

Article

First Insights into the Mitochondrial DNA Diversity of the Italian Sea-Slater Across the Strait of Sicily

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Abstract

The Strait of Sicily represents a biogeographically rich and complex region. The diverse geological origin and past continental connection of its islands have shaped a highly heterogeneous fauna, mainly composed of both African and European taxa. The Italian sea-slater, *Ligia italica* (Fabricius, 1798), is a small isopod inhabiting rocky shores of the Mediterranean Sea, Black Sea, and Atlantic Ocean. Despite its wide distribution, the phylogeography of this species is poorly understood, with limited available data suggesting a remarkable level of cryptic diversity. In this study, we investigated the mitochondrial genetic diversity (COX1) of *L. italica* across nine Italian and Maltese islands across the Strait of Sicily, aiming to clarify the biogeographic patterns underlying the distribution of these insular populations. Our results reveal an unexpectedly high genetic diversity within our study area, with eight different haplogroups, each characterized by low internal genetic variation and mutual distances ranging from 5.5% to 17.9%. These values are comparable to those associated with species-level rank within the genus *Ligia*. Overall, the phylogenetic relationships between the lineages appear well supported; however, the same relationships are not clearly correlated with geographic proximity or connectivity among the sampled localities. The distribution patterns of some of the detected haplogroups suggest possible passive dispersal mechanisms (e.g., rafting), while others indicate more intricate biogeographic scenarios. The overall diversity of *L. italica* within the Strait of Sicily, as well as the unclear origin of some insular populations, cannot be fully explained with the current data. In particular, the high genetic structure observed within the Maltese Archipelago, may partially reflect human-mediated dispersal (e.g., maritime transport), possibly involving source populations that remain unsampled or genetically uncharacterized. Our results highlight that the Strait of Sicily can be considered a diversity hot spot for *L. italica* and support the designation of this taxon as a putative species complex, with a cryptic diversity worthy of an exhaustive taxonomic revision.

Keywords: *Ligia italica*; phylogeography; rocky shores; coastal species; Mediterranean Sea

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1. Introduction

The genus *Ligia* (Fabricius, 1798) includes over 50 species of isopods with a cosmopolitan distribution [1–3]. This group is notable for its highly restricted habitat, which is largely confined to the uppermost reaches of the intertidal zone along rocky shores [4], equivalent to the mediolittoral and lower supralittoral zone in microtidal seas like the Mediterranean. In these restricted biotopes, sea-slaters complete their entire life cycle, feeding on algae or scavenging for dead organisms [5]. Unlike other semiterrestrial coastal crustaceans with a planktonic larval stage [6–8], the developmental stages of sea-slaters occur in brood sacs, carried by females, and after birth the juveniles share the same habitat with adults [9]. Many sea-slaters species dive into rock pools to escape predators and to rehydrate [10]. However, sea-slaters are unable to remain submerged for extended periods and must surface to breathe. At the same time, they cannot stray far from water sources, wave-splash zones or moist crevices as they rapidly dehydrate [11,12].

The extreme specialization of their ecological niche, combined with the absence of a planktonic larval stage, results in a very limited dispersal ability [11]. Consequently, these isopods are highly susceptible to population fragmentation caused by the emergence of barriers such as sandy shorelines or episodes of marine transgression, such as flooding [13]. The unique ecological adaptations of the genus *Ligia*, along with the recurrent occurrence of allopatric conditions over evolutionary time, have disrupted gene flow between populations, promoting genetic diversification and speciation phenomena [13,14]. For these reasons, this taxon is currently considered an ideal model for investigating evolutionary processes through a biomolecular approach, as highlighted by recent studies revealing extensive cryptic diversity within its species [14–17].

The Italian sea-slater *Ligia italica*, Fabricius, 1798, exhibits a wide distribution that includes the coasts of the Mediterranean Sea, Black Sea, and Atlantic Ocean, including Macaronesia, Cape Verde, Morocco, and Western Sahara coasts [1,3]. Despite its wide range, the phylogeographic structure of this species remains poorly understood. Nonetheless, the few studies addressing its genetic variability have revealed substantial cryptic diversity, such as the two deeply divergent clades identified among Tunisian populations [17].

