

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

3
4 Pedro E Vieira^{1,2*}, Andrea Desiderato^{3,4,5}, Sofia L Azevedo¹, Patricia Esquete¹, Filipe O Costa^{2,6}, Henrique
5 Queiroga¹

6
7 ¹Centre for Environmental and Marine Studies (CESAM), Department of Biology, University of Aveiro,
8 Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

9 ²Centre of Molecular and Environmental Biology (CBMA), Department of Biology, University of Minho,
10 Campus de Gualtar, 4710-057 Braga, Portugal

11 ³Department of Invertebrate Zoology and Hydrobiology, University of Lodz, Banacha 12/16, 90-237
12 Lodz, Poland

13 ⁴Programa de Pós-graduação em Zoologia (PGZOO), Universidade Federal do Paraná, CP 19020,
14 Curitiba, Paraná 81531-980, Brazil

15 ⁵Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Department of Functional
16 Ecology, Am Handelshafen 12, 27570 Bremerhaven, Germany

17 ⁶Institute of Science and Innovation for Bio-Sustainability (IB-S), University of Minho, Portugal

18

19 **Corresponding author:**

20 Pedro E Vieira

21 pedroefrvieira@gmail.com

22 <https://orcid.org/0000-0003-4880-3323>

23 **Abstract**

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

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

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

41

42

43 **Keywords**

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

45

46 **Introduction**

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

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

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

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

74

75 **Material and Methods**

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

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

91 DNA extraction, COI amplification, PCR products purification and sequencing were performed
92 for each specimen following [14]. The other sequences were obtained in our previous works
93 [13,14,16,17] and from [18] (see supplementary material, Tables S1-S2, for list of primers, number of
94 specimens in each species and source). A common fragment of 520 base pair was obtained and used
95 in subsequent analyses. Maximum and mean pairwise distances (p-distances) for COI within each
96 morphospecies were calculated in general and within groups in MEGA 7.0 [19].

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

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

108

109 **Results**

110 Molecular analyses and MOTUs delimitation

111 A total of 483 sequences were analysed, of which 173 were produced in this study, belonging to
112 23 morphospecies. Mean intraspecific distance (ISD) varied between 1.81% (*Ampithoe ramondi*
113 Audouin, 1826) and 17.16% (*Janira maculosa* Leach, 1814), while Maximum ISD was higher than 3%

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

116 The molecular species delimitation methods retrieved between 79 (ABGD) and 95 (TCS) MOTUs
117 (Table 2). Between 41 and 53 MOTUs were present among the 13 amphipods, between 25 and 29
118 MOTUs in the seven isopods and between 11 and 14 MOTUs in the three tanaidaceans (Table 2,
119 supplementary material Figs. S1-3). The consensus number of MOTUs was 89 (Table 2), with a
120 minimum of two in six morphospecies and maximum of 11 in *Apothyale stebbingi* Chevreux, 1888 (Table
121 2, supplementary material, Fig. S4).

122 Peracarid community analysis

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

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

137

138 **Discussion**

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

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

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152 The 23 species here examined displayed completely sorted MOTUs between Iberia and
153 Webbnesia, which were well supported by multiple clustering methods. The large amount of COI data
154 available for animals indicates that COI-based MOTUs commonly correspond to separate species
155 [26,27]. The genetic distances within morphospecies we observed are above the intraspecific range
156 reported in comprehensive studies with crustaceans [17,18,28]. Even considering the top range of COI
157 evolutionary rates estimated for crustaceans [29], these distances indicate long-term evolutionary
158 divergence and suggest that these may be separate species.

