140 +/- 0.0342	0.0042 +/- 0.0025
BATMAR	<i>I. cuenoti</i>	9	7	0.9167 +/- 0.0920	0.0062 +/- 0.0038

Table 4. Molecular variance analyses (AMOVA) for *Iphitime cuenoti*. First approach, four groups: CADI; WEST AOF; south to IC (ALAC); NORTH to IC. Second approach, two groups: CADI+west AOF vs. north to AOF.

STRUCTURE	Source of variation	d.f.	Sum of squares	Variance components	Variation %	Fixation indices	P-value
First approach, four groups	Among groups	3	11.618	0.1002	4.88	Fct:0.0594	0.1300
	Among populations within groups	2	3.404	0.0079	1.06	Fsc:0.0111	0.2678
	Within populations	102	159.932	1.5646	94.06	Fst:0.0594	0.0029
Second approach, two groups	Among groups	1	8.283	0.1321	7.67	Fct:0.0767	0.1241
	Among populations within groups	5	8.769	0.0067	0.74	Fsc:0.0079	0.2052
	Within populations	101	157.902	1.5679	91.60	Fst:0.0841	0.0000

Table 5. Molecular variance analyses (AMOVA) for *Ophryotrocha mediterranea*. First approach Alborán region vs. western Mediterranean. Second approach: EALB vs. all other populations.

STRUCTURE	Source of variation	d.f.	Sum of squares	Variance components	Variation %	Fixation indices	P-value
Alborán region Vs. W. Mediterranean	Among groups	1	8.986	0.17804	8.52	Fct: 0.0852	0.1144
	Among populations within groups	4	9.983	0.05554	2.66	Fsc:0.0291	0.2170
	Within populations	66	122.517	1.85632	88.82	Fst:0.1117	0.0362
EALB Vs all other populations	Among groups	1	9.686	0.3673	16.23	Fct: 0.1623	0.1808
	Among populations within groups	4	9.283	0.0397	1.75	Fsc: 0.0209	0.2805
	Within populations	66	122.517	1.8563	82.02	Fst: 0.1798	0.0195

Table 6. Pairwise estimates of F_{st} between samples of *Iphitime cuenoti* (below diagonal) and *Ophryotrocha mediterranea* (above diagonal) based on mtDNA COI. Significant values ($p < 0.05$) indicated in bold. For *I. cuenoti*, NORTH includes DELT+CCAT+NCAT. Abbreviations of localities as in Table 1.

Localities	CADI	ALBI	WALB	EALB	ALAC	VALE	NORTH	NCAT
CADI	-	-	-	-	-	-	-	-
ALBI	0.0092	-	0.0158	0.0000	0.06362	0.02353	-	0.12993
WALB	0.0000	0	-	0.17066	0.03273	0	-	0.00994
EALB	0.0187	0.06091	0.0007	-	0.15315	0.21667	-	0.32788
ALAC	0.1578	0.0716	0.1316	0.0447	-	0.00323	-	0
VALE	0.1281	0.0512	0.0999	0.0213	0.0423	-	-	0
NORTH	0.1091	0.0466	0.0702	0.0000	0.0169	0.0364	-	-
NCAT	-	-	-	-	-	-	-	-
VALE+NORTH	0.12715	0.0424	0.10171	0.0123	0.01256	-	-	-

Table 7. Neutrality tests for all populations together of *Iphitime cuenoti* and *Ophryotrocha mediterranea*, and for both lineages of *O. mediterranea*. Significance values by $P < 0.02$.

	Tajima's D	Tajima's D p-value	Fu's Fs	Fs p-value
<i>I. cuenoti</i>	-2.3095	0.001	-78.350	0.0000
<i>O. mediterranea</i>	-0.3731	0.422	-6.787	0.0180
<i>O. mediterranea</i> L1	-1.1119	0.142	-5.637	0.0020
<i>O. mediterranea</i> L2	-0.8164	0.234	-3.927	0.0091

Table 8. Review of papers related to studies on the phylogeography of Atlantic-Mediterranean species. GT: Strait of Gibraltar. AOF: Almería-Orán Front. PEL: Peloponnese break. ISB: Isolation by distance. IC: Ibiza Channel. ST: Siculo-Tunisian Strait. Localities: WM: Western Mediterranean; EM: Eastern Mediterranean; NWM: North Western Mediterranean; At: Atlantic; Nat; North Atlantic; NEAt: North East Atlantic; Wat: Western Atlantic; AS: Adriatic Sea; BS: Black Sea

