



REPORT

# Biogeography of endosymbionts (Symbiodiniaceae) associated with zoantharian species (Hexacorallia: Anthozoa) from the Macaronesia and Cape Verde ecoregions

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Received: 11 March 2021 / Accepted: 13 April 2022 / Published online: 10 May 2022  
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**Abstract** Populations of some zoantharian species (Cnidaria: Anthozoa) have been able to proliferate in locations where abiotic conditions hinder scleractinian corals' survival. One of the contributing factors might be the advantageous host-symbiont associations that could lead holobionts to be more resilient to environmental variation, even in marginal-environments. However, few studies have addressed these Zoantharia–Symbiodiniaceae associations, especially little is known about their nature and distribution in the Atlantic Ocean. In this study, we use the large ribosomal subunit (LSU-rDNA) and Internal Transcribed Spacer 2 (ITS2) to examine the biodiversity and distribution of Symbiodiniaceae species within zoantharians that inhabit along the latitudinal gradient Madeira (40°N)—Cape Verde (16°N). Moreover, we determine the number of different endosymbionts genera inhabiting the same specimen, in order to estimate their ability to face alterations in the

environment. The results showed that Symbiodiniaceae diversity increases towards the tropics, with a total of six ITS2 types belonging to *Symbiodinium* and *Cladocopium*, the latter being the most frequent genus. Furthermore, we have found a possible undescribed species inhabiting *P. aff. clavata* collected at Madeira Island, the northernmost limit of brachycnemic zoantharian distributions in the East Atlantic. These results, combined with the literature reviewed, constitute the first mention of the genus *Symbiodinium* in a species host that is not *Zoanthus* spp and for the archipelago of the Canary Islands. An appendix summarizing Zoantharia–Symbiodiniaceae distributions around the Atlantic is included to facilitate future research on these holobiont associations.

**Keywords** Zooxanthellae · *Symbiodinium* · *Cladocopium* · Zoantharia · Atlantic Ocean · ITS2-rDNA

Topic Editor James Davis Reimer

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00338-022-02260-9>.

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## Introduction

Marine ecosystems worldwide are facing the consequences of climate change (Harley et al. 2006; Fabry et al. 2008). Ocean warming is the most noticeable effect as it modifies abundance and distribution patterns of marine species, depending on their thermal tolerance and ability to adapt (Harley et al. 2006; Heron et al. 2018). Biological responses to higher temperatures differ among organisms, and cnidarian species living in symbiosis with photosynthetic dinoflagellates are some of the most affected by thermal stress. Ocean warming combined with higher ultraviolet radiation (UV) causes what is known as bleaching events, which consist in the loss of zooxanthellae and/or their photosynthetic pigments from host coral tissues (Hughes et al. 2003; Berkelmans and Oppen 2006; Van Oppen and

Lough 2009). This loss of functional symbionts leaves the host without its main source of food, since zooxanthellae provide most of the metabolic energy used by the holobiont (Muscatine and Porter 1977; Trench 1987). Therefore, this symbiotic relationship entails an evolutionary advantage that has led to the formation of coral reef ecosystems in oligotrophic tropical waters worldwide (Trench 1979; Muscatine 1990; Loh et al 2001), where zooxanthellae (Family Symbiodinaceae) play a critical role in hermatypic coral survival (Trench 1979; Muscatine and Porter 1977; Rowan et al. 1997; Rowan 1998; Veron and Stafford-Smith 2000). Because of the current global warming, bleaching events are becoming more frequent and severe, which, combined with other anthropogenic disturbances, are leading to increased scleractinian coral mortalities (Reimer et al. 2007; Heron et al. 2016; Hughes et al. 2018a, b). As a consequence, new alternative communities are emerging as different organisms recolonize open spaces after a massive coral mortality event (Hughes 1994; McManus and Polsenberg 2004; Noström et al. 2009; Dudgeon et al. 2010; Cruz et al. 2015; Slattery et al. 2019; Reimer et al. 2021).

Soft corals, sponges and zoantharians have demonstrated to be more resistant and some species are being able to take advantage in sites where environmental conditions hinder scleractinian corals' survival (Noström et al. 2009; Inoue et al. 2013; Cruz et al. 2014; Cruz et al. 2015; Reimer et al. 2021). The emergence of these new non-calcareous communities is mainly related to their faster growth (Silva et al. 2015), and greater resistance to thermal and UV stresses (Yang et al. 2013). Disparate resilience capacities depend on both the physiological characteristics of the host (Grottoli et al. 2006; Baird et al. 2009) and the symbiont type (Berkelmans and Van Oppen 2006; Sampayo et al. 2008; LaJeunesse et al. 2010), which can differ even among zooxanthellae species within the same genus of Symbiodiniaceae (LaJeunesse et al. 2018). Symbiodiniaceae encompass at least up to ten genera (formerly known as clades) (LaJeunesse et al. 2018; Mathew et al. 2020; Yorifuji et al. 2021), composed of several ITS2 genotypes that differ in their physiological responses to abiotic factors such as temperature and irradiation ranges (Ulstrup and Van Oppen 2003; Sampayo et al. 2008; Hume et al. 2015). These differences to cope with changes in environmental conditions determine zooxanthellae distributions around the globe and can vary among host species inhabiting the same location (Loh et al. 2001; Toller et al. 2001; Tonk et al. 2013; LaJeunesse 2018). Host-zooxanthellae relationships are frequently specific and can vary even within the same host species but inhabiting different depths (Rowan and Knowlton, 1995; Baker et al. 1997; Kamezaki et al. 2012). In this sense, specimens living in high irradiance environments may host *Symbiodinium* spp., capable of producing mycosporine-like aminoacids (MAAs) against

UV (Banaszak et al. 2000; Silva Lima et al. 2020), while at deeper environments *Breviolum* or *Cladocopium* genera are more frequent (LaJeunesse 2002; Kamezaki et al. 2012).

