Fire ! The spread of the Caribbean fire coral *Millepora alcicornis* in the Eastern Atlantic.

Peter Wirtz & Carla Zilberberg

Peter Wirtz (email peterwirtz2004@yahoo.com) Centro de Ciências do Mar, Universidade do Algarve, Campus de Gambelas, PT 8005-139 Faro, Portugal. Carla Zilberberg (email carla.zilberberg@gmail.com) Núcleo em Ecologia e Desenvolvimento Ambiental de Macaé, Universidade Federal do Rio de Janeiro, Macaé, Brazil.

The Western Atlantic fire coral *Millepora alcicornis* is recorded from Madeira Island in the Eastern Atlantic for the first time. A large colony of this species has apparently been present at a very exposed site at the northern shore of Madeira Island for more than 15 years. Genetic analyses suggest that the colonies of this tropical fire coral at a mid-Atlantic location (Ascension Island) and at each of three Eastern Atlantic locations (Cape Verde Islands, Canary Islands, Madeira Island) originated from independent long-distance dispersal events from the Caribbean area.

Key words: Long-distance dispersal, Millepora alcicornis, phylogeography, habitat change

INTRODUCTION

Long-distance dispersal events occur rarely but, lying at the heart of island biogeography theory, they play a fundamental role in shaping species large-scale biogeography (Smith et al. 2018). The arrival of a species into a new area can have profound impacts on local ecological communities, leading altered environmental conditions and novel interactions, in particular if that species is a habitat forming one and competing with local endemics.

Fire corals (*Millepora* species) are well known for being important reef builders because of their large calcareous skeletons and for inflicting painful stings to humans (Lewis 2006). The genus is limited to 50 m depth in tropical seas, with a clear distinction between Atlantic and Pacific species (Razak & Hoeksema 2003). There are seven species in the Atlantic Ocean and until recently these were only reported from the tropical western Atlantic and from the Cape Verde Islands in the Eastern Atlantic (Laborel 1974, de Weerd 1984). The species found at the Cape Verde Islands, *Millepora alcicornis* Linnaeus, 1758, is common in the western Atlantic and has recently also been documented from Ascension Island in the middle of the Atlantic Ocean (Hoeksema et al. 2017) and from the Canary Islands in the Eastern Atlantic, where it apparently arrived only a short time ago (Clemente et al. 2011, López et al. 2017).

In summer 2016, a fisherman from Madeira Island showed a large dried branch of a fire coral (*Millepora* sp.) to the first author and claimed that it came from the north coast of this island. The previous finding of fire coral at the Canary Islands (28°N) was already quite unexpected; the presence of *Millepora* in the even colder waters of Madeira Island (32°N) would be an even greater surprise.

Here we report that there is indeed a large colony of fire coral at Madeira's north coast, the type of habitat it occupies, the identity of the species, and the probable origin of the colony.

MATERIAL AND METHODS

The *Millepora* colony was photographed under water and several branches were collected in August 2016. Some of these branches are now deposited in the Natural History Museum of Funchal, Madeira, with the registration number MMF 46310. Fragments were preserved in 96% ethanol and sent to the second author for molecular analyses.

Total DNA extraction followed the phenol-chlorophorm method performed by Fukami et al. (2004), placing the sample in a CHAOS solution one week prior to extraction. DNA quality and concentration were assessed on a 0.8% agarose gel stained with GelRed (Biotium) and visualized under UV light, using the pattern Lambda DNA (125 ng/μL).

For species identification, the 16S rRNA gene of mitochondrial DNA (16S) was obtained from the Madeira *Millepora* colony and compared with previously reported sequences from other Atlantic *Millepora* specimens and species from the NCBI database (<https://www.ncbi.nlm.nih.gov>). A 537bp fragment of the large ribosomal subunit of the mitochondrial DNA (16S) was amplified using the following pair of primers: SHA 5'-ACGGAATGAACTCAAATCATGT-3'; SHB 5'-TCGACTGTTTACCAAAAACATA-3' (Cunningham & Buss 1993).

The polymerase chain reactions (PCR) consisted of PCR buffer 1X, dNTP (2 mM), bovine serum albumin (1 mg/ml), MgCl₂ (1.5 mM), Taq polymerase (1U), primers (0.5 uM), ~1ng of template DNA. Thermal cycling conditions started with a denaturing step at 95°C for 3 min, followed by 10 cycles of 94°C for 1min, 40°C for 1min and 72°C for 1min, 40 cycles at 94°C for 1min, 52°C for 1min and 72°C for 1min and a final extension step at 72°C for 5 min. The amplified product was purified with ExoSAP-IT PCR Product Cleanup (Thermo Fisher Scientific) following manufacturer's instructions and Sanger sequencing was performed in both directions at GATC Biotech (Germany). Electropherograms were edited and a consensus sequence was created with Geneious R7 (<http://www.geneious.com>, Kearse et al., 2012). Alignment was performed using the ClustalW package in Geneious R7. Maximum likelihood (ML) phylogenetic reconstruction analyses and substitution models' calculations were performed with PhyML 3.0 (Guignon et al. 2010). Substitution model selection was calculated using Smart Model Selection (Lefort et al. 2017) with the Akaike

Information Criterion and the ML reconstruction. The substitution model used for the phylogenetic tree reconstruction was the HKY85 +G+I. Maximum likelihood analysis started with a neighbour joining tree followed by a Nearest Neighbour Interchange searching criterion and 1000 bootstraps for branch support.

