Molecular evidence for sweeping discontinuity between peracarid (Crustacea) fauna of Macaronesian islands and nearby continental coasts: over fifty candidate endemic species

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Abstract

Oceanic islands are recognized evolutionary hotspots for terrestrial organisms, but little is known about their impact on marine organisms' evolution and biogeography. The volcanic archipelagos of Macaronesia occupy a vast and complex region which is particularly suitable to investigate marine island biogeography.

In this study, we used mitochondrial DNA sequences to investigate the genetic differentiation between the populations from Webbnesia (i.e. Madeira, Selvagens and Canaries) and adjacent coasts, of 23 intertidal peracarid species. All species had unexpectedly high intraspecific genetic distances, reaching more than 20% in some cases. Between 79 and 95 Molecular Operational Taxonomic Units (MOTUs) were found in these species. Webbnesia populations displayed an impressive genetic diversity and high endemism, with 83% of the MOTUs being private to these islands, particularly La Palma and Madeira. Network analyses suggested higher similarity between Webbnesia and Azores than with adjacent continental coasts.

These results reveal an unanticipated and sweeping biogeographic discontinuity of peracaridean fauna between Webbnesia and the Iberian Peninsula, raising suspicion about the possible occurrence of identical patterns in other groups of marine invertebrates in the region. We emphasize the unique genetic heritage hosted by these islands, underlining the need to consider the fine scale endemism in marine conservation efforts.

Keywords

Biogeographic discontinuity, Islands, Peracarida, cryptic species, DNA barcoding, Northeast Atlantic.

Introduction

The marine realm is generally considered to have lower habitat diversity and higher connectivity than terrestrial habitats [1,2]. Non-marine biota inhabiting oceanic islands have to cross the ocean to disperse and are more prone to isolation than marine organisms [3]. However, several studies have been indicating an increasing number of discontinuities between and within marine bioregions, possibly driven by constraints in dispersal and gene flow, that only recently started to be noticed and reported (e.g. [4,5]). Moreover, it is known that even geographically close islands [6–8] may comprise distinct marine coastal communities in response to local biotic and abiotic factors.

Recent studies on the marine biota of Macaronesia, sustain that this group of 31 islands belonging to five archipelagos (i.e. Azores, Madeira, Selvagens, Canaries, Cape Verde) in the Northeast Atlantic (NEA), comprise in fact not one, but three distinct bioregions. For example, Cabo Verde differs significantly from the other Macaronesian archipelagos and appears to be a subprovince within the West African Transition province [9–11], while the remaining archipelagos may belong to the Lusitanian province [11,12]. Because Madeira, Selvagens and Canaries share a higher affinity in their biota, it was proposed that these archipelagos should be grouped in a separate ecoregion named “Webbnesia”, leaving the Azores as an independent ecoregion by itself [11].

Recently, with the support of molecular tools, we have found cryptic diversity within the isopod *Dynamene edwardsi* (Lucas, 1849) [13] and in the amphipod family Hyalidae [14] occurring in Macaronesia. Our studies suggested segregation among islands and a possible discontinuity between Webbnesian fauna and the adjacent continental landmasses.

Peracarids are abundant benthic crustaceans in marine coasts that have presumably lower dispersal capacities due to lacking planktonic larvae, thereby being particularly suited to investigate biogeographic discontinuities in the open ocean. In this study, we aimed to use the cytochrome c oxidase subunit I (COI) DNA barcoding region [15] to conduct a comprehensive parallel screening of genetic differentiation across populations from the NEA of 23 morphospecies of Amphipoda, Isopoda and Tanaidacea. In particular, we aim to probe the occurrence of cryptic diversity by investigating the suspected genetic and taxonomic discontinuities between the above-mentioned presumptive bioregions.

Material and Methods

Peracarid specimens were collected in the archipelagos of Azores, Madeira, Selvagens and Canaries and in the continental coasts of Morocco and Iberian Peninsula (figure 1). Morphology-based taxonomic identification was performed consulting specialized literature. Sampling details and literature used can be accessed in the supplementary material.