Host species can also influence holobiont adaptations by using different mechanisms to prevent photodamage of the symbiont cells (Baird et al. 2009). For instance, anemones reduce the irradiation flux reaching the symbiont cells by covering themselves with detritus (Dykens and Shick 1984; LaJeunesse and Trench 2000). Another strategy used by several hermatypic corals, which has also been described in zoantharians (LaJeunesse 2002; Fujiwara et al. 2021), consists in hosting different symbiont types simultaneously. This mechanism may allow the holobiont to cope with stressful conditions as the symbiont community composition can under certain circumstances shift to favor a more tolerant symbiont type (Baker 2003; Ulstrup and Van Oppen 2003; Baker et al. 2004; Berkelmans and Van Oppen 2006). Some authors consider the bleaching processes as an adaptive response whereby hosts expel the less tolerant zooxanthellae and acquire more resistant types (Buddemeier and Fautin 1993; Baker 2001). These different symbiont shuffling strategies, acquiring more temperature-tolerant types, might enable holobionts survival in extreme conditions, which could be particularly interesting in the current context of climate change (Hume et al. 2015; Silverstein et al. 2014; Cuning et al. 2018).

Coral reefs are one of the most biodiverse and productive marine ecosystems worldwide, but also, as mentioned above, the most threatened by ocean warming (Moberg and Folke 1999; Wilkinson 2006; Heron et al. 2018). For these reasons, many studies have focused on analyzing the nature of symbiotic associations in hermatypic corals in order to understand their future survival trajectories (LaJeunesse et al. 2003; Littman et al. 2010; Mashini et al. 2015). However, few studies have addressed alternative host-zooxanthellae associations involving soft corals, sponge or zoantharians, although the number of studies is starting to increase (Reimer et al. 2010; López et al. 2019; Slattery et al. 2019; Fujiwara et al. 2021). Because some zoantharian species have been able to proliferate after large coral reef mortalities (Cruz et al. 2016; Wee et al. 2017; Reimer et al. 2021), the study of zoantharian-endosymbiont associations could be a key factor to understand the mechanisms that make them more resistant and to better understand the future of tropical reef communities. Furthermore, characterizing symbionts associated with zoantharians could facilitate the understanding of holobionts' ecology and biogeography, and their ability to adapt to different ecological conditions.

Previous studies have addressed the zooxanthellae inhabiting zoantharians from the East Atlantic (Reimer et al. 2010; López et al. 2019) by analyzing 23S and ITS-rDNA molecular markers, distinguishing only up to Symbiodiniaceae genera. However, it has been demonstrated

that the ability of coping with environmental conditions can vary among ITS2 types within the same Symbiodiniaceae genus (Fisher et al. 2012; Wang et al. 2012; Hume et al. 2015). For this reason, our aim was to identify the predominant symbionts associated with zoantharians in the Macaronesia and Cape Verde ecoregions, at approximately species level, using a more variable molecular marker (ITS2-rDNA). The studied archipelagos present different environmental features related to their latitudinal position, according to previous studies (Spalding et al. 2007; Freitas et al. 2019). Furthermore, we determined the number of different endosymbiont genera inhabiting the same host, as an approximation of their ability to flexibly associate with different symbionts. Our final goal was to establish the biogeographic pattern of the different zoantharian-endosymbiont associations from the East Atlantic and their relationship with the natural temperature gradient along the Madeira (32°N)—Cape Verde (16°N) latitudinal gradient.

## Materials and methods

### Molecular analysis

A total of 63 DNA samples from nine zoantharian species, collected in the Macaronesia and Cape Verde ecoregions were analyzed in this study. The samples included four specimens from Madeira, 37 specimens from the Canary Islands and 22 from Cape Verde, belonging to *Palythoa* ( $n=37$ ): *P. caribaeorum* ( $n=16$ ), *P. aff. clavata* ( $n=13$ ), *P. grandiflora* ( $n=6$ ), *P. grandis* ( $n=2$ ); *Zoanthus* ( $n=24$ ): *Z. pulchellus* ( $n=9$ ), *Z. aff. pulchellus* ( $n=9$ ), *Z. sociatus* ( $n=3$ ), *Z. solanderi* ( $n=3$ ); and *Isaurus* genera ( $n=2$ ): *I. tuberculatus* ( $n=2$ ). Specimens were given an identification code composed of the initials of the geographic location where they were collected (MA Madeira, CN Canary Islands and CV Cape Verde), followed by the sample number. Sample data, including collection depths, are summarized in Online Resource 1.

### DNA extraction, PCR amplification and RFLP analyses

In all cases, genomic DNA was extracted from 50 mg of zoantharian tissue following the phenol/chloroform procedure (Sambrook et al. 1989).

Two nuclear regions were amplified in order to determine the number and type of possible endosymbionts inhabiting each sample, the nuclear large ribosomal subunit (LSU-rDNA) and the Internal Transcribed Spacer 2 (ITS2-rDNA). For the LSU-rDNA, 63 samples were amplified with the primers LSU-F (5'-CCCGCTGAATTTAAGCAT ATAAGT AAGCGG-3') and LSU-R (5'-GTTAGACTCCTTGGTCCG TGTTTCAAGA-3') designed by Zardoya et al. (1995). The

