1 **Molecular evidence for sweeping discontinuity between peracarid (Crustacea) fauna of**
2 **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 diferentiation 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 endemicity, 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 endemicity 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 inabithing 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 discountinuities 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 discountinuities 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.

80   According to the main hypothesys, 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 commom 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 *Apohyale 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 intituively 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 metaspecies screening of genetic differentiation between Atlantic

150 continental and islands populations.

151 *Diversification and evolution of peracarids and other marine invertebrates in Macaronesia*

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 intrapecific 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 caracteristics (e.g. [41–

169 44]) may play a major role in the geographic seggregation 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 endemicity 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 incorportate 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 archipleagos, it is crucial that the fine-scale endemicity 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 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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233   **Acknowledgements**

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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/pedroemanuelvieira/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.

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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.
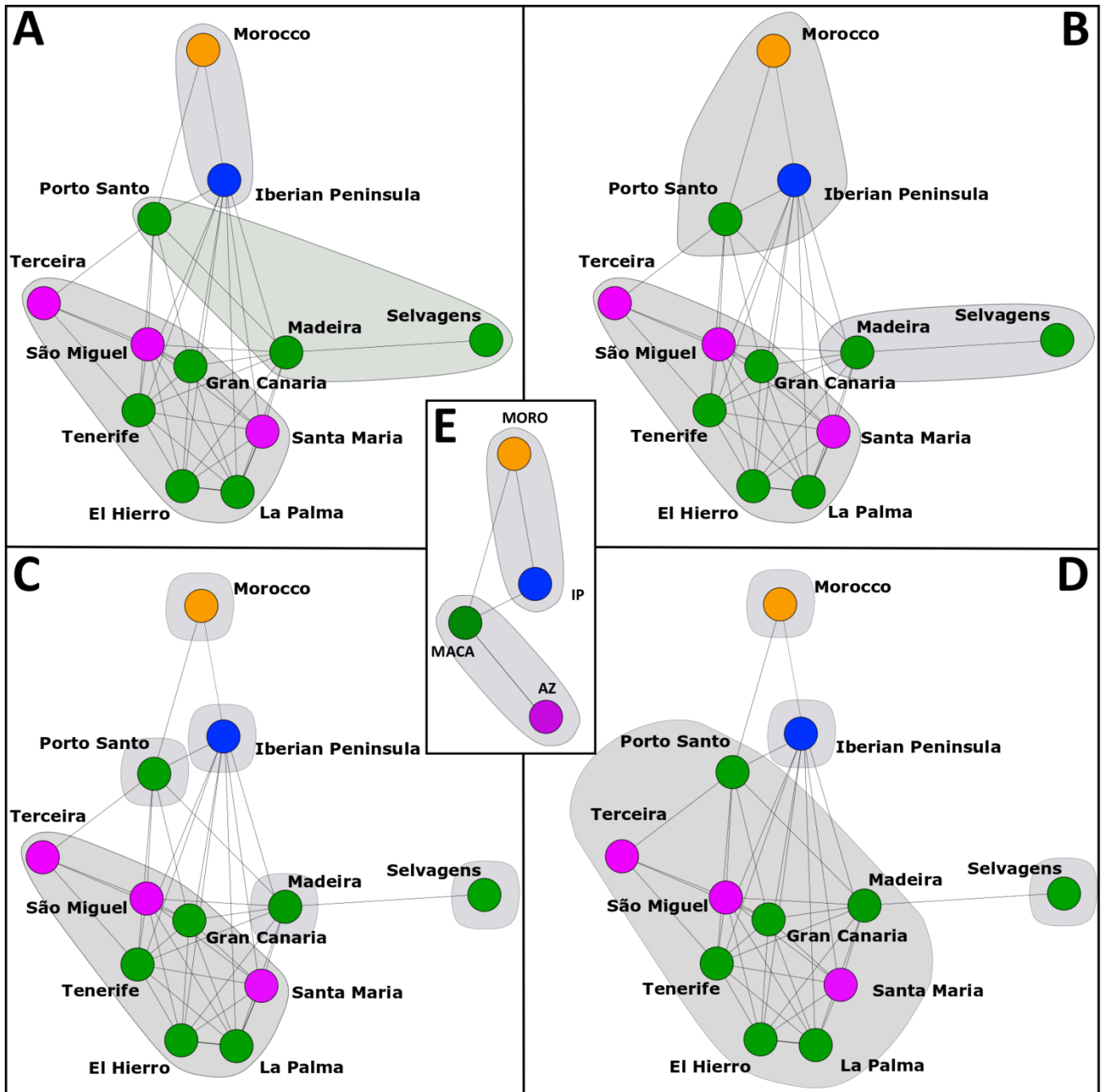411 AZ – Azores; MORO – Morocco.
412