The Strait of Sicily and its islands represent a complex system of great biogeographic interest. The diverse geological origin, their intermittent connections with the mainland, and the intricate surface water circulation in the area [18] have shaped a remarkably diverse native fauna. This fauna includes a mixture of taxa of African [19–21] and European [22,23] origin, as well as a high rate of endemism [24–26]. Moreover, the central Mediterranean has been subject to intense anthropogenic influence, both ancient and modern, which has further shaped the current biodiversity of its islands through numerous biotic introductions [27,28].

Here, we investigated the mitochondrial genetic diversity of *Ligia italica* across several Italian and Maltese islands of the Strait of Sicily, including the Maltese Archipelago, the Aegadian Islands, the Pelagian Islands, and Pantelleria, aiming to assess the biogeographical pattern that led to the formation of these isolated populations. For this purpose, we also included comparative data from the mainland boundaries of this area, including novel generated sequences from Sicily and already published data from Tunisia [17]. Furthermore, in order to explore within-island genetic variation, we genetically characterized multiple populations sampled along the coasts of the main Maltese islands.

2. Materials and Methods

Between 2024 and 2025, we sampled 19 localities distributed across seven different islands within three Sicilian Strait archipelagos (Maltese, Pelagian, and Aegadian) and four localities along the southern and northern coasts of Sicily. Additional samples were collected in previous years from the island of Pantelleria and from two further localities in northwestern Sicily. The sampling sites are listed in Table 1 and mapped in Figure 1, produced with QGIS v. 3.30.2 [29].

Table 1. Sampled localities. The “Locality” column reports the name of the island followed by the toponym of the site (where available). The ISO 3166-1 alpha-3 codes are provided to indicate the corresponding country (i.e., MLT: Malta; ITA: Italy).

Country	Locality	Latitude	Longitude	Sampling Date	COI A.N.	Clade
MLT	Malta, Baħar iċ-Ċagħaq	35.9496	14.4464	27/09/2024	PX239617-PX239618	A
MLT	Malta, Qawra Point	35.9599	14.4254	27/09/2024	PX239610, PX239619	A
MLT	Malta, Birżebbuġa	35.8325	14.5315	28/09/2024	PX239620	A
MLT	Comino	36.0052	14.3351	02/10/2024	PX239616, PX239629	A
MLT	Malta, Ċirkewwa	35.9869	14.3439	02/10/2024	PX239615, PX239628	A
MLT	Gozo, Dahlet Qorrot	36.0494	14.3171	16/10/2024	PX239630	A
MLT	Malta, Marsaxlokk	35.8331	14.5458	28/09/2024	PX239611, PX239621	B
MLT	Malta, Wied iż-Żurrieq	35.8208	14.4521	28/09/2024	PX239612, PX239622	B
MLT	Malta, Xagħra	35.8846	14.5542	30/09/2024	PX239613, PX239623	B
MLT	Malta, Zonqor	35.8692	14.5727	30/09/2024	PX239624-PX2396245	B
ITA	Sicily, Cava D’Aliga	36.7174	14.7015	13/10/2024	PX239635-PX2396356	C
MLT	Malta, Gnejna	35.9216	14.3441	01/10/2024	PX239614, PX239627	D
MLT	Gozo, Dwejra	36.0507	14.1901	16/10/2024	PX239631-PX2396312	D
MLT	Gozo, Xlendi	36.0289	14.2149	16/10/2024	PX239637	D
MLT	Malta, Popeye Cliffs	35.9583	14.3383	18/10/2024	PX239633-PX239634	D
ITA	Lampedusa, Cala Croce	35.5011	12.5933	27/05/2025	PX239651-PX239652	E
ITA	Lampedusa, Punta Sottile	35.4951	12.6307	27/05/2025	PX239649-PX23964950	E
ITA	Sicily, San Vito Lo Capo	38.1796	12.7333	19/09/2011	PX239626, PX239644	F
ITA	Sicily, Capo D’Orlando	38.1655	14.7482	02/11/2024	PX239640-PX2396401	F
ITA	Sicily, Barcarello	38.2092	13.2819	01/06/2019	PX239609	F
ITA	Pantelleria, Punta Bue Marino	36.8378	11.9632	01/05/2018	PX239605-PX2396058	H
ITA	Sicily, Torretta Granitola	37.5667	12.6608	01/11/2024	PX239638-PX2396389	H
ITA	Favignana, Calafumere	37.9331	12.3215	11/12/2024	PX239642-PX239643	H
ITA	Sicily, Licata	37.1012	13.8834	04/02/2025	PX239645-PX2396456	H
ITA	Marettimo, Scugghiazzu	37.9721	12.0700	11/05/2025	PX239653-PX2396534	H
ITA	Linosa, Molo Mannarazza	35.8748	12.8649	01/06/2025	PX239647-PX2396478	H