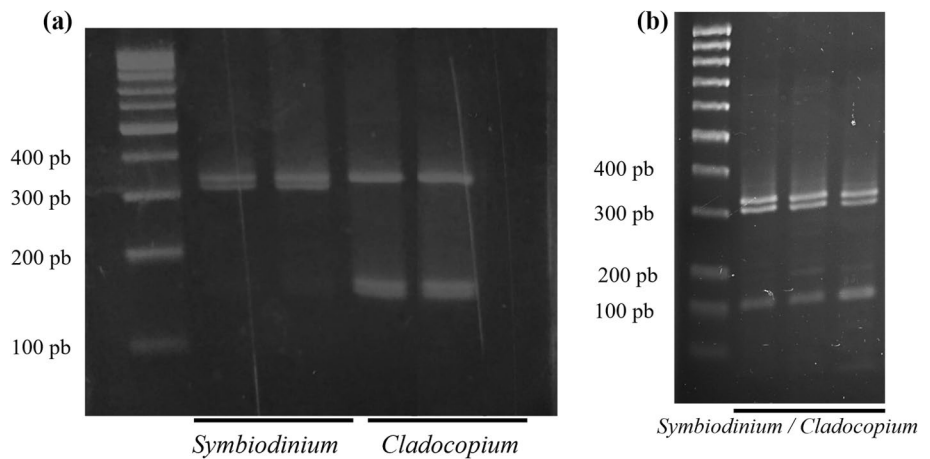
ITS2-rDNA region was amplified to identify the numerically dominant symbiont ITS2 type. For this fragment, ITS2-F (5'-GAATTGCAGAACTCCGTGAACC-3') and ITS2-R (5'-TCACTTGTCTGACTTCATGC-3') primers were designed based on the highly conserved flanking rDNA 5'8S and 28S sequences (present study).

PCR amplifications were performed in a total volume of 20  $\mu$ l, containing 1X BioTaq buffer (BioLine GmbH, Germany), 3 mM of  $MgCl_2$ , 150  $\mu$ M of dNTPs, 0.5 pmol  $\mu$ l<sup>-1</sup> of each primer, 0.5  $\mu$ g of bovine serum albumin (BSA), 1 U of BioTaq DNA polymerase (BioLine GmbH, Germany) and 20 ng of genomic DNA. PCR cycle conditions consisted of an initial step at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 10 s, annealing at 67 °C for LSU-rDNA and at 58 °C for ITS2-rDNA for 20 s, extension at 72 °C for 30 s and a final extension at 72 °C for 10 min. The amplifications were carried out in a BioRad T100 thermocycler (BioRad, Hercules, CA, USA). Aliquots of reaction products were electrophoresed on 1.7% agarose gel to verify DNA amplifications.

The genus level diversity of endosymbionts hosted by all zoantharian specimens was determined using restriction fragment length polymorphism (RFLP) analyses of LSU-rDNA. This method can resolve the presence of multiple genera in a single sample, though it cannot resolve the number of species present within each genus. The restriction patterns expected, from digestions with Taq I enzyme, were previously performed in silico in the Webcutter web server (<http://www.firstmarket.com/cutter/cut2.html>) (Heiman 1997). The LSU-rDNA sequences analyzed belonged to *Symbiodinium* (AB704058), *Breviolum* (LK934670) and *Cladocopium* (KR996308), which are the three genera currently known to inhabit zoantharian species from the East Atlantic Ocean. Generated band patterns were characteristic of each genus (Online Resource 2). *Symbiodinium* (former clade A) was easily identified due to its double band of 323 and 304 bp, whereas *Cladocopium* (former C clade) presented a double band of 155 and 149 bp and maintained the 323 bp band. Finally, *Breviolum* (former clade B) presented four well separated bands of 323, 149, 98 and 57 bp. Besides, the three genera exhibited a minor band of 35 bp.

Reaction products of LSU-rDNA were digested with FastDigest Taq I restriction enzyme (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Digested products were separated by electrophoresis in 2.2% agarose gels at 12 V  $cm^{-1}$  for 1 h. Gels were stained with ethidium bromide solution for 15 min, washed in distilled water for 10 min and visualized on a UV transilluminator. Additionally, two LSU-rDNA samples were sequenced in order to check the amplified region.

**Fig. 1** **A** Taq I digest of LSU-rDNA of *Symbiodinium* (Lanes 2 and 3) and *Cladocopium* (Lanes 4 and 5) genera in four of the samples analyzed. **B** *Symbiodinium-Cladocopium* mixed pattern in the CV12, CV13 and CN201 samples. Lane 1, 100 bp DNA ladder marker and Lane 6, negative control



On the other hand, ITS2-rDNA amplified fragments were enzymatically purified with Illustra ExoProStar kit (Ge Healthcare Life Science, PA, USA) according to the manufacturer's instructions, and sequenced, using Sanger method, at the Genomic Service (SEGAI) of the University of La Laguna. This method can resolve the numerically dominant endosymbiont type, but it cannot resolve if additional types within the same genus are present. In addition, the sequences were used to establish the phylogenetic relationships among them and to accurately identify the endosymbiont type or closest relative using the Basic Local Alignment Search Tool (BLAST) in GenBank.

### Phylogenetic analysis

The nucleotide sequences for the ITS2-rDNA were edited and assembled using MEGA7 software (Molecular Evolutionary Genetics Analysis version 7.0) (Kumar et al. 2016). Sequence alignment was performed using CLUSTAL W (Thompson et al. 1994) as implemented in the same software. A total of 74 sequences were analyzed and their corresponding accession number can be found in Online Resource 3. Of these sequences, 57 were obtained in this study, 11 from Reimer et al. (2010) and the remaining six are reference sequences from NCBI. The best-fitting model of nucleotide substitution for the data set was selected in jModelTest 2 (Darriba et al. 2012) according to the Bayesian Information Criterion (Schwarz 1978). Phylogenetic trees were inferred by maximum likelihood (ML) and Bayesian inference (BI), using the previously determined model of nucleotide evolution JC (Jukes-Cantor, 1969) and F81 (Felsenstein, 1981) for *Symbiodinium* and *Cladocopium* sequences, respectively. All phylogenetic analyses were performed through the CIPRES Science Gateway V 3.3 (Miller et al. 2010). ML analyses were conducted using RAxML 7.2.8 software (Stamatakis 2014) with 1000 replicates of bootstrap (BS). For BI, we used MrBayes

v.3.2.1 software (Ronquist et al. 2012). In this case, two independent runs were performed with default prior values, running  $10^7$  generations with sampling frequency every 100 generations. Convergence of all parameters in the two independent runs was assessed using Tracer 1.5 software (Rambaut and Drummond, 2007). After removing 25% of the resulting trees, the remaining were used to obtain the topology and parameters of the consensus tree and only those nodes with Bayesian posterior probabilities (BPP) higher than 0.95 were considered significant (Huelsenbeck et al. 2001). Finally, trees were visualized and edited with Figtree v1.4.0 (Rambaut 2009).