Order	Species	MACA	IP	AZ	MORO	Mean ISD	Max ISD	Mean p-distances between IP and MACA
Amphipoda	*Ampithoe helleri*	●	●			0.0715	0.1327	0.1230
	Ampithoe ramondi	●	●	●		0.0181	0.0385	0.0341
	Ampithoe riedli	●	●		●	0.0439	0.0827	0.0782
	Apohyale perieri	●	●	●		0.0483	0.1135	0.0770
	Apohyale stebbingi	●	●	●	●	0.1243	0.2000	0.1574
	Caprella acanthifera	●	●	●	●	0.0805	0.1462	0.1374
	Elasmopus pectenicrus	●	●		●	0.0381	0.0635	0.0583
	Jassa herdmani	●	●	●		0.0751	0.1362	0.1237
	Podocerus variegatus	●	●			0.0613	0.1019	0.0974
	Protohyale schmidtii	●	●	●	●	0.0693	0.1346	0.1087
	Quadrimaera inaequipes	●	●			0.0911	0.1596	0.1357
	Serejohyale spinidactylus	●	●	●		0.1152	0.1769	0.1348
	*Stenothoe monoculoides**	●	●			0.1637	0.2765	0.2765
Isopoda	*Anthura gracilis*	●	●	●	●	0.1521	0.2846	0.2800
	Campecopea lusitanica	●	●			0.1012	0.1981	0.1226
	Cymodoce truncata	●	●	●		0.1263	0.2019	0.1619
	Dynamene edwardsi	●	●	●	●	0.1140	0.1865	0.1643
	Gnathia maxillaris	●	●			0.1324	0.2038	0.2000
	Janira maculosa	●	●			0.1715	0.2673	0.2564
	Joeropsis brevicornis	●	●			0.1252	0.2500	0.2462
Tanaidacea	*Apseudopsis latreilii*	●	●			0.1674	0.2404	0.2372
	Tanais dulongii	●	●		●	0.0840	0.1192	0.1150
	Tanais grimaldii	●	●	●		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	*Ampithoe helleri*	3	3	3	3	3	3
	Ampithoe ramondi	1	2	2	2	2	2
	Ampithoe riedli	2	2	3	2	2	2
	Apohyale perieri	4	4	4	4	4	4
	Apohyale stebbingi	9	13	11	11	11	11
	Caprella acanthifera	3	4	4	4	4	4
	Elasmopus pectenicrus	2	3	4	3	4	3
	Jassa herdmani	2	2	3	3	3	3
	Podocerus variegatus	3	3	3	3	3	3
	Protohyale schmidtii	2	5	6	2	6	2
	Quadrimaera inaequipes	3	3	3	3	3	3
	Serejohyale spinidactylus	5	5	5	5	5	5
	Stenothoe monoculoides	2	2	2	2	2	2
Isopoda	*Anthura gracilis*	4	5	5	5	5	5
	Campecopea lusitanica	2	3	2	3	2	2
	Cymodoce truncata	4	4	4	4	4	4
	Dynamene edwardsi	7	9	9	9	9	9
	Gnathia maxillaris	3	3	3	3	3	3
	Janira maculosa	3	3	3	3	3	3
	Joeropsis brevicornis	2	2	2	2	2	2
Tanaidacea	*Apseudopsis latreilli*	3	3	3	3	3	3
	Tanais dulongii	6	6	6	4	6	6
	Tanais grimaldii	4	5	5	4	5	5
	Total	79	94	95	87	94	89

419

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 interative 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.

431

Figure 2. Network scenarios resulted from the best algoritms 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.