Sampling was conducted on rocky shores inhabited by *Ligia italica* (Figure 2). Sea-slaters were captured by hand or with forceps, if hidden in rocky crevices, and immediately preserved in 96% alcohol.

One to four specimens (65 individual in total) were selected from each population for DNA extraction. A single leg was excised from each specimen and subsequently soaked in distilled water for 5 min to remove any residual ethanol. DNA extraction was then carried out using the BIORON GmbH “Ron’s Tissue DNA Mini Kit”, following the manufacturer’s standard protocol.

The selective amplification of the mitochondrial gene encoding Cytochrome c oxidase subunit 1 (COX1) was carried out by the polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 [30]. The PCR mix consisted of 19.3 µL of distilled water, 2.5 µL of Buffer 10X which includes 20 mM of MgCl₂, 0.3 µL of dNTPs (10 mM for each), 0.3 µL of each of the primers (10 µM), 0.3 µL of Taq polymerase (5 U/µL) and 2 µL of template DNA, for a total volume of 25 µL. The thermal cycle consisted of 35 cycles of

denaturation (94 °C for 45 s), annealing (45 °C for 45 s) and extension (72 °C for 1 min), followed by 7 min at 72 °C for the final extension step.

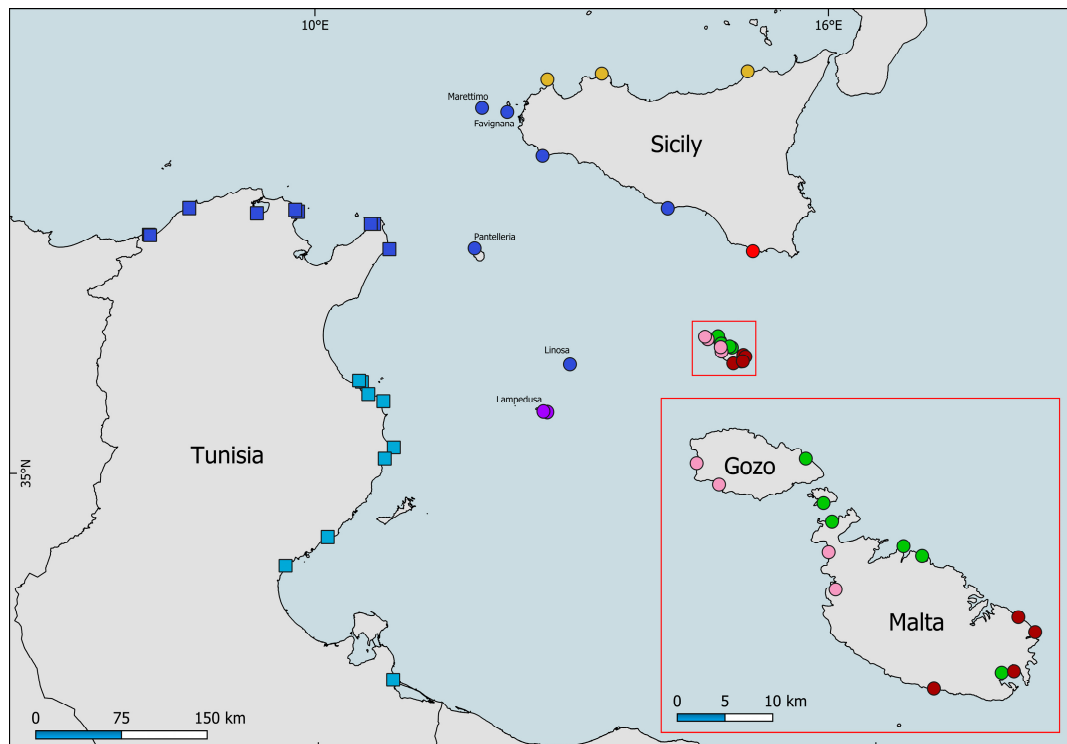