### Results

The resulting LSU-rDNA Taq I digestion patterns (Fig. 1) showed that all the analyzed samples harbored zooxanthellae belonging to the genera *Symbiodinium* (former clade A) or *Cladocopium* (former clade C) (Fig. 1), with the latter being the most frequent genus. *Cladocopium* was present in 51 of the 63 samples analyzed, which included all *Palythoa* specimens and sample locations. Nine samples contained *Symbiodinium* that was associated mainly to specimens of *Zoanthus* spp. collected at the intertidal zone and shallow waters (< 1 m depth) of Cape Verde archipelago (Online Resource 1), except for one sample of *Palythoa* aff. *clavata* (CN201) from the Canary Islands.

The remaining samples ( $n=3$ ) (CN201, CV12 and CV13) presented a mixed pattern indicating the presence of two genera (*Symbiodinium* and *Cladocopium*) both present in the same host (Fig. 1).

Neither of the samples showed the smallest fragment (35 bp) predicted for both genera (Online Resource 2). This may be due to the running time of electrophoresis applied in order to accurately identify the two bands of similar weight that distinguish each genus. Consequently, and due to its low



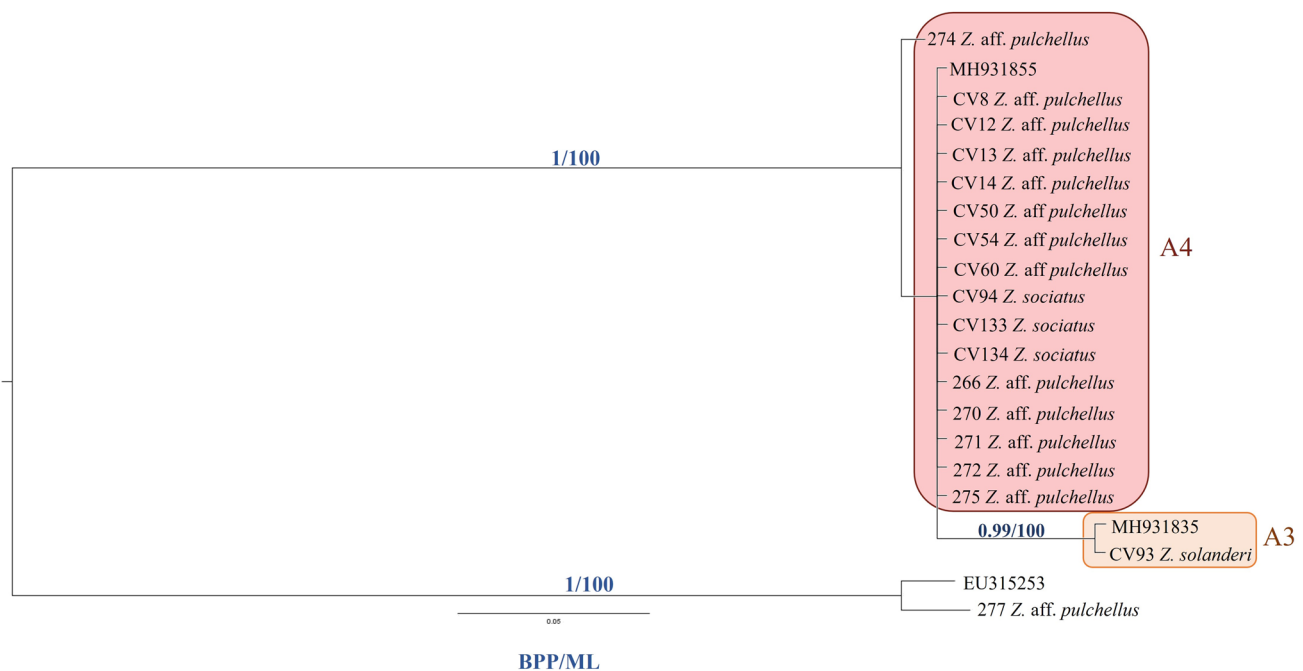
molecular weight, the fragment would have leaked out of the electrophoresis gel.

Analyses of the ITS2-rDNA region were carried out in an attempt to uncover more variation not detected with LSU-rDNA restriction analysis. Only four samples could not be successfully amplified. ITS2-rDNA sequences displayed clean and free of ambiguity electropherograms, except for two samples (CN201 and CN206) which presented double peaks within the sequence. We interpreted these results as intragenomic variation (Wee et al. 2020), or the presence of two different endosymbionts in the sample. Among them, 55 were successfully identified at ITS2 type or species level, while two (MA137 and MA138) could be only identified at genus level (Online Resource 1). New sequences for the ITS2-rDNA region obtained in the present study were deposited in GenBank (Accession Numbers MW843375-MW843396 and OL377962-OL377997).

