

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.

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 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

233 **Acknowledgements**

234 The authors wish to thank the colleagues who helped during fieldwork, sample processing
235 and/or laboratory work: Tavares M and Santos R (University of Algarve, Portugal), Ladeiro B, Peteiro L,
236 Gomes I, Albuquerque R, Guimarães B and Fuente N (University of Aveiro, Portugal) and Gomes N
237 (University of Minho, Portugal). Additionally, thanks to Carvalho D in name of the Portuguese Museum
238 of Natural History and Science of Lisbon for supplying material from the
239 EMEPC/M@rBis/Selvagens2010 and EMAM/PEPC_M@rBis/2011 campaigns to Selvagens. Thanks to
240 Bellisario B for feedback regarding network analysis. Finally, thanks to Ferreira EL for the use of some
241 equipments.

242 This work was supported by the project "DiverseShores - Testing associations between genetic
243 and community diversity in European rocky shore environments (PTDC/BIA-BIC/114526/2009)"
244 funded by the Fundação para a Ciência e Tecnologia (FCT) under the COMPETE programme supported
245 by the European Regional Development Fund. FCT also supported a PhD grant to PEV
246 (SFRH/BD/86536/2012). Thanks to FCT/MCTES are also due for the financial support to CESAM
247 (UIDP/50017/2020+UIDB/50017/2020), through national funds. PE was funded through FCT in the
248 scope of the framework contract foreseen in the numbers 4, 5 and 6 of the article 23 of the Decree-
249 Law 57/2016, of August 29, changed by Law 57/2017, of July 19.

