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Genetic Variability and Connectivity in the Western Mediterranean Populations of the Bathyal Crab *Geryon longipes*

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Abstract: *Geryon longipes* is a crab species that inhabits the muddy bottoms of the middle and lower slopes, as well as bathyal bottoms ranging from 400 to 2000 m in depth. To assess its molecular diversity, a fragment of 572 bp of the *COI* (*Cytochrome Oxidase subunit I*) mitochondrial gene was sequenced in eight Western Mediterranean locations. Within the studied area, two oceanographic fronts are present (Almeria-Oran Front and Ibiza Channel). From the 124 sequences obtained, only 7 distinct haplotypes were identified. The population distribution indicated three well-differentiated regions: the Alboran Sea, the Gulf of Vera and the Levantine/Catalan coasts. The molecular diversity was compared with that obtained in the same year for the same gene in *Liocarcinus depurator*, a crab species that is captured on the continental shelf and upper slope (40 to 500 m). The estimates of molecular diversity parameters for the *COI* gene fragment were rather similar between both species, but the number of haplotypes was higher for *L. depurator*. Finally, the obtained *COI* sequences of *G. longipes* were compared to those from other populations of the species distribution range, recovered from the DNA repository. Only one additional, different haplotype was reported (Sicily), whereas all the rest were common with those described in our study. Therefore, the *COI* gene fragment would indicate that all the sequences analysed in the Mediterranean and NE Atlantic belong to the same species, *G. longipes*.

Keywords: *COI*; heteroplasmy; haplotypes; diversity; gene flow; *Liocarcinus depurator*; oceanic fronts

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1. Introduction

There was a time when evolutionary biologists tended to consider that marine organisms would not have major restrictions in their mobility and dispersion since the oceans showed no directly evident physical barriers like those present in the terrestrial environment [1]. As a result, it was assumed that marine organisms could move rather freely in the water mass, leading to a high degree of gene flow and minimal differentiation between populations. However, this assumption was found to be far from reality as marine currents create oceanographic fronts that disrupt the connectivity between populations. As such, in addition to isolation by distance, permanent and semi-permanent oceanic barriers are now recognised as one of the primary factors contributing to population genetic differentiation [2–6]. Nonetheless, the situation is markedly different for deep-sea species, as deep current patterns are poorly understood and the environmental conditions in these regions tend to be relatively stable, except for occasional and local high-energy processes [7]. Deep-sea fronts between water masses and geological deep-sea structures, such as mountain ranges, may create isolation for benthic and epibenthic populations unless the species exhibit epiplanktonic larval stages that allow them to surmount these barriers. Furthermore, although knowledge of deep-sea larval stages is limited, evidence suggests that at least

some species have epiplanktonic larval stages that may, depending on their developmental stage, be affected by epipelagic fronts and currents, while later developmental stages are located in deeper waters [8,9].

The Mediterranean Sea is an ideal marine system for studying these essential evolutionary processes due to its rich biodiversity and well-characterised currents and epipelagic oceanographic fronts [6,10–14]. Specifically, on its western basin, the Mediterranean contains four oceanographic barriers of interest: Gibraltar Strait (GS), Almeria-Oran Front (AOF), Ibiza Channel (IC) and Balearic Front (BF). In previous studies, the effect of the first three discontinuities was analysed by using the portunid crab *Liocarcinus depurator* as a model to study geographic variability of the *Cytochrome Oxidase subunit I* (*COI*) gene [15,16]. These studies identified two haplogroups, one from Atlantic-influenced waters and the other predominant in Mediterranean waters. Additionally, it was possible to study its spatial and temporal distribution along different populations of the Atlanto-Mediterranean transition. The GS and AOF were found to be the primary oceanographic discontinuities that differentiated populations with Atlantic or Mediterranean influence. Notably, the strength of the AOF displays significant seasonal and interannual variability that affects connectivity at the population level [17,18].

Building on this knowledge, an interesting evolutionary question arises as to whether we would obtain similar results using a crab species living in a deep-sea environment. *L. depurator* is a portunid crab dwelling on the continental shelf and upper slope muddy bottoms in the Mediterranean Sea and NE Atlantic, where it is commonly caught as bycatch in demersal trawl fisheries [19,20]. This species is characterised by the occurrence of a relatively long series of epipelagic planktonic larval stages [21,22], which are affected and transported by coastal currents [23]. This mechanism is considered the primary means of population dispersion for the species. To make a comparison, we selected a deep-sea species, the bathyal crab *Geryon longipes* (Geryonidae), which is also distributed along the Atlantic-Mediterranean transition but differs from *L. depurator* in that it inhabits much deeper muddy bottoms on the middle and lower continental slope, from around 400 m to 2000 m [24–26].

