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REPORT

Genetic relationships of the hydrocoral *Millepora alcicornis* and its symbionts within and between locations across the Atlantic

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Abstract Although the hydrocoral *Millepora alcicornis* is a prominent and ecologically relevant amphi-Atlantic reef builder, little attention has been given to its endosymbionts which are also involved in the survival and adaptation success of the species in different environments. In this study, we resolve the genetic relationships between *M. alcicornis* and its symbionts (*Symbiodiniaceae*) within both sides and across the Atlantic. The COI and 16S-rDNA regions were selected for the host tissues, and the 23S-rDNA and ITS regions were chosen for the symbionts. Phylogenetic networks consistently showed that host populations from the eastern Atlantic archipelagos (Canary and Cape Verde Islands) were more related to western Atlantic populations than they were between them. However, results for Symbiodiniaceae species varied according to the molecular marker used. Samples from Mexico were grouped as *Symbiodinium* sp. (formerly *Symbiodinium* clade A) by both markers. Specimens from Puerto Rico were grouped as either *Symbiodinium* sp. or *Breviolum* sp. (formerly *Symbiodinium* clade B), according to the molecular marker used. Most samples from the eastern Atlantic were identified as *Breviolum* sp. by both markers, except for one sample from the Canary Islands and two samples from the Cape Verde Islands, which were identified as *Cladocopium* sp. (formerly *Symbiodinium* clade C) using ITS-rDNA. These results suggest that these two genera of Symbiodiniaceae may cohabit the same *M. alcicornis* colony. Because hydrocorals from the Canary Islands were phylogenetically related to the western Atlantic, but symbionts were more related to those of the Cape Verde Islands, the origin of the coral and its symbionts is probably different. This may be explained either by "horizontal" transmission, i.e. acquisition from the environment, or by a change in the dominant symbiont composition within the host. The flexibility of this hydrocoral to select symbionts, depending on environmental conditions, can provide new insight to understand how this coral may face ongoing climate change.

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Keywords Climate change · Coral–symbiont flexibility · Hydrozoa · Molecular marker · Symbiodiniaceae transmission

Introduction

Coral reefs are among the most biologically diverse and economically important marine ecosystems, providing vital services to humans, including fisheries, coastal protection, medicines and tourism activities (Hoegh-Guldberg et al. 2007; Srividhya and Chellaram 2012). The main reef framework is typically built by calcifying corals (phylum Cnidaria), such as scleractinians and hydrocorals (Buddemeier et al. 2004). Their three-dimensional structure provides multiple microhabitats, leading to a great diversity and abundance of associated organisms (Graham and Nash 2013). Many corals host photosynthetic symbionts, i.e. dinoflagellates, belonging to the family Symbiodiniaceae (LaJeunesse et al. 2018). This association is of fundamental ecological importance, the host provides inorganic nutrients and refuge from herbivory to its symbionts and, in turn, the symbionts contribute to host nutrition by providing photosynthetically fixed carbon and enhancing coral skeletogenesis and reef development (Weis et al. 2001; Yellowlees et al. 2008; Davy et al. 2012).

Symbiotic hydrocorals of the genus *Millepora* are important reef builders, covering extensive areas in shallow tropical reefs around the world (Boschma 1948; de Weerdt 1984). They are commonly found in both the Indo-Pacific and Atlantic Oceans (Razak and Hoeksema 2003; Amaral et al. 2008; Ruiz-Ramos et al. 2014, Takama et al. 2018). In the Atlantic, most *Millepora* species inhabit the western basin (Caribbean and Brazilian biogeographic provinces), with the exception of *Millepora alcicornis* (Linnaeus 1758), which has an amphi-Atlantic distribution as evidenced by its presence in the Cape Verde Islands (Laborel 1974), Ascension Island (Hoeksema et al. 2017) and the Canary Islands (Clemente et al. 2010). Asexual reproduction by fragmentation is the main mechanism to form new zooids of *M. alcicornis*, because they breed infrequently, releasing sexual medusae seasonally (Lewis 1989, 2006). This hydrocoral has high growth and recruitment rates (Lewis 2006) and is capable of colonizing both natural and artificial substrates, from dead gorgonians and rocks to the hull of ships (Bertelsen and Ussing 1936; Wahle 1980). This ability to inhabit different substrates and its rapid colonization rates (Clemente et al. 2010) provide a competitive advantage for potential habitat expansions.

Symbiotic dinoflagellates of the family Symbiodiniaceae live in association with *Millepora* spp. (van Oppen et al. 2009). Recently, the differentiation of 7 genera (previously referred to as clades of *Symbiodinium* spp.) has been described for the family Symbiodiniaceae (LaJeunesse et al. 2018). Some symbiont genera are widely distributed across extensive oceanographic regions and at different depths (Baker 2003; Rodriguez-Lanetty and Hoegh-Guldberg 2003). Others have narrow geographic distributions, e.g. Temperate "Clade A" (previously termed *Symbiodinium* "Temperate A"), mainly found in the Mediterranean (Visram et al. 2006; Casado-Amezúa et al. 2016). Some corals present high levels of symbiont specificity, e.g. *Acropora palmata* with *Symbiodinium "fitti"*, nomen nudum (A3) (Pinzón et al. 2011), while others can host several Symbiodiniaceae genera within the same colony (Rowan and Knowlton 1995; Apprill and Gates 2007; Kemp et al. 2015; Grajales and Sanchez 2016). Symbiont acquisition differs among corals. Some transmit their symbionts directly to their offspring (vertical transmission), ensuring the viability of the juvenile coral. Other species acquire symbionts from the environment (horizontal transmission) through phagocytosis of symbionts (Hirose et al. 2001; Weis et al. 2001; Stat et al. 2006, 2008) expelled from other hosts, such as corals, molluscs, foraminifera, anemones (Rodriguez-Lanetty 2003), flatworms (Kunihiro and Reimer 2018), or they may uptake free-living symbionts (e.g. Reimer et al. 2010b; Nitschke et al. 2016). Others utilize both strategies, acquiring physiologically advantageous novel symbionts through horizontal transmission, which are perpetuated via vertical transmission (Byler et al. 2013). When corals suffer stressful conditions, some of them can replace their symbionts, via horizontal transmission, with other "host-compatible" symbionts. However, once the stressful situation is over, they usually recover their original symbiont species (Sampayo et al. 2016), reducing the stability of the new symbiotic association over the long term (Coffroth et al. 2010; Sampayo et al. 2016). Others can also change their internal abundance and composition of symbionts under adverse conditions (Berkelmans and van Oppen 2006), or even seasonally (Gates 1990; Yang et al. 2000; Warner et al. 2002; Costa et al. 2013). Other species do not change their symbionts under stressful conditions (Goulet 2006, 2007).