Figure 1. Distribution of *Ligia italica* samples collected in Sicily (Italy) and Malta. Squares and circles indicate published [17] and novel sample sites, respectively. Different colours refer to the different clades reported in Figure 3. See Table 1 for the coordinates of the sampled sites. Map created using the Free and Open Source QGIS software v. 3.30.2.

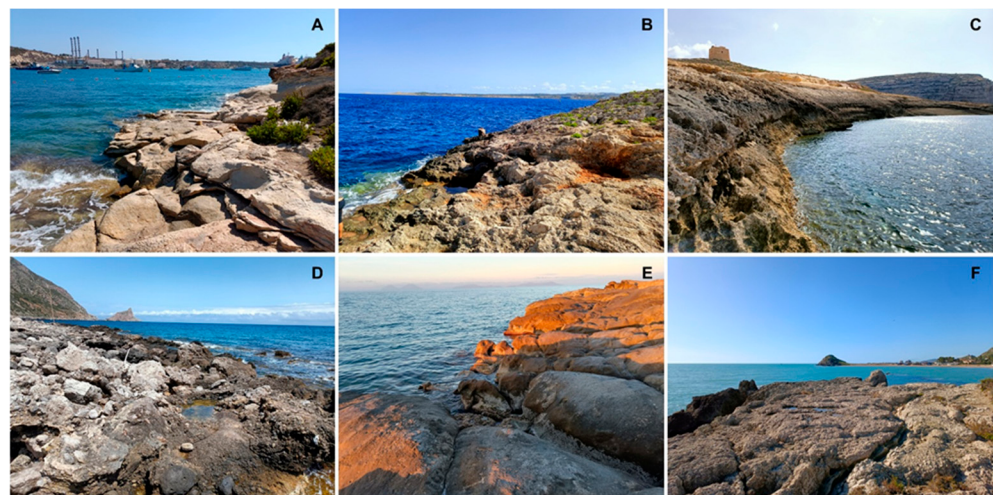


Figure 2. Habitats sampled in some study areas: (A) Malta, Marsaxlokk; (B) Comino; (C) Gozo, Dwejra; (D) Marettimo, Scugghiazzu; (E) Sicily, Capo d'Orlando; (F) Sicily, Licata.

After PCR amplification, 4 µL of each PCR product was loaded onto a 1% agarose gel pre-stained with ethidium bromide and subjected to electrophoresis at 90 V for 25 min. DNA fragments were visualized under a UV transilluminator, and only products showing a clear single band of the expected size were purified using the Exo-SAP-IT® kit (Affymetrix USB, USA). Purified amplicons were then sent for sequencing to MacroGen Europe (Italy) on an ABI 3130xl genetic analyser (Applied Biosystems). The same primer

pairs used for PCR were employed for direct sequencing. Sequence quality was evaluated based on Phred scores [31], and only sequences with continuous stretches of high-quality bases (QV > 20) were retained for subsequent analyses. Resulting chromatograms were inspected and manually curated using MEGA12 software [32].