Phylogenetic trees of *Symbiodinium* and *Cladocopium* were created separately, due to the large divergence between sequences from different genera (Figs. 2 and 3). In the case of *Symbiodinium*, the phylogenetic trees using BI and ML approaches recovered the same topology, showing two well-supported clades (Fig. 2, 0.99 BPP/100% BS). Specimens CV8, CV12, CV13, CV14, CV60, CV50, and CV54 from *Zoanthus* aff. *pulchellus*, and CV94, CV133, CV134 from *Z. sociatus*, were identical to sequences belonging to ITS2 type A4 (MH931855) and grouped with sequences of *Z. aff. pulchellus* obtained by Reimer et al.

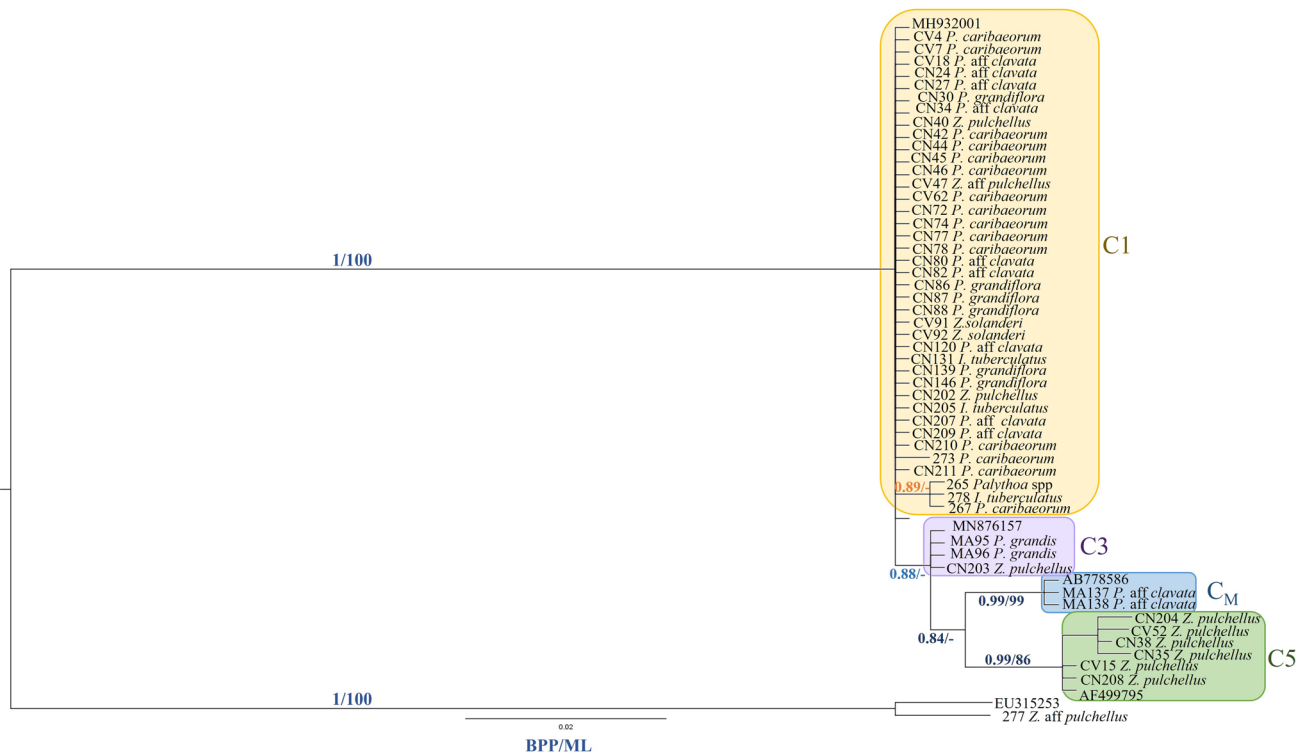
(2010), in samples collected in Cape Verde (Fig. 2). The other well-supported clade (0.99 BPP/100% BS) grouped a specimen of *Z. solanderi* collected at Maio Island (CV93), with the sequence MH931835 taken as reference of the A3 ITS2 type (Fig. 2). All the *Symbiodinium* ITS2 types identified and related in the phylogenetic analysis were associated with *Zoanthus* spp. and collected from the intertidal and subtidal shallow zones (< 1 m deep) of Cape Verde, the lowest latitudinal sampled archipelago (Online Resource 1; Fig. 2).

Regarding the *Cladocopium* genus, the BI and ML phylogenetic trees also displayed the same topology, showing four clusters (Fig. 3). The majority of the species analyzed in the Canary Islands and eleven from Cape Verde, showed a close relationship with the sequence MH932001, representative of the C1 ITS2 type (Fig. 3 and Online Resource 1). Two samples of *P. grandis* collected at Madeira (MA95 and MA96) and one sample of *Z. pulchellus* from the Canary Islands (CN203) (Fig. 3), grouped with the reference C3 sequence (MN876157) obtained from GenBank (0.88 BPP/- % BS). Another ITS2 type was constituted by four samples of *Z. pulchellus* from the Canary Islands (CN35, CN38, CN204 and CN208) and two from Cape Verde (CV52 and CV15) in a close relationship with the reference sequence AF499795, representative of C5 ITS2 type (0.99 BPP/86- % BS) (Fig. 3). Finally, sequences of *P. aff. clavata* collected at Madeira Island (MA137 and MA138) were identical to a *Cladocopium* specimen obtained from



**Fig. 2** Phylogenetic tree of *Symbiodinium* ITS2-rDNA gene obtained by Bayesian Inference (BI). Numbers above branches represent Bayesian posterior probabilities (BPP) and maximum likelihood

bootstraps (BS), respectively. ID sequences from this study and GenBank Accession Numbers from previous studies are displayed in the analysis



**Fig. 3** Phylogenetic tree of *Cladocopium* ITS2-rDNA gene obtained by Bayesian Inference (BI). Numbers above branches represent Bayesian posterior probabilities and maximum likelihood bootstraps,

respectively. ID sequences from this study and GenBank Accession Numbers from previous studies are displayed in the analysis

a bivalve (*Corculum cardissa*) (AB294624) (Fig. 3). This sequence, which is closely related to ITS2 type C5, may be a new, undescribed ITS2 type (designated here as C<sub>M</sub>) (0.99 BPP/99% BS). The *Cladocopium* ITS2 phylogenetic tree includes specimens collected from the three locations of study and at various depths, but mainly associated with *Palythoa* spp. hosts (Online Resource 1; Fig. 3).