Two species are presently recognised in the genus *Geryon*, namely *G. longipes*, distributed in the Mediterranean and adjacent Atlantic waters, and *G. trispinosus*, present in the Eastern Central Atlantic waters off the northern European coasts [27–30]. *G. longipes* is frequently collected from the red shrimp (*Aristeus antennatus*) fishery, along with other co-occurring species such as the squat lobster *Munida tenuimana*, polychelid lobsters, and caridean shrimps [19,31]. *G. longipes* is often commercialised as a demersal trawling bycatch of the red shrimp fishery in the Western Mediterranean ports [32]. While its larval morphology has been described [33], information concerning the depths of its larval occurrence or its behaviour and dispersion is still scarce [34,35]. Concerning other geryonid species, information on this subject is also scarce, but it shows that the first larval stages are also epipelagic [34,36,37] and that early-stage larvae show physiological and behavioural mechanisms that allow them to ascend in the water column, while late stages descend into deep waters [8]. The size-depth relationship reported for juveniles and adults of the closely related species *G. trispino*sus (but see below) suggests that larval settlement takes place in deeper areas than those at which adult specimens occur [27]. Larval ecology and behaviour may therefore be very different between the continental shelf crab *L. depurator* and the deep-sea crab *G. longipes*.

The main aim of this research is to check for possible genetic structuration in the western Mediterranean populations of *G. longipes,* taking into account the different oceanographic fronts present, by analysing a fragment of the *COI* mitochondrial gene, equivalent to that previously sequenced in *L. depurator*. For this genetic marker, its molecular diversity was estimated in *G. longipes*, and the population distribution of the recorded haplotypes was studied. Since the *G. longipes* samples were obtained from the same areas where we previously analysed the samples of *L. depurator* [18], a comparison of the molecular diversity and geographic distribution of haplotypes between both species in the same

year was possible. Additionally, *COI* mitochondrial gene fragments from other alleged *G. longipes* specimens downloaded from DNA databases were analysed for comparison, and the geographic patterns of all described haplotypes were also analysed.

2. Materials and Methods

2.1. Samples and Sequencing

Samples of *G. longipes* were collected during the April and May 2016 MEDITS_ES fishery research cruise using a standardised fisheries research bottom trawl gear [38]. The studied populations were: West Alboran (WA), East Alboran (EA), Vera (VE), Ibiza Is. (EI), Alicante (AC), Valencia (VA), Central Catalonia (CC) and North Catalonia (NC) (Figure 1). The precise sampling locations and depths are described in Table 1.

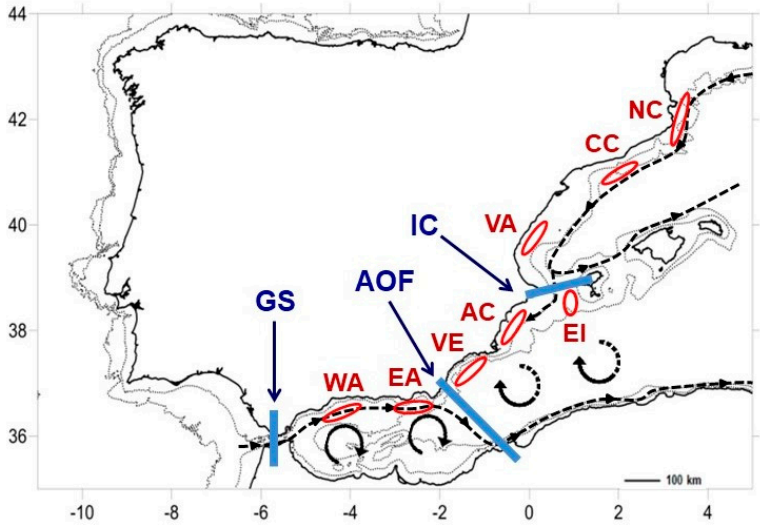

Figure 1. Sampling populations of *G. longipes* (red colour) in the Western Mediterranean area. Populations are identified by the following abbreviations: West Alboran (WA), East Alboran (EA), Vera (VE), Ibiza Is. (EI), Alicante (AC), Valencia (VA), Central Catalonia (CC) and North Catalonia (NC). Dashed black lines identify the location of the main currents and gyres in the studied area. Oceanographic fronts are shown as solid blue lines: Gibraltar Strait (GS), Almeria-Oran Front (AOF) and Ibiza Channel (IC).

Table 1. Population name (with its abbreviation), latitude, longitude, depth (in meters), number of analysed individuals and reference of all populations studied. Information from samples obtained by other researchers is also included.