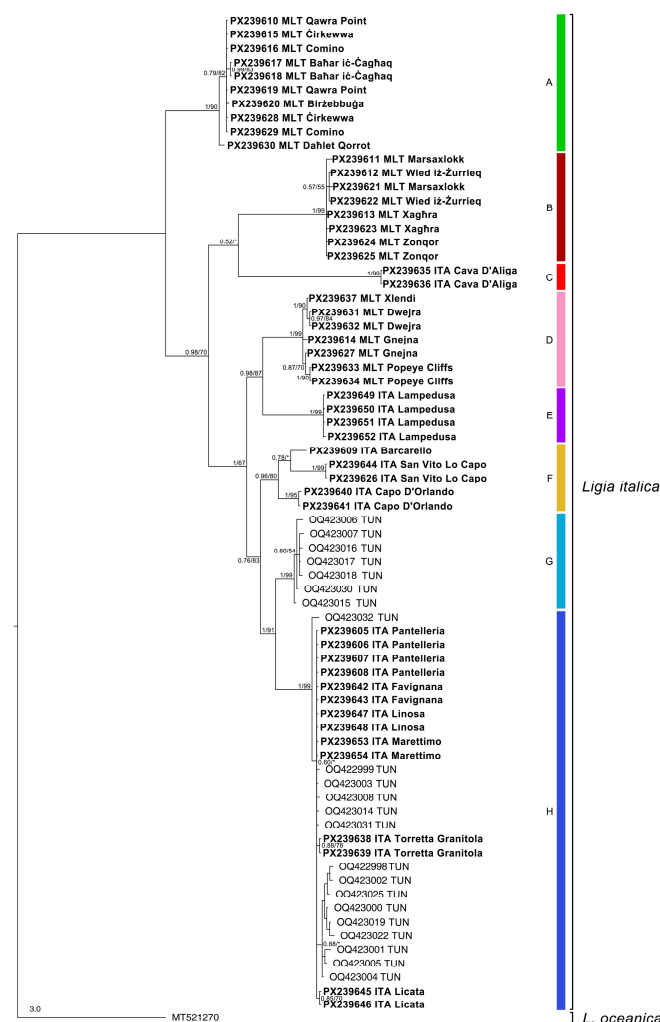


Figure 3. Bayesian phylogram of the studied *Ligia italica* samples based on the mtDNA I dataset. *Ligia oceanica* (Linnaeus, 1767) was used as an outgroup to root the tree. Node supports are reported as posterior probabilities (BI)/bootstrap values (ML). Asterisks indicate support values lower than 50. Square brackets group samples according to their current taxonomy. Letters A–H identify the main clades discussed in the text. The analyzed specimens are reported using the GenBank accession numbers listed in Table 1 and the ISO 3166-1 alpha-3 code for the country. Novel sequences are reported in bold.

Novel sequences were deposited in GenBank (see Table 1 for their Accession Numbers, A.N.'s). Furthermore, aiming to compare the novel produced COX1 sequences with a selection of those publicly available, 27 *Ligia italica* COX1 sequences (including one sequence for each known haplotype of the species) and a sequence of *L. oceanica* (Linnaeus, 1767) (used as an outgroup) were downloaded from GenBank and included in the analyses. All COX1 sequences were aligned with MEGA12 software through the ClustalW method. All analyzed COX1 sequences were translated into amino acids to check for any possible presence of frameshifts or stop codons, eventually highlighting the presence of sequencing errors or pseudogenes. The uncorrected *p*-distance values among COX1 sequences were calculated using MEGA12.

Based on available sequences, two datasets were built. The first one included COX1 *Ligia italica* sequences only from the study area (“mtDNA I dataset”). This allowed to include 73 sequences, i.e., the Tunisian specimens investigated in the literature (see [17]), the novel ones investigated in the present study and the outgroup. A second dataset included all the COX1 sequences available on GenBank (“mtDNA II dataset”—see Figure S1).