The ancient association complex of Cnidaria–Symbiodiniaceae is extremely diverse and provides multiple functional traits for the holobiont as a whole (e.g. Mieog et al. 2009; LaJeunesse et al. 2018). For example, the thermal tolerance of some corals may depend on the Symbiodiniaceae species that it hosts, while the physiological response of the symbiont may depend on the host population origin, or its genetic background (Mieog et al. 2009; Parkinson et al. 2015). Thus, the coral and its symbionts need to be considered as a single unit to better understand their biological and ecological responses to

environmental conditions, which is fundamental to predict how coral reefs will face climate change (Parkinson and Baums 2014; Grajales and Sanchez 2016).

Hydrocorals are sessile marine invertebrates, and their medusoid larval stage and asexual fragments represent their opportunity for dispersal (Lewis 2006; Ortiz-González et al. 2017); however, they are difficult to track. Molecular tools provide an ideal technique for an indirect assessment of their phylogenetic relationships, connectivity and population structure (Hunter et al. 1997; Van Oppen et al. 2001; Vollmer and Palumbi 2004). Govindarajan et al. (2005) suggested that the cytochrome oxidase subunit I (COI) and the 16S-rDNA are useful molecular markers for hydrozoan phylogeographic studies. Zheng et al. (2014) recommended the use of 16S-rDNA for DNA barcoding and phylogenetic analyses of hydrozoans at the genus level. Both markers demonstrated high genetic diversity for *Millepora* spp. in the Caribbean Sea, which are the source populations of the eastern Atlantic *M. alcicornis* found in the Cape Verde and Canary Islands (López et al. 2015; de Souza et al. 2017). Most of the studies based on symbiont identification point towards the use of multiple markers (e.g. LaJeunesse et al. 2012). For example, Santos et al. (2002) used the chloroplast large-subunit 23S-rDNA to infer its molecular phylogeny. LaJeunesse (2001) used the internal transcribed spacer (ITS) region for the same purpose and to investigate the biodiversity and ecology of the symbionts. Santos et al. (2001) also used the ITS for comparing cultured *versus* freshly isolated zooxanthellae, detecting that a single host can contain heterogeneous symbiont populations. Within those, the ITS region is often analysed to differentiate genera in the family Symbiodiniaceae (Coffroth and Santos 2005; LaJeunesse et al. 2010, 2012, 2018). In particular, the ITS2 region has been used to resolve symbiont diversity at the classical subclade level (LaJeunesse 2001; LaJeunesse et al. 2004; Sampayo et al. 2009; Stat et al. 2011; Arif et al. 2014). Despite the abundant scientific literature based on genetic analyses of corals or symbionts, only a few studies have combined genetic analyses of both components of the Cnidaria–Symbiodiniaceae association, e.g. Alcyonacea–Symbiodiniaceae (Coffroth et al. 2001), Actiniaria–Symbiodiniaceae (Thornhill et al. 2013), Zoantharia–Symbiodiniaceae (Reimer et al. 2010a, 2017) and Scleractinia–Symbiodiniaceae (Thornhill et al. 2006; Mieog et al. 2009; Pettay et al. 2011; Casado-Amezúa et al. 2014; Shinzato et al. 2014; Picciani et al. 2016).

To date, three studies have analysed the genetic structure of *M. alcicornis* across the Atlantic (López et al. 2015; Hoeksema et al. 2017; de Souza et al. 2017). However, all of them have only considered the host and have employed analyses based on a single gene, without including their endosymbionts in the analyses. Including symbionts in the analyses could improve the resolution for delineating the genetic structure of the hydrocoral–symbiont association. In this study, we used four molecular markers, two for *M. alcicornis* (COI and 16S-rDNA) and two for its symbionts (23S-rDNA and ITS-rDNA), with the aim of resolving the genetic relationships for the hydrocoral–Symbiodiniaceae association within and between populations from across the Atlantic. This combined analysis (host and symbiont) might help to understand the origin and colonization process of this holobiont into new regions, such as the Canary Islands, and how it may respond to varying environments. This information might be relevant when evaluating possible global warming scenarios that may result in shifts in species distribution towards the eastern Atlantic (e.g. González-Delgado et al. 2018).

Materials and methods

Sample collection

A total of 62 fragments of *M. alcicornis* were collected by scuba diving or snorkelling, between the intertidal zone down to 14 m depth (Table S1) at 15 sites across the Atlantic (Fig. 1). The corresponding national permits were obtained when needed (e.g. Mexico, Conapesca 01-013). All samples were preserved in dimethyl sulfoxide (DMSO) until further analyses.

DNA extraction and PCR conditions

DNA from 50 mg of tissue, scraped from each specimen, was extracted using the DNeasy Blood & Tissue Kit (Qiagen®, Redwood City, CA, USA), following the manufacturer's instructions. The polymerase chain reaction (PCR) was used to amplify two fragment genes from the host tissue (COI and 16S-rDNA) and from their Symbiodiniaceae symbionts (23S-rDNA and ITS-rDNA). PCR amplifications were performed in a total volume of 25 µl, containing 1X buffer (GeneAll Biotechnology, South Korea), 0.2 mM of each dNTP, 0.4 µM of each primer, 1U of Taq DNA polymerase (GeneAll Biotechnology, South Korea) and 20 ng of total genomic DNA.

PCR cycle conditions consisted of an initial cycle at 94 °C for 2 min, followed by 40 cycles at 94 °C for 10 s, an annealing temperature specific to each primer (see Table 1) during 20 s, and 30 s at 72 °C, with a final extension at 72 °C for 10 min. Amplified products were checked by 1% agarose gel electrophoresis and, subsequently, the unincorporated primers and nucleotides were removed with the ExoSAP-IT kit (GE Healthcare Illustra, Sweden). Finally, the samples were sequenced by the Genomic Service (SEGAI) of the University of La