Population	Latitude	Longitude	Depth	n	Reference
West Alboran (WA)	36.312 N	4.340 W	770	15	This study
East Alboran (EA)	36.581 N	2.498 W	528	14	This study
Vera (VE)	36.856 N	1.759 W	714	16	This study
Ibiza Is. (EI)	38.838 N	0.842 E	681	15	This study
Alicante (AC)	38.069 N	0.040 W	581	16	This study
Valencia (VA)	39.453 N	0.156 E	561	16	This study
Central Catalonia (CC)	41.161 N	2.358 E	665	17	This study
North Catalonia (NC)	41.391 N	3.269 E	622.5	15	This study
Nahariyya, Israel (IS)	33.050 N	34.830 E	1043	1	Tel Aviv University
Castellammare del Golfo, Sicily (CG)	35.730 N	14.050 E	605	2	Matzen da Silva et al., 2011
South coast of Portugal (SP)	36.600 N	8.030 W	752	5	Matzen da Silva et al., 2011
SW coast of Portugal (WP)	37.540 N	9.190 W	612	2	Matzen da Silva et al., 2011
NW of St. Kilda, Scotland (SC)	58.170 N	9.000 W	600	3	Matzen da Silva et al., 2011

All studied samples were adults, mainly males (85% males and only 15% females), with carapace lengths between 20.7 mm and 71.2 mm (mean = 50.5 ± 9.8 mm). From each crab, a piece of muscular tissue (0.1 g) from a leg (a tissue rich in mitochondria) was preserved in absolute alcohol on board the ship. In the laboratory, DNA was extracted using the Qiagen Puregene® Cell Kit (2 × 108) kit. The *Cytochrome Oxidase subunit I* (*COI*) gene fragment was amplified through the universal primers LCO1490 (forward) and HCO2198 (reverse) [39]. PCR reactions were carried out in 20 µL final volume, containing 1 µL sample DNA and 19 µL mix: 12.5 µL H_2O, 4 µL buffer X5, 1 µL $MgCl_2$, 0.5 µL dNTPs (1 mM), 0.4 µL primer forward (10 µM), 0.4 µL primer reverse (10 µM) and 0.2 µL Taq polymerase (Go Taq 5 U/µL, Promega). The PCR protocol was 4 min at 94 °C, 30 cycles of 1 min at 94 °C, 1 min at 50 °C, 1 min at 72 °C, and a final extension of 7 min at 72 °C. The resulting PCR products were cleaned with ExoSAP (1.2 U of Exonuclease and 1.2 U of Shrimp Alkaline Phosphatase) in a 2:1 proportion for 15 min at 37 °C. Finally, the samples were dried at 80 °C for 15 min and sent to Serveis Científics i Tecnològics de la Universitat de Barcelona for sequencing.

2.2. Data Analysis

A total of 124 samples were sequenced (Table 1), which were initially aligned and trimmed to obtain a final alignment of 622 bp using the BioEdit v7.2.6.1 [40]. Only one strand was sequenced, and the possible presence of stop codons or indels was checked. However, these sequences were later aligned with those deposited in DNA data repositories (13 in total) to obtain the maximum common fragment for all sequences, which had a length of 572 bp. The description of the downloaded sequences from databases is also shown in Table 1. The haplotype sequences obtained in the present research were deposited in GenBank under accession numbers MK720650–MK720669 and OQ283874–OQ283977. The accession numbers for the sequences downloaded from GenBank were: JQ305902 and JQ305903 (Castellammare del Golfo, Sicily, Italy), JQ306198–JQ306202 (South coast of Portugal), JQ306134 and JQ306135 (SW coast of Portugal) and JQ305968–JQ305970 (NW of St. Kilda, Scotland). The sequence from Nahariyya (Israel) was downloaded from the BOLD database (accession number: BIM369-13.COI-5P BOLD). The number of different haplotypes (*h*), the number of polymorphic sites (*S*), the haplotype diversity (*Hd*) and the nucleotide diversity (π) were computed with the DnaSP v6.12 software [41]. A network of haplotypes was constructed using the Median Joining network algorithm from the Network v5.0.1.1 software [42] and a phylogenetic tree was generated using the Neighbour-Joining method of MEGA X [43]. Moreover, with the Western Mediterranean samples, a comparison between the genetic (GammaST) and geographic distances was carried out. Genetic distances were computed with the DnaSP v6.12 software [41], whereas geographic distances were obtained using the Karto v5.2 software [44], following an isobathic line at 200 m depth. The comparison between the groups of populations was carried out with an AMOVA analysis. The genetic and geographic distance matrices were compared using a Mantel test. Furthermore, a PCoA was carried out with the genetic distances to study the distribution of the Western Mediterranean samples. These computations were carried out using the R package vegan [45].

3. Results

The parameters estimating the molecular diversity for 124 sequences of the studied populations are presented in Table 2. Neither stop codons nor indels were detected. West Alboran and Vera presented the highest values for haplotype and nucleotide diversity, whereas the lowest were recorded at Alicante and North of Catalonia. It is worth comparing the molecular diversity between *G. longipes* and *L. depurator* samples in the same localities (West Alboran, East Alboran, Alicante, Valencia and Central Catalonia) collected in 2016 (Table 3).