According to the main hypothesis, specimens of each species were chosen from two main regions (Iberian Peninsula and Webbnesia), following the genetic differentiation observed between these regions in previous works [13,14]. The first group included the specimens sampled in Iberian Peninsula (IP) and the second included the specimens collected in Webbnesia, i.e. Madeira, Selvagens and Canaries archipelagos (MACA). Only the sequences of *Stenothoe monoculoides* (Montagu, 1813) were from the North Sea, because there were no public data available from the Iberian Peninsula. However, our unpublished data derived from metabarcoding already detected this species in Northwest of Spain and was confirmed as the same haplotype as the one from North Sea. Therefore, we are confident that this morphospecies occurs in Iberian Peninsula. In addition, when present, specimens from Morocco (MORO) and Azores (AZ), were added to the main experimental design (figure 1; supplementary material, Table S1).

DNA extraction, COI amplification, PCR products purification and sequencing were performed for each specimen following [14]. The other sequences were obtained in our previous works [13,14,16,17] and from [18] (see supplementary material, Tables S1-S2, for list of primers, number of specimens in each species and source). A common fragment of 520 base pair was obtained and used in subsequent analyses. Maximum and mean pairwise distances (p-distances) for COI within each morphospecies were calculated in general and within groups in MEGA 7.0 [19].

To assess the presence of cryptic species (i.e. multiple molecular operational taxonomic units - MOTUs) in each morphospecies [20], five methods were applied to the dataset: automatic barcode gap analysis (ABGD), BOLD (BINs), bayesian Poisson Tree Partition (bPTP), Generalized Mixed Yule Coalescent (GMYC) and TCS (details can be consulted in the supplementary material). A majority rule (i.e. most common number of MOTUs for each species) was applied and, in case of a tie, a conservative approach was applied choosing the lowest number of MOTUs.

Chord diagrams were built in R 3.5.0 [21] with the package 'chorddiag' [22] to inspect the number of MOTUs endemic to each island (including Iberia and Morocco) and region (i.e. MACA, IP, AZ, MORO), and amount of shared ones. Community detection representations (based on shared and private MOTUs between/within locations) were calculated with the R packages 'igraph' [23] and 'visNetwork' [24].

Results

Molecular analyses and MOTUs delimitation

A total of 483 sequences were analysed, of which 173 were produced in this study, belonging to 23 morphospecies. Mean intraspecific distance (ISD) varied between 1.81% (*Ampithoe ramondi* Audouin, 1826) and 17.16% (*Janira maculosa* Leach, 1814), while Maximum ISD was higher than 3%

for all species (Table 1). Mean p-distances between IP and MACA regions were always higher than 3%, with the highest value observed in the isopod *Anthura gracilis* (Montagu, 1808) (28%, Table 1).

The molecular species delimitation methods retrieved between 79 (ABGD) and 95 (TCS) MOTUs (Table 2). Between 41 and 53 MOTUs were present among the 13 amphipods, between 25 and 29 MOTUs in the seven isopods and between 11 and 14 MOTUs in the three tanaidaceans (Table 2, supplementary material Figs. S1-3). The consensus number of MOTUs was 89 (Table 2), with a minimum of two in six morphospecies and maximum of 11 in *Apohyale stebbingi* Chevreux, 1888 (Table 2, supplementary material, Fig. S4).

Peracarid community analysis

The MACA region harboured more MOTUs than the IP region (56 and 26 respectively; figure 1, supplementary material, Fig. S4), with the islands of La Palma (19), Madeira (17) and Gran Canaria (14) with the highest number of MOTUs. No more than four MOTUs were shared between islands and only three were shared between MACA and IP (figure 1). La Palma and Madeira were the islands with the highest number of private MOTUs (12 and 8 respectively), with MACA displaying 49 endemic MOTUs and IP only 18 (figure 1; supplementary material, Fig. S4).

The artificial networks of the islands including Morocco and Iberian Peninsula, retrieved Multilevel (modularity:0.080; figure 2A), Spinglass (modularity:0.110; figure 2B), Edge betweenness (modularity:0.020; figure 2C) and Walktrap (modularity:0.023; figure 2D) as the most fitting community detection algorithms to our data. All these algorithms grouped Canaries and Azores together, with Madeira and Selvagens showing different patterns (depending on the algorithm), and Morocco and IP in separate clusters. When regions were used, the network retrieved Multilevel and Spinglass (modularity: 0.061; figure 2E) algorithms. Both retrieved the same topology (figure 2E), with pairs MORO-IP and AZ-MACA clustering together.