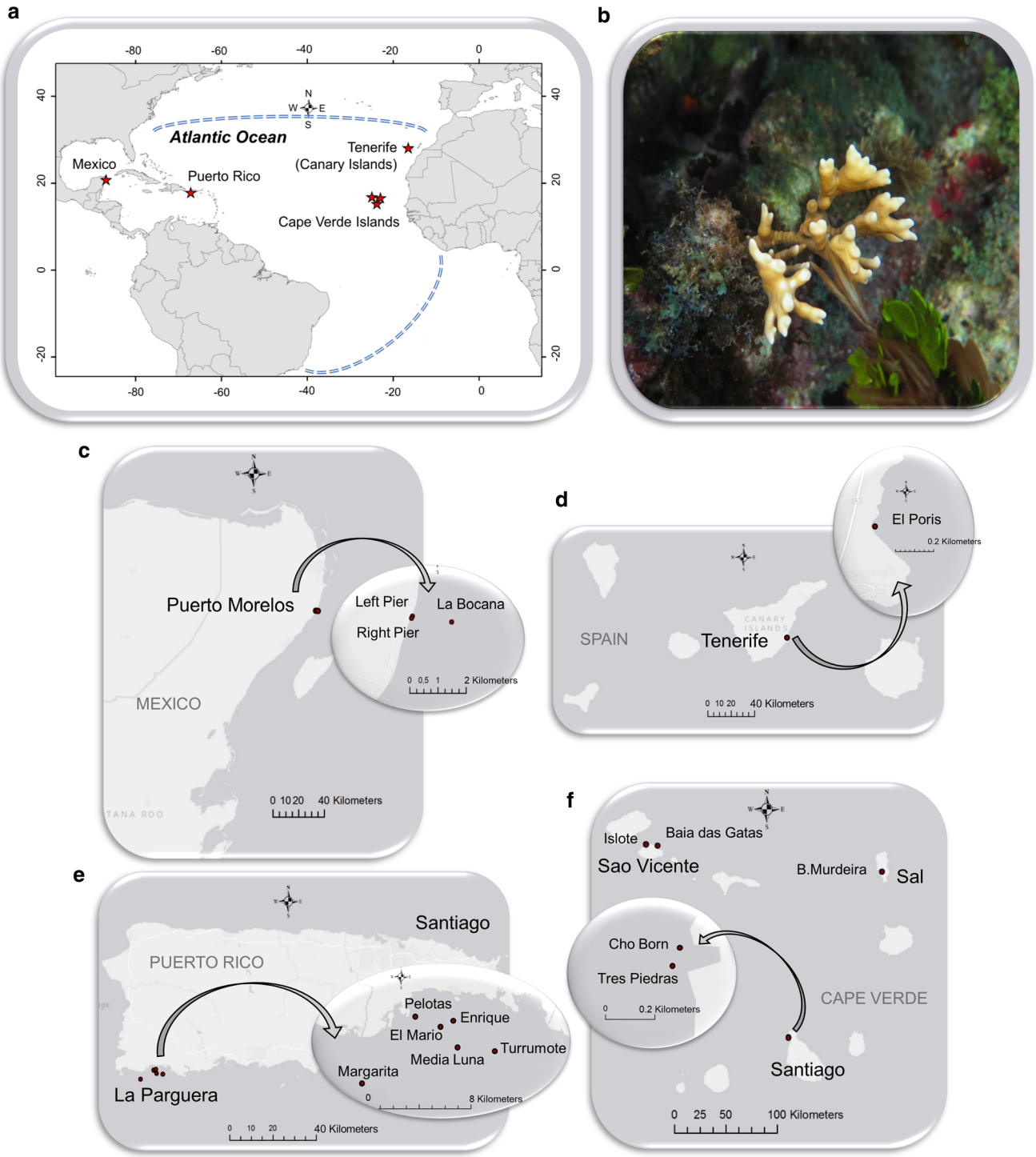


Fig. 1 **a** Map denoting sample collection sites in the Atlantic Ocean (red stars). Limits of the current distribution of *Millepora alcicornis* are included by double dashed blue lines. **b** *M. alcicornis* from La Bocana (Mexico). Photograph credit: Laura Rodríguez. Sample sites in **c** Mexico, **d** Spain (Tenerife), **e** Puerto Rico and **f** Cape Verde Islands

Laguna. The sequences obtained in this study were deposited in GenBank (see accession numbers in Table S1).

Sequence editing, alignment and analysis

DNA sequences were edited and assembled using MEGA7: Molecular Evolutionary Genetic Analysis, version 7.0 (Kumar et al. 2016). Sequence alignments were performed

Table 1 Summary of the organisms and primers used for genetic analyses

Organism	Region	Primer	Primer sequence	Size (bp)	Ta (°C)	Substitution model	References
Millepora alcicornis	COI (mitochondrial)	COIF	5′TAG-AAT-TAG-CTG-GGC-CAG-GA-3′	462	50	–	Modified from Ruiz-Ramos 2009 MMX: PureTaq Ready-To-Go PCRbeads (GE Healthcare, Uppsala, Sweden)
		COIR	5′CCT-GTC-TGT-AAG-CAG-CAT-GG-3′				
Millepora alcicornis	16S-rDNA (mitochondrial)	16SAR	5-TCGACTGTTTACCAAAAACATAGC-3	469	52	–	Cunningham and Buss (1993)
		16SBR	5-ACGGAATGAACTCAAATCATGTAAG-3				Modified from Santos et al. (2001)
Symbiodinium sp.	23S-rDNA (plasmid)	23S2M13	5-CACGACGTTGTAAAACGACGGCTG TAACTATAACGGTCC-3	514	51	GTR + G	Santos et al. (2002)
		23S2M13	5-GGATAACAATTTCACACAGGCCA TCGTATTGAACCCAGC-3				
Symbiodinium sp.	ITS (nuclear)	ZITSUPM13	5-CACGACGTTGTAAAACGACCCGGTG AATTATTCGG ACTGACGCAGTGCT-3	638	55	GRT + I+ G	Modified from Santos et al. (2002) MMX: PureTaq Ready-To-Go PCRbeads (GEHealthcare, Uppsala, Sweden)
		ZITSDNM13	5-GGATAACAATTTCACACAGG CTGTTTA GTTCCTTTTCCTCCGC-3				

Substitution model obtained from the *J*ModelTest used in MRBAYES to construct the phylogenetic analyses of the symbiont samples

Bp base pair, *Ta* annealing temperature

using CLUSTAL W (Thompson et al. 1994), as implemented in MEGA7, and further revised by eye. In all cases, both sequence ends were trimmed manually to remove low-quality regions. Additionally, a total of 81 sequences of COI and 28 sequences of 16S-rDNA from the Caribbean, the coast of Brazil and the central Atlantic were obtained from GenBank to complete a representative sample size of *M. alcicornis* across the Atlantic. Network 5.0.0.3 software (Fluxus Technology©, www.fluxus-engineering.com) was used to generate one haplotype network for each marker.