159 Phylogeographic discontinuities have been reported in marine environments worldwide, e.g.
160 [5,30–34], as the notorious case of the Wallace’s line in the Makassar Strait [4], but little is known for
161 the NEA. Differentiation between populations of Webbnesia and those from Iberian Peninsula was also
162 found in taxa with a planktonic phase such as sponges [35], molluscs [36,37] and fish [38], suggesting
163 a phylogeographic discontinuity for marine fauna in general, despite the potential for larval transport
164 by recurring oceanographic features. Long-distance dispersal in peracarideans is mainly due to
165 stochastic events through rafting on floating objects or mediated by human vectors [39,40].
166 Notwithstanding the different lifestyles of each peracaridean species, a deep genetic differentiation
167 was transversal between the populations from Webbnesia and adjacent continental coasts, suggesting
168 that other factors such as "post-colonization monopolization" or the islands characteristics (e.g. [41–
169 44]) may play a major role in the geographic segregation of these species. Moreover, considering the
170 deep genetic divergences found, it is probable that the populations’ differentiation preceed the last
171 glaciation maximum [45] and occurred thousands to millions of years ago [13].

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

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

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

187 Implications for marine biodiversity conservation and management

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

199 The preservation of genetic diversity is an essential factor in the design of marine conservation
200 areas which should therefore include domains that incorporate fundamental evolutionary processes
201 [57]. In the marine environment, priority should be given to the conservation of those species most
202 vulnerable to human activities and those with populations dangerously affected. Due to their small
203 size and isolation, island and endemic species are more likely to extinguish than continental or non-
204 endemic species [58]. Moreover, the human activities that mainly affect the marine environment
205 usually take place in coastal areas, whose extension is limited and where the highest marine
206 productivity is reached [57,59].

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

218

219 Conclusions

220 This study provides compelling evidence for a sweeping discontinuity in shallow-water peracarid
221 fauna between Webbesia 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.

232

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254 **Data Accessibility**

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

259

260 **Author Contributions**

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

264

265 **Competing interests**

266 The authors declare no conflict of interest.

267

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408 **Table 1.** Presence (●) of the peracaridean species used in this study in each region.
 409 Mean and Maximum (Max) intraspecific distance (ISD) for each species. The Mean p-distance between the
 410 Iberian Peninsula (IP) and Webbnesia (MACA) populations for each morphospecies is also displayed.
 411 AZ – Azores; MORO – Morocco.
 412

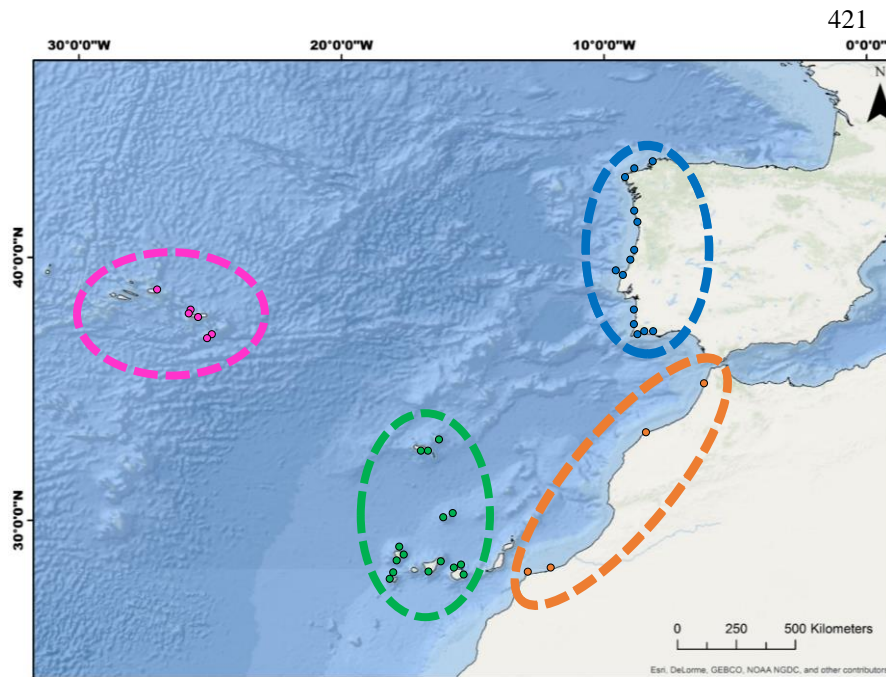
Order	Species	MACA	IP	AZ	MORO	Mean ISD	Max ISD	Mean p-distances between IP and MACA
Amphipoda	<i>Ampithoe helleri</i>	●	●			0.0715	0.1327	0.1230
	<i>Ampithoe ramondi</i>	●	●	●		0.0181	0.0385	0.0341
	<i>Ampithoe riedli</i>	●	●		●	0.0439	0.0827	0.0782
	<i>Apohyale perieri</i>	●	●	●		0.0483	0.1135	0.0770
	<i>Apohyale stebbingi</i>	●	●	●	●	0.1243	0.2000	0.1574
	<i>Caprella acanthifera</i>	●	●	●	●	0.0805	0.1462	0.1374
	<i>Elasmopus pecteniscrus</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>Quadrimaera 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>Campecopea 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