250

251

252

253

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

268 **References**

- 269 1. Gray JS. 1997 Marine biodiversity: patterns, threats and conservation needs. *Biodivers. Conserv.* **6**, 153–175.
270 2. Appeltans W *et al.* 2012 The Magnitude of Global Marine Species Diversity. *Curr. Biol.* **22**, 2189–2202.
271 3. Cox SC, Carranza S, Brown RP. 2010 Divergence times and colonization of the Canary Islands by Gallotia
272 lizards. *Mol. Phylogenet. Evol.* **56**, 747–757. (doi: 10.1016/j.ympev.2010.03.020).
273 4. Barber PH, Palumbi SR, Erdmann MV, Moosa MK. 2000 A marine Wallace's line? *Nature* **406**, 692–693.
274 5. Bellisario B, Camisa F, Abbattista C, Cimmaruta R. 2019 A network approach to identify bioregions in the
275 distribution of Mediterranean amphipods associated with *Posidonia oceanica* meadows. *PeerJ* **7**, e6786. (doi:
276 10.7717/peerj.6786).
277 6. Fernández-Palacios JM *et al.* 2016 Towards a glacial-sensitive model of island biogeography. *Glob. Ecol.*
278 *Biogeogr.* **25**, 817–830. (doi: 10.1111/geb.12320).
279 7. Rijdsdijk KF *et al.* 2014 Quantifying surface-area changes of volcanic islands driven by Pleistocene sea-level
280 cycles: biogeographical implications for the Macaronesian archipelagos. *J. Biogeogr.* **41**, 1242–1254. (doi:
281 10.1111/jbi.12336).
282 8. Ávila SP *et al.* 2018 Global change impacts on large-scale biogeographic patterns of marine organisms on
283 Atlantic oceanic islands. *Mar. Pollut. Bull.* **126**, 101–112. (doi: 10.1016/j.marpolbul.2017.10.087).
284 9. Spalding MD *et al.* 2007 Marine Ecoregions of the World: A Bioregionalization of Coastal and Shelf Areas.
285 *BioScience* **57**, 573–583. (doi: 10.1641/B570707).
286 10. Tuya F, Haroun RJ. 2009 Phylogeography of Lusitanian Macaronesia: biogeographic affinities in species
287 richness and assemblage composition. *Eur. J. Phycol.* **44**, 405–413. (doi: 10.1080/09670260902836246).
288 11. Freitas R *et al.* 2019 Restructuring of the 'Macaronesia' biogeographic unit: A marine multi-taxon
289 biogeographical approach. *Sci. Rep.* **9**, 15792. (doi: 10.1038/s41598-019-51786-6).
290 12. Almada VC *et al.* 2013 Complex origins of the Lusitania biogeographic province and northeastern Atlantic
291 fishes. *Front. Biogeogr.* **5**, 1. (doi: 10.21425/F5FBG14493).
292 13. Vieira PE *et al.* 2019 Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for low
293 dispersal marine invertebrates. *Mol. Ecol.* **28**, 1784–1800. (doi: 10.1111/mec.15052).
294 14. Desiderato A *et al.* 2019 Macaronesian islands as promoters of diversification in amphipods: The remarkable
295 case of the family Hyalidae (Crustacea, Amphipoda). *Zool. Scr.* **48**, 359–375. (doi: 10.1111/zsc.12339).
296 15. Hebert PDN, Cywinska A, Ball SL, deWaard JR. 2003 Biological identifications through DNA barcodes. *Proc.*
297 *R. Soc. Lond. B Biol. Sci.* **270**, 313–321. (doi: 10.1098/rspb.2002.2218).
298 16. Lobo J *et al.* 2013 Enhanced primers for amplification of DNA barcodes from a broad range of marine
299 metazoans. *BMC Ecol.* **13**, 34. (doi: 10.1186/1472-6785-13-34).
300 17. Lobo J *et al.* 2016 Contrasting morphological and DNA barcode-suggested species boundaries among shallow-
301 water amphipod fauna from the southern European Atlantic coast. *Genome* (doi:10.1139/gen-2016-0009).
302 18. Raupach MJ *et al.* 2015 The Application of DNA Barcodes for the Identification of Marine Crustaceans from
303 the North Sea and Adjacent Regions. *PLOS ONE* **10**, e0139421. (doi: 10.1371/journal.pone.0139421).
304 19. Kumar S, Stecher G, Tamura K. 2016 MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for
305 Bigger Datasets. *Mol. Biol. Evol.* **33**, 1870–1874. (doi: 10.1093/molbev/msw054).
306 20. Fišer C, Robinson CT, Malard F. 2018 Cryptic species as a window into the paradigm shift of the species
307 concept. *Mol. Ecol.* **27**, 613–635. (doi: 10.1111/mec.14486).
308 21. R Core Team. 2018 R: A language and environment for statistical computing. R Foundation for Statistical
309 Computing, Vienna, Austria. <https://www.R-project.org/>.
310 22. Flor M. 2016 chorddiag: Interactive Chord Diagrams. R package version 0.1.2.
311 <http://github.com/mattflor/chorddiag/>.
312 23. Csardi G, Nepusz T. 2006 The igraph software package for complex network research. InterJournal Complex
313 Systems. <http://igraph.org>.
314 24. Almende B, Thieurmel B, Robert T. 2018 visNetwork: Network Visualization using 'vis.js' Library. R package
315 version 2.0.4. <https://CRAN.R-project.org/package=visNetwork>.
316 25. Dawson MN. 2016 Island and island-like marine environments. *Glob. Ecol. Biogeogr.* **25**, 831–846. (doi:
317 10.1111/geb.12314).
318 26. Teixeira MAL. *et al.* 2020 Molecular and morphometric analyses identify new lineages within a large *Eumida*
319 (Annelida) species complex. *Zool. Scr.* **49**, 222–235. (doi: 10.1111/zsc.12397).
320 27. Blaxter M. 2016 Imagining Sisyphus happy: DNA barcoding and the unnamed majority. *Philos. Trans. R. Soc.*
321 *B Biol. Sci.* **371**, 20150329. (doi: 10.1098/rstb.2015.0329).
322 28. Costa F, Henzler C, Lunt D, Whiteley NM, Rock J. 2009 Probing marine *Gammarus* (Amphipoda) taxonomy
323 with DNA barcodes. *Syst. Biodivers.* **7**, 365–379. (doi: 10.1017/S147200009990120).
324 29. Loeza-Quintana T *et al.* 2018 Recalibrating the molecular clock for Arctic marine invertebrates based on DNA
325 barcodes. *Genome* **62**, 1–17. (doi: 10.1139/gen-2018-0107).