## Discussion

A total of six types of endosymbionts were identified, five of them already described in the published literature of zoantharians for the Atlantic Ocean (Online Resource 4). Despite the wide range of locations (Madeira (32°N)—Cape Verde (16°N)) and host species examined, *Cladocopium* was the dominant genus although some *Symbiodinium* spp. were also present mainly closer to the equator.

Prior investigations have demonstrated the efficiency of using RFLPs analysis of LSU-rDNA band patterns to determine zooxanthellae genera in hard corals and anemones (LaJeunesse and Trench 2000; Baker, 2001; Loh et al. 2001). This analysis even allows to detect several genera of symbionts within the same host (LaJeunesse and Trench 2000; Teschima et al. 2019). The results of our RFLP

analyses showed that most zoantharian specimens (60/63) hosted either *Symbiodinium* sp. or *Cladocopium* sp., except for three samples (CN201, CV12 and CV13) which hosted both genera simultaneously. However, due to the fact that even zooxanthellae belonging to the same genus differ on their sensitivity to abiotic factors (Reimer et al. 2006; Sampayo et al. 2008; Fisher et al. 2012; LaJeunesse et al. 2018), and the fact that RFLP analyses cannot discard the presence of more than one symbiont species, subsequent analyses could be applied to determine their inner symbiont composition. In fact, five studies using ITS2-rDNA fingerprints (DGGE) have described the presence of several endosymbiont types co-inhabiting zoantharians species, three of them associated with *Zoanthus* spp. and the other two to *P. caribaeorum* (e.g., La Jeunesse, 2002; Costa et al. 2013; Finney et al. 2010). These exceptions suggest that the inner abundance of the symbiont community might be modified in zoantharians as it has been previously described for hermatypic corals and sea anemones (Baker et al. 2004). However, recent evidence suggests symbiont community modification via shuffling or switching may be unlikely to occur in some zoantharians under environmental stress (Fujiwara et al. 2021). Future studies could elucidate the symbiont exchange capacity of other zoantharian species and

the inner dominance and abundance of certain ITS2 types under climate stress conditions.

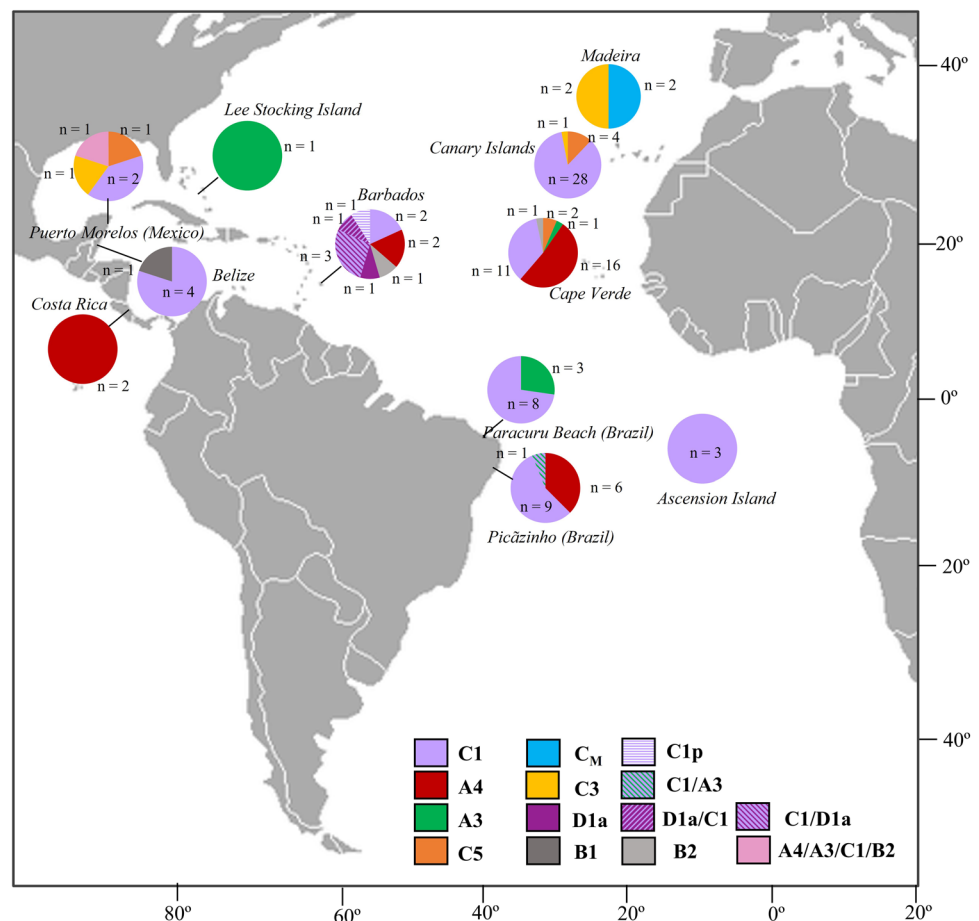
In recent years, the ITS2-rDNA region has been broadly used as a genetic marker to identify symbionts at a species level due to its high variability (LaJeunesse 2001). However, to accurately characterize the inner holobiont community using this molecular marker, genetic sequence methods such as NGS are needed (Hume et al. 2019). Therefore, in this study the ITS-rDNA analyses allowed us to determine the numerically dominant zooxanthellae genotype inhabiting each zoantharian specimen.