A median-joining haplotype network was constructed using the software Networ v4.6.1.1 (Fluxus Technology Ltd.). This haplotype network included all *M. alcicornis* sequences used in de Souza et al. (2017), in addition to the sequence generated from the *Millepora* sample from Madeira Island in this study: Forty-four *Millepora alcicornis* colonies from the Caribbean Province, 109 colonies from the Brazilian Province, nine colonies from the Cape Verde Islands, a single colony from the Canary Islands, and two colonies from Ascension Island (Hoeksema et al. 2017) were compared with the colony from Madeira Island (Table 1).

RESULTS

Colony site and colony morphology

The *Millepora* colony of Madeira Island was found in shallow water (3m depth at low tide), in a small bay at a very exposed site on the north coast of Madeira Island (approximately 32°45'N, 16°43'E). Water temperature at this site varies from 16 to 23 degrees C annually (personal observations by the first author).

The main colony had a roughly rectangular shape, approximately 4 m long and 3 m wide (figure 1). There were numerous small colonies (hand-sized or smaller) scattered around the large main colony. The fisherman, who guided the first author to this site, reported that there were fewer small colonies previously but that the main colony was already this large size when he first encountered it more than 15 years ago.

Figure 1: *Millepora* colony at the north coast of Madeira Island. The fins of the diver are 80 cm long.

The central part of the colony was characterized by erect branches up to 18 cm high, flattened laterally at the tips (Figures 2-3); at the edges and at its base, the colony was encrusting. The strong branches were very solid and difficult to break off, being able to resist the heavy wave action typical for the north coast of Madeira Island.

The growth form of the Madeiran *Millepora* colony was typical of - but not restricted to - the species *Millepora alcicornis* Linnaeus, 1748 (de Weerd 1984, Hoeksema et al. 2017).

118

119 Fig 2: Detail of the main colony, showing the branching pattern and the erect branches typical of *M.*
120 *alcorni*, with laterally flattened tips.

121

122

123 Figure 3: 16 cm high branch of the main colony.

Figure 4. Maximum likelihood phylogenetic reconstruction of the partial 16S rRNA region of *Millepora* species. An arrow shows the sample from Madeira Island, grouping with *M. alcicornis*. Numbers at nodes represent the bootstrap support values (> 60%). All sequences downloaded from GenBank are presented with their access number. Genbank number of Madeira sample is MH660402.

b) Relationship of the colony

Figure 5 shows the haplotype network of the 16S fragment analyzed. Forty-three different haplotypes were found. The haplotype of the Madeiran colony corresponds to a Caribbean haplotype (with identical haplotype recorded at Bermuda and Florida) but not to a haplotype recorded from any of the other areas. This haplotype is only 2bp different from the Ascension Island haplotype.

Figure 5. Haplotype network of *Millepora alcicornis* using sequences from de Souza et al. (2017; Table 1), including the *M. alcicornis* sample from Madeira Island. Circle sizes correspond to the number of haplotypes and colours illustrate major regions of occurrence. The four black dots represent ancestral or not sampled haplotypes and line length corresponds to distance between haplotypes, the smallest distance indicating one mutational event.

154

155 DISCUSSION

156

157 In the Western Atlantic, *Millepora* species are most abundant in shallow, turbulent environments
158 (Lewis 1989), i.e. in places similar (in these aspects) to the site where *Millepora alcicornis* has now
159 been found at Madeira Island. To find the tropical fire coral *Millepora alcicornis* in the comparatively
160 cold waters of Madeira Island (32°N) comes as a surprise and extends the known range of the species
161 in the Eastern Atlantic about 550 km to the north.

162 In the Western Atlantic, the northern limit of *M. alcicornis* is at the Bermuda Islands, i.e. also at
163 32°N, but in a location with considerably warmer water temperatures than those at Madeira Island,
164 winter minimum temperature there being 20°C (Bertelsen & Ussing 1936); this is four degrees
165 warmer than at the north coast of Madeira Island. Thus, the Madeiran colony of *M. alcicornis* is
166 living in the coldest environment ever reported for this species and this may well be the limit of the
167 temperature tolerance of the species.