413
 414 **Stenothoe monoculoides* was retrieved from North Sea instead of IP.
 415

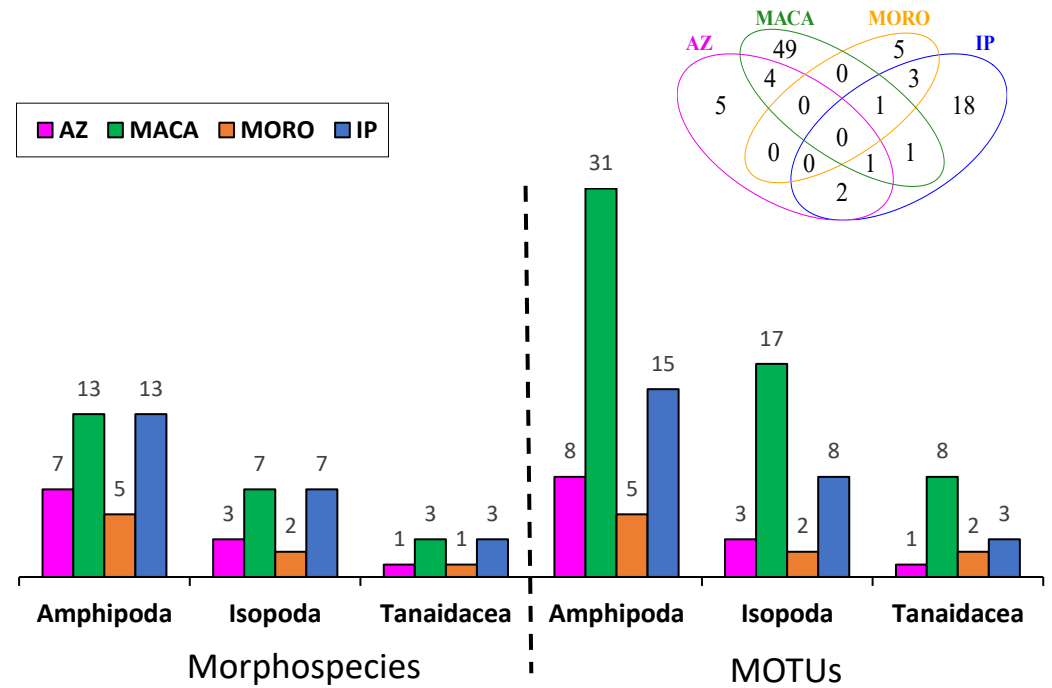
416 **Table 2.** Number of MOTUs accordingly to different molecular species delineation methods for each
 417 morphospecies.
 418

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 pecteniscrus</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>Quadrimaera inaequipus</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>Campeopea 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