Table 2. Molecular diversity of the *G. longipes* populations: West Alboran (WA), East Alboran (EA), Vera (VE), Ibiza Is. (EI), Alicante (AC), Valencia (VA), Central Catalonia (CC) and North Catalonia (NC). (*n*) number of sequences, (*h*) the number of different haplotypes, (*S*) the number of polymorphic sites, (*Hd*) the haplotype diversity with the standard deviation and ($\pi \times 100$) the nucleotide diversity multiplied by 100 with the standard deviation.

Population	*n*	*h*	*S*	*Hd*	$\pi \times 100$
WA	15	4	2	0.714 ± 0.081	0.223 ± 0.020
EA	14	4	2	0.495 ± 0.151	0.146 ± 0.044
VE	16	5	5	0.708 ± 0.094	0.246 ± 0.065
EI	15	3	2	0.590 ± 0.106	0.117 ± 0.027
AC	16	2	1	0.458 ± 0.095	0.080 ± 0.017
VA	16	3	2	0.658 ± 0.075	0.137 ± 0.024
CC	17	3	2	0.699 ± 0.049	0.154 ± 0.020
NC	15	2	1	0.343 ± 0.128	0.060 ± 0.022

Table 3. Molecular diversity of the *G. longipes* and *L. depurator* populations sampled in 2016: West Alboran (WA), East Alboran (EA), Alicante (AC), Valencia (VA) and Central Catalonia (CC). (*n*) number of sequences, (*h*) the number of different haplotypes, (*h*/*n*) ratio of different haplotypes to number of sequences, (*Hd*) the haplotype diversity with the standard deviation and ($\pi \times 100$) the nucleotide diversity multiplied by 100 with the standard deviation. *G. long.* and *L. dep.* stand for *Geryon longipes* and *Liocarcinus depurator*, respectively. *L. depurator* data are from [18].

Population	Species	*n*	*h*	*h*/*n*	*Hd*	$\pi \times 100$
WA	*G. long.*	15	4	0.267	0.714 ± 0.081	0.223 ± 0.020
	L. dep.	24	11	0.458	0.815 ± 0.063	0.431 ± 0.055
EA	*G. long.*	14	4	0.286	0.495 ± 0.151	0.146 ± 0.044
	L. dep.	23	8	0.348	0.581 ± 0.120	0.246 ± 0.075
AC	*G. long.*	16	2	0.125	0.458 ± 0.095	0.080 ± 0.017
	L. dep.	25	7	0.280	0.633 ± 0.104	0.301 ± 0.402
VA	*G. long.*	16	3	0.188	0.658 ± 0.075	0.137 ± 0.024
	L. dep.	41	8	0.195	0.316 ± 0.095	0.093 ± 0.039
CC	*G. long.*	17	3	0.176	0.699 ± 0.049	0.154 ± 0.020
	L. dep.	6	2	0.333	0.333 ± 0.215	0.063 ± 0.083
TOTAL	*G. long*	78	5	0.064	0.734 ± 0.024	0.203 ± 0.011
	L. dep.	119	24	0.202	0.592 ± 0.052	0.300 ± 0.034

The values of *Hd* and π are higher for *L. depurator* in West Alboran, East Alboran and Alicante, and the opposite result was observed in Valencia and Central Catalonia (although the sample size for *L. depurator* was rather small). Considering all five populations together, *Hd* is higher in *G. longipes* than in *L. depurator* (0.734 ± 0.024 and 0.592 ± 0.052, respectively), but not for π (0.203 ± 0.011 in *G. longipes* and 0.300 ± 0.034 in *L. depurator*). However, the *h*/*n* ratio was always higher for *L. depurator*, considering the five populations separately or together (0.064 for *G. longipes* and 0.202 for *L. depurator*). A summary of the molecular diversity parameters, computed for all 137 *G. longipes* sequences (124 from the present study and 13 from databases), is shown in Table S1. These sequences belonged to eight haplotypes, which were named Glon_1 to Glon_8 (Table S2). They were characterised by 6 polymorphic positions, of which 286 and 367 presented heteroplasmy in different individuals. Positions showing heteroplasmy were not excluded from the computations. The presence of these eight haplotypes in the studied populations is presented in Table 4.

Table 4. Distribution of the eight haplotypes detected in *G. longipes* in the studied populations. The presence of a particular haplotype is indicated by "+" and its absence by "−". Abbreviations of populations: WA (West Alboran), EA (East Alboran), VE (Vera), EI (Ibiza Is.), AC (Alicante), VA (Valencia), CC (Central Catalonia), NC (North Catalonia), IS (Nahariyya, Israel), CG (Castellammare del Golfo, Sicily), SP (South coast of Portugal), WP (SW coast of Portugal) and SC (NW of St. Kilda, Scotland).