In order to compare our biogeographic results with previous research, we performed an overall revision of the available studies that address zoantharian symbiont identification from the Atlantic Ocean throughout ITS-rDNA (LaJeunesse 2002; Finney et al. 2010; Reimer et al. 2010, 2014; Costa et al. 2013; Rabelo et al. 2014; López et al. 2019) (Fig. 4 and Online Resource 4). Our phylogenetic results of ITS2-rDNA showed that four ITS2 genotypes of *Cladocopium* and two of *Symbiodinium* are the numerically dominant endosymbionts inhabiting zoantharians along the latitudinal gradient Madeira (32°N)—Cape Verde (16°N) (Figs. 2 and 3). Although our study area covered almost

2000 km along the East-Atlantic and included 9 zoantharian species from three different genera (Figs. 2 and 3; Online Resource 1), *Cladocopium* was the dominant genus (51/63). *Cladocopium* has been described as the most species-rich, ecologically abundant and broadly distributed genus within Symbiodiniaceae (LaJeunesse et al. 2018) and, in fact, four of the six ITS2 types found belonged to *Cladocopium* (C1, C5, C3 and CM) ( $n = 46/57$ ), and C1 was the most frequent ( $n = 35/46$ ) (Figs. 3 and 4). Specifically, C1 is known to inhabit a wide range of hosts, including zoantharians (LaJeunesse 2002; Thornhill et al. 2013; Teschima et al. 2019), and has been described as a broadly distributed variant (Fig. 4). This *Cladocopium* ITS2 type has been found in eight of the eleven locations revised (Fig. 4), associated with a wide variety of zoantharian host species, including *Palythoa grandis*, *P. caribaeorum*, *P. aff. clavata*, *P. grandiflora*, *Zoanthus pulchellus*, *Z. aff. pulchellus*, *Z. solanderi*, *Z. sociatus*, *Protopalythoa variabilis*, and *Isaurus tuberculatus*.

Most of the *Palythoa* specimens analyzed in this study hosted C1 as the predominant symbiont, even the ones collected at the intertidal zone of the tropical region of Cape Verde. This archipelago constitutes a well-differentiated

**Fig. 4** Review of the biogeographic distribution of Symbiodiniaceae ITS2 types associated with zoantharians from the Atlantic. The relative abundance of zooxanthellae ITS2 types and their distribution around the Atlantic are represented by pie charts. Sampling sites are indicated on cursive and the information about host species, number of colonies, genetic markers and analyses performed can be found on Online Resource 4 and the corresponding papers (LaJeunesse 2002; Finney et al. 2010; Reimer et al. 2010, 2014; Costa et al. 2013; Rabelo et al. 2014; López et al. 2019)



ecoregion characterized by higher irradiance and SST levels, compared to the rest of the archipelagos studied (Madeira and Canary Islands) (Spalding et al. 2007; Brito 2010; Freitas et al. 2019; López et al. 2019), The ability of *Palythoa* spp to incorporate sand and detritus into their mesoglea (Haywick and Mueller 1997; Reimer et al. 2010), might act as protective layer against UV light in Cape Verde, as it has been shown in sea anemones, which regulate the light flux that reaches their endosymbionts by covering themselves with debris (LaJeunesse and Trench 2000). On the other hand, it has been suggested that the absence of photoprotectants (MAAs) in *Cladocopium* (Shoguchi et al. 2020) could be supplied if the metabolic pathway existed in *Palythoa* hosts (Silva Lima et al. 2020). Furthermore, in most of the geographic locations of the Atlantic Ocean reviewed in the literature, *Palythoa* spp. specimens were always associated with the C1 ITS2 type (Online Resource 4), except for the colonies located at Barbados and Florida that presented, either alone or simultaneously, members of the genus *Durusdinium* (D1a; C1/D1a) (Kemp et al. 2006; Finney et al. 2010) some of which may be particularly resistant to bleaching events (LaJeunesse et al. 2018). Regarding the presence of this genus in *Palythoa caribaeorum*, the colonies that hosted D1 were under high environmental stress due to elevated sea temperature levels, leading to mass bleaching events in Barbados (Finney et al. 2010; Kemp et al. 2006). These observations reinforce our idea that *Palythoa* spp. constitute resistant zoantharians that might present a species-specific association among C1 and certain lineages (Thornhill et al. 2013), adapted to higher irradiance rates and temperatures.

Among the remaining five zooxanthellae ITS2 types identified, four of them were commonly found inhabiting zoantharians from the Atlantic Ocean. (C3, C5, A3 and A4) (Fig. 4). However, the ITS2 type inhabiting two *Palythoa* aff. *clavata* specimens from Madeira (CM) (MA137 and MA138), had not been recorded in zoantharians yet. The ITS2-rDNA sequence of this undescribed symbiont type matched with a sequence of *Cladocopium* inhabiting the bivalve *Corculum cardissa* (Cardiidae; Bivalvia) from Okinawa Island, Japan (accession number AB294624). Genetic and morphological evidences will determine this possible new type of *Cladocopium* ( $C_M$ ) in zoantharians which inhabit their northernmost limit of distribution (López et al. 2019).

Regarding the *Cladocopium* C3, it has also been described as host generalist and predominantly occurring in deeper environments than C1 (Finney et al. 2010; Thornhill et al. 2013). Despite the fact that in the phylogenetic analyses of our study the C3 clade was not well supported (0.88 BPP/—% BS), all sequences identified as C3 were associated with specimens inhabiting deeper environments than C1. In this sense, this ITS2 type has only been found associated

with one *Zoanthus pulchellus* of the Canary Islands (CN203) at 10 m depth and three specimens of *Palythoa grandis*, two samples from Madeira collected at 14 m depth (MA95 and MA96, this study) (Online Resource 1) and one at 12 m depth in Puerto Morelos (Mexico) (LaJeunesse, 2002) (Fig. 4). However, since many *Cladocopium* lineages share an ancestral state (Thornhill et al. 2013), analyses of additional molecular markers would be necessary to confirm the belonging to C1/C3 ITS2 types.