168 It is tempting to ascribe the recent discovery of a tropical species outside its known range to the
169 effects of global warming (cf. Hansen et al. 2010). While this may be true for the new and rapidly
170 growing colony at the Canary Islands (Clemente et al. 2011, López et al. 2015), this is a less likely
171 explanation for the presence of fire coral at Madeira Island. The main colony already was large (about
172 4x3m area) when detected more than 15 years ago. Clemente et al. (2011) and González (2017) report
173 on the growth rate of the colony at the Canary Islands. In eight years, this colony increased from
174 1.100 cm² to 34.704 cm². Assuming a linear growth rate, this would mean that the Madeiran colony
175 (about 120.000 cm²) would have an age of about 28 years; however, the growth rate of older colonies
176 is likely to be lower than that of younger colonies and growth in the colder waters of Madeira is
177 likely to be slower than at the Canary Islands. Therefore, the *Millepora alcicornis* colony at the north
178 coast of Madeira is probably considerably more than 30 years old. The appearance of *Millepora* at
179 Madeira Island is thus unlikely to have been facilitated by Global Warming.

180 Successful settlement is limited not only by favourable conditions on arrival; it is limited first of all
181 by the probability of arrival. Genetic analysis suggested that the Madeiran *M. alcicornis* colony
182 originated in a long distance dispersal event directly from the Caribbean area. Additionally, genetic
183 analysis already suggested that the colonies of this tropical fire coral species at the mid-Atlantic
184 location (Ascension Island) and at the Cape Verde Island, and at the Canary Island have also
185 originated from settlement events from the Caribbean area (López et al. 2015, Hoeksema et al. 2017,
186 de Souza et al. 2017). As the Madeira colony is only 2bp different from the Ascension Island colony,
187 there remains a small possibility that it may have originated from there.

188 To date, no other marine species at Madeira Island has been shown to be directly related to the
189 Caribbean area. The possible ways of arrival of *M. alcicornis* at the north coast of Madeira Island are
190 natural larval dispersal, rafting of an adult colony, or transport by humans (in ballast water or attached
191 to a ship hull). Human transport appears unlikely, as there are only small, local fishing harbours on
192 the north coast of Madeira, and none of them near the colony: no ship from the Caribbean is likely to
193 have passed there. Natural larval dispersal depends on currents. The North Equatorial Countercurrent
194 directly connects the Caribbean and the Cape Verde archipelago. There are, however, no currents
195 directly linking the Caribbean and Madeira Island. Madeira is bathed by a branch of the Gulf Stream,
196 which first passes the Azores far in the north and then turns southward as the Canary Current. This
197 would be a very long journey indeed for a *Millepora* larva; given the short life span of the medusa
198 stage of *Millepora* (Lewis 2006), larval transport from the Caribbean to Madeira therefore also
199 appears unlikely. *M. alcicornis* is capable of growing on artificial substrates (de Souza et al. 2017)
200 and *Millepora* species have been reported to raft on ship hulls (Bertelsen and Ussing 1936) and
201 pumice (Jokiel 1989). Transatlantic rafting from the Western Atlantic Ocean - as demonstrated
202 already for various mollusc species (Holmes et al 2015) and the coral *Astrangia poculata* (Hoeksema
203 et al. 2012, 2015) - appears to be the most likely explanation for the origin of the Madeira *Millepora*
204 colony.

Long distance dispersal – long considered to be extremely rare (de Queiroz 2005) – is more common than previously thought and may even become more common under climate change conditions (Batista et al. 2018).

ACKNOWLEDGEMENTS

Many thanks to the fisherman, who showed the colony to the first author and described its history; he wishes to remain anonymous. For helpful comments on an early draft of the manuscript, we are grateful to C. Lopez. This study received Portuguese national funds through FCT - Foundation for Science and Technology - through project UID/Multi/04326/2013.

LITERATURE CITED

- Batista, M. B., A. B. Anderson, P. F. Sanches, P. S. Polito et al. 2018. Kelps' long-distance dispersal: role of ecological/oceanographic processes and implications to marine forest conservation. *Diversity* 2018, 10, 11; doi:10.3390/d10010011
- Bertelsen, E. & H. Ussing 1936. Marine tropical animals carried to the Copenhagen Sydhavn on a ship from the Bermudas. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn* 100:237–245
- Clemente, S., A. Rodríguez, A. Brito, A. Ramos, O. Monterroso & J. C. Hernández 2011. On the occurrence of the hydrocoral *Millepora* (Hydrozoa: Milleporidae) in the subtropical eastern Atlantic (Canary Islands): is the colonization related to climatic events? *Coral Reefs* 30/1, 237-240
- Cunningham, C. W. & L. W. Buss 1993. Molecular evidence for multiple episodes of paedomorphosis in the Family Hydractiniidae. *Biochemical Systematics and Ecology* 21:57–69

230 de Queiroz, A. 2005. The resurrection of oceanic dispersal in historical biogeography. *Trends in*
231 *Ecology and Evolution* 20(2), 68–73.