Population	Haplotype							
	Glon_1	Glon_2	Glon_3	Glon_4	Glon_5	Glon_6	Glon_7	Glon_8
WA	+	+	+	−	−	−	+	−
EA	+	−	+	−	−	+	+	−
VE	+	−	−	−	+	+	+	+
EI	+	+	−	−	−	−	+	−
AC	+	+	−	−	−	−	−	−
VA	+	+	−	−	−	−	+	−
CC	+	+	−	−	−	−	+	−
NC	+	+	−	−	−	−	−	−
IS	−	−	+	−	−	−	−	−
CG	−	+	−	+	−	−	−	−
SP	−	+	+	−	−	−	−	−
WP	−	−	+	−	−	−	−	−
SC	+	+	−	−	−	−	+	−

Haplotypes Glon_1, Glon_2, Glon_3 and Glon_7 were frequent, whilst Glon_6 was only found in two populations (East Alboran and Vera) and Glon_4, Glon_5 and Glon_8 were detected only once (the first in Castellamare del Golfo and the other two in Vera). Vera was the population presenting more different haplotypes (five). The haplotype network showing the similarity regarding the sequence of the eight haplotypes and their relative abundance is presented in Figure 2.

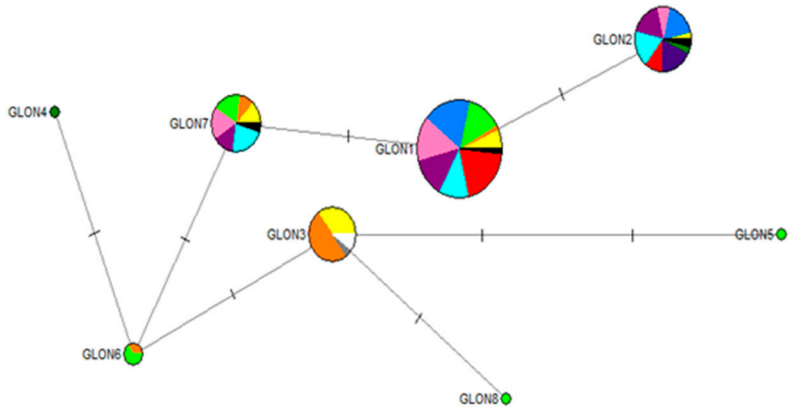

Figure 2. Haplotype network using the eight haplotypes detected in *G. longipes*. Circle sizes are proportional to the abundance of each haplotype. Transversal small lines in branches indicate one nucleotide change between the connected haplotypes. Colours indicate the origin of the haplotypes: yellow (West Alboran), brown (East Alboran), light green (Vera), pink (Ibiza Is.), blue (Alicante), purple (Valencia), light blue (Central Catalonia), red (North Catalonia), grey (Israel), dark green (Sicily), white (West Portugal), dark blue (South Portugal) and black (Scotland).

This network was rather linear with few ramifications (only two), with Glon_1, Glon_2 and Glon_7 haplotypes being the most frequent. There was only one nucleotide change between Glon_1 and Glon_2 and Glon_1 and Glon_7. The next frequent haplotype was Glon_3, but it was related to Glon_7 through Glon_6. Finally, Glon_4, Glon_5 and Glon_8 were infrequent and located at the tips of the network. The neighbour-joining tree confirmed the resemblances between the sequences of the different haplotypes (Figure 3).

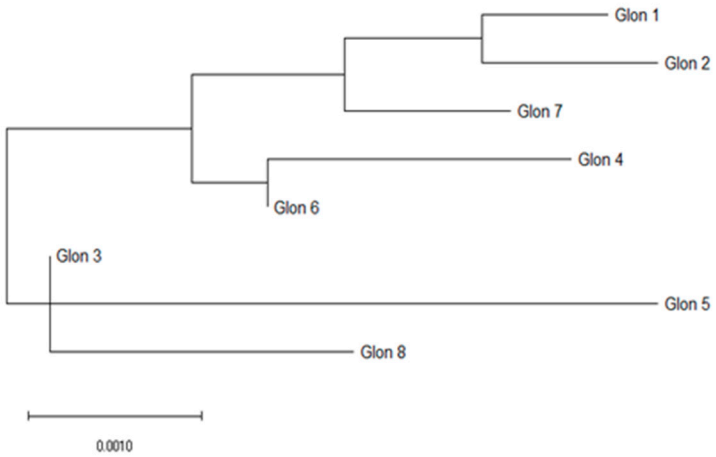

Figure 3. Phylogenetic tree for the eight different haplotypes of *G. longipes*. This result is due to the fact that all the sequences are rather similar.