Author	Especie	Group	Molecular Marker	Locality	Structure	Barriers	Depth
Borrero Pérez <i>et al.</i> (2011)	<i>Holothuria (H.) mammata</i>	Echinodermata	16S, COI	WM, EM, At.	No	ST	0-30 m
Calderon <i>et al.</i> (2008)	<i>Paracentrotus lividus</i>	Echinodermata	16S, nuclear ANT	NEAt., At., WM, EM	YES	GS, AOF	0-7 m
Perez-Portela <i>et al.</i> (2010)	<i>Marthasterias glacialis</i>	Echinodermata	COI	NWM, Nat.,	NO	NO	5-25 m
Pérez-Portela <i>et al.</i> (2013)	<i>Ophiothrix fragilis, O. quinquemaculata</i>	Echinodermata	16S, COI	NWM, NEAt	NO	NO	5-65 m
Maltagliati <i>et al.</i> (2010)	<i>Paracentrotus lividus</i>	Echinodermata	<i>Cyt b</i>	EM, Wat.	NO	ISB	0,5-7 m.
Zulliger <i>et al.</i> (2009)	<i>Astropecten aranciacus</i>	Echinodermata	COI, Microsatellite loci	EM, WM, At.	YES	ISB	0-200 m
Boissin <i>et al.</i> (2011)	<i>Ophioderma longicauda</i>	Echinodermata	COI	WM, EM, At.	NO	PEL	Shallow water
Costantini <i>et al.</i> (2011)	<i>Corallium rubrum</i>	Cnidaria	Microsatellite loci	NWM	YES	Depth cline	20-70 m
Mokhtar-Jamai <i>et al.</i> (2011)	<i>Paramuricea clavata</i>	Cnidaria	Microsatellite loci	NEAt., WM	YES	IC: Strong AOF: weak	10-40 m
Palero <i>et al.</i> (2011)	<i>Palinurus elephas</i>	Crustacea	Microsatellite loci	NEAt, WM, EM	NO	AOF: Weak	20-80 m
Palero <i>et al.</i> (2008)	<i>Palinurus elephas</i>	Crustacea	COI	NEAt, WM, EM	NO	GS	20-80 m
Zane <i>et al.</i> (2000)	<i>Meganyctiphanes norvegica</i>	Crustacea	COI	NEAt., CADI, WM	YES	AOF	100-400 m.
García-Merchán <i>et al.</i> (2012)	<i>L. depurator</i>	Crustacea	COI	CADI	NO	GS, IC	25-400 m
García-Merchán <i>et al.</i> (2012)	<i>M. tuberculatus</i>	Crustacea	COI	CADI, WM	NO	GS	150-500 m.
Fernandez <i>et al.</i> (2011)	<i>Aristeus antennatus</i>	Crustacea	16S - COI	CADI, WM, EM	NO	GS	Deep-water
Luttikhuisen <i>et al.</i> (2008)	<i>Crangon crangon</i>	Crustacea	COI	NEAt, WM, AS, BS	YES	GS, AOF	< 2 m.
Sardá <i>et al.</i> (2010)	<i>Aristeus antennatus</i>	Crustacea	16S	NWM	NO	-	350-1500 m
Maggio <i>et al.</i> (2009)	<i>Aristeus antennatus</i>	Crustacea	mtDNA non-coding CR	WM, EM.	NO	NO	450-550 m.
Lo Brutto <i>et al.</i> (2012)	<i>Aristeus antennatus</i>	Crustacea	AFLP	At., WM, EM	NO	NO	450-550 m.
Pérez-Portela & Turon (2008)	<i>Pycnoclavella communis</i>	Ascidacea	COI	WM, At.	YES	SG, AOF	5-30 m.
Pérez-Losada <i>et al.</i> (2007)	<i>Sepia officinalis</i>	Molusca	COI	NEAt., WM, EM	YES	AOF	2-200 m.
Iannotta <i>et al.</i> (2009)	<i>Lysidice ninetta</i>	Polychaeta	16S	WM, EM	NO	NO	11-28 m
Iannotta <i>et al.</i> (2007)	<i>Lysidice ninetta</i>	Polychaeta	COI, ITS1	WM, EM	NO	NO	11-28 m
Dominguez <i>et al.</i> (2008)	<i>Parablennius parvicornis (A), P. sanguinolentus (M)</i>	Fish	COI	NEAt. WM	NO	-	0-2 m
Dominguez <i>et al.</i> (2007)	<i>Coryphoblennius galerita</i>	Fish	12S, 16S rDNA	EAt., WM	YES	GS, AOF	collected in tidepools
Galarza <i>et al.</i> (2009)	7 littoral fish	Fish	Microsatellite loci	WM	YES	AOF (6 species), BL (5 species)	5-10 m
Sala-Bozano <i>et al.</i> (2009)	<i>Lithognathus mormyrus</i>	Fish	MtDNA, Microsatellite loci	WM; EM; At	YES	AOF	< 50 m
Schunter <i>et al.</i> (2011)	<i>Serranus cabrilla</i>	Fish	Microsatellite loci	WM, EM	YES	OAF, IC	< 30 m

Table 9. Main differences observed between *Ophryotrocha mediterranea* and *O. geryonicola* according to Martin et al. (1991).

	<i>O. mediterranea</i>	<i>O. geryonicola</i>
Distribution	Mediterranean Sea	Atlantic Ocean
Host species	<i>Geryon tridens</i> , <i>Geryon longipes</i> , <i>Geryon quinquedens</i> , <i>Cancer borealis</i>	<i>Geryon longipes</i>
Maxillary plates	7 (7th one bidentate)	6 (from 3 to 14 but bidentate plates absent)
Maxillae	posterior end with a thick aileron	thick aileron absent
Maxillary carriers	oblong and wide laterally, typically thin in frontal view	typically thin
Chaetae	strongly spinulate	smooth
Anal cirrus	3 (middle one similar in size to the lateral ones)	2-3 (when present, middle one shorter than lateral ones)
Gut	annular (one ring per segment)	two laterally extended branches per segment
Dorsal cirri	conical, longer than ventral cirri and parapodial lobes	conical or ovoid, but shorter than ventral cirri and parapodial lobes
Ventral cirri	ovoid, shorter than dorsal cirri	absent (if present ovoid or conical, but longer than dorsal cirri)