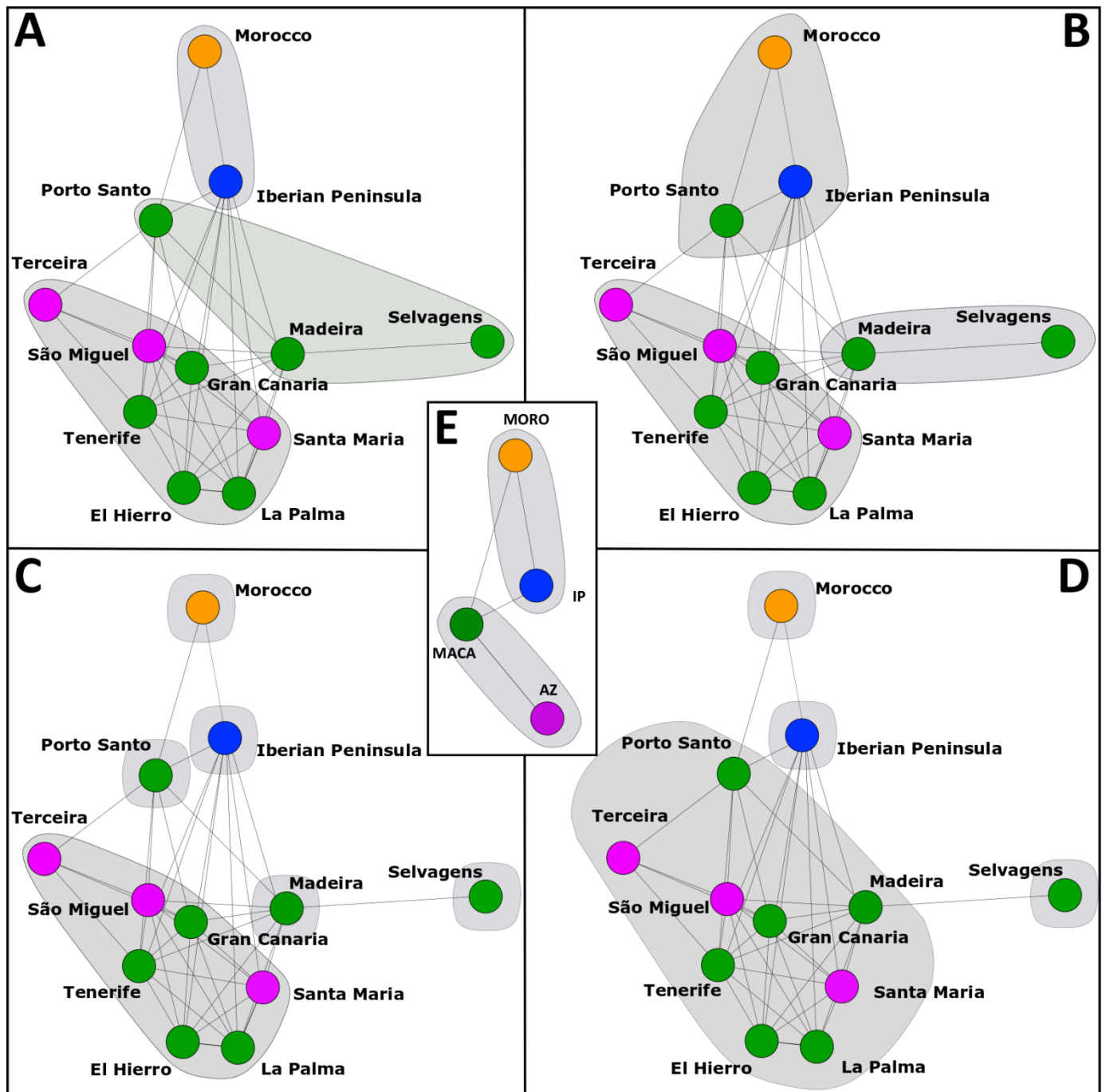
419



424



425 **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
 426 endemic and shared MOTUs between regions.
 427 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
 428 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*
 429 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].
 430 Az - Azores; MACA - Webbnesia; MORO - Morocco; IP - Iberian Peninsula.



431

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

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

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

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

436 Az- Azores; MACA - Webnesia; MORO - Morocco; IP - Iberian Peninsula.