232 De Souza, J. N. , F. L. D. Nunes, C. Zilberberg, J. A. Sanchez, A. E. Migotto, B. W. Hoeksema, X.
233 M. Serrano, A. C. Baker & A. Lindner 2017. Contrasting patterns of connectivity among endemic
234 and widespread fire coral species (*Millepora* spp.) in the tropical Southwestern Atlantic. *Coral Reefs*
235 36/3, 701-716

236 De Weerdt, W. H. 1984. Taxonomic characters in Caribbean *Millepora* species (Hydrozoa,
237 Coelenterata). *Bijdragen tot de Dierkunde* 54, 243–262.

238 Fukami, H., A. F. Budd, D. R. Levitan, J. Jara , R. Kersanach & N. Knowlton 2004. Geographic
239 differences in species boundaries among members of the *Montastraea annularis* complex based on
240 molecular and morphological markers. *Evolution* 58:324–337

241 González, E. P. 2017. Estudio del hidrocoral *Millepora alcicornis* Linnaeus, 1758, en la isla de
242 Tenerife. Thesis, University of La Laguna, Tenerife, Canary Islands, Spain. 26 pages

243 Hansen, J. , R. Ruedy, M. Sato & K. Lo 2010. Global surface temperature change. *Reviews of*
244 *Geophysics* 48, RG4004, doi:10.1029/2010RG000345

245 Hoeksema, B. W. , F. L. D. Nunes, A. Lindner & J. N. de Souza 2017. *Millepora alcicornis*
246 (Hydrozoa: Capitata) at Ascension Island: confirmed identity based on morphological and molecular
247 analyses. *Journal of the Marine Biological Association of the United Kingdom* 97(4), 709–712.

248 Hoeksema, B. W. , P. J. Roos & G. C. Cadée 2012. Trans-Atlantic rafting by the brooding reef coral
249 *Favia fragum* on man-made flotsam. *Marine Ecology Progress Series* 445, 209–218.

250 Hoeksema, B. W. , P. J. Roos & G. C. Cadée 2015. Corrigendum: Trans-Atlantic rafting by the
251 brooding reef coral *Favia fragum* on man-made flotsam. *Marine Ecology Progress Series* 541, 279.

252 Holmes, A. M. , P. G. Oliver, S. Trewheella, R. Hill & D. T. G. Quigley 2015. Trans-Atlantic rafting of
253 inshore mollusca on macro-litter: American molluscs on British and Irish shores. *Journal of*
254 *Conchology* 42/1, 1-9.

255 Jokiel, P. L. 1989. Rafting of reef corals and other organisms at Kwajalein Atoll. *Marine Biology*
256 101:483–493

257 Kearse, M. , R. Moir, A. Wilson A, S. Stones-Havas , M. Cheung, S. Sturrock, S. Buxton, A. Cooper,
258 S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Mentjies & A. Drummond 2012. Geneious Basic:
259 an integrated and extendable desktop software platform for the organization and analysis of sequence
260 data. *Bioinformatics* 28(12), 1647-1649.

261 Laborel, J. 1974. West African reef corals: A hypothesis on their origin. In: *Proceedings of the 2nd*
262 *International Coral Reef Symposium*, pp. 425-442.

263 Lefort, V., J. E. Longueville & O. Gascuel 2017. SMS: Smart Model Selection in PhyML. *Molecular*
264 *Biology and Evolution* msx149

265 Lewis, J. B. 1989. The ecology of *Millepora*. *Coral Reefs* 8: 99-107.

266 Lewis, J. B. 2006. The biology and ecology of the hydrocoral *Millepora* on coral reefs. *Advances in*
267 *Marine Biology* 50:1–55.

268 López, C. , S. Clemente, C. Almeida, A. Brito, M. Hernández 2015. A genetic approach to the origin
269 of *Millepora* sp. in the eastern Atlantic. *Coral Reefs* 34 (2), 631–638.

270 Razak, T. B. & B. W. Hoeksema 2003. The hydrocoral genus *Millepora* (Hydrozoa: Capitata:
271 Milleporidae) in Indonesia. *Zoologische Verhandelingen Leiden* 345, 313–336.

272 Smith, T. M. , P. H. York, B. R. Broitman, M. Thiel, G. C. Hays, E. van Sebille , N. F. Putman, P. I.
273 Macreadie & C. D. H. Sherman 2018. Rare long distance dispersal of a marine angiosperm across the
274 Pacific Ocean. *Global Ecology and Biogeography* 27/4, 487-496.

275
276
277

278 Table 1. List of the 16S rRNA sequences of *Millepora alcicornis* used in this study with their
279 respective sampling sites and GenBank accession numbers.