Discussion

Compared to terrestrial fauna, very little is known about the biogeography and evolution of insular marine fauna [25]. The common perception for Macaronesia's marine invertebrate fauna is that many species are shared with mainland coasts of NW Africa and Iberia, hence a basal faunistic continuity is assumed. The absence of any obvious geographic barriers for marine organisms' dispersal in the region intuitively reinforces this perception. Our findings appear to contradict this view, at least in what concerns peracarids. We have found: i) extensive and profound genetic differentiation between peracarid populations from Iberia and Webbnesia; ii) extensive peracarid endemic diversity in Webbnesia, patent in 48 well-supported and highly divergent MOTUs; and iii) geographic segregation among Webbnesia's MOTUs, including many private to only one or a few islands. Our study captured for the first time this faunistic discontinuity because, to this date, it is probably the most

extensive marine invertebrate metasppecies screening of genetic differentiation between Atlantic continental and islands populations.

Diversification and evolution of peracarids and other marine invertebrates in Macaronesia

The 23 species here examined displayed completely sorted MOTUs between Iberia and Webbnesia, which were well supported by multiple clustering methods. The large amount of COI data available for animals indicates that COI-based MOTUs commonly correspond to separate species [26,27]. The genetic distances within morphospecies we observed are above the intraspecific range reported in comprehensive studies with crustaceans [17,18,28]. Even considering the top range of COI evolutionary rates estimated for crustaceans [29], these distances indicate long-term evolutionary divergence and suggest that these may be separate species.

Phylogeographic discontinuities have been reported in marine environments worldwide, e.g. [5,30–34], as the notorious case of the Wallace’s line in the Makassar Strait [4], but little is known for the NEA. Differentiation between populations of Webbnesia and those from Iberian Peninsula was also found in taxa with a planktonic phase such as sponges [35], molluscs [36,37] and fish [38], suggesting a phylogeographic discontinuity for marine fauna in general, despite the potential for larval transport by recurring oceanographic features. Long-distance dispersal in peracarideans is mainly due to stochastic events through rafting on floating objects or mediated by human vectors [39,40]. Notwithstanding the different lifestyles of each peracaridean species, a deep genetic differentiation was transversal between the populations from Webbnesia and adjacent continental coasts, suggesting that other factors such as "post-colonization monopolization" or the islands characteristics (e.g. [41–44]) may play a major role in the geographic segregation of these species. Moreover, considering the deep genetic divergences found, it is probable that the populations’ differentiation preceed the last glaciation maximum [45] and occurred thousands to millions of years ago [13].

While a clear differentiation between Webbnesia and Iberian populations was patent in all the examined species, the populations from Azores and Morocco displayed specificities depending on the taxon. The biota of Azores and Webbnesia is usually presumed similar [46], due to the currents’ patterns during interglacial periods [11,47]. Previous studies showed genetic similarities between marine invertebrates of these archipelagos [35,48], while others suggest stronger affinities between Azorean and Iberian populations [36]. In this study, both patterns were observed, although with higher support for the Azores-Webbnesia connection than for the Azores-Iberian Peninsula. Moreover, our data suggests a higher genetic proximity between Moroccan and Iberian populations, contradicting other studies that relate the populations from Morocco with those from Macaronesia due to their vicinity [32,46,49,50].

During glacial periods, the isolation of Webbnesia’s islands may have been significantly reduced when compared to present geographic distances [51], due to a lower sea level, greater surface area

and exposure of the currently submerged islands that could have served as stepping-stones. This factor, together with the similarity of habitats and geographic proximity between Madeira, Selvagens and Canaries, may explain the high number of shared MOTUs within Webbnesia (figure 2).

Implications for marine biodiversity conservation and management

Species are commonly used as framework for conservation strategies since they constitute the basic units for distributional and habitat studies in biodiversity assessments. However, with the emergence of molecular methods, the importance of molecular evidence for species delineation [52] arose as a critical contribution to understand evolution and inform conservation strategies. Using molecular methods, MOTUs could be considered as the functional units of biodiversity and might act as proxies for estimating diversity [53]. Concepts such as "Evolutionary Significant Units" (ESU) may help surpass the limitations imposed by rigid species boundaries [54], enabling the recognition of pertinent infraspecific units for the purpose of biodiversity conservation [55] and connectivity [56]. Hence, regardless of the formal species boundaries of the peracarids here investigated, it appears there is at least an extraordinary level of endemism of genetic lineages with very small ranges, frequently no larger than the island that harbours them.