The distribution and abundance of the haplotypes in the Western Mediterranean populations (Figure 4) qualitatively suggests the existence of three different geographic areas or groups of populations: (1) the region with Atlantic water influence (West and East Alboran), represented by the presence of Glon_3 in high frequency; (2) the Levantine/Catalan zone with predominant Mediterranean waters (Alicante, Ibiza Is., Valencia, Central and North Catalonia), in which Glon_1 and Glon_2 were predominant; and (3) Vera population, likely influenced by both Atlantic and Mediterranean waters and presenting five haplotypes: Glon_1 in a frequency similar to that observed in the Levantine/Catalan region; Glon_7, which is found in the Atlantic and most Mediterranean populations; Glon_6, which was also detected in East Alboran; and finally, Glon_5 and Glon_8 detected only in this population and related with Glon_3 by two and three changes, respectively. The obtained results by the AMOVA analysis indicated a significant differentiation between the Alboran Sea populations and those from the Levantine/Catalan zone ($p = 0.047$). However, no significant differentiation was observed between Vera and the Levantine/Catalan populations ($p = 0.217$) or between Vera and the Alboran Sea populations ($p = 0.667$). Interestingly, Glon_1 was detected in all studied Western Mediterranean populations but was most abundant from Vera to North Catalonia and was also found in St. Kilda, Scotland. In all the populations studied in this research, with the exception of Alicante and North Catalonia, the haplotype Glon_7 was rather common and was also reported in St. Kilda. Finally, and also concerning the haplotype distribution, it is remarkable that seven of the eight described haplotypes were detected in our Western Mediterranean populations (Glon_1, Glon_2, Glon_3, Glon_5, Glon_6, Glon_7 and Glon_8). Most important, all sequences from different geographic origins belonged to any of these haplotypes (Table S2), with the only exception of Glon_4, which was only found in Castellamare del Golfo (Sicily); however, this population also presented Glon_2. Thus, the reported haplotypes are usually shared between different populations.

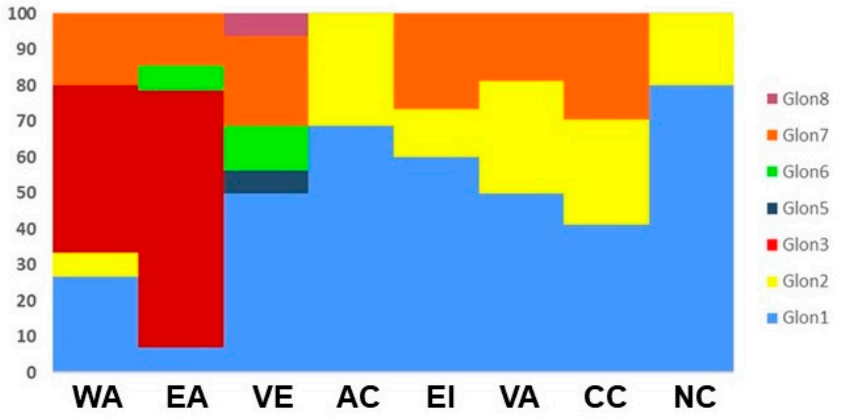

Figure 4. Frequencies of the haplotypes for each western Mediterranean population. Colours indicate each haplotype: blue (Glon_1), yellow (Glon_2), dark red (Glon_3), dark green (Glon_5), light green (Glon_6), brown (Glon_7) and purple (Glon_8). The abbreviations stand for the following populations: WA (West Alboran), EA (East Alboran), VE (Vera), AC (Alicante), EI (Ibiza Is.), VA (Valencia), CC (Central Catalonia) and NC (North Catalonia).

There was a significant correlation between genetic and geographic distances (Mantel test r = 0.473, p = 0.036). The PCoA analysis allowed for a graphical representation of all Western Mediterranean populations (Figure 5). The first and second coordinates explain 98.36% and 1.64% of the variability, respectively. Thus, the first coordinate explains almost all variability. It separates the previously mentioned three areas: the Alboran Sea populations, Vera and the remaining populations. However, in the third group (Alicante, Ibiza Is., Valencia, Central and North Catalonia), populations do not follow a geographic pattern because Alicante and North Catalonia were grouped on the far right of the graph, likely due to the absence of Glon_7 in them.