Sample name	Sampling site	Accession number	Reference
-------------	---------------	------------------	-----------

1	ABD03	Caribbean Province, Bermuda	KT891016	de Souza et al. 2017
2	ABD16	Caribbean Province, Bermuda	KT891017	de Souza et al. 2017
3	ABD23	Caribbean Province, Bermuda	KT891018	de Souza et al. 2017
4	ABD51	Caribbean Province, Bermuda	KT891019	de Souza et al. 2017
5	ABD54	Caribbean Province, Bermuda	KT891020	de Souza et al. 2017
6	ABD218	Caribbean Province, Bermuda	KT891021	de Souza et al. 2017
7	ABD282	Caribbean Province, Bermuda	KT891022	de Souza et al. 2017
8	ABD363	Caribbean Province, Bermuda	KT891024	de Souza et al. 2017
9	ABD382	Caribbean Province, Bermuda	KT891023	de Souza et al. 2017
10	ACAPA03	Caribbean Province, Panama	KT891025	de Souza et al. 2017
11	ACAPA04	Caribbean Province, Panama	KT891026	de Souza et al. 2017
12	ACAPA05	Caribbean Province, Panama	KT891027	de Souza et al. 2017
13	ACAFL01	Caribbean Province, Florida	KT891028	de Souza et al. 2017
14	ACAFL02	Caribbean Province, Florida	KT891029	de Souza et al. 2017
15	ACAFL03	Caribbean Province, Florida	KT891030	de Souza et al. 2017
16	ACAFL04	Caribbean Province, Florida	KT891031	de Souza et al. 2017
17	ACAFL05	Caribbean Province, Florida	KT891032	de Souza et al. 2017
18	ACAFL06	Caribbean Province, Florida	KT891033	de Souza et al. 2017
19	ACAFL07	Caribbean Province, Florida	KT891034	de Souza et al. 2017
20	ACAFL08	Caribbean Province, Florida	KT891035	de Souza et al. 2017
21	ACAFL09	Caribbean Province, Florida	KT891036	de Souza et al. 2017
22	ACAFL10	Caribbean Province, Florida	KT891037	de Souza et al. 2017
23	ACAFL11	Caribbean Province, Florida	KT891038	de Souza et al. 2017
24	ACAFL12	Caribbean Province, Florida	KT891039	de Souza et al. 2017
25	ACAFL15	Caribbean Province, Florida	KT891040	de Souza et al. 2017
26	ACAFL16	Caribbean Province, Florida	KT891041	de Souza et al. 2017
27	ACAFL17	Caribbean Province, Florida	KT891042	de Souza et al. 2017
28	ACAFL18	Caribbean Province, Florida	KT891043	de Souza et al. 2017
29	ACAFL27	Caribbean Province, Florida	KT891044	de Souza et al. 2017
30	ACAFL28	Caribbean Province, Florida	KT891045	de Souza et al. 2017
31	ACAFL29	Caribbean Province, Florida	KT891046	de Souza et al. 2017
32	ACAFL30	Caribbean Province, Florida	KT891047	de Souza et al. 2017
33	ACAFL31	Caribbean Province, Florida	KT891048	de Souza et al. 2017
34	ACACO451	Caribbean Province, Colombia	KT891049	de Souza et al. 2017
35	ACACO454	Caribbean Province, Colombia	KT891050	de Souza et al. 2017
36	ACACO455	Caribbean Province, Colombia	KT891051	de Souza et al. 2017
37	ACACO456	Caribbean Province, Colombia	KT891052	de Souza et al. 2017
38	ACACO458	Caribbean Province, Colombia	KT891053	de Souza et al. 2017
39	ACACO4512	Caribbean Province, Colombia	KT891054	de Souza et al. 2017
40	ACACO4514	Caribbean Province, Colombia	KT891055	de Souza et al. 2017
41	ACACO826	Caribbean Province, Colombia	KT891056	de Souza et al. 2017
42	ACACO8211	Caribbean Province, Colombia	KT891057	de Souza et al. 2017
43	ACACO8213	Caribbean Province, Colombia	KT891058	de Souza et al. 2017
44	ACACO8214	Caribbean Province, Colombia	KT891059	de Souza et al. 2017
45	AM01	Ascension Province, Ascension Island	KT891060	de Souza et al. 2017
46	AMSAS02	Ascension Province, Ascension Island	KT891061	de Souza et al. 2017
47	AAFA01	Lusitania Province, Canary Islands	KT891062	de Souza et al. 2017
48	MADI01	Lusitania Province, Madeira Island	MH660402	Present study