The preservation of genetic diversity is an essential factor in the design of marine conservation areas which should therefore include domains that incorporate fundamental evolutionary processes [57]. In the marine environment, priority should be given to the conservation of those species most vulnerable to human activities and those with populations dangerously affected. Due to their small size and isolation, island and endemic species are more likely to extinguish than continental or non-endemic species [58]. Moreover, the human activities that mainly affect the marine environment usually take place in coastal areas, whose extension is limited and where the highest marine productivity is reached [57,59].

Marine invertebrates are rarely contemplated in marine protected areas, despite benefitting greatly from these programs [60], and little information is available about the status of each species/MOTU/ESU/population in Macaronesia. Remarkably, neither Webbnesia or the Azores were included yet in the "Ecologically or Biologically Significant Marine Areas" (EBSAs; www.cbd.int/ebsa/ebsas) [61]. However, both would probably qualify if there was a wider awareness of their unique endemic marine diversity and vulnerability. Indeed, the extent of the taxonomic and genetic diversity harboured by the marine invertebrates from Macaronesia is still poorly known [11,62] and there is an urgent need to accelerate its inventory, particularly using molecular tools, given that at current rates it will take decades till completion [63]. To protect, manage and conserve the unique biological heritage of these archipelagos, it is crucial that the fine-scale endemism of marine organisms is considered in the design of more effective networks of marine protected areas.

219 Conclusions

220 This study provides compelling evidence for a sweeping discontinuity in shallow-water peracarid
 221 fauna between Webbnesia and nearby continental coasts. We also found rampant endemic peracarid
 222 diversification in these archipelagos, and multiple cases of clear geographic sorting of MOTUs even
 223 among islands separated by no more than 60 km. These findings challenge the intuitive perception of
 224 faunistic continuity of marine organisms between islands and nearby mainland, somewhat
 225 downplaying the role of contemporary dispersal and connectivity as a main explanation for the
 226 biogeography of insular marine organisms. Indeed, founder effects, mechanisms of monopolization
 227 and preemptive exclusion [13,41,64], coupled with the islands' configuration [44], may have a more
 228 prevalent role in the elucidation of contemporary biogeographies of islands' shallow water
 229 invertebrates than previously acknowledged. We hope these results may rise the awareness on the
 230 need of considering a larger variety of taxa for the identification of protected areas shedding light into
 231 the poorly known island biogeography of marine organisms.

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Data Accessibility

All new DNA sequences generated in this work were deposited in BOLD under the projects (PMACA: “Peracarida Macaronesia vs IberiaPeninsula” and PERAC: “Peracarida New data”). All the data used in this work is available in the BOLD dataset DS-PMACA: “Peracarida Macaronesia vs IberiaPeninsula”. All R scripts are available at <https://github.com/pedromanuelvieira/Macaronesiadiscontinuity>.

Author Contributions

PEV, FOC and HQ designed the research plan; PEV, AD and SLA performed the research and analysed the data; PEV, PE and AD identified the specimens; PEV wrote the original manuscript; all the authors contributed with suggestions, to the manuscript structure and reviewed the manuscript final version.

Competing interests

The authors declare no conflict of interest.

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Table 1. Presence (●) of the peracaridean species used in this study in each region. Mean and Maximum (Max) intraspecific distance (ISD) for each species. The Mean p-distance between the Iberian Peninsula (IP) and Webbnesia (MACA) populations for each morphospecies is also displayed. AZ – Azores; MORO – Morocco.

Order	Species	MACA	IP	AZ	MORO	Mean ISD	Max ISD	Mean p-distances between IP and MACA
Amphipoda	<i>Ampithoe helleri</i>	●	●			0.0715	0.1327	0.1230
	<i>Ampithoe ramondi</i>	●	●	●		0.0181	0.0385	0.0341
	<i>Ampithoe riedli</i>	●	●		●	0.0439	0.0827	0.0782
	<i>Apohyale perieri</i>	●	●	●		0.0483	0.1135	0.0770
	<i>Apohyale stebbingi</i>	●	●	●	●	0.1243	0.2000	0.1574
	<i>Caprella acanthifera</i>	●	●	●	●	0.0805	0.1462	0.1374
	<i>Elasmopus pecteniscus</i>	●	●		●	0.0381	0.0635	0.0583
	<i>Jassa herdmanni</i>	●	●	●		0.0751	0.1362	0.1237
	<i>Podocerus variegatus</i>	●	●			0.0613	0.1019	0.0974
	<i>Protohyale schmidtii</i>	●	●	●	●	0.0693	0.1346	0.1087
	<i>Quadrinemaera inaequipes</i>	●	●			0.0911	0.1596	0.1357
	<i>Serejohyale spinidactylus</i>	●	●	●		0.1152	0.1769	0.1348
	<i>Stenothoe monoculoides</i> *	●	●			0.1637	0.2765	0.2765
Isopoda	<i>Anthura gracilis</i>	●	●	●	●	0.1521	0.2846	0.2800
	<i>Campepeopea lusitanica</i>	●	●			0.1012	0.1981	0.1226
	<i>Cymodoce truncata</i>	●	●	●		0.1263	0.2019	0.1619
	<i>Dynamene edwardsi</i>	●	●	●	●	0.1140	0.1865	0.1643
	<i>Gnathia maxillaris</i>	●	●			0.1324	0.2038	0.2000
	<i>Janira maculosa</i>	●	●			0.1715	0.2673	0.2564
	<i>Joeropsis brevicornis</i>	●	●			0.1252	0.2500	0.2462
Tanaidacea	<i>Apseudopsis latreilii</i>	●	●			0.1674	0.2404	0.2372
	<i>Tanais dulongii</i>	●	●		●	0.0840	0.1192	0.1150
	<i>Tanais grimaldii</i>	●	●	●		0.0919	0.1481	0.1065