For all datasets phylogenetic relationships were inferred using the software packages MrBayes v. 3.2.7 [33] and PhyML v. 3.0 [34], employing Bayesian Inference (BI) and Maximum Likelihood (ML) approaches, respectively. Node support was assessed by calculating bootstrap values (BS) with 1000 replicates for the ML trees, while posterior probability values (PP) were reported for the BI trees. For each marker, the optimal evolutionary model was determined from those available by MrBayes, using the Bayesian model choice criteria (nst = mixed, rates = invgamma) and subsequently applied in the ML analysis. The best-fit model for all datasets was identified as the General Time-Reversible model of sequence evolution with a proportion of invariable sites and gamma-distributed rate variation among sites (GTR + I + Γ).

In the BI analyses, two independent Markov chain Monte Carlo analyses were carried out for 1,000,000 generations (temp.: 0.2; default priors) with sampling every 1000 generations, the first 2500 trees were discarded as a burn-in process and a consensus tree was constructed (Effective Sample Size (ESS) greater than 200 was reached in all the analyses performed).

3. Results

Overall, 50 COX1 novel *Ligia italica* sequences were produced. After aligning novel and GenBank sequences, as well as trimming the tails which were not present in all the specimens, we obtained a 590 bp COX1 fragment (for both mtDNA datasets). Moreover, no stop codons were detected through the translation of the novel and published COX1 sequences into amino acids.

The BI and ML trees based on the mtDNA I dataset, overall, showed eight well-supported haplogroups clustering the sequences based on their geographical origin (Figures 1 and 3): (A) Maltese Archipelago (northern coasts); (B) Maltese Archipelago (eastern coasts); (C) Sicily (south-eastern coast); (D) Maltese Archipelago (western coasts); (E) Lampedusa island; (F) Sicily (northern coasts); (G); Tunisia (southern coast); (H) Tunisia (northern coast), Aegadian islands (Marettimo and Favignana), Pantelleria island, Linosa island, Sicily (southern coasts). In contrast, the BI and ML trees based on the mtDNA II dataset showed a topology which was less congruent with the mtDNA I results. Haplogroups A-H from the study area appeared with lower support and several polytomies, suggesting a more unresolved pattern when compared with the broader dataset available (Figure S1). The mean uncorrected *p*-distance values within and between haplogroups are reported in Table 2.

Table 2. Estimates of Evolutionary Divergence (uncorrected *p*-distance) over sequence pairs within and between groups (haplogroups).

Within Groups		Between Groups						
		A	B	C	D	E	F	G
A	0.004							
B	0.003	0.161						
C	0.000	0.170	0.176					
D	0.006	0.142	0.132	0.179				
E	0.001	0.150	0.139	0.171	0.090			

F	0.041	0.135	0.127	0.177	0.089	0.092		
G	0.005	0.137	0.128	0.171	0.087	0.089	0.063	
H	0.006	0.150	0.132	0.175	0.096	0.102	0.076	0.055

4. Discussion

Our results highlight an unexpectedly high genetic diversity and phylogenetic structure within the study area (Figure 3). We found eight distinct haplogroups (i.e., A–H, see Figure 3 and Table 1) with low internal diversity and high divergence between them (Table 2). The distances between haplogroups, ranging from 5.5% to 17.9% (Table 2), are at the higher end of the reported range comparable to those assigned to a specific rank within the genus *Ligia* [17,35,36], while their average within-groups distance is 0.8%. This finding further supports the interpretation of *L. italica* as a putative species complex [17], harbouring cryptic diversity worthy of taxonomic revision. Similar complex patterns of hyperdiversity have also been documented in other arthropods typical of Mediterranean coastal shores and intertidal zones as Coleoptera and Copepoda [37–40].