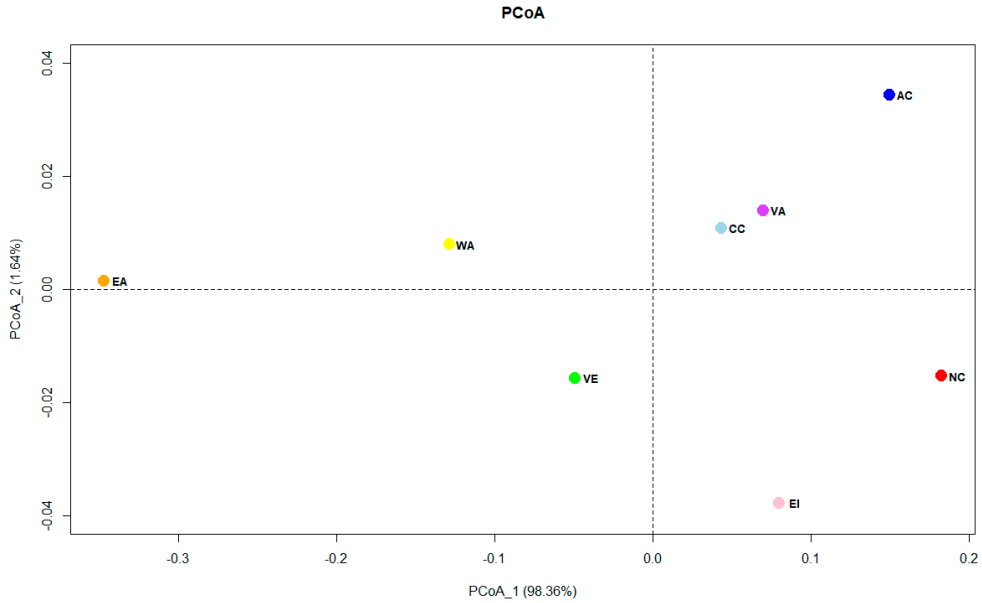

Figure 5. Graphical representation of the PCoA. The abbreviations stand for the following populations: WA (West Alboran), EA (East Alboran), VE (Vera), AC (Alicante), EI (Ibiza Is.), VA (Valencia), CC (Central Catalonia) and NC (North Catalonia).

4. Discussion

For the 572-bp fragment of the *G. longipes COI* gene analysed, the observed number of polymorphic sites (only 6) was scarce considering all 137 sequences together. Of these

positions, 4 presented sporadic substitutions (49, 292, 424 and 496), whereas the other 2 (286 and 367) showed heteroplasmy in many individuals. This heteroplasmy could be produced by a mixture of different mtDNA molecules from the same species in the same individual, an introgression between different species or a simulation of heteroplasmy generated by the presence of Numts (nuclear mitochondrial pseudogenes) [46]. It has been described that Numts are rather common in crustaceans [47,48], and they have been previously reported from *G. longipes* [49], although this was in a specimen preserved in a museum, and, if preservation in origin has not been optimal, DNA extraction and PCR amplification can yield anomalous results [46]. We hypothesize that in our case, heteroplasmy is a consequence of a mixture of mtDNA molecules belonging to the same species, as we used fresh tissue rich in mitochondria for mtDNA extraction, stop codons have not been detected in any of our sequences, the polymorphic position for heteroplasmy showed high variability among the analysed individuals, and both peaks (for G and A) showed similar height in heteroplasmic individuals, which could likely indicate a current source of species variation.

In the comparison of the same populations (West Alboran, East Alboran, Alicante, Valencia and Central Catalonia) and for the same year (Table 3), *L. depurator* presented higher molecular variability for the *COI* gene fragment than *G. longipes*. Moreover, the global estimates of haplotype and nucleotide diversities obtained for *G. longipes* (Table S1) were similar but lower than those observed in samples of *L. depurator* and *M. intermedia* collected in the same region in other surveys [15]. Furthermore, our values of *Hd* and π from *G. longipes* were similar to those obtained from the deep-sea red shrimp *Aristeus antennatus* (0.624 ± 0.050 and 0.0017 ± 0.0002, respectively). In the latter species, the molecular variability was estimated from both the information provided by a fragment of the *COI* gene and the *S16* subunit gene in 137 shrimps from the Western Mediterranean [50]. It is worth pointing out that *G. longipes* and *A. antennatus* widely co-occur on the middle and lower continental shelves in the Mediterranean, and their habitats are therefore highly coincident [51,52]. In summary, the levels of molecular variation detected in the sampled populations of *G. longipes* would indicate that deep-sea species would present lower genetic diversity than those present in shallower waters, in agreement with [15]. These results provide additional and new information on the relevant topic of the role of depth as an evolutionary factor [53,54].

The haplotype network for *G. longipes* (Figure 2) showed one main haplotype (Glon_1), together with three other rather abundant haplotypes (Glon_2, Glon_3 and Glon_7), and the four remaining haplotypes being sporadic (Glon_4, Glon_5, Glon_6 and Glon_8). The obtained network is rather lineal, presenting only a couple of ramifications, a pattern not found in other networks generated from the *COI* fragments in other decapod crustaceans from the study area, such as the hermit crabs *Pagurus excavates* (shallow-water species) and *Pagurus alatus* (deep-sea species), the caridean shrimp *Plesionika heterocarpus* (deep-sea), the penaeid shrimp *Parapenaeus longirostris* (deep-sea), the portunid crab *Macropipus tuberculatus* (deep-sea) [15], or in other decapods from European waters, such as the crawfish *Palinurus elephas* (shallow-water) [4], the crabs *Pachygrapsus marmoratus* (shallow-water) [55], *Carcinus aestuarii* (shallow-water) [56], *Acanthonyx brevifrons* and *A. lunulatus* (both shallow-water) [57] and the deep-sea red shrimp *Aristaeomorpha foliacea* (deep-sea) [58].