- 326 30. Leese F, Kop A, Wägele JW, Held C. 2008 Cryptic speciation in a benthic isopod from Patagonian and Falkland
327 Island waters and the impact of glaciations on its population structure. *Front. Zool.* **5**, 19. (doi: 10.1186/1742-
328 9994-5-19).
- 329 31. Markow TA, Pfeiler E. 2010 Mitochondrial DNA evidence for deep genetic divergences in allopatric
330 populations of the rocky intertidal isopod *Ligia occidentalis* from the eastern Pacific. *Mol. Phylogenet. Evol.*
331 **56**, 468–473. (doi: 10.1016/j.ympev.2009.12.002).
- 332 32. Xavier R *et al.* 2011 Phylogeography of the marine isopod *Stenosoma nadejda* (Rezig, 1989) in North African
333 Atlantic and western Mediterranean coasts reveals complex differentiation patterns and a new species. *Biol. J.*
334 *Linn. Soc.* **104**, 419–431. (doi: 10.1111/j.1095-8312.2011.01718.x).
- 335 33. Arnaud-Haond S *et al.* 2007 Vicariance patterns in the Mediterranean Sea: east–west cleavage and low
336 dispersal in the endemic seagrass *Posidonia oceanica*. *J. Biogeogr.* **34**, 963–976. (doi: 10.1111/j.1365-
337 2699.2006.01671.x).
- 338 34. Varela AI, Haye PA. 2012 The marine brooder *Excirrolana braziliensis* (Crustacea: Isopoda) is also a complex
339 of cryptic species on the coast of Chile. *Rev. Chil. Hist. Nat.* **85**, 495–502. (doi: 10.4067/S0716-
340 078X2012000400011).
- 341 35. Sá-Pinto A, Branco M, Sayanda D, Alexandrino P. 2008 Patterns of colonization, evolution and gene flow in
342 species of the genus *Patella* in the Macaronesian Islands. *Mol. Ecol.* **17**, 519–532. (doi: 10.1111/j.1365-
343 294X.2007.03563.x).
- 344 36. Xavier JR, Soest RWM, van Breeuwer AJ, Martin, AMF, Menken SBJ. 2010 Phylogeography, genetic diversity
345 and structure of the poecilosclerid sponge *Phorbas fictitius* at oceanic islands. *Contrib. Zool.* **79**, 119–129.
- 346 37. Quinteiro J, Rodríguez-Castro J, Rey-Méndez M, González-Henríquez N. 2020 Phylogeography of the insular
347 populations of common octopus, *Octopus vulgaris* Cuvier, 1797, in the Atlantic Macaronesia. *PLOS ONE* **15**,
348 e0230294. (doi: 10.1371/journal.pone.0230294).
- 349 38. Domingues VS *et al.* 2008 Tropical fishes in a temperate sea: evolution of the wrasse *Thalassoma pavo* and
350 the parrotfish *Sparisoma cretense* in the Mediterranean and the adjacent Macaronesian and Cape Verde
351 Archipelagos. *Mar. Biol.* **154**, 465–474. (doi: 10.1007/s00227-008-0941-z).
- 352 39. Beermann J *et al.* 2020 Ancient globetrotters—connectivity and putative native ranges of two cosmopolitan
353 biofouling amphipods. *PeerJ*. **8**, e9613. (doi: 10.7717/peerj.9613).
- 354 40. Thiel M, Gutow L. 2005 The ecology of rafting in the marine environment. II. The rafting organisms and
355 community. **43**, 279–418.
- 356 41. Waters JM, Fraser CI, Hewitt GM. 2013 Founder takes all: density-dependent processes structure biodiversity.
357 *Trends Ecol. Evol.* **28**, 78–85. (doi: 10.1016/j.tree.2012.08.024).
- 358 42. Torre G, Fernández-Lugo S, Guarino R, Fernández-Palacios JM. 2019 Network analysis by simulated
359 annealing of taxa and islands of Macaronesia (North Atlantic Ocean). *Ecography* **42**, 768–779. (doi:
360 0.1111/ecog.03909).
- 361 43. Hachich NF *et al.* 2015 Island biogeography: patterns of marine shallow-water organisms in the Atlantic Ocean.
362 *J. Biogeogr.* **42**, 1871–1882. (doi: 10.1111/jbi.12560).
- 363 44. Norder SJ *et al.* 2019 Beyond the Last Glacial Maximum: Island endemism is best explained by long-lasting
364 archipelago configurations. *Glob. Ecol. Biogeogr.* **28**, 184–197. (doi: 10.1111/geb.12835).
- 365 45. Jenkins TL, Castilho R, Stevens JR. 2018 Meta-analysis of northeast Atlantic marine taxa shows contrasting
366 phylogeographic patterns following post-LGM expansions. *PeerJ* **6**, e5684. (doi: 10.7717/peerj.5684).
- 367 46. Santos RS, Hawkins S, Monteiro LR, Alves M, Isidro EJ. 1995 Marine research, resources and conservation in
368 the Azores. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **5**, 311–354.
- 369 47. Arístegui J *et al.* 2009 Sub-regional ecosystem variability in the Canary Current upwelling. *Prog. Oceanogr.* **83**,
370 33–48. (doi: 10.1016/j.pocean.2009.07.031).
- 371 48. Hawkins SJ, Corte-Real HBSM, Pannacciulli FG, Weber LC, Bishop JDD. 2000 Thoughts on the ecology and
372 evolution of the intertidal biota of the Azores and other Atlantic islands. *Hydrobiologia* **440**, 3–17. (doi:
373 10.1023/A:1004118220083).
- 374 49. Xavier R, Branco M, dos Santos AM. 2016 Using a phylogeographic approach to investigate the diversity and
375 determine the distributional range of an isopod (Crustacea: Peracarida), *Stenosoma nadejda* (Rezig, 1989) in
376 the Atlantic-Mediterranean region. *Hydrobiologia* **768**, 315–328. (doi: 10.1007/s10750-015-2559-8).
- 377 50. Cabezas MP, Cabezas P, Machordom A, Guerra-García JM. 2013 Hidden diversity and cryptic speciation refute
378 cosmopolitan distribution in *Caprella penantis* (Crustacea: Amphipoda: Caprellidae). *J. Zool. Syst. Evol. Res.*
379 **51**, 85–99. (doi: 10.1111/jzs.12010).
- 380 51. Fernández-Palacios JM *et al.* 2011 A reconstruction of Palaeo-Macaronesia, with particular reference to the
381 long-term biogeography of the Atlantic Island laurel forests. *J. Biogeogr.* **38**, 226–246. (doi: 10.1111/j.1365-
382 2699.2010.02427.x).
- 383 52. Radulovici AE, Archambault P, Dufresne F. 2010 DNA Barcodes for Marine Biodiversity: Moving Fast
384 Forward? *Diversity* **2**, 450–472. (doi: 10.3390/d2040450).
- 385 53. Hey J. 2006 On the failure of modern species concepts. *Trends Ecol. Evol.* **21**, 447–450.