**Stenothoe monoculoides* was retrieved from North Sea instead of IP.

Table 2. Number of MOTUs accordingly to different molecular species delineation methods for each morphospecies.

Order	Species	ABGD K2P	BOLD	TCS 95%	bPTP	GMYC	Consensus
Amphipoda	<i>Ampithoe helleri</i>	3	3	3	3	3	3
	<i>Ampithoe ramondi</i>	1	2	2	2	2	2
	<i>Ampithoe riedli</i>	2	2	3	2	2	2
	<i>Apohyale perieri</i>	4	4	4	4	4	4
	<i>Apohyale stebbingi</i>	9	13	11	11	11	11
	<i>Caprella acanthifera</i>	3	4	4	4	4	4
	<i>Elasmopus pecteniscus</i>	2	3	4	3	4	3
	<i>Jassa herdmani</i>	2	2	3	3	3	3
	<i>Podocerus variegatus</i>	3	3	3	3	3	3
	<i>Protohyale schmidtii</i>	2	5	6	2	6	2
	<i>Quadrinemaera inaequipis</i>	3	3	3	3	3	3
	<i>Serejohyale spinidactylus</i>	5	5	5	5	5	5
	<i>Stenothoe monoculoides</i>	2	2	2	2	2	2
Isopoda	<i>Anthura gracilis</i>	4	5	5	5	5	5
	<i>Campecopea lusitanica</i>	2	3	2	3	2	2
	<i>Cymodoce truncata</i>	4	4	4	4	4	4
	<i>Dynamene edwardsi</i>	7	9	9	9	9	9
	<i>Gnathia maxillaris</i>	3	3	3	3	3	3
	<i>Janira maculosa</i>	3	3	3	3	3	3
	<i>Joeropsis brevicornis</i>	2	2	2	2	2	2
Tanaidacea	<i>Apseudopsis latreilli</i>	3	3	3	3	3	3
	<i>Tanais dulongii</i>	6	6	6	4	6	6
	<i>Tanais grimaldii</i>	4	5	5	4	5	5
	Total	79	94	95	87	94	89

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Figure 1. Sampling locations (left) and number of morphospecies and MOTUs retrieved for each region for each order (right). The Venn diagram shows the total number of endemic and shared MOTUs between regions. Number of consensus MOTUs accordingly with Table 2. Co-ordinates can be consulted in Table S1. For interactive map, see <http://rpubs.com/Vieira/PeracaridaNE>. In the interactive map, in the right-top corner, it is possible to choose the different species and verify the sampling locations for each species. The records of *Stenothoe monoculoides* from North Sea are only displayed in the interactive map. The interactive map was created with the package “leaflet” [65], through the software R 3.5.0 [21]. Az - Azores; MACA - Webbnesia; MORO - Morocco; IP - Iberian Peninsula.

Figure 2. Network scenarios resulted from the best algorithms tested using locations (A-D) and regions (E).

A: multi level; B: spinglass; C: edge betweenness; D: walktrap; E: multi level and spinglass.

Interactive networks can be accessed at <https://rpubs.com/Vieira/Peracaridab> and at

<https://rpubs.com/Vieira/Peracaridab>.

Az- Azores; MACA - Webbnesia; MORO - Morocco; IP - Iberian Peninsula.