Although only eight different haplotypes were detected in our study, they showed a particular distribution in the studied Mediterranean populations (Figure 4). Thus, three areas could be defined: the Alboran Sea (West and East), Vera, and Levantine/Catalan area (Alicante, Ibiza, Valencia, Central and North Catalonia). The Alboran Sea populations showed a high frequency of the Glon_3 haplotype, which was absent in the rest of the studied populations. This suggests that the Glon_3 haplotype is characteristic of Atlantic waters since it is present in both the Alboran Sea and Portuguese populations sampled, but it was also found in Israel (Table 4). The case of Vera is particular, with five different haplotypes and a frequency of Glon_1 close to 50%. It could be a population with waters from different

origins—the Atlantic and Mediterranean. The remaining populations presented only two haplotypes (Glon_1 and Glon_2), as is the case of Alicante and North Catalonia, or three haplotypes (Glon_1, Glon_2 and Glon_7), with Glon_1 being the most common. These three groupings are corroborated by the results of PCoA (Figure 5). However, the haplotype distribution pattern in *L. depurator* for the same year was rather different. In this species, two haplogroups (Atlantic and Mediterranean) were well defined [15–18], and their distribution was likely conditioned by the gene flow mediated by the pattern of currents and the position and intensity of the oceanic fronts [16–18]. In 2016, the haplotype distribution of *L. depurator* showed a clear differentiation between the Western and Eastern Alboran, with the Atlantic haplotype being predominant in Cadiz and West Alboran and the Mediterranean haplotype in the remaining Mediterranean populations (East Alboran, Alicante, Valencia, Ebro Delta and Central Catalonia). It is now well known that the molecular composition of adult *L. depurator* populations depends on the larval movements in the plankton during the previous year [15,16]. However, there is no distribution and behavioural information on the larval behaviour of *G. longipes*. A fundamental question arises: whether the molecular differentiation found in our Mediterranean samples is adaptive or not. If it is adaptive, the observed distribution of the haplotypes should be the result of natural selection acting on the larvae, adults or both larvae and adults. These are open questions that deserve more research to be properly answered.

Another remarkable result is the haplotype distribution of the ***COI*** fragment in all populations so far sequenced (Table 4). None of the common haplotypes is restricted to particular geographic areas. Furthermore, all the sequences obtained are very similar, with just a few nucleotide changes (Figures 2 and 3). Accordingly, all individuals analysed in this research (137 sequences) would likely belong to the same species. The identification of geryonid crabs based on morphological characters is relatively difficult, as Reference [59] already pointed out, so molecular markers can produce new insights into the taxonomy of this group [60]. From our study of the 572 bp of *COI*, the three sequences obtained from NE Atlantic individuals (attributed to *Geryon trispinosus*) belonged to the most common haplotypes present in Mediterranean *G. longipes*. This result agrees with the remarks reported by Reference [61] and would therefore indicate that the Atlantic individuals examined in the present study would belong to the same species present in the Mediterranean *G. longipes*. However, the *COI* fragment used is relatively small, and other regions and additional molecular markers would be useful to fully confirm this conclusion.

Ecologically, larval behaviour is considered the main mechanism of dispersion for populations of benthic and epibenthic species occurring in the marine benthos [62,63]. In particular, larval transport along systems of currents and counter-currents coupled to larval migrations has been particularly suggested as a possible mechanism of larval dispersal for deep-sea geryonid species [8,60]. Thus, migration of ovigerous females to the upper distribution limits of *G. trispinosus* and its role as a mechanism for larval release to take place at appropriate depth locations to minimise the larval treks to epipelagic waters have been reported [27]. Similarly, Reference [64] showed that large-sized individuals clearly dominated the population present at the upper distribution range of *G. longipes*.

Our results clearly suggest that there is a need for further comparative studies of population biology patterns, including population genetics and behavioural aspects in deep-sea species. These studies should assess the geographical and temporal patterns that may help to appropriately understand deep marine habitats, providing essential information for their management.

5. Conclusions

The estimates of molecular diversity parameters for the *COI* gene fragment are similar in *G. longipes* and *L. depurator*, although molecular diversity is slightly higher in the latter species. However, the number of haplotypes detected is different for both species. In *G. longipes*, only eight haplotypes were detected considering both our sequences and those downloaded from the databases. In the Western Mediterranean region and according

to the haplotypes for the *COI* gene fragment, the *G. longipes* populations were clearly distributed in three major regions: the Alboran Sea, Vera and Levantine/Catalan area. This result can be relevant to properly defining the Marine Protected Areas and for the correct implementation of fishery conservation and management policies. Finally, the *COI* gene fragment indicated that all sequences obtained in this research and from genetic databases belong to the same species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15040534/s1, Table S1: Molecular diversity for all *G. longipes* sequences (124 from the present study and 13 from databases). (*n*) number of sequences, (*h*) the number of different haplotypes, (*S*) the number of polymorphic sites, (*Hd*) the haplotype diversity with the standard deviation and ($\pi \times 100$) the nucleotide diversity multiplied by 100, with the standard deviation.; Table S2: Description of the eight haplotypes of *G. longipes* observed based on their nucleotide composition at the polymorphic sites. All sequences were aligned presenting a total length of 572 bp. Polymorphic sites are referred to as this length.

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