Poorly aligned regions from the alignment of 23S-rDNA and ITS-rDNA fragment genes from Symbiodiniaceae were removed using Gblocks® (Integrated DNA Technologies, Inc) (Castresana 2000). GenBank Symbiodiniaceae sequences for these genes (11 for the 23S-rDNA and 13 for the ITS-rDNA) defining the classical clades (A, B, C, D, E, F and G) were added to the analyses. The best-fitting DNA substitution model for each Symbiodiniaceae data set was determined according to the Bayesian information criterion (Schwarz 1978) in *j*ModelTest (Darriba et al. 2012) (Table 1). Phylogenetic trees of the symbiont samples were inferred using MrBayes 3.2.6 (Ronquist et al. 2012). The Markov chain Monte Carlo (MCMCs) were run for 5,000,000 generations and sampled every 100 generations. Two independent analyses were run, and the similarity of the tree sample diagnostics was calculated every 100 generations. We left the default setting of MrBayes, which discards the first 25% samples from the cold chain, to obtain values of the potential scale reduction factor (PSRF) close to 1. Consensus trees were visualized through Fig.Tree v1.4.2 (Rambaut 2009). A suitable outgroup (*Gymnodinium* sp.) to each alignment was added to root the phylogram and identify the correct evolutionary pathway. To assess topological congruences between the 23S-rDNA and the ITS-rDNA phylogenetic trees, the congruence index (Icong) and its associated *p* value were calculated through the online programme http://max2.ese.u-psud.fr/bases/upresa/pages/devienne/index.help.html (de Vienne et al. 2007).

To identify the correspondent species (formerly subclades) hosted by each population of *M. alcicornis*, we analysed the ITS2 region. Once the genera hosted by each population were defined, independent phylogenetic trees were constructed for each genus: *Symbiodinium* (A), *Breviolum* (B) and *Cladocopium* (C), including sequences (subclades) from GenBank. The same methodology used for the analyses of ITS-rDNA and 23S-rDNA was followed for the ITS2.

Finally, restriction fragment length polymorphism (RFLP) analyses were developed following LaJeunesse and Trench (2000) to detect if two or more symbiont genera were cohabiting within the same *M. alcicornis* colony. Briefly, large-subunit RNA (hereafter LSUrDNA) was amplified following the conditions and primers described by Wilcox (1998) and PCR products were verified by agarose gel electrophoresis. Subsequently, amplification products were digested using Dpn II (New England Biolabs, USA), following the manufacturer's protocol, and products were visualized in an agarose gel (2%) stained with GelRed. The restriction digests of LSUrDNA were performed only for a subset of samples from Mexico (samples 1R, 1M, 1Ds, 2R, 2P and 4Ds), the Cape Verde Islands (samples CV5, CV15, CV18, CV20 and CV50) and Tenerife (samples CN21 and CN22).

Results

Millepora alcicornis genetic analyses

From the 134 sequences analysed for the COI fragment, 53 from this research (Table S1) and 81 obtained from GenBank (Table S2), a total of 89 haplotypes were obtained: 79 belonged to the Caribbean Sea, one to the Canary Islands, and nine to the Cape Verde Islands. Haplotypes from both eastern Atlantic archipelagos (the Canary and the Cape Verde Islands) were more related to the Caribbean than they were between them (Fig. 2a), with the most frequent haplotypes shared between the Caribbean and the Cape Verde Islands.

From the 74 sequences analysed for the 16S-rDNA region, 44 from our research (Table S1) and 30 from GenBank (Table S2), a total of 28 haplotypes were obtained: 14 from the Caribbean, one from the Canary Islands, nine from the Cape Verde Islands, three from Brazil and one from Ascension Island. The haplotype network for the 16S-rDNA fragment (Fig. 2b) confirms the results obtained for the COI region. Again, both eastern Atlantic archipelagos were more related to the western Atlantic than between them, and two haplotype groups were detected in the Cape Verde Islands. These two Cape Verde Islands haplotypes were more related to the western Atlantic than they were between them, with one of them also closely related to Ascension Island and distantly related to the Brazilian samples.

Symbiodiniaceae phylogenetic analyses

Symbiodiniaceae alignments contained 36 sequences for the ITS-rDNA, 23 from our study (Table S1) and 13 from GenBank (Table S2) representing all the existing genera described in the family. Symbiodiniaceae alignments also contained 45 sequences for the 23S-rDNA, 34 from our study (Table S1) and 11 from GenBank (Table S12). ITS-rDNA and 23S-rDNA analyses determined that different

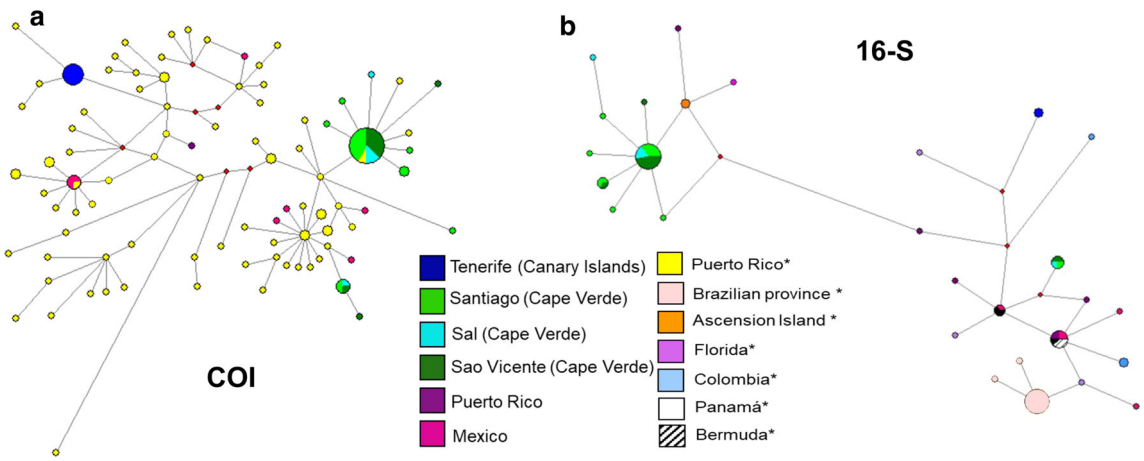


Fig. 2 Haplotype network based on **a** COI and **b** 16S-rDNA sequences of *Millepora alcicornis*. Additional GenBank sequences from those collected in this study were added to the network, e.g. western Atlantic and Ascension Island (Table S2)

Symbiodiniaceae genera are hosted by *M. alcicornis* (Fig. 3a, b, Table S1). The phylogenetic results of the ITS-rDNA (Fig. 3a) showed that all individuals from Mexico (representing 30.5% of the samples) hosted *Symbiodinium* sp. (formerly clade A) (Bayesian posterior probability, BPP = 1); in particular, type A4 as unveiled by the ITS2 analysis (Fig. S1b). Individuals from Puerto Rico, and most of the samples collected from the Cape Verde Islands (i.e. 56.5% of our samples), hosted *Breviolum* sp. (formerly clade B) (BPP = 1). The ITS2 phylogeny showed a high genetic diversity within this genus. Some individuals from the Cape Verde Islands (samples 5 and 7) grouped with type B23 and B30, while the rest of the samples were included within a group formed by types B1, B37, B10, B16, B17, B8 and B20 (Fig. S1a). Samples 20 and 18 from Santiago (Cape Verde Islands) and 21 from Tenerife (Canary Islands) (i.e. 13% of the samples) hosted *Cladocopium* sp. (BPP = 1 (Fig. 3a), in particular type C1 as unveiled by the ITS2 analysis (Fig S1c).