Finally, the five samples identified in this study as C5 ITS2 type, were associated with *Z. pulchellus* hosts from Cape Verde (CV15 and CV52, at the subtidal zone), and from the Canary Islands (CN35, CN38 and CN208 from the intertidal zone, and CN204 at 10 m). The C5 constitutes an uncommon symbiont that had only been described for zoantharians in an unidentified *Zoanthus* sp. collected at 15 m in Puerto Morelos (Mexico) (LaJeunesse 2002) (Fig. 4).

Members of the family Zoanthidae (*Zoanthus* and *Isaurus*) are characterized by the lack of particles in their mesoglea, leaving the endosymbionts more exposed to UV light, contrary to what happens in Sphenopidae (*Palythoa* and *Sphenopus*). For this reason, it is expected that symbiont distributions within Zoanthidae species are strongly correlated to the surrounding environment, and, in fact, the specimens of *Zoanthus* from the Canary Islands hosted *Cladocopium* spp., while in most of the *Zoanthus* specimens collected at the tropical region of Cape Verde *Symbiodinium* spp. were the dominant types (Figs. 2 and 3; Online Resource 1). The species of the genus *Symbiodinium* (former clade A) have been described as highly UV and thermal resistant (Banaszak et al. 2000, 2006; Reimer et al. 2006), being capable of producing MAAs (Silva Lima et al. 2020; Shoguchi et al. 2020). Until now, *Symbiodinium* had been exclusively described inhabiting *Zoanthus* spp. (LaJeunesse, 2002; Finney et al. 2010; Reimer et al. 2010; Costa et al. 2013; Rabelo et al. 2014; Fujiwara et al. 2021), probably due to the lack of capacity of the host to produce MAAs (Silva Lima et al. 2020). However, our results of the LSU-rDNA RFLPs analysis indicated the presence of *Symbiodinium* in a specimen of *P. aff. clavata* (CN201). This finding would constitute the first record of *Symbiodinium* spp. in the Atlantic Ocean associated with a zoantharian host species not being *Zoanthus* spp. and the first record of this genus in the Canary Islands (López et al. 2019; Rodriguez et al. 2019).

The A3 has been previously described associated with *Zoanthus sociatus* (LaJeunesse, 2002; Rabelo et al. 2014), and cohabiting with ITS2 types A4, C1, and/or B1 (*Breviolum* spp.) (LaJeunesse 2002; Costa et al. 2013) (Fig. 4; Online Resource 4). Regarding A4, it has also been detected on *Z. sociatus* (LaJeunesse, 2002; Reimer et al. 2010; Costa et al. 2013) and on *Z. pulchellus* (Finney et al.



2010) (Fig. 4). ITS2 types A3 and A4 are always found in shallow zones (Online Resource 1 and 3) and usually cohabiting with other ITS2 types, which may provide a greater resilience to the holobiont. In this regard, two samples of *Z. aff pulchellus* (CV12 and CV13), collected at the intertidal zone of Cape Verde, displayed the predominant ITS2 type A4 together with various undetermined types of *Cladocopium* sp. Despite the greater vulnerability of *Zoanthus* spp. against extreme environmental conditions, the results of this study reveal that two specimens of *Zoanthus solanderi* (CV91 and CV92) and one of *Z. aff pulchellus* (CV47), from shallow areas of Cape Verde were associated with *Cladocopium* C1. It has been noted that intertidal colonies of *Z. sansibaricus* can host *Symbiodinium* A1z and/or *Cladocopium* C1z-intertidal depending on whether the colonies were directly exposed to light or sheltered inside shaded fissures (Kamezaki et al. 2012; Fujiwara et al. 2021). These differences in symbiont types due to microhabitat variability might explain why *Zoanthus* colonies showed distinct endosymbionts genotypes, despite being collected at the same location in Cape Verde.

The results of this study emphasize how environmental conditions such as temperature and irradiance may shape the ecological distribution of Symbiodiniaceae that associate with zoantharians in the latitudinal gradient Madeira (40°N)—Cape Verde (16°N). In most cases, the predominant symbiont inhabiting each holobiont seemed to be directly related to the local environmental condition, collection depth, and characteristics of the host genus. Understanding these different types of relationships among host-Symbiodiniaceae associations and their relation to the environmental conditions is becoming an increasingly necessary issue under the current context of climate change and human impact over marine ecosystems. The environmental pressure of increased ocean temperature and ultraviolet radiation is favoring those holobionts which are most resistant, that is, organisms with an advantageous combination of host species and symbiont type (Berkelmans and Van Oppen 2006; Swain et al. 2018). The results of the RFLP analyses of LSU-rDNA fragment, combined with the ITS2-rDNA marker, demonstrated that the majority of the holobionts studied were formed by a zoantharian species associated with only one zooxanthellae genus, belonging either to *Cladocopium* or *Symbiodinium* (bearing in mind that this method cannot resolve the presence of multiple species within the same genus, as has been observed in zoantharians using other techniques). Furthermore, previous studies evaluating symbiont associations in corals from the Canary Islands (López et al. 2019; Rodríguez et al. 2019) only detected the presence of *Cladocopium* in several hosts. Therefore, this study constitutes the first mention of *Symbiodinium* in corals of the Canary Islands. Future research could elucidate whether this observation constitutes

an adaptation promoted by the increase in ocean temperature experienced in the archipelago over the last decades (Batista et al. 2019; López 2019).

**Acknowledgements** The research was supported by the Canarian Agency for Research, Innovation and Information Society of the Ministry of Economy, Industry, Trade and Knowledge and by