The sea-slater populations of the Maltese archipelago present an unexpectedly complex phylogeographic condition. Three different haplogroups (A, B, D) were identified, exhibiting with very high mutual genetic distances ranging between 13.2% and 16.1% (Table 2). The three lineages have a paraphyletic condition, with no sister relationships (Figure 3). No cases of mixed population were identified and their distribution in the archipelago is apparently parapatric (Figure 1), with the sole exception of a single sample of haplogroup A (A.N., PX239620), which represented the only specimen observed along the entire 160 m concrete dock of the Birżebbuġa Marina, which falls within the range of haplogroup B (Figure 1). The isolation and unsuitable habitat (e.g., lack of shelters and pools) of this single sample suggests that it may have been unintentionally introduced via “shipping” (see [41]) from the range of haplogroup A.

The origin and relationships of the Maltese *Ligia italica* populations are very difficult to interpret, based on our results. Although Malta has a rather complex geological history [42], which likely influenced the origin and distribution of its native fauna [24,43,44], vicariance events within the archipelago are currently not supported for *L. italica*, due to the evident paraphyletic relationships between haplogroups A, B, and D (Figures 3 and S1); this condition could suggest a mixture of colonization processes from different sources. Furthermore, active dispersal events from the Hyblaean Plateau (southeastern Sicily) during Pleistocene marine regression occurrences are unlikely, due to the weakly supported relationships between the Maltese haplogroup B and the closer “mainland” one C (Figures 3 and S1). In addition, the hypothesis of passive dispersion via rafting is difficult to apply since the Maltese archipelago is at the centre of a complex surface circulation, affected by the Malta–Sicily anticyclonic gyre to the north, and by the Medina cyclonic gyre to the south [45]. Similarly, origin through several introduction events (e.g., by shipping) cannot currently be ruled out, due to the limited knowledge of genetic variation within *L. italica* across its entire range, which could hide possible sources of translocation. Greater sampling coverage could, in fact, further clarify the relationships between populations and further identify the causes and drivers of recent dispersal events, both natural and human-mediated.

Haplogroup E includes sequences obtained from two localities on the island of Lampedusa (Table 1, Figure 1). It presents consistent mean uncorrected *p*-distance values with respect to the other groups (9–17%, Table 2) and sister relationships with haplogroup D (western cliffs of the Maltese Archipelago), with the latter haplotype location directly facing the island of Lampedusa, albeit at an approximate distance of 110 km. Thus, unexpectedly, the populations of *Ligia italica* from Lampedusa do not appear to have direct phylogenetic relationships with those of the geographically closest Tunisian

mainland (*G* and *H* haplogroups). Lampedusa and Tunisia, in fact, share the same continental shelf and were connected by land bridges during the Pleistocene intermittent marine regressions [46]; this has shaped, on the island, a unique faunal assemblage largely of North African origin [47,48]. Furthermore, Lampedusa is affected (similarly to the small islands of the *H* lineage) by a superficial marine circulation that would favour a passive dispersal of *L. italica* from the coasts of North Africa [45]. These factors do not support a hypothesized long-term isolation of this population as well as the phylogenetic relationships that emerge from our analysis. Therefore, they deserve to be explored further by increasing sampling coverage in the wider central Mediterranean area, to more precisely define the origin, affinities and possible source of the haplogroup *D*.