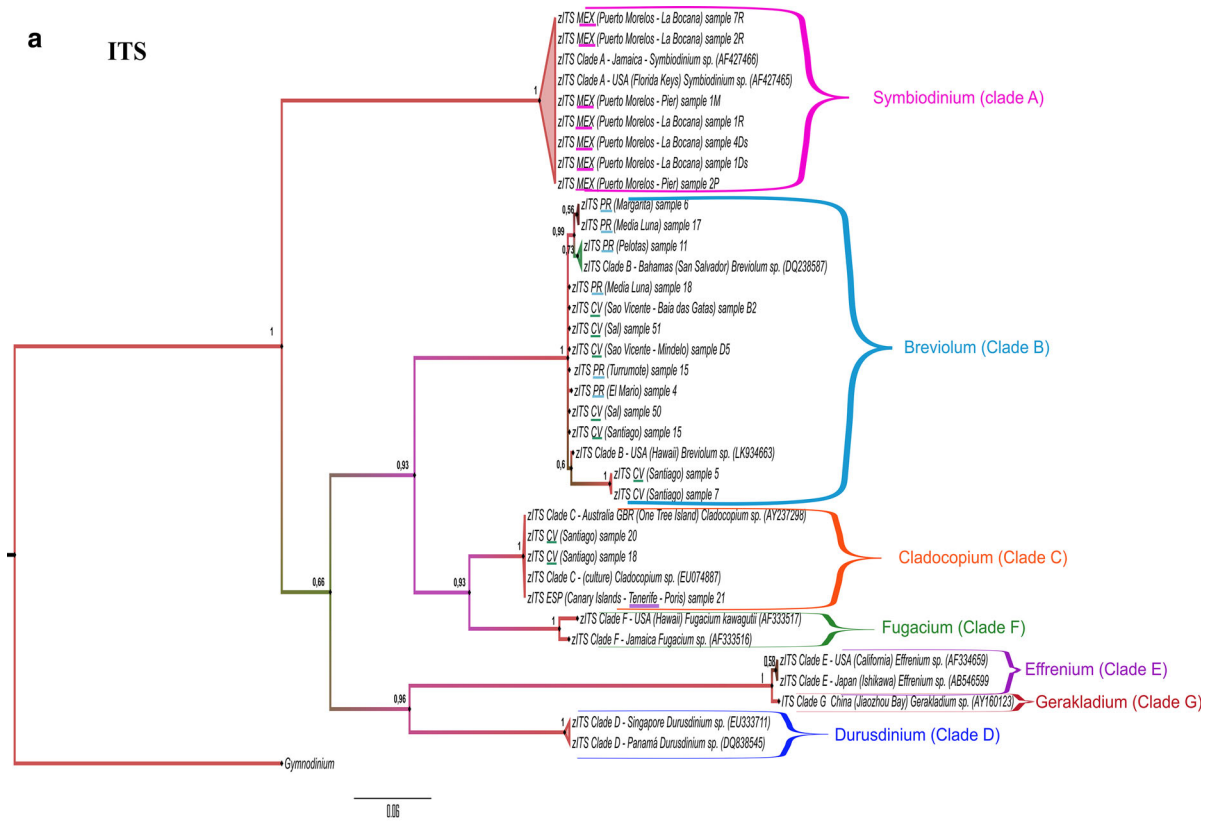
The 23S-rDNA (Fig. 3b) was able to separate two well-supported clades, grouping all samples from Mexico and Puerto Rico (Caribbean region), accounting for 41% of the samples, within *Symbiodinium* sp. (BPP = 0.91). The other group (BPP = 1) included all samples from the Cape Verde and Canary Islands, 59% of the samples, which fit with previously published sequences of *Breviolum* sp.

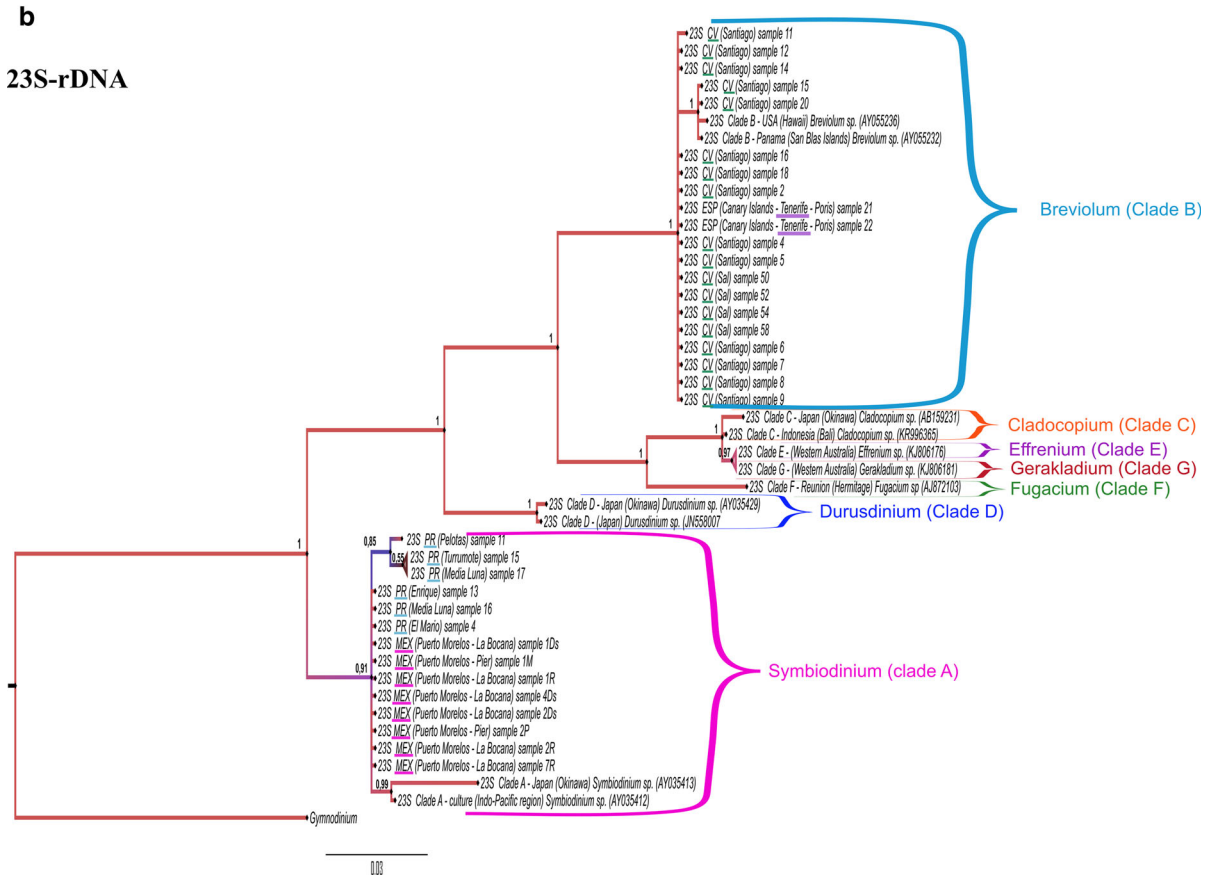
The LSUrDNA RFLP analyses indicated that samples from Mexico presented the same fragment pattern, showing two bands: one at 300 bp and another at 600 bp. All the Cape Verde Islands samples also presented the same band pattern among them (150 bp and 800 bp), indicating that all hosted the same symbiont type and different to those from Mexico. This outcome was congruent with the results of the phylogenetic trees. Regarding the two samples from Tenerife (Canary Islands), they presented the same band pattern as Cape Verde samples, but one of them also included an additional band. This fact may indicate the presence of more than one symbiont within the same host.