49	AAFCV237	Tropical Eastern Atlantic Province, Cape Verde	KT891063	de Souza et al. 2017
50	AAFCV239	Tropical Eastern Atlantic Province, Cape Verde	KT891064	de Souza et al. 2017
51	AAFCV241	Tropical Eastern Atlantic Province, Cape Verde	KT891065	de Souza et al. 2017
52	AAFCV243	Tropical Eastern Atlantic Province, Cape Verde	KT891066	de Souza et al. 2017
53	AAFCV245	Tropical Eastern Atlantic Province, Cape Verde	KT891067	de Souza et al. 2017
54	AAFCV247	Tropical Eastern Atlantic Province, Cape Verde	KT891068	de Souza et al. 2017
55	AAFCV249	Tropical Eastern Atlantic Province, Cape Verde	KT891069	de Souza et al. 2017
56	AAFCV251	Tropical Eastern Atlantic Province, Cape Verde	KT891070	de Souza et al. 2017
57	AAFCV253	Tropical Eastern Atlantic Province, Cape Verde	KT891071	de Souza et al. 2017
58	ABRFN01	Brazilian Province, Fernando de Noronha	KT891072	de Souza et al. 2017
59	ABRFN02	Brazilian Province, Fernando de Noronha	KT891073	de Souza et al. 2017
60	ABRFN03	Brazilian Province, Fernando de Noronha	KT891074	de Souza et al. 2017
61	ABRFN04	Brazilian Province, Fernando de Noronha	KT891075	de Souza et al. 2017
62	ABRFN05	Brazilian Province, Fernando de Noronha	KT891076	de Souza et al. 2017
63	ABRFN06	Brazilian Province, Fernando de Noronha	KT891077	de Souza et al. 2017
64	ABRFN07	Brazilian Province, Fernando de Noronha	KT891078	de Souza et al. 2017
65	ABRFN08	Brazilian Province, Fernando de Noronha	KT891079	de Souza et al. 2017
66	ABRFN09	Brazilian Province, Fernando de Noronha	KT891080	de Souza et al. 2017
67	ABRFN10	Brazilian Province, Fernando de Noronha	KT891081	de Souza et al. 2017
68	ABRFN109	Brazilian Province, Fernando de Noronha	KT891082	de Souza et al. 2017
69	ABRFN111	Brazilian Province, Fernando de Noronha	KT891083	de Souza et al. 2017
70	ABRFN113	Brazilian Province, Fernando de Noronha	KT891084	de Souza et al. 2017
71	ABRFN115	Brazilian Province, Fernando de Noronha	KT891085	de Souza et al. 2017
72	ABRFN117	Brazilian Province, Fernando de Noronha	KT891086	de Souza et al. 2017
73	ABRRN01	Brazilian Province, Rio Grande do Norte	KT891087	de Souza et al. 2017
74	ABRRN02	Brazilian Province, Rio Grande do Norte	KT891088	de Souza et al. 2017
75	ABRRN03	Brazilian Province, Rio Grande do Norte	KT891089	de Souza et al. 2017
76	ABRRN04	Brazilian Province, Rio Grande do Norte	KT891090	de Souza et al. 2017
77	ABRRN05	Brazilian Province, Rio Grande do Norte	KT891091	de Souza et al. 2017
78	ABRRN06	Brazilian Province, Rio Grande do Norte	KT891092	de Souza et al. 2017
79	ABRRN08	Brazilian Province, Rio Grande do Norte	KT891093	de Souza et al. 2017
80	ABRRN09	Brazilian Province, Rio Grande do Norte	KT891094	de Souza et al. 2017
81	ABRRN10	Brazilian Province, Rio Grande do Norte	KT891095	de Souza et al. 2017
82	ABRPE92	Brazilian Province, Pernambuco	KT891096	de Souza et al. 2017
83	ABRPE123	Brazilian Province, Pernambuco	KT891097	de Souza et al. 2017
84	ABRPE124	Brazilian Province, Pernambuco	KT891098	de Souza et al. 2017
85	ABRPE212	Brazilian Province, Pernambuco	KT891099	de Souza et al. 2017
86	ABRPE263	Brazilian Province, Pernambuco	KT891100	de Souza et al. 2017
87	ABRAS06	Brazilian Province, Southern Alagoas (Maceió)	KT891101	de Souza et al. 2017
88	ABRAS09	Brazilian Province, Southern Alagoas (Maceió)	KT891102	de Souza et al. 2017
89	ABRAS10	Brazilian Province, Southern Alagoas (Maceió)	KT891103	de Souza et al. 2017
90	ABRAS31	Brazilian Province, Southern Alagoas (Maceió)	KT891104	de Souza et al. 2017
91	ABRAS55	Brazilian Province, Southern Alagoas (Maceió)	KT891105	de Souza et al. 2017
92	ABRAS58	Brazilian Province, Southern Alagoas (Maceió)	KT891106	de Souza et al. 2017
93	ABRBN04	Brazilian Province, Northern Bahia (Salvador)	KT891107	de Souza et al. 2017
94	ABRBN05	Brazilian Province, Northern Bahia (Salvador)	KT891108	de Souza et al. 2017
95	ABRBN08	Brazilian Province, Northern Bahia (Salvador)	KT891109	de Souza et al. 2017
96	ABRBN10	Brazilian Province, Northern Bahia (Salvador)	KT891110	de Souza et al. 2017