Haplogroup *H* is the most widespread in our study area, including samples from northern Tunisia [17], much of the southern coast of Sicily, the Aegadian archipelago and the volcanic islands of Linosa (Pelagian Archipelago) and Pantelleria (Figures 1 and 3). Low internal variability (mean uncorrected *p*-distance < 1%; Table 2), a poor geographic structure and the sharing of haplotypes between allopatric populations (Figure 1) are observed inside this group, despite its wide distribution and the occurrence of many potential barriers between populations. Haplogroup *H*, in fact, occurs on isolated (open-water) islands (i.e., Pantelleria and Linosa), and along the predominantly sandy coast of southern Sicily [49] between Torretta Granitola and the western part of the Gulf of Gela (Figure 1), where *Ligia italica* has a fragmented distribution along a few scattered rocky shores, factors that should increase diversity by interrupting gene flow (see [13]). In a genus known for its poor dispersal ability and high intraspecific variability [13,14,17], it is difficult to interpret a monophyletic unit that combines a wide and fragmented distribution and a low internal variability. However, it cannot be ruled out that the occurrence of passive dispersion may play a role in the distribution and internal gene flow of haplogroup *H*, thus diluting any putative internal genetic variation that may arise. In particular, rafting is known as one of the main processes that can maintain connectivity within coastal species under allopatric conditions [50,51]; this was also often hypothesized for sea-slatters [41,52,53]. The sea surface circulation across the Strait of Sicily, described by Reyes-Suarez et al. [45], suggests that rafting events could be favoured by the Atlantic Ionian Stream (Tunisia to the Aegadian islands and to southern Sicily), as well as by the Atlantic Tunisian Current (Tunisia to Pantelleria and Linosa). Similarly, haplogroup *F*, which encompasses samples from the northern Sicilian coast and presents phylogenetic relationships with the Southern Tunisian (*G* haplogroup) [17] and the *H* haplogroup, may have been influenced by the Atlantic/Algerian bifurcation current.

The genetic variation in *Ligia italica* across the southern coasts of Sicily is not solely represented by haplogroup *H*. The southeastern area is in fact affected by haplogroup *C* (Figure 1), phylogenetically unrelated to *H* (Figure 3), from which it presents a significant genetic distance (17.5%) (see Table 2). This result suggests, once again, a non-obvious relationship between diversity and geographic proximity. Our sampling coverage does not allow us to precisely establish the geographical limits between haplogroups *C* and *H*, furthermore, the actual extension of the former haplogroup deserves further investigation. Given the limited data available on *L. italica* in this area, it is currently difficult to explain the possible factors that determined its diversity. It could be influenced by vicariance phenomena, given the particular geological history of the Hyblaean Plateau and its fauna [20,23,54], as well as by dispersal, given the peculiar pattern of surface marine circulation that affects this area [45].

Overall, our findings do not reveal unambiguous patterns of dispersal, isolation, or differentiation across the Strait of Sicily. Instead, they point to a highly complex biogeographic scenario, likely shaped by multiple, independent factors. While the phylogenetic relationships among lineages within the study area are generally well

supported (Figure 3), these relationships do not appear to correspond clearly to geographic distance or to connectivity between localities. These discrepancies, along with a reduced clarity in phylogenetic relationships, become more pronounced when *Ligia italica* sequences available from other areas are included in the analysis (Figure S1). This likely reflects the high degree of genetic diversity within the species, combined with the incomplete and geographically scattered nature of the available dataset across the distribution range of *L. italica*.

5. Conclusion

In conclusion, the remarkable cryptic diversity that emerges amongst the Italian sea-slater populations (see also [17]) emphasizes the need to expand sampling and genetic investigation across its entire geographic range in order to acquire a better understanding of their evolutionary relationships between the identified haplotypes. To further explore its taxonomy, it will also be necessary to compare populations using additional genetic markers (e.g., nuclear DNA) and by assessing any variation detected at the molecular level through an examination of morphological traits too.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d17090622/s1>, Figure S1: Bayesian phylogram based on the mtDNA II dataset. *Ligia oceanica* (Linnaeus, 1767) was used as an outgroup to root the tree. Node supports are reported as posterior probabilities (BI)/bootstrap values (ML). Asterisks indicate support values lower than 50. Square brackets group samples according to their current taxonomy. The analyzed specimens are reported using the GenBank accession numbers listed in Table 1 and the ISO 3166-1 alpha-3 code for the country. Novel sequences are reported in bold.

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Data Availability Statement: The data presented in this study are available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/>, accessed on 28 August 2025 (COI A.N.'s: PX239605-PX239654).

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