In summary, all the specimens from Mexico were found to contain *Symbiodinium* sp. for the two molecular markers, i.e. BPP $>$ 0.90 (Fig. 3a, b). Furthermore, we found that the Puerto Rico specimens were grouped within either *Symbiodinium* sp. and *Breviolum* sp., depending on the molecular region used, 23S-rDNA (BPP = 0.91) *versus* ITS (BPP = 1). Regarding the eastern Atlantic samples (Canary Islands and Cape Verde Islands), *Breviolum* sp. was the dominant genus when the phylogenetic tree was constructed with the 23S-rDNA marker (BPP = 1), while three sequences (one from Tenerife and two from Santiago) matched *Cladocopium* sp. according to the ITS-rDNA region (BPP = 1). In other words, *Cladocopium* sp. was only detected by the ITS region, in the eastern Atlantic specimens, from the recently established colonies of the Canary Islands, and in two of the nine samples from Cape Verde Islands (Fig. 3a, b). Meanwhile, the same samples matched with *Breviolum* sp. according to the 23S-rDNA region. Despite these differences, the congruence test value (Icong = 1.85) and its associated *p*-value of 4.5×10^{-5} indicated that the 23S-rDNA and the ITS trees were more congruent than expected by chance.

Differences between *Millepora alcicornis* and Symbiodiniaceae genetic analyses

The results extracted from the haplotype networks of the hydrocoral and the phylogenetic trees of its symbiont show different relationships among populations. While the analyses of the hydrocoral sequences confirm that samples from the Canary Islands are more related to the Caribbean than to those from the Cape Verde Islands, the


a
ITS
zITS MEX (Puerto Morelos - La Bocana) sample 7R
zITS MEX (Puerto Morelos - La Bocana) sample 2R
zITS Clade A - Jamaica - Symbiodinium sp. (AF427466)
zITS Clade A - USA (Florida Keys) Symbiodinium sp. (AF427465)
zITS MEX (Puerto Morelos - Pier) sample 1M
zITS MEX (Puerto Morelos - La Bocana) sample 1R
zITS MEX (Puerto Morelos - La Bocana) sample 4Ds
zITS MEX (Puerto Morelos - La Bocana) sample 1Ds
zITS MEX (Puerto Morelos - Pier) sample 2P
Symbiodinium (clade A)
zITS PR (Margarita) sample 6
zITS PR (Media Luna) sample 17
zITS PR (Pelotas) sample 11
zITS Clade B - Bahamas (San Salvador) Breviolum sp. (DQ238587)
zITS PR (Media Luna) sample 18
zITS CV (Sao Vicente - Baia das Gatas) sample B2
zITS CV (Sal) sample 51
zITS CV (Sao Vicente - Mindelo) sample D5
zITS PR (Turrumote) sample 15
zITS PR (El Mario) sample 4
zITS CV (Sal) sample 50
zITS CV (Santiago) sample 15
zITS Clade B - USA (Hawaii) Breviolum sp. (LK934663)
zITS CV (Santiago) sample 5
zITS CV (Santiago) sample 7
Breviolum (Clade B)
zITS Clade C - Australia GBR (One Tree Island) Cladocopium sp. (AY237299)
zITS CV (Santiago) sample 20
zITS CV (Santiago) sample 18
zITS Clade C - (culture) Cladocopium sp. (EU074887)
zITS ESP (Canary Islands - Tenerife - Poris) sample 21
Cladocopium (Clade C)
zITS Clade F - USA (Hawaii) Fugacium kawagutii (AF333517)
zITS Clade F - Jamaica Fugacium sp. (AF333516)
Fugacium (Clade F)
zITS Clade E - USA (California) Effrenium sp. (AF334659)
zITS Clade E - Japan (Ishikawa) Effrenium sp. (AB546599
ITS Clade G China (Jiaozhou Bay) Gerakladium sp. (AY160123)
Effrenium (Clade E)
Gerakladium (Clade G)
zITS Clade D - Singapore Durusdinium sp. (EU333711)
zITS Clade D - Panamá Durusdinium sp. (DQ838545)
Durusdinium (Clade D)
Gymnodinium
0.06