- 386 54. Coyne JA, Orr HA. 2004 *Speciation*. Oxford University Press.
- 387 55. Casacci LP, Barbero F, Balletto E. 2014 The “Evolutionarily Significant Unit” concept and its applicability in
388 biological conservation. *Ital. J. Zool.* **81**, 182–193. (doi: 10.1080/11250003.2013.870240).
- 389 56. Pante E *et al.* 2015 Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Mol. Ecol.*
390 **24**, 525–544. (doi: 10.1111/mec.13048).
- 391 57. Allendorf F, Luikart G, Aitken S. 2012 *Conservation and the Genetics of Populations*. Wiley.
- 392 58. Frankham R. 1998 Inbreeding and Extinction: Island Populations. *Conserv. Biol.* **12**, 665–675.
- 393 59. Korpinen S *et al.* 2021 Combined effects of human pressures on Europe’s marine ecosystems. *Ambio.*
394 (doi:10.1007/s13280-020-01482-x).
- 395 60. Cacabelos E *et al.* 2020 Limited effects of marine protected areas on the distribution of invasive species, despite
396 positive effects on diversity in shallow-water marine communities. *Biol. Invasions* **22**, 1169–1179. (doi:
397 10.1007/s10530-019-02171-x).
- 398 61. Rogers AD *et al.* 2020 Critical Habitats and Biodiversity: Inventory, Thresholds and Governance.
399 www.oceanpanel.org/blue-papers/critical-habitats-and-biodiversity-inventory-thresholds-and-governance.
- 400 62. Quinteiro J *et al.* 2012 Rede BANGEMAC: Banco genético marinho de Macaronésia (Memória técnica).
- 401 63. Vieira *et al.* 2021. Gaps in DNA sequence libraries for Macaronesian marine macroinvertebrates imply decades
402 till completion and robust monitoring. *Divers. Distrib.* Accepted.
- 403 64. Hupało K *et al.* 2019 Persistence of phylogeographic footprints helps to understand cryptic diversity detected
404 in two marine amphipods widespread in the Mediterranean basin. *Mol. Phylogenet. Evol.* **132**, 53–66. (doi:
405 10.1016/j.ympev.2018.11.013).
- 406 65. Cheng J, Karambelkar B, Xie Y. 2018 leaflet: Create Interactive Web Maps with the JavaScript ‘Leaflet’
407 Library. R package version 2.0.1. <https://CRAN.R-project.org/package=leaflet>.

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

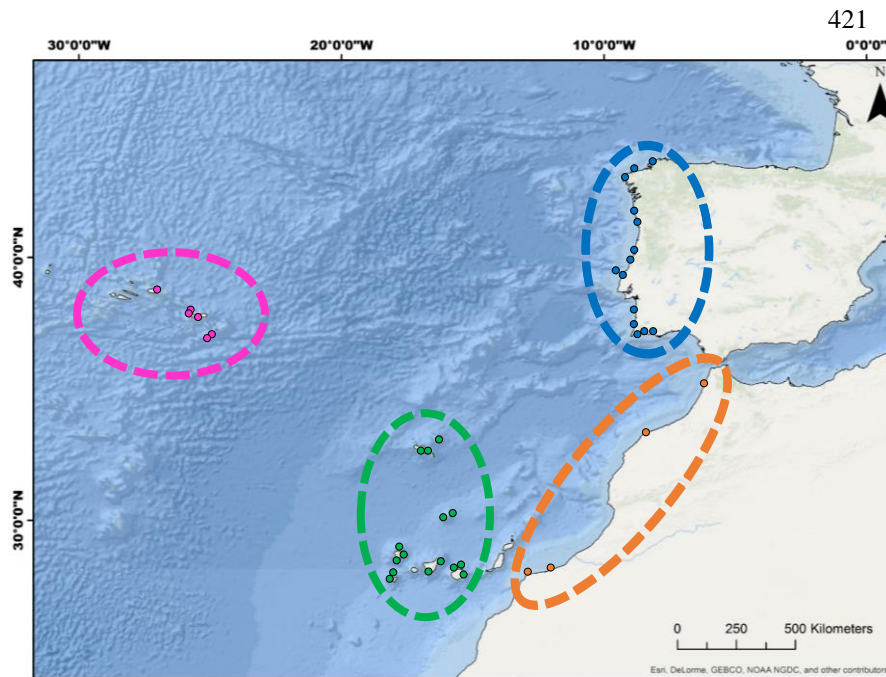
Order	Species	MACA	IP	AZ	MORO	Mean ISD	Max ISD	Mean p-distances between IP and MACA
Amphipoda	<i>Ampithoe helleri</i>	●	●			0.0715	0.1327	0.1230
	<i>Ampithoe ramondi</i>	●	●	●		0.0181	0.0385	0.0341
	<i>Ampithoe riedli</i>	●	●		●	0.0439	0.0827	0.0782
	<i>Apohyale perieri</i>	●	●	●		0.0483	0.1135	0.0770
	<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

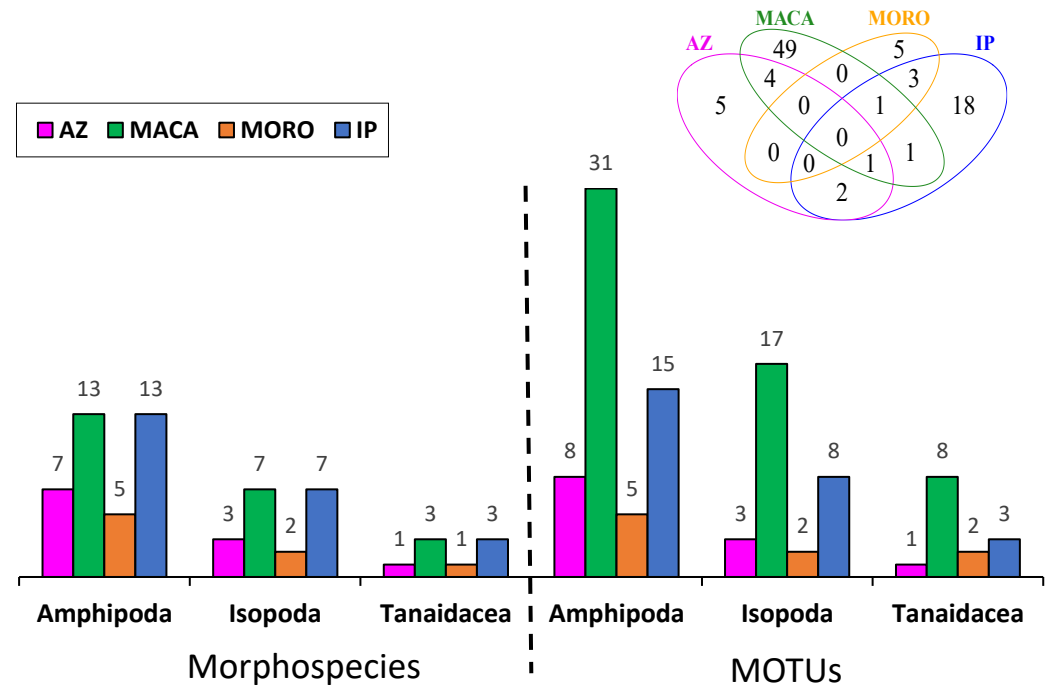
416 **Table 2.** Number of MOTUs accordingly to different molecular species delineation methods for each
 417 morphospecies.
 418

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>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

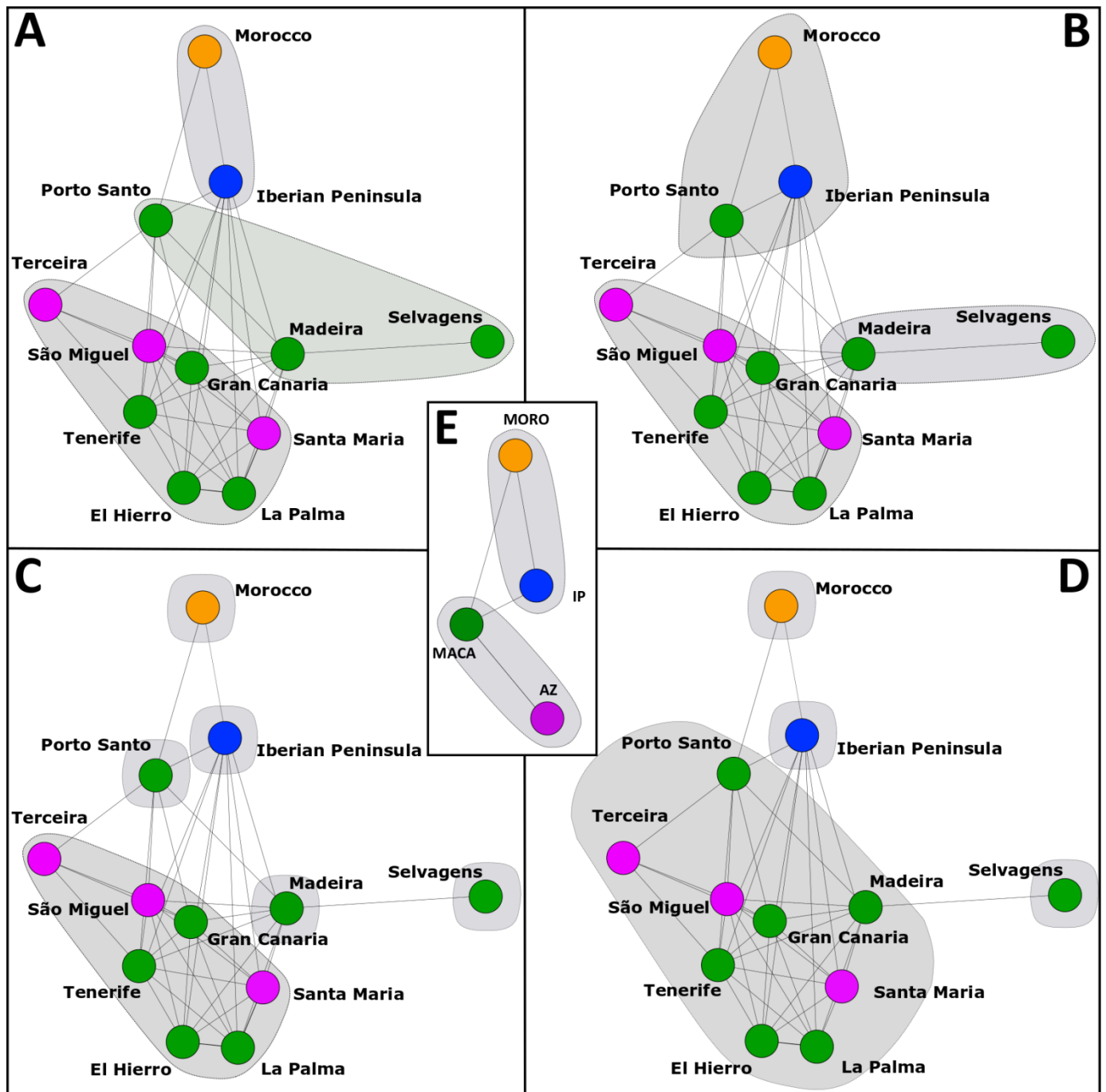
419



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.