97	ABRBN12	Brazilian Province, Northern Bahia (Salvador)	KT891111	de Souza et al. 2017
98	ABRBN16	Brazilian Province, Northern Bahia (Salvador)	KT891112	de Souza et al. 2017
99	ABRBN17	Brazilian Province, Northern Bahia (Salvador)	KT891113	de Souza et al. 2017
100	ABRBN20	Brazilian Province, Northern Bahia (Salvador)	KT891114	de Souza et al. 2017
101	ABRBN24	Brazilian Province, Northern Bahia (Salvador)	KT891115	de Souza et al. 2017
102	ABRBN25	Brazilian Province, Northern Bahia (Salvador)	KT891116	de Souza et al. 2017
103	ABRBN26	Brazilian Province, Northern Bahia (Salvador)	KT891117	de Souza et al. 2017
104	ABRBN28	Brazilian Province, Northern Bahia (Salvador)	KT891118	de Souza et al. 2017
105	ABRBN34	Brazilian Province, Northern Bahia (Salvador)	KT891119	de Souza et al. 2017
106	ABRBN35	Brazilian Province, Northern Bahia (Salvador)	KT891120	de Souza et al. 2017
107	ABRBN41	Brazilian Province, Northern Bahia (Salvador)	KT891121	de Souza et al. 2017
108	ABRBN42	Brazilian Province, Northern Bahia (Salvador)	KT891122	de Souza et al. 2017
109	ABRBN44	Brazilian Province, Northern Bahia (Salvador)	KT891123	de Souza et al. 2017
110	ABRBN46	Brazilian Province, Northern Bahia (Salvador)	KT891124	de Souza et al. 2017
111	ABRBN51	Brazilian Province, Northern Bahia (Salvador)	KT891125	de Souza et al. 2017
112	ABRBN57	Brazilian Province, Northern Bahia (Salvador)	KT891126	de Souza et al. 2017
113	ABRBN67	Brazilian Province, Northern Bahia (Salvador)	KT891127	de Souza et al. 2017
114	ABRBN71	Brazilian Province, Northern Bahia (Salvador)	KT891128	de Souza et al. 2017
115	ABRBN75	Brazilian Province, Northern Bahia (Salvador)	KT891129	de Souza et al. 2017
116	ABRBN79	Brazilian Province, Northern Bahia (Salvador)	KT891130	de Souza et al. 2017
117	ABRBP87	Brazilian Province, Southern Bahia (Porto Seguro)	KT891131	de Souza et al. 2017
118	ABRBP89	Brazilian Province, Southern Bahia (Porto Seguro)	KT891132	de Souza et al. 2017
119	ABRBP91	Brazilian Province, Southern Bahia (Porto Seguro)	KT891133	de Souza et al. 2017
120	ABRBP199	Brazilian Province, Southern Bahia (Porto Seguro)	KT891134	de Souza et al. 2017
121	ABRBP201	Brazilian Province, Southern Bahia (Porto Seguro)	KT891135	de Souza et al. 2017
122	ABRBP203	Brazilian Province, Southern Bahia (Porto Seguro)	KT891136	de Souza et al. 2017
123	ABRBP205	Brazilian Province, Southern Bahia (Porto Seguro)	KT891137	de Souza et al. 2017
124	ABRBP207	Brazilian Province, Southern Bahia (Porto Seguro)	KT891138	de Souza et al. 2017
125	ABRBP301	Brazilian Province, Southern Bahia (Porto Seguro)	KT891139	de Souza et al. 2017
126	ABRBP306	Brazilian Province, Southern Bahia (Porto Seguro)	KT891140	de Souza et al. 2017
127	ABRBP319	Brazilian Province, Southern Bahia (Porto Seguro)	KT891141	de Souza et al. 2017
128	ABRBP401	Brazilian Province, Southern Bahia (Porto Seguro)	KT891142	de Souza et al. 2017
129	ABRBP406	Brazilian Province, Southern Bahia (Porto Seguro)	KT891143	de Souza et al. 2017
130	ABRBP419	Brazilian Province, Southern Bahia (Porto Seguro)	KT891144	de Souza et al. 2017
131	ABRBP501	Brazilian Province, Southern Bahia (Porto Seguro)	KT891145	de Souza et al. 2017
132	ABRBP506	Brazilian Province, Southern Bahia (Porto Seguro)	KT891146	de Souza et al. 2017
133	ABRBP606	Brazilian Province, Southern Bahia (Porto Seguro)	KT891147	de Souza et al. 2017
134	ABRBA209	Brazilian Province, Southern Bahia (Abrolhos)	KT891148	de Souza et al. 2017
135	ABRBA211	Brazilian Province, Southern Bahia (Abrolhos)	KT891149	de Souza et al. 2017
136	ABRBA213	Brazilian Province, Southern Bahia (Abrolhos)	KT891150	de Souza et al. 2017
137	ABRES01	Brazilian Province, Espírito Santo	KT891151	de Souza et al. 2017

138	ABRES03	Brazilian Province, Espírito Santo	KT891152	de Souza et al. 2017
139	ABRES13	Brazilian Province, Espírito Santo	KT891153	de Souza et al. 2017
140	ABRES18	Brazilian Province, Espírito Santo	KT891154	de Souza et al. 2017
141	ABRES21	Brazilian Province, Espírito Santo	KT891155	de Souza et al. 2017
142	ABRES27	Brazilian Province, Espírito Santo	KT891156	de Souza et al. 2017
143	ABRES29	Brazilian Province, Espírito Santo	KT891157	de Souza et al. 2017
144	ABRES45	Brazilian Province, Espírito Santo	KT891158	de Souza et al. 2017
145	ABRES52	Brazilian Province, Espírito Santo	KT891159	de Souza et al. 2017
146	ABRES57	Brazilian Province, Espírito Santo	KT891160	de Souza et al. 2017
147	ABRES61	Brazilian Province, Espírito Santo	KT891161	de Souza et al. 2017
148	ABRES64	Brazilian Province, Espírito Santo	KT891162	de Souza et al. 2017
149	ABRES65	Brazilian Province, Espírito Santo	KT891163	de Souza et al. 2017
150	ABRES66	Brazilian Province, Espírito Santo	KT891164	de Souza et al. 2017
151	ABRES71	Brazilian Province, Espírito Santo	KT891165	de Souza et al. 2017
152	ABRES96	Brazilian Province, Espírito Santo	KT891166	de Souza et al. 2017
153	ABRES97	Brazilian Province, Espírito Santo	KT891167	de Souza et al. 2017
154	ABRES101	Brazilian Province, Espírito Santo	KT891168	de Souza et al. 2017
155	ABRES151	Brazilian Province, Espírito Santo	KT891169	de Souza et al. 2017
156	ABRES155	Brazilian Province, Espírito Santo	KT891170	de Souza et al. 2017
157	ABRRJ51	Brazilian Province, Rio de Janeiro	KT891171	de Souza et al. 2017
158	ABRRJ52	Brazilian Province, Rio de Janeiro	KT891172	de Souza et al. 2017
159	ABRRJ159	Brazilian Province, Rio de Janeiro	KT891173	de Souza et al. 2017
160	ABRRJ161	Brazilian Province, Rio de Janeiro	KT891174	de Souza et al. 2017
161	ABRRJ163	Brazilian Province, Rio de Janeiro	KT891175	de Souza et al. 2017
162	ABRRJ166	Brazilian Province, Rio de Janeiro	KT891176	de Souza et al. 2017
163	ABRRJ167	Brazilian Province, Rio de Janeiro	KT891177	de Souza et al. 2017
164	ABRRJ169	Brazilian Province, Rio de Janeiro	KT891178	de Souza et al. 2017
165	ABRRJ173	Brazilian Province, Rio de Janeiro	KT891179	de Souza et al. 2017
166	ABRRJ175	Brazilian Province, Rio de Janeiro	KT891180	de Souza et al. 2017
167	ABRRJ177	Brazilian Province, Rio de Janeiro	KT891181	de Souza et al. 2017

280

281

282 Text to Figures:

283 Figure 1: *Millepora* colony at the north coast of Madeira Island. The fins of the diver are 80 cm long.

284 Fig 2: Detail of the main colony, showing the branching pattern and the erect branches typical of *M.*

285 *alcicornis*, with laterally flattened tips.

286 Figure 3: 16 cm high branch of the main colony.

287 Figure 4. Maximum likelihood phylogenetic reconstruction of the partial 16S rRNA region of

288 *Millepora* species. An arrow shows the sample from Madeira Island, grouping with *M. alcicornis*.

289 Numbers at nodes represent the bootstrap support values (> 60%). All sequences downloaded from
 290 GenBank are presented with their access number.

291 Figure 5. Haplotype network of *Millepora alcicornis* using sequences from de Souza et al. (2017;
 292 Table 1), including the *M. alcicornis* sample from Madeira Island. Circle sizes correspond to the
 293 number of haplotypes and colours illustrate major regions of occurrence. The four black dots
 294 represent ancestral or not sampled haplotypes and line length corresponds to distance between
 295 haplotypes, the smallest distance indicating one mutational event.