b
23S-rDNA
23S CV (Santiago) sample 11
23S CV (Santiago) sample 12
23S CV (Santiago) sample 14
23S CV (Santiago) sample 15
23S CV (Santiago) sample 20
23S Clade B - USA (Hawaii) Breviolum sp. (AY055236)
23S Clade B - Panama (San Blas Islands) Breviolum sp. (AY055232)
23S CV (Santiago) sample 16
23S CV (Santiago) sample 18
23S CV (Santiago) sample 2
23S ESP (Canary Islands - Tenerife - Poris) sample 21
23S ESP (Canary Islands - Tenerife - Poris) sample 22
23S CV (Santiago) sample 4
23S CV (Santiago) sample 5
23S CV (Sal) sample 50
23S CV (Sal) sample 52
23S CV (Sal) sample 54
23S CV (Sal) sample 58
23S CV (Santiago) sample 6
23S CV (Santiago) sample 7
23S CV (Santiago) sample 8
23S CV (Santiago) sample 9
Breviolum (Clade B)
23S Clade C - Japan (Okinawa) Cladocopium sp. (AB159231)
23S Clade C - Indonesia (Bali) Cladocopium sp. (KR996365)
Cladocopium (Clade C)
23S Clade E - (Western Australia) Effrenium sp. (KJ806176)
Effrenium (Clade E)
23S Clade G - (Western Australia) Gerakladium sp. (KJ806181)
Gerakladium (Clade G)
23S Clade F - Reunion (Hermitage) Fugacium sp (AJ872103)
Fugacium (Clade F)
23S Clade D - Japan (Okinawa) Durusdinium sp. (AY035429)
23S Clade D - (Japan) Durusdinium sp. (JN558007
Durusdinium (Clade D)
23S PR (Pelotas) sample 11
23S PR (Turrumote) sample 15
23S PR (Media Luna) sample 17
23S PR (Enrique) sample 13
23S PR (Media Luna) sample 16
23S PR (El Mario) sample 4
23S MEX (Puerto Morelos - La Bocana) sample 1Ds
23S MEX (Puerto Morelos - Pier) sample 1M
23S MEX (Puerto Morelos - La Bocana) sample 1R
23S MEX (Puerto Morelos - La Bocana) sample 4Ds
23S MEX (Puerto Morelos - La Bocana) sample 2Ds
23S MEX (Puerto Morelos - Pier) sample 2P
23S MEX (Puerto Morelos - La Bocana) sample 2R
23S MEX (Puerto Morelos - La Bocana) sample 7R
23S Clade A - Japan (Okinawa) Symbiodinium sp. (AY035413)
23S Clade A - culture (Indo-Pacific region) Symbiodinium sp. (AY035412)
Symbiodinium (clade A)
Gymnodinium
0.03

◀**Fig. 3** Phylogenetic analyses inferred from **a** 23S-rDNA and **b** ITS regions of *Symbiodinium* sp. hosted by *Millepora alcicornis* across the Atlantic: Mexico (Puerto Morelos), Puerto Rico, Cape Verde Islands (Sal, Santiago, Sao Vicente) and Canary Islands (Tenerife)

Symbiodiniaceae results indicate that the symbiont sequences from the Canary Island samples are more related to the Cape Verde Islands than the Caribbean. These results indicate that the origin of the hydrocoral, recently settled in the Canary Islands, and the origin of its symbionts are different.

Discussion

This is the first study focusing on the genetic relationships of both *M. alcicornis* and its symbionts within and across the Atlantic. Our results initially confirm the genetic variation of this hydrocoral and identify the Caribbean as the region of origin for the recently established *M. alcicornis* colonies from the Canary Islands (López et al. 2015; de Souza et al. 2017) and provide further evidence for two independent founder events occurring in the Cape Verde Islands, previously reported by López et al. (2015). Importantly, we found a genetic mismatch between the hydrocoral–Symbiodiniaceae associations, as the genetic relationships between both sides of the Atlantic varied between these two components. Hydrocoral haplotypes from the eastern Atlantic archipelagos (Canary Islands and Cape Verde Islands) were more related to the western Atlantic than they were between them, showing the same population structure previously found by López et al. (2015) and de Souza et al. (2017). However, sequences of Symbiodiniaceae from the two eastern Atlantic archipelagos were considerably more similar than to those from the Caribbean. According to our results, the origin of the symbionts from the Canary Islands colonies did not follow the same pattern as the hydrocoral, probably due to acquisition of the symbionts from the environment, or because changes in the internal symbiont abundances have taken place in colonies from the Canary Islands.

Millepora alcicornis has two free-living stages during sexual reproduction, a jellyfish and a fertilized planula larva to further disperse the species (Lewis 2006). However, the period of time they spend in the water column is short, impeding long-distance dispersion (Hickson 1899; Mayer 1910; de Weerdt and Glynn 1991; de Souza et al. 2017). Therefore, the success of *M. alcicornis* resides in asexual reproduction by small fragments, which are able to travel in ballast water, encrusted on the hull of ships, or on floating materials (López et al. 2015; de Souza et al. 2017). *Millepora alcicornis* arrived at the Canary Islands via the Gulf Stream (López et al. 2015), and we would expect that those colonies brought their symbionts with them, but our results suggest a shift in symbionts within the Canary Island population. *M. alcicornis* hosted different species within the Symbiodiniaceae across the Atlantic, without a congruent genetic pattern between the host and its symbiont. Specifically, eastern Atlantic Symbiodiniaceae populations seemed to be genetically related, in contrast to the pattern obtained for the hydrocoral. The topological congruence test between the chloroplast and the nuclear phylogenies of Symbiodiniaceae was significant, as previously reported by Santos et al. (2002), Baker (2003) and Takishita et al. (2003). This reinforces the idea of low divergence (Santos et al. 2002) and a parallel evolution of these two genes (Takishita et al. 2003). However, the results obtained from Symbiodiniaceae analyses differed according to the molecular marker used. It has been found that *Breviolum* sp. and *Cladocopium* sp. are closely related and difficult to differentiate based on rDNA markers (Wilcox 1998; Baker 2003). This could explain why some of the eastern Atlantic samples were related to *Cladocopium* sp., according to the ITS region, but not according to the 23S-rDNA. Similarly, Grajales and Sanchez (2016) also found difficulties in the identification of these two Symbiodiniaceae genera, even at the ITS2 resolution (see also LaJeunesse 2005). Multi-locus DNA analysis has been previously used to investigate the symbiont composition and variability within the host (e.g. Goulet and Coffroth 2003). The fact that the same coral samples showed different symbionts from different genera, according to the analysed genetic region, suggests that a heterogeneous pool of symbionts may exist within the same colony (see Fay and Weber 2012). Grajales and Sanchez (2016) found that *M. alcicornis* from the Caribbean hosted *Cladocopium* sp. and *Breviolum* sp. within the same colony. Similarly, our samples from the Canary and Cape Verde Islands showed *Cladocopium* sp. and *Breviolum* sp. in the same specimens. In addition, our specimens from Puerto Rico also showed a mixture of *Symbiodinium* sp. according to the 23S-rDNA marker, and *Breviolum* sp. according to the ITS-rDNA marker. In this sense, the results of RFLP of LSUrDNA analysis suggest the possibility of several symbionts in the same host, at least in one sample from Tenerife.

