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Deep segregation in the open ocean: Macaronesia as an evolutionary hotspot for low dispersal marine invertebrates.

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Deep segregation in Macaronesian marine fauna

Abstract

Diversification and speciation of terrestrial organisms is anticipated in oceanic islands such as Macaronesia, a group of Atlantic islands that have remained unconnected to continental landmasses. Hitherto, the diversification of marine organisms in oceanic islands, especially those with low vagility, has received little direct empirical analysis using molecular markers. Here, we focus on such a case study, through applying a multi-locus molecular approach to investigate the diversity and evolution of a group that lacks a planktonic larval stage, the isopod genus *Dynamene*, in Macaronesia and Northeast Atlantic. Sequences of two mitochondrial (cytochrome c oxidase subunit I and 16S rRNA) and two nuclear (18S rRNA and 28S rRNA) loci were obtained from specimens of *Dynamene edwardsi* (Lucas, 1849), *Dynamene magnitorata* Holdich, 1968 and *Dynamene bidentata* (Adams, 1800) collected along the Northeast Atlantic and Macaronesia. Although no major phylogeographic structure was detected in *D. bidentata* and *D. magnitorata*, from five to nine deeply divergent lineages were evident within *D. edwardsi*. The divergent lineages displayed genetic distances comparable to those found among established species of peracarids. *Dynamene edwardsi* exhibits a long, rich and complex phylogeographic history in Macaronesia, where the geodynamics of the islands possibly associated with founder effects and subsequent lack of gene flow among populations, confounds patterns based on geographical proximity of targeted populations. Our findings collectively suggest a much larger role of oceanic islands in the diversification of marine invertebrates than previously anticipated. The work provides insights into the origins and dynamics of ongoing geographic segregation and associated deep divergence among sister evolutionary lineages in Macaronesia.

Keywords

Dynamene, Macaronesia, cryptic species, oceanic islands, endemism, Northeast Atlantic.

Introduction

Islands have always caught the attention of researchers as ideal and unique natural laboratories to study evolutionary and phylogeographic processes (Emerson, 2002; Valente, Etienne, & Phillimore, 2014). Some archipelagos are notorious for the biodiversity they harbor, such as the Galapagos where Darwin (1859) recorded the occurrence of extensive levels of endemic taxa. Another famous group of islands, associated with unique biodiversity, is Macaronesia; a group of oceanic archipelagos of volcanic origin in the North Atlantic (Azores, Madeira, Canaries and Cabo Verde) that have distinct geneses and geological timelines. For instance, Selvagens emerged more than 25 million years (Myr) ago, Gran Canaria and Porto Santo around 15 Myr, while El Hierro and some islands of Azores appeared less than 1.5 Myr (for details of origin and geological times of each island and archipelago, see Carine & Schaefer, 2010; Fernández-Palacios et al., 2011; Geldmacher, Hoernle, Bogaard, Duggen, & Werner, 2005; Geldmacher, Hoernle, Bogaard, Zankl, & Garbe-Schönberg, 2001). Moreover, Macaronesian islands experience a wide range of climatic conditions and possess a highly diverse marine biota that have experienced dynamic geological and climatic changes over relatively long periods (e.g., Pleistocene glaciations; Fernández-Palacios, 2016; Fernández-Palacios et al., 2016; Norder et al., 2018). Because Macaronesian archipelagos have never been connected to the mainland, their biota is the result of dispersal from distant geographical sources and *in situ* diversification (Fernández-Palacios et al., 2011). Macaronesia therefore provides an ideal, yet understudied system to investigate evolution and phylogeography in marine benthic organisms with limited dispersal capacity (Maggs et al., 2008; Wares & Cunningham, 2001).

While patterns of diversification in Macaronesia, mainly in terrestrial organisms, have been reviewed by Borregaard et al. (2017), colonization and gene flow of marine benthic organisms have only been studied in those with a pelagic phase (Chevolot, Hoarau, Rijnsdorp, Stam, & Olsen, 2006; Sá-Pinto, Branco, Sayanda, & Alexandrino, 2008; Xavier, van Soest, Breeuwer, Martins, & Menken, 2010). Marine benthic invertebrates that inhabit intertidal coastal areas are

81 unique relative to both terrestrial organisms and other marine taxa (Hachich et al., 2015). Many
82 intertidal species have pelagic larvae which may promote dispersal over open water,
83 potentially circumventing habitat discontinuity (Grantham, Eckert, & Shanks, 2003; Scheltema,
84 1986). However, some small invertebrates, such as free-living isopods, are more prone to
85 isolation due to life histories characterised by direct development and putatively reduced
86 vagility, compared to other marine species with pelagic larvae (Thiel & Gutow, 2005). Long-
87 distance dispersal may occur through random events such as transport by shipping (Rumbold
88 et al., 2018) or by rafting on detached macroalgae or floating debris (Thiel & Gutow, 2005).
89 Effective recruitment following such mechanisms is, however, typically low due to high
90 mortality and competition in the new habitat (Highsmith, 1985; Thiel & Gutow, 2005).

91 Understanding the diversity of such organisms can be hampered either by lack of
92 comprehensive data on species distribution, i.e., the Wallacean shortfall (e.g., Cardoso, Erwin,
93 Borges & New, 2011) or the lack of relevant phylogenetic information, i.e., the Darwinian
94 shortfall (e.g., Diniz-Filho, Loyola, Raia, Mooers & Bini, 2013). In addition, it may be difficult to
95 describe species based solely on morphological characters (Beheregaray & Caccone, 2007;
96 Fišer, Robinson, & Malard, 2018; Knowlton, 1993). In peracarids, for example, even family-
97 level diagnostic characters can change with development and sex, making identification
98 problematic (Larsen, 2001; Larsen & Wilson, 1998). Such ambiguity is enhanced further by the
99 occurrence of cryptic species, as for example among different terrestrial organisms in
100 Macaronesia (e.g., Borges et al., 2017; López, Hernández-Teixidor, Macías-Hernández, Juan
101 & Oromí, 2013) and in marine organisms elsewhere (Miglietta, Faucci, & Santini, 2011),
102 especially among those with reduced vagility (Cook, Page, & Hughes, 2008; Knowlton, 1993).

103 In order to resolve taxonomic ambiguity, molecular taxonomy has been successfully applied
104 to isopods. Examples include the validation of new species (Khalaji-Pirbalouty & Raupach,
105 2014; Xavier et al., 2011), discrimination of morphologically similar species (Radulovici et al.,
106 2009; Xavier et al., 2012) or unravelling multiple cryptic species complexes (Brix, Svavarsson,

& Leese, 2014; Markow & Pfeiler, 2010; Raupach et al., 2014; Raupach & Wägele, 2006; Varela & Haye, 2012).

The existence of cryptic species is suspected, though not yet investigated, within most of the large genera in the isopod family Sphaeromatidae such as *Cilicaea*, *Cymodoce*, *Dynamenella*, *Exosphaeroma*, *Pseudosphaeroma* and *Dynamene* (Poore & Bruce, 2012). Members of the genus *Dynamene* Leach, 1814 are common and abundant on rocky intertidal and shallow subtidal habitats of the Northeast (NE) Atlantic Ocean, Mediterranean and Black Seas. Although eight *Dynamene* species were reported worldwide (six in the Northern Hemisphere), only three species are present in the NE Atlantic (Holdich, 1970; Vieira, Queiroga, Costa, & Holdich, 2016): *D. bidentata* (Adams, 1800), *D. edwardsi* (Lucas, 1849) and *D. magnitorata* Holdich, 1968. Consequently, in this study, we aim to exploit an ideal opportunity to investigate the role of Macaronesian islands in shaping the phylogeography and evolutionary history of marine invertebrates lacking a pelagic dispersal stage. We examine the genetic diversity and phylogeography of *Dynamene* morphospecies from the Macaronesian archipelagos of Azores, Madeira and Canaries in the NE Atlantic using a multi-locus approach, and reveal an evolutionary history of extensive radiation followed by long-term lineage segregation, which would not be anticipated in an island-dwelling marine invertebrate.

Material and Methods

Specimen sampling and taxonomic identification

Specimens were collected in the NE Atlantic (Vieira, Queiroga, Costa, & Holdich, 2016) between 2009 and 2015 among algae in the rocky shore intertidal (Figure 1; Table S1). Three species were sampled: *D. bidentata*, *D. magnitorata* and *D. edwardsi*. *Dynamene bidentata* was collected in Scotland, the Iberian Peninsula, Morocco and Gran Canaria (Figure 1; Table S1); *D. magnitorata* was found in the Iberian Peninsula, Morocco and in the islands of Santa Maria, Terceira, São Miguel and La Palma (Figure 1; Table S1) and *D. edwardsi* in the Iberian Peninsula, Madeira, Porto Santo, Selvagens, Gran Canaria, La Palma, Tenerife, El Hierro,

134 São Miguel and Morocco (Figure 1; Table S1). Two additional individuals sampled in 2014
135 (see Acknowledgements) from algae present at 1 m depth in two harbors from the
136 Mediterranean were also incorporated, one from France and another from Croatia (Figure 1;
137 Table S1). A detailed interactive map can be accessed at
138 <https://rpubs.com/Vieira/DynameneSamplingLocations>, in which the sampling locations and
139 the distances between them can be observed for each *Dynamene* species. The interactive
140 map was created with the package 'leaflet' (Cheng, Karambelkar, & Xie, 2018), through the
141 software R 3.5.0 (R core team 2018; www.r-project.org).

142 After collection, specimens were immediately preserved in 96% ethanol. Morphology-based
143 taxonomic identification was supported by specialized literature (Holdich, 1968a; Vieira,
144 Queiroga, Costa, & Holdich, 2016) and David Holdich, a recognized expert in *Dynamene*
145 taxonomy (e.g., Holdich, 1968a, 1968b, 1976), confirmed the classification of the specimens
146 used in the analyses and their assignment to morphospecies. Identifications were reviewed
147 before and after obtaining the DNA sequences to ensure correct identification of the
148 specimens. A total of 2815 *Dynamene* specimens were observed morphologically (1144 *D.*
149 *edwardsi* specimens, 497 *D. bidentata* specimens and 1174 *D. magnitorata* individuals). A
150 complete and detailed list of the material observed (and other *Dynamene* specimens/species)
151 by David Holdich and/or Pedro Vieira can be consulted in Vieira, Queiroga, Costa, & Holdich
152 (2016) Supplementary material 1. Representative specimens from each location were
153 deposited in the CESAM Biological Research Collection of Marine Invertebrates (Aveiro,
154 Portugal) under the accession number DBUA0002261- DBUA0002273.

155 Scanning electron microscope images were produced for one representative adult male for
156 each species by David Holdich whilst at the University of Nottingham – see Holdich (1976) for
157 details of preparation and equipment used. The *Dynamene bidentata* adult male was collected
158 in a mid-shore crevice at St Brides Haven, Wales, in October 1965. The *Dynamene*
159 *magnitorata* adult male was also collected in a mid-shore crevice, at Roscoff, France, in April
160 1966. The *Dynamene edwardsi* adult male was collected amongst tube worms at 1 m depth in

Naples, Italy, in June 1967. The specimens of the three species no longer exist as they had been dried for the SEM work in 1976. Consequently, no accession numbers are available.

DNA extraction, amplification and sequencing

DNA was extracted using the E.Z.N.A. Mollusc DNA extraction Kit according to manufacturer's instructions. Depending on specimen size, only a small amount of tissue (pereopods or/and part of the pereon) or the whole animal was used. We used the cytochrome c oxidase subunit I (COI) DNA barcode region as the primary locus for investigating the genetic diversity of *Dynamene*. A total of 179 sequences were obtained (40 for *D. bidentata*, 101 for *D. edwardsi* and 38 for *D. magnitorata*; Table S1). Based on the COI phylogeny we selected representative specimens from each geographic region and species for further sequence analysis using part of the mitochondrial gene 16s rRNA (N=43), a partial segment of the nuclear gene coding for 28s rRNA (N=46) and the variable regions 2-5 of 18s rRNA (N=120). All PCR reactions were performed in a total of 25 µl volume, containing 12.5 µl supreme *Taq* (Nzytech), 0.5-1.25 µl of each primer (10mM) and 1-4 µl DNA extraction. The remaining volume comprised ultrapure water. PCR conditions and primers used are detailed in Table S2.

A 658-base pair (bp) region of COI was amplified using LoboF1/LoboR1 (Lobo et al., 2013) or LCO1490/HCO2198 (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) primers, and a fragment of 16S rRNA was amplified using 16Sar/16Sbr (Palumbi et al., 2002) or D16SAR/D16SBR (Geller, Walton, Grosholz, & Ruiz, 1997) primers depending on PCR reaction success. The nuclear genes coding 18S rRNA were amplified with the primers 18sAi/18sBi (Whitting, 2002) and 28S rRNA with the primers AM-28S-H/AM-28S-T (Tomikawa, Kobayashi, Morino, & Mawatari, 2007). Amplification success was verified in a 1.5% agarose gel. PCR products were purified (with Roche purification kit according to manufacturer instructions) and sequenced bidirectionally by an external service supplier (STABVida), using an ABI 3730 sequencer and following standard chain-termination sequencing protocols.

Data analysis

188 All sequences were analysed and edited using MEGA 7.0 (Kumar, Stecher, & Tamura, 2016).
189 Trace files were checked manually; unreadable zones and primers were removed and
190 ambiguous bases corrected. For the 658 bp COI region, the edited sequences were aligned
191 using Clustal W (Thompson, Higgins, & Gibson, 1994) as implemented in MEGA 7.0 (Kumar,
192 Stecher, & Tamura, 2016) and the translation verified for stop codons or indels.
193 Sequences of 16S rRNA, 18S rRNA and 28S rRNA were aligned separately using Clustal W
194 (Thompson, Higgins, & Gibson, 1994) tool in MEGA 7.0 (Kumar, Stecher, & Tamura, 2016).
195 Highly variable regions were deleted from the analysis using Gblocks 0.91b
196 (http://molevol.cmima.csic.es/castresana/Gblocks_server.html; Castresana, 2000). Alignment
197 using Clustal W and subsequent removal of poorly aligned positions and divergent regions in
198 rRNA sequences is advisable so that it becomes more suitable for phylogenetic analysis
199 (Castresana, 2000; Talavera & Castresana, 2007).

200 Phylogenetic analyses

201 Phylogenetic analyses for each locus and for the concatenated data (merged together in
202 DNASP 5.10; Librado & Rozas, 2009), were performed using maximum likelihood (ML) and
203 Bayesian inference (BI). The software MEGA 7.0 (Kumar, Stecher, & Tamura, 2016) was used
204 to determine the best model of evolution (ML based on AICc information and BI based on BIC
205 information; see Table S3 for list of models). The ML tree was reconstructed using PhyML 3.0
206 (Guindon et al., 2010; <http://www.atgc-montpellier.fr/phyml/>). Branch support was estimated
207 using 1,000 bootstrap replicates. The Bayesian tree was built using MrBayes on XSEDE 3.2.6
208 (Ronquist et al., 2012) through the CIPRES Science Gateway (Miller, Pfeiffer, & Schwartz,
209 2010). Two independent runs were conducted with 200,000,000 generations each. Parameters
210 were sampled every 1,000 generations. In the end, a majority rule consensus tree was
211 reconstructed with a burn-in of 25% and the convergence checked (close to or equal to 1).
212 Haplotype genealogy was investigated by building median networks for COI, 16S rRNA, 18S
213 rRNA and 28S rRNA data using the reduced median algorithm (Bandelt, Forster, Sykes, &
214 Richards, 1995) in Network 5.0.0.3 (<http://www.fluxus-engineering.com>).

Molecular-based species delineation

Five methods of molecular-based species delineation were applied to define molecular operational taxonomic unit (MOTUs). They were applied to each locus and to the concatenated data, except for the BIN system that relies only on COI. The first two methods were based on distance measures. First, COI sequences were automatically subject to the BIN system implemented in BOLD. This approach clusters barcode sequences algorithmically to calculate MOTUs that show high concordance to species (Ratnasingham & Hebert, 2013). Then, the Automatic Barcode Gap Discovery (ABGD) species delineation tool on a web interface (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>) was applied with default settings using the Kimura-2-parameter (K2P) distance matrix. This tool, based on the barcode gap detection (i.e., break between the distribution of intraspecific and interspecific distances of the barcode region), sorts the sequences into hypothetical species (Puillandre, Lambert, Brouillet, & Achaz, 2012). Finally, three tree-based methods were applied: GMYC (Generalized Mixed Yule Coalescent) single and multi-threshold models (Fujisawa & Barraclough, 2013) and bPTP (Poisson Tree Processes; Zhang, Kapli, Pavlidis, & Stamatakis, 2013). The GMYC method is based on the examination of the branching patterns in an ultrametric tree, and the recognition of the transitions from branching patterns attributable to speciation (one lineage per species) to those that can be attributed to intra-species coalescent process (multiple lineages per species). We applied both the single threshold (Pons et al., 2006) and multiple thresholds (Monaghan et al., 2009) variants of this method. Since the GMYC methods require an ultrametric tree, we first calculated a Bayesian ultrametric phylogenetic tree. The tree was generated in BEAST 2.4.6 (Bouckaert et al., 2014) with the appropriate best model (based on BIC criteria; Table S3), and four independent runs for 70,000,000 Markov chain Monte Carlo (MCMC) generations, sampled every 10,000 generations, were performed. Effective sampling sizes (ESSs > 200 for all parameters) and convergence of the parameter estimates were evaluated using Tracer 1.6 software (Rambaut, Suchard, Xie, & Drummond, 2014). The consensus tree was annotated using TreeAnnotator 2.4.6 (Bouckaert et al., 2014). The

consensus tree was loaded into the R software package 'SPLITS' (Species Limits by Threshold Statistics; Ezard, Fujisawa, & Barraclough, 2009) through the software R 3.5.0 (R core team 2018; www.r-project.org) and analysed using the single- and multiple-threshold models. The bPTP method incorporates the number of substitutions in the model of speciation and assumes that the probability that a substitution gives rise to a speciation event follows a Poisson distribution. The branch lengths of the input tree are supposed to be generated by two independent Poisson process classes, one corresponding to speciation and the other to coalescence (Zhang, Kapli, Pavlidis, & Stamatakis, 2013). In contrast to GMYC, bPTP uses non-ultrametric phylograms. For the input tree, we used the ML phylogenies (for each locus and for the concatenated data) obtained above. Species delimitation analysis was performed using the python code available at: www.exelixis-lab.org/software.html (Zhang, Kapli, Pavlidis, & Stamatakis, 2013) with 1,000,000 iterations of MCMC and 25% burn-in.

Genetic diversity and structure

Mean and maximum pairwise distances (p-distances) were calculated across the 16S rRNA, 18S rRNA and 28S rRNA loci after the application of Gblocks for each species (intraspecific distances) using MEGA 7.0 (Kumar, Stecher, & Tamura, 2016). Additionally, p-distances for COI and 16S rRNA (after the application of Gblocks) within and between MOTUs were also calculated in MEGA 7.0 (Kumar, Stecher, & Tamura, 2016). Indices of genetic diversity, namely haplotype diversity (h) and nucleotide diversity (π), were estimated for each locus and for each species and MOTU using DNASP 5.10 (Librado & Rozas, 2009).

Using the COI data, F_{st} estimations were made in Arlequin 3.5 (Excoffier & Lischer, 2010). Significance of pairwise F_{st} values was determined by performing 10,000 permutations between locations, under the null hypothesis of no differentiation. Locations with less than three individuals were excluded from the F_{st} analyses. Analysis of molecular variance (AMOVA) was performed in Arlequin 3.5 (Excoffier & Lischer, 2010) to assess the population structure of each species.

268 Different phylogeographic and demographic evolutionary scenarios were tested using the R
269 package BioGeoBEARS (Matzke, 2013a; [https://cran.r-](https://cran.r-project.org/web/packages/BioGeoBEARS/index.html)
270 [project.org/web/packages/BioGeoBEARS/index.html](https://cran.r-project.org/web/packages/BioGeoBEARS/index.html); for detailed information, methodology
271 and associated packages see Matzke, 2013b, 2014) through the software R 3.5.0 (R core team
272 2018; www.r-project.org). Since this tool relies on a tree with only one representative sequence
273 per lineage, we selected randomly one sequence per MOTU to build the input tree in MEGA
274 7.0 (Kumar, Stecher, & Tamura, 2016), consisting on a concatenated ML tree based on the
275 GTR+G+I substitution model and using 1,000 bootstraps.

276 In order to test for a model of isolation by distance we applied a Mantel test to Fst (as a genetic
277 distance measure) between regions and geographical distance matrices on the IBDWS web
278 interface (<http://ibdws.sdsu.edu/~ibdws/distances.html>; Jensen, Bohonak, & Kelley, 2005).
279 Geographic distances were accessed through the GeoDataSource platform
280 (www.geodatasource.com/distance-calculator).

281 Estimation of approximate time of divergence

282 Phylogenetic reconstruction and age estimates of the different clades were also performed in
283 BEAST 2.4.6 (Bouckaert et al., 2014) using COI data. We used both a relaxed molecular clock
284 (uncorrelated lognormal) and a strict clock with an HKY85 model of molecular evolution (Table
285 S3) and gamma distributed rates (10 gamma categories). The analyses ran for a total of
286 50,000,000 states in the Markov chain. Effective sampling sizes (ESSs > 200 for all
287 parameters) and convergence of the parameter estimates were evaluated using Tracer 1.6
288 software (Rambaut, Suchard, Xie, & Drummond, 2014). The consensus tree was annotated
289 using TreeAnnotator 2.4.6 (Bouckaert et al., 2014). The COI divergence rate has not been
290 previously estimated for *Dynamene* species. However, there are estimates for other isopods:
291 2.5% per Myr for *Stenasellus* (Ketmaier, Argano, & Caccone, 2003) and *Asellus* (Konec,
292 Prevorčnik, Sarbu, Verovnik, & Trontelj, 2015; Verovnik, Sket, & Trontelj, 2005) and 1.56 –
293 1.72% per Myr for *Orthometopon* (Poulakakis & Sfenthourakis, 2008). Because there is not an
294 available fossil for *Dynamene* for calibration of the molecular clock, we selected five normally

distributed priors for the age estimation and the calibration of the rate of evolution. One of the priors was the evolutionary rate itself that we centered at 2% with a 95% confidence interval ranging from 1.5% to 2.5% representing the lowest and the highest estimated COI rates in isopods in the literature (Ketmaier, Argano, & Caccone, 2003; Konec, Prevorčnik, Sarbu, Verovnik, & Trontelj, 2015; Poulakakis & Sfenthourakis, 2008; Verovnik, Sket, & Trontelj, 2005). These rates were also applied successfully by Xavier et al. (2012) and Panova, Nygren, Jonsson, & Leidenberger (2016) in other two isopod genera (*Stenosoma* and *Idotea* respectively) and fit within the range of COI rates estimated for other marine arthropods (Knowlton & Weigt, 1998; Markow & Pfeiler, 2010; Sponer & Lessios, 2009; Wares & Cunningham, 2001). The second and third priors were based on the hypotheses that *D. edwardsi* colonized La Palma (formed at around 1.7 Myr; Fernández-Palacios et al., 2011; Guillou, Carracedo, & Duncan, 2001) and El Hierro (1.1 Myr; Carracedo, 2011; Fernández-Palacios et al., 2011) shortly after the formation of the islands, as used by Machado, Rodríguez-Expósito, López, & Hernández (2017). The corresponding clades were set to priors of 1.7 Myr (standard error of 0.17 Myr) and 1.1 Myr (standard error of 0.11 Myr). A normal distribution fits the hypotheses as the colonization by *D. edwardsi* could have occurred after the islands' formation, or the clades could also be older as the first settlers could have already carried ancestral polymorphisms. The fourth and fifth priors were based on paleontological records, namely on data inferred from isopod fossils. No direct fossil evidence could be found for the *Dynamene* genus, nor for any member of the family Sphaeromatidae. However, Lins, Ho, Wilson, & Lo (2012) were able to use isopod fossils (from deep-sea habitats) and molecular data (COI and 18s rRNA) to estimate the timing of colonization events of several isopod species. Based on their calculations, we were able to estimate the divergence between the *Dynamene* genus' sister clades *Campecopea hirsuta* and *C. lusitanica* (102 Myr; 95% CI [35-175 Myr]) and between *Sphaeroma serratum* and *Lekanesphaera hookeri* (75 Myr; 95% CI [25-135 Myr]), setting up the prior standard error in a way that the 95% confidence interval would fit the above. Because these estimates are less accurate and have wide confidence

intervals, we assessed the effect of the different calibration points applying the relaxed clock under three settings: using only the rate of evolution, using both islands' colonization priors, and using both fossil-based estimates. Results were generated considering a burn-in of 10% and 25%. Detailed information regarding the species and outgroups used in the phylogenetic reconstruction and age estimates can be consulted in Table S4.

Results

Morphological analysis

Morphologically, *D. edwardsi*, *D. bidentata* and *D. magnitorata* are relatively easy to discriminate among adult males, but harder to separate between sub-adult males, juveniles and females (Holdich, 1968a; Vieira, Queiroga, Costa, & Holdich, 2016). Figure 2 shows scanning electronmicrographs of the three species and highlights diagnostic morphological characters. The main morphological difference is in the form of the pleotelsonic boss (thinner arrows), which in *D. edwardsi* is an upright cleft plate with peg-like structures at the corners (Figure 2a, b). In *D. bidentata* it is bilobed and the two halves are separated by a wide v-shaped groove (Figure 2c, d). In *D. magnitorata* the pleotelsonic boss is also bilobed, but the two halves are more angular and separated by a narrow groove (Figure 2e, f). Also important is the shape of the arms of the bidentate process (thicker arrows), which arises from the posterior margin of the sixth pereonite – a feature unique amongst sphaeromatid isopods. In *D. edwardsi* the end of each arm has a downwardly-directed spur (Figure 2a), whilst that of *D. bidentata* tapers to a point (Figure 2c), and that of *D. magnitorata* is more tuberculate and ends bluntly (Figure 2e). No stable morphological differences in the known diagnostic traits were found among individuals of the same species between different locations.

Molecular analyses and MOTUs delimitation

A total of 179 sequences of COI, 43 of 16S rRNA, 120 of 18S rRNA and 46 of 28S rRNA were obtained for the three *Dynamene* species (Table S1). After the use of Gblocks, 16S rRNA, 18S rRNA and 28S rRNA comprised, respectively for each species: *D. bidentata* (458 bp, 1120 bp,

781 bp); *D. edwardsi* (451 bp, 1084 bp, 801 bp) and *D. magnitorata* (426 bp, 1125 bp, 780 bp). All loci (individually and concatenated) discriminated the three species (Figures S1-5) and the BI and ML analyses produced similar topologies. Consequently, we present the BI tree with posterior probabilities from each analysis, complemented with ML bootstrap support (Figures S1-5). *Dynamene edwardsi* showed higher values of intraspecific variance (Table 1) and genetic diversity indices (Table S5) compared with *D. bidentata* or *D. magnitorata*, regardless of the locus used.

The total number of MOTUs obtained varied between 7 and 20 depending on the locus and delimitation method applied (Figure 3 for COI data; Figures S2-5 only show consensus MOTUs for the other loci and for the concatenated data). *Dynamene edwardsi* displayed the highest number of MOTUs (between 5 and 11), followed by *D. magnitorata* (between 1 and 5) and *D. bidentata* (between 1 and 4). To simplify further analyses, a consensus number of MOTUs was decided based on the majority rule (i.e., most common number of MOTUs across different delimitation methods for each locus and for the concatenated data). The consensus number of MOTUs for COI was 12 (Figure 3), for 16S rRNA was 7 and 12 (a tie; Figure S2), for 18S rRNA was 9 (Figure S3), for 28S rRNA was 8 (Figure S4) and for the concatenated data was 11 and 13 (a tie; Figure S5). Delimitation methods applied to COI and the concatenated data identified the same MOTUs within *D. edwardsi* and *D. bidentata*, but not within *D. magnitorata* (2 using COI and between 1 and 3 using concatenated data). Because extensive evidence indicates that COI among the four markers, is the one that better reflects species boundaries (Grabowski, Wysocka, & Mamos, 2017; Jörger, Norenburg, Wilson, & Schrödl, 2012; Jörger & Schrödl, 2013), we considered the value of 12 MOTUs the most plausible, partitioned into 9 MOTUs for *D. edwardsi*, 2 for *D. magnitorata* and 1 for *D. bidentata* (Figure 3). We consider this number of MOTUs as the most plausible and robust, given the data (the number delimited by COI, 16S rRNA and concatenated data), and use it as a reference for the remainder of the study.

All MOTUs showed low intraspecific genetic variance (<1%) but high genetic distance between different MOTUs (2.02-23.55% for COI and 0.40-30.02% for 16S rRNA; Table 2). Within *D. edwardsi* and *D. magnitorata*, the individual MOTUs were exclusive to specific regions and, in several instances, single islands (Table S1). MOTU 2 (*D. edwardsi*) displayed the highest value of nucleotide diversity, and MOTU 11 (*D. magnitorata*) had the highest values of haplotype diversity and segregating sites. MOTUs 3 (*D. edwardsi*) and 12 (*D. magnitorata*) were unique in displaying single haplotypes (Table S5), with the latter being the only singleton retrieved in our analyses.

Phylogeographic structure

Results of the AMOVA (Table S6) showed that most of the variation within each species was among geographic regions. Here, we show the best geographic region partitioning that retrieved the highest values of variation for each *Dynamene* species. In *D. edwardsi*, 98.14% of the variation is explained by the genetic differentiation between the following 12 geographic regions: Iberian Peninsula (which includes Galicia, Portugal West and Portugal South), Morocco North, Morocco South, Mediterranean (which includes France and Croatia), São Miguel, Porto Santo, Madeira, Selvagens, Tenerife, Gran Canaria, La Palma and El Hierro (Table S6). Observed pairwise F_{st} values among locations (from different regions) were generally high, showing significant differentiation between regions (Table S7). In *D. magnitorata*, the variation was best explained by the differentiation among the geographic regions of Iberian Peninsula (which includes Galicia, Portugal West and Portugal South), Azores (which includes Terceira, São Miguel and Santa Maria) and La Palma; while the F_{st} values were higher than 0.6 between locations from different regions (Iberian Peninsula and Azores), but less than 0.4 between locations within these regions. The AMOVA of *D. bidentata* data yielded highest variation (67.58%) between the geographic regions: Iberian Peninsula (which includes Galicia, Portugal West and Portugal South), Scotland, Morocco North and Gran Canaria. Observed pairwise F_{st} values between locations from different regions (Iberian

Peninsula, Scotland and Morocco) were higher than 0.6 and lower than 0.3 between locations within each region (Table S7).

Networks of the three species (Figure 1; Figure S6) supported the AMOVA results and clearly discriminated the same populations, with no shared haplotypes among regions. Moreover, the networks (for each locus) displayed similar topologies, thereby reinforcing the closer genetic similarity within *D. magnitorata* and *D. bidentata* compared to *D. edwardsi*.

The analysis of the dispersal method with BioGeoBEARS was only possible for *D. edwardsi*, because the method uses trees with clearly discriminated lineages, which did not occur in *D. magnitorata* and *D. bidentata*. The most probable model retrieved was DIVALIKE+J, which comprises a likelihood interpretation of the parsimony DIVA (Dispersal-vicariance analysis; Ronquist, 1997). This model was constructed by modelling DIVA's processes in order to restrict them to pre-established allowed schemes (widespread vicariance: yes; subset sympatry: no; see Ronquist & Sanmartin, 2011 for details and explanation regarding DIVA). Also, *j*, was added, representing the relative per-event weight of founder-event/jump speciation events at cladogenesis events. The higher *j* is, the more probable these events are, and less probable are the standard cladogenesis events (Matzke, 2014). From the total events (9.52), 6.64 were founder events with anagenetic dispersal (1.52) and with vicariance (1.36) also playing a role. A Table with detailed information about the different dispersal models can be consulted at http://phylo.wdfiles.com/local--files/biogeobears/BioGeoBEARS_supermodel.png (accessed on 01 February 2018; see also Matzke, 2013a, 2013b, 2014).

Results of the isolation by distance test showed no significant correlation between genetic distance and geographic distance in any species ($p > 0.05$).

Approximate time of divergence

We calculated Bayes factors using Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014), comparing the likelihood and posteriors of the analyses using the strict and relaxed molecular clocks (Soares et al., 2013). Bayes factors indicated that a relaxed clock was more appropriate than a strict clock for the analysis (following Kass & Raftery, 1995). The heterogeneity of the

rates was evident in the estimated coefficient of variation of the evolutionary rate, that was 1.267 (0.886-1.761, 95% confidence interval), already on the verge of being too high for being consistent with the use of a clock (Drummond & Bouckaert, 2015). The estimated mutation rate for the 5 priors' analysis (25% burn-in) was 1.943% per Myr for COI (with a 1.750-2.150 95% confidence interval). The COI-based divergence time estimates for all the MOTUs were higher than 9 Myr. The most recent estimated divergence was between MOTU 7 and MOTU 8: around 10 Myr and the first and oldest split within *D. edwardsi* dated to around 36 Myr (Figure 3). The divergence between *D. magnitorata* and *D. bidentata* was estimated at approximately 29 Myr and the three species most likely shared a common ancestor more than 54 Myr b.p. (Figure 3). Figure S7 displays the analysis using the different models based on the rate alone and using either the islands' colonization priors or the paleontological calibration priors. It is clear that the paleontological calibration led to much older age estimates than the other three models (Figure S7A). The five priors' calibration displayed a more objective and weighted analysis and provided an estimate of the COI evolutionary rate clearly in line with previous estimates, independently of the divergent estimates provided by individual set of priors, and also resulting in an estimate with substantially narrower confidence intervals than the ones provided as a prior (Figure S7B). In this analysis (with 5 priors), the paleontological calibration was the one responsible for much older estimates on the coalescence of deeper lineages (on the genus and species' level) than on younger clades (islands' clades). Such an obtained feature of the relaxed clock, allowing a more rapid evolutionary rate for younger clades and a slower one for deep clades is very plausible, considering that it has been extensively demonstrated for the molecular clock of various markers and species (Ho et al., 2011).

Discussion

Here we report a new perspective on the drivers of evolution and underlying evolutionary mechanisms promoting diversification and segregation of marine invertebrates in the North

Atlantic islands of Macaronesia. This new perspective resulted from the most comprehensive phylogeographic study to date applied to a larvae-lacking peracarid crustacean inhabiting this region. The archipelagos of Macaronesia harbour high genetic diversity within the morphospecies *D. edwardsi* (hereafter designated as the *Dynamene edwardsi* complex), comprising multiple, deeply divergent evolutionary lineages, geographically separated, and displaying high levels of island endemism. Such findings would be anticipated in terrestrial organisms from oceanic islands, for whom the ocean constitutes a barrier to between-island dispersal, but are somewhat less expected in an intertidal marine invertebrate. Although the potential dispersal ability of *D. edwardsi* can be considered low compared to other invertebrates with planktonic larvae, no obvious geographic barriers can be identified that justify the lack of dispersal and effective migration between some geographically close populations (e.g., from as little as 50 km between Porto Santo and Madeira or between Tenerife and Gran Canaria); especially over the course of millions of years, since the establishment of founding populations. Alternative explanations must therefore be sought for the pronounced patterns of diversification observed.

Re-appraisal of Dynamene diversity and incidence of cryptic species

The diversity and distribution of the genus *Dynamene* in the Northern Hemisphere has been recently reviewed and updated (Vieira, Queiroga, Costa, & Holdich, 2016). The revised information was based on morphology, as well as on new and published occurrence records, including the Macaronesian archipelagos. Just six species are known from the Northern Hemisphere and, among these, only three are found in the NE Atlantic: *D. bidentata*, *D. magnitorata* and *D. edwardsi*. Our findings, based on DNA sequence data from multiple mitochondrial and nuclear loci, challenge such assertions; the diversification reported here suggests the existence of at least seven *Dynamene* species in the NE Atlantic alone, and possibly up to nearly four times more species than are currently recognized in this oceanic region.

The evidence for the striking diversification of *Dynamene* derives exclusively from molecular data and is essentially due to the *D. edwardsi* complex; notably, the species with the widest distribution in the Macaronesian islands. Combined and isolated data from the four-analysed mitochondrial DNA (mtDNA) and nuclear loci provide compelling evidence for the existence of at least five deeply divergent evolutionary units within the *D. edwardsi* complex, which have been genetically isolated for a long period (more than 9 Myr and up to 36 Myr; Figure 3). Such entities likely coincide with recognition as separate species, at least at a molecular level (De Queiroz, 2007). The five genetic lineages are completely sorted, consistently recognized as separate MOTUs in all loci, and geographically arranged in such a way that, within the same island, only one lineage is represented at most, thus excluding any indication of occurrence of sympatric speciation.

The number of recognizable sorted lineages and MOTUs within the *D. edwardsi* complex were variable, depending of the delimitation methods used, and whether the locus under consideration is a slow evolving nuclear gene (maximum five lineages), or a rapidly mutating mitochondrial gene (up to nine lineages). Lack of divergence or fixed substitutions in the nuclear loci between some of the lineages, which in turn are apparent with mtDNA, does not necessarily exclude the possibility that they represent separate cryptic species. Since rates of substitution are much slower in the nuclear loci, often highly divergent COI lineages (e.g., >20%) may coincide with very little differentiation in rDNA sequences (e.g., <1%; Borges et al., 2012). In fact, both nuclear loci used here are notoriously known for poor species-level discrimination ability in many animal taxa (e.g., Jörger, Norenburg, Wilson, & Schrödl, 2012), despite their robustness for reconstructing deeper phylogenies (e.g., Wetzter, Pérez-Losada, & Bruce, 2013). Therefore, a combination of mitochondrial and nuclear multi-locus approach is advised to better assess species boundaries and unravel cryptic diversity (Grabowski, Wysocka, & Mamos, 2017; Jörger & Schrödl, 2013).

Taking COI data as a benchmark for comparison of genetic distances among crustacean species (Costa et al., 2007; Lobo et al., 2016), even the shortest distances between *D.*

edwardsi complex MOTUs (3.79%) would fall beyond the recorded distribution of intraspecific distances in numerous well established morphospecies, including this study's results for the other two congeneric species *D. bidentata* and *D. magnitorata* (maximum 1.54% and 2.65% respectively; Table 1). On the other hand, the highest average distance observed between the nine MOTUs within *D. edwardsi* complex (21.92%) surpasses the average distance between *D. bidentata* and *D. magnitorata* (16.55%). The range of COI genetic distances observed within the *D. edwardsi* complex are similar to values reported for a number of complexes of cryptic species of isopods, such as the *Ligia occidentalis* complex comprising 15 putative cryptic species with a divergence range of 13% to 27% (Markow & Pfeiler, 2010); *Excirolana braziliensis* with three putative species, ranging from 14% to 19% (Varela & Haye, 2012); *Chelator insignis*, five lineages with > 20% divergence (Brix, Svavarsson, & Leese, 2014) and *Sphaeroma terebrans* with four distinct clades diverging from 15 to 18% (Baratti, Filippelli, & Messana, 2011; Baratti, Goti, & Messana, 2005).

Phylogeography and evolution of the D. edwardsi complex

The extensive population structure revealed in the *D. edwardsi* complex was strongly reinforced by observed differences among regions in the AMOVA analysis (98.14% - Table S6). Each MOTU was geographically delimited (Table S1), with the archipelagos of Madeira and Canaries comprising most of the diversity (80% of the total haplotypes). Moreover, the high recorded *F*_{st} values (Table S7) also suggest that gene flow is rare or absent among populations from different regions. This conjecture is further supported by the observed congruence in the lineage structure among different loci (Figures S1-5). Even though a lower number of specimens per MOTU was analysed for rRNA loci data compared with COI, the former has notably low intraspecific variation and hence sample size is not expected to impact the basal phylogenetic structure, nor the MOTU delimitation obtained with those loci (Borges et al., 2012). Indeed, as suggested by Bachtrog, Thornton, Clark, & Andolfatto (2006), in 'ideal' systems like islands, under strict allopatry (i.e., no gene flow), all regions of the genome will have a single divergence history and vary only in their coalescence times.

535 The complete genetic segregation of populations among most of the islands is therefore well
536 established, and originated over millions of years (spanning from a few Myr to possibly as
537 much as 50 Myr b.p.; Figure 3) until the present. Such patterns are unexpected due to the
538 geographic proximity among many of the sampled populations. Although the dispersal ability
539 of *Dynamene* specimens is thought to be fairly constrained, juveniles can be transported
540 somewhat far from their birthplace by factors such as rough weather (Holdich, 1968b, 1970,
541 1976). Moreover, it is well documented that isopods can also be dispersed passively away
542 from the coast (Ingólfsson & Agnarsson, 2003; Thiel, 2002) through rafting on floating objects
543 (usually detached macro algae; Scheltema, 1986; Thiel & Gutow, 2005), via anthropogenic
544 transfer (Kiessling, Gutow, & Thiel, 2015; Rumbold et al., 2018) or, more rarely, if specimens
545 remain attached to other species (Sponer & Lessios, 2009), including marine birds (Frisch,
546 Green, & Figuerola, 2007). The occurrence of *Dynamene* species in a wide range of locations
547 also confirms their dispersal capacity (Vieira, Queiroga, Costa, & Holdich, 2016).

548 However, the fact that we did not find MOTUs sharing the same island (i.e., within each
549 morphospecies, namely *D. edwardsi*), suggests that any migrants, over geologically extended
550 periods of millions of years, did not have the capacity to establish in locations where other
551 populations/MOTUs were present, or, at least, that their genetic signature has been lost
552 through genetic drift. Such an evolutionary phenomenon can occur by competitive exclusion,
553 high density blocking (Waters, Fraser, & Hewitt, 2013) or high mortality of physiologically
554 stressed migrants (Barber, Palumbi, Erdmann, & Moosa, 2002). Because *Dynamene* species
555 require specific habitats to reproduce (e.g., barnacles or crevices), intense reproductive
556 competition among *Dynamene* individuals is suspected (Holdich, 1976; Vieira, Queiroga,
557 Costa, & Holdich, 2016). The pattern observed in the *D. edwardsi* complex seems to fit the
558 ‘founder takes all’ density-dependent process (Waters, Fraser, & Hewitt, 2013), where the first
559 founders rapidly colonize a new habitat, but subsequent migrants are incapable of successfully
560 colonizing the pre-empted space. Pre-emptive competition occurs when a species recruits to
561 and dominates a habitat, monopolizing all available space and precluding the establishment

of potential competitors, i.e., when a species' reproductive ability relative to its longevity is higher than that of potential competitors (Amarasekare, Hoopes, Mouquet, & Holyoak, 2004). Importantly, pre-emptive competition has also been invoked in structuring space-limited habitats such as the rocky intertidal (Dudgeon, Steneck, Davison, & Vadas, 1999; Sutherland & Karlson, 1977). Hence, the first colonist obtains an advantage over subsequent competitors. Successful exclusion then depends on the time lag before other immigrants arrive, growth rate of the resident population and local carrying capacity (De Meester, Vanoverbeke, Kilsdonk, & Urban, 2016). Dispersal between coasts separated by deep-water over geological time-scales must have occurred (Cowie & Holland, 2006) and, therefore, it seems remarkable that such discrete phylogeographic structure among islands was preserved apparently over millions of years. In such cases, it appears that the first lineage to colonize a new island has effectively excluded the secondary dispersers that must also have subsequently dispersed to the island. This phenomenon was previously described in marine (e.g., Barber, Palumbi, Erdmann, & Moosa, 2000; Meyer & Paulay, 2005; Waters, 2008) and terrestrial organisms (e.g., Cowie, 1992) over fine geographic scales, despite the lack of contemporary physical barriers. In fact, *Dynamene* isopods have relatively short life cycles which enable them to attain large census populations sizes in a short period (Holdich, 1968b, 1976). Our personal observations and population density estimates during quantitative sampling campaigns (specimen counts per unit of area) indicate that Macaronesia islands comprise very large census populations of *Dynamene*, in the order of thousands of specimens per site. Hence, even if a specimen or a brood from a foreign population could settle, it would be unlikely to affect and impact resident genetic pools of putatively high effective size.

It is noteworthy that no significant correlation was observed, either between geographic distance or island emergence time (data not shown) and the distribution of the multiple lineages of *D. edwardsi* complex. Dispersal episodes of this isopod probably occurred by accidental events associated with founder effects, suggesting that complex random patterns were the primary underlying mechanism of evolution. Such stochastic events have been suggested

among various marine invertebrates in the Canaries and the Azores (e.g., Riel et al., 2005; Sá-Pinto et al., 2008; Xavier et al., 2010). Within the *D. edwardsi* complex, the most interesting case of stochasticity is Madeira, whose haplotypes are very close to those of Tenerife, and embedded in the same MOTU (Figure 3). The island of Madeira emerged much more recently (around 5 Myr) than Tenerife (around 11 Myr) or Porto Santo islands (about 15 Myr ago; see Fernández-Palacios et al., 2011; Geldmacher et al., 2005 for details), but it is located hundreds of kilometers away from Tenerife, whereas Porto Santo is located only 50 km away. Therefore, the Madeira population is likely to have originated from the Tenerife lineage through a relatively recent dispersal event that persisted, despite potential immigrant flow from the nearby Porto Santo lineage.

Macaronesian islands have been proposed as an offshore refugium for several marine organisms during the quaternary glaciations (e.g., Chevolot et al., 2006; Domingues, Almada, Santos, Brito, & Bernardi, 2007; Domingues, Santos, Brito, & Almada, 2006; Domingues, Stefanni, Brito, Santos, & Almada, 2008; Xavier et al., 2010). These groups of islands appeared millions of years ago (see Fernández-Palacios et al., 2011; Fernández-Palacios & Whittaker, 2008 for details), at different geological times and were shaped by the Pleistocene glaciations (2.58 Myr-present). During the Pleistocene, sea level changed several times and, more recently, raised more than 100 m (over the last 20,000 years; Mix, Bard, & Schneider, 2001), submerging several islands that could have served as stepping stones in the past, namely the Paleo Madeira and Paleo Canaries (Ávila, 2000; Fernández-Palacios, Kueffer, & Drake, 2015; Fernández-Palacios et al., 2011). Stepping stone colonizations are common in many terrestrial invertebrates in Macaronesia (reviewed by Juan, Emerson, Oromí, & Hewitt, 2000) and such a scenario cannot be discarded for marine invertebrate species. Such processes may explain the relative genetic proximity of the *D. edwardsi* population from Porto Santo Island with those from the Iberian Peninsula, which seem to have separated around 5 Myr (Figure 3), before the last glaciation periods (Fernández-Palacios et al., 2011). How the change in sea surface temperature and sea level during quaternary glaciations, although small

and gradual (Crowley, 1981; Santos, Hawkins, Monteiro, Alves, & Isidro, 1995), affected marine (and coastal) populations is still not well understood (Barton et al., 1998; Cunha et al., 2014; Fernández-Palacios, 2016). It is probable that a combination of factors (e.g., littoral area, distance to continental coasts and island geological time) played a role in driving the observed evolution and the diversification of marine invertebrates in Macaronesia (see Ávila et al., 2018; Fernández-Palacios et al., 2016; Shaw & Gillespie, 2016). However, in the case of the *D. edwardsi* complex, such factors appear to have little detectable impact on the observed deep phylogeographic footprint in a number of putatively cryptic species, which is better interpreted through alternative, though potentially complementary, mechanisms (e.g., ‘founder takes all’ density-dependent process).

Final remarks

The present study revealed twelve consistent MOTUs within the genus *Dynamene* in NE Atlantic, nine of which belong to the *D. edwardsi* complex and display an allopatric distribution. It is noteworthy that *Dynamene* is a genus with only six known species present in the Northern Hemisphere and just three in the NE Atlantic (Vieira, Queiroga, Costa, & Holdich, 2016). If the putative cryptic species here reported are confirmed by further studies (e.g., ultrastructure or histological studies, geometric morphometry, ecology and behavior), it would represent a remarkable increase of 300% in known species diversity in the Northern Hemisphere for this species-poor genus. Moreover, since it is almost the case that each island holds its own putatively endemic cryptic species of *D. edwardsi*, this implies that each suspected species has a very narrow range and limited genetic diversity, and therefore constitute a particularly vulnerable species from the viewpoint of conservation (Pimm et al., 2014). If the evolutionary history of Macaronesia led to similar diversification processes in other peracarids (our unpublished data) or other marine invertebrates, there are novel and important implications to be considered in the coastal management and conservation strategies of such oceanic regions. Not only would the endemic diversity of marine invertebrates in the islands be much

higher than previously recorded (i.e., Linnaean Shortfall), but also the design and planning of marine protected areas may need to contemplate the protection of island-specific marine biodiversity.

The segregation of cryptic species of *D. edwardsi* in Macaronesia islands have endured over millions of years, with clearly long-established divergence patterns. Complete lack of dispersal and migrants among islands over such a long period is highly unlikely. The patterns we observed are better explained by founder effects, followed by low survival and/or recruitment of posterior immigrants, due to genetic drift and density-driven exclusion in competition with the resident founder population. Such evolutionary mechanisms likely account for current diversity and distribution of the *D. edwardsi* complex in Macaronesia, in contrast with the often-assumed processes for marine invertebrates, such as dispersal and connectivity, geographic distance and isolation. Priority effects of the type here illustrated may therefore provide an alternative explanation to the generation of deep segregation in the open ocean, and should be further investigated in other peracarids and marine invertebrate species present in oceanic islands.

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Data Accessibility

All DNA sequences produced in the scope of this study were deposited in BOLD and submitted to GenBank. All data are publicly available through the BOLD dataset DS-DYNAMENE (dx.doi.org/10.5883/DS-DYNAMENE).

DNA sequences of *Dynamene* specimens before alignment using Clustal W and subsequent removal of poorly aligned positions and divergent regions in rRNA sequences with Gblocks can be accessed at <https://doi.org/10.5281/zenodo.1464764>.

Author Contributions

P.E.V., D.M.H., F.O.C. and H.Q. designed the research plan; P.E.V. and D.M.H. performed the research; P.E.V., A.D. and P.S. analysed the data in collaboration with S.C. and G.R.C; P.E.V., F.O.C., H.Q., S.C. and G.R.C. wrote the paper; all the authors contributed with suggestions to the manuscript structure and reviewed the manuscript final version.

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1086 **Table 1.** Mean and maximum pairwise genetic distances within each *Dynamene* species and for each of the four analysed loci.

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Species	COI mean/ maximum	16S rRNA mean/ maximum	18S rRNA mean/ maximum	28S rRNA mean/ maximum
<i>Dynamene edwardsi</i>	0.1414/0.2192	0.0829/0.1596	0.0093/0.0236	0.0117/0.0282
<i>Dynamene bidentata</i>	0.0043/0.0154	0.0018/0.0044	0.0002/0.0036	0.0000/0.0000
<i>Dynamene magnitorata</i>	0.0074/0.0265	0.0025/0.0047	0.0052/0.0117	0.0013/0.0064

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1094 **Table 2.** Average pairwise distances between MOTUs (1 to 9 *D. edwardsi*; 10 *D. bidentata* and 11-12 *D. magnitorata*) for COI (lower diagonal) and 16S rRNA
1095 (upper diagonal). The mean COI pairwise distances within each MOTU are highlighted in bold in the diagonal.

	MOTU 1	MOTU 2	MOTU 3	MOTU 4	MOTU 5	MOTU 6	MOTU 7	MOTU 8	MOTU 9	MOTU 10	MOTU 11	MOTU 12
MOTU 1	0.0003	0.0304	0.0622	0.1314	0.1312	0.1356	0.1272	0.1292	0.1277	0.2884	0.2713	0.2699
MOTU 2	0.1222	0.0091	0.0631	0.1265	0.1263	0.1307	0.1200	0.1198	0.1228	0.2875	0.2775	0.2786
MOTU 3	0.1506	0.1424	0.0000	0.1378	0.1354	0.1419	0.1321	0.1333	0.1319	0.3002	0.2706	0.2692
MOTU 4	0.1805	0.1727	0.1746	0.0012	0.0267	0.0244	0.0501	0.0535	0.0453	0.2902	0.2709	0.2747
MOTU 5	0.1846	0.1842	0.1772	0.0755	0.0042	0.0155	0.0533	0.0556	0.0474	0.2920	0.2729	0.2767
MOTU 6	0.1838	0.1930	0.1852	0.0909	0.1178	0.0037	0.0555	0.0578	0.0497	0.2918	0.2727	0.2764
MOTU 7	0.1717	0.1713	0.1747	0.1308	0.1404	0.1282	0.0040	0.0207	0.0207	0.2910	0.2646	0.2635
MOTU 8	0.1690	0.1730	0.1781	0.1438	0.1402	0.1302	0.0482	0.0005	0.0379	0.2946	0.2661	0.2651
MOTU 9	0.1776	0.1778	0.1732	0.1341	0.1414	0.1281	0.0408	0.0178	0.0031	0.2909	0.2693	0.2683
MOTU 10	0.2355	0.2343	0.2158	0.2115	0.2083	0.2223	0.2101	0.2207	0.2166	0.0042	0.1074	0.1111
MOTU 11	0.2229	0.2258	0.2198	0.2079	0.2027	0.2174	0.2140	0.2159	0.2048	0.1658	0.0064	0.0040
MOTU 12	0.2204	0.2264	0.2234	0.2042	0.2061	0.2232	0.2110	0.2130	0.2023	0.1651	0.0227	-

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Figure 1. A. Sampling locations for each *Dynamene* species. **B.** Reduced median network based on COI data for the *Dynamene* morphospecies. Size of the circles is proportional to the number of haplotypes. Number of mutations separating each haplotype and inferred ancestors (median vectors) are displayed in black. Mutation numbers not shown in links displaying a single mutation.

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Figure 2. Stereoscan electronmicrographs of the three species of *Dynamene* showing differences in the posterior pereon and pleotelson. **A** - pereon and pleotelson of stage 8 male *Dynamene edwardsi*. **B** - pleotelsonic boss in dorsal view. **C** - posterior pereon, and pleotelson of stage 8 male *Dynamene bidentata*. **D** - pleotelsonic boss in dorsal view. **E** - posterior pereon and pleotelson of stage 8 male *Dynamene magnitorata*. **F** - pleotelsonic boss in dorsal view. Scale bars of 0.5 and 0.1 mm respectively for left and right figures. Adapted from Holdich (1976).

Figure 3. Bayesian, time-calibrated, phylogenetic reconstruction for the three *Dynamene* species, inferred from partial sequences (658 base pair) of the COI gene. For simplicity, only the outgroup species *Campecopea hirsuta*, *C. lusitanica*, *Sphaeroma serratum* and *Lekanesphaera hookeri* are displayed (in Table S4, a full list of the outgroups used can be consulted). All nodes have Bayesian posterior probability values of 1.0. Blue horizontal bars indicate 95% confidence intervals (within MOTUs not shown). Stars indicate islands' formation priors and circles the paleontological calibrations. Islands genesis also displayed in dashed lines. Vertical black bars correspond to MOTUs determined from COI sequence data by the following methods. **A.** BINs delimitation. **B.** bPTP analyses. **C.** ABGD analyses. **D.** GMYC single threshold analyses. **E.** GMYC multiple threshold analyses. **F.** Consensus MOTUs.