Symbiont acquisition by a coral can occur via vertical transmission, where symbionts are transferred to the offspring, or via horizontal transmission, where symbionts are taken up from the environment through phagocytosis (Hirose et al. 2001; Weis et al. 2001; Stat et al. 2006, 2008). In the case of *M. alcicornis*, the medusae released during sexual reproduction harbour symbionts (vertical transmission) (Mangan 1909). However, our results indicated that the symbionts from the Canary Islands were more related to those from the Cape Verde

Islands than to those from the Caribbean, contrary to the hydrocoral results. This suggests that horizontal transmission may dominate, as a mechanism to acquire symbionts from the environment. The presence of different types of symbionts in the waters of the Canary and the Cape Verde Islands, and in some of their marine organisms, could be the potential sources for *M. alcicornis* uptake. For example, some zoanthids from the Canary Islands harbour the genus *Cladocopium* (C. López, personal communication), while *Symbiodinium natans* is present in the waters around the Canary Islands as a free-living dinoflagellate (Hansen and Daugbjerg 2009). Additionally, the genera *Symbiodinium*, *Brelovium* and *Cladocopium* are also associated with some zoanthids from the Cape Verde Islands (Reimer et al. 2010a, 2017), and at least one stony coral (*Siderastrea radians*) also harbours the genus *Cladocopium* from the island of Sal (Monteiro et al. 2013).

An alternative explanation could be the existence of background symbionts of *Cladocopium* species that were not detected in the Caribbean samples, but that became more abundant, and therefore, detectable in the eastern Atlantic samples. This may occur because the abundance of the symbiont community within a single colony is not necessarily static. The colony can be flexible, changing their symbiont community by shifting existing background symbiont types, or by uptaking or expelling their symbionts depending on environmental conditions related to depth, light intensity or temperature (LaJeunesse 2002; Baker 2003; Iglesias-Prieto et al. 2004; Berkelmans and van Oppen 2006; Finney et al. 2010). According to Rowan and Knowlton (1995), *Symbiodinium* sp. and *Breviolum* sp. were normally associated with shallow depths in the Caribbean for *Orbicella* (= *Montastraea*) spp., whereas *Cladocopium* sp. was found at greater depths. Our results showed no particular trend of *M. alcicornis* symbionts with depth, similar to Grajales and Sanchez (2016). However, the latitudinal gradient and the varying oceanographic conditions between the populations in our study should be considered when inferring the appearance of genera. *Cladocopium* sp. and *Breviolum* sp. have been associated with high-latitude corals, which resist cold temperatures and extreme seasonal changes (Thornhill et al. 2008; Silverstein et al. 2011; Lien et al. 2012). These Symbiodiniaceae genera appeared in samples from the Canary Islands (28°N) and Cape Verde Islands (14 to 16°N), located in areas influenced by the African upwelling and the cold Canary Current, where temperatures tend to be lower than expected for their latitudes, ranging from 18 to 25 °C (Clemente et al. 2010; Faye et al. 2015). After settlement in the Canary Islands, likely facilitated by temperature increases (Brito et al. 2005; Clemente et al. 2010; López et al. 2015), coral growth was probably possible due to the acquisition or "shuffling" of these cold-tolerant symbiont types (detected as *Breviolum* sp. by the 23S-rDNA and *Cladocopium* sp. by the ITS region). *Symbiodinium* sp. seemed to show a preference for shallow and warm environments, since it appeared in Mexico and Puerto Rico samples, all located at depths < 7 m. The occurrence of *Symbiodinium* sp. in shallow waters coincides with LaJeunesse (2002) and Finney et al. (2010) and could be associated to its ability to protect itself from ultraviolet radiation (Banaszak et al. 2000; Reynolds et al. 2008). Although some of the samples from the Cape Verde Islands were also collected in the intertidal and shallow subtidal zones, no *Symbiodinium* sp. was found there. Despite the fact that the Cape Verde Archipelago was our southernmost eastern Atlantic study site, and that solar radiation is high at these latitudes, the mild temperature caused by the cold African upwelling and the Canary Current (Faye et al. 2015) may explain the absence of *Symbiodinium* sp. This fact also reinforces the idea of horizontal transmission or changes in background symbiont composition by *M. alcicornis* in the Cape Verde Islands.

There is some evidence that symbiont communities are changing due to climate change, so stress tolerance traits are selected by the holobiont (Baker et al. 2004; Parkinson and Baums 2014). These physiological traits vary between species of Symbiodiniaceae and also between strains (cultured isolates or subclades) within species (Díaz-Almeyda et al. 2017; Grégoire et al. 2017). For example, the dominant *Cladocopium* sp. was replaced by *Durusdinium* sp. in *Acropora millepora*, when seawater temperature increased in experimental conditions (Berkelmans and van Oppen 2006). *Durusdinium* sp. and some species of *Cladocopium* sp. (previously subclade C17) seem to be resistant to bleaching (Coffroth and Santos 2005), and *Symbiodinium* sp., *Durusdinium* sp. or *Effrenium* sp. usually appear in corals that have recovered from bleaching (Toller et al. 2001; Baker 2003; LaJeunesse et al. 2003). The aforementioned *Cladocopium* sp. (C1) showed tolerance to low temperatures in corals from Japan (Lien et al. 2012) and from South Korea (de Palmas et al. 2015), explaining its presence at high latitudes. Similarly, some species (previously from subclade B2) of the genus *Breviolum* resisted temperatures of 10 °C under laboratory conditions (Thornhill et al. 2008), and the subclade B18 appeared in corals from temperate south-western Australia (33°S) (Silverstein et al. 2011). Therefore, corals capable of changing their symbionts (through exogenous uptake or shuffling its internal symbiont abundance), depending on environmental conditions, can select the best genera to provide resilience to climate change (Baker et al. 2004; Berkelmans and van Oppen 2006). However, the ability of corals to exchange symbionts is reduced to a limited number of species (Iglesias-Prieto et al. 2004; Goulet

2006), and only a few coral genotypes will be able to cope with extreme climatic changes (Goulet 2006).

Our results indicate that *M. alcicornis* has the capacity to uptake symbionts from the environment ("horizontal transmission"), or to change the abundance of its internal symbionts types. Either of these mechanisms allows the species to acclimatize to the surrounding environmental conditions. Poleward coral expansions have been reported in the last decades due to climate change (Vargas-Ángel et al. 2003; Greenstein and Pandolfi 2008; Yamano et al. 2011). If sea water temperature keeps increasing according to the IPCC scenarios, the expansion of *M. alcicornis* to other subtropical or temperate regions could also occur. Therefore, the presence of this species in new regions, and the flexibility of the coral to adapt to the new conditions, would depend on the pool of available symbionts, whether inside the host or in the surrounding seawater. The collection and further DNA analyses of samples, taken at different seasons and over several years, would help to elucidate whether the hydrocoral has a rapid ability to change their symbionts under different conditions (Stat et al. 2006). Additionally, further research on the transcriptome of *M. alcicornis* (see Ortiz-González et al. 2017), as well as the extraction of information about its symbionts, would improve the scientific knowledge about the holobiont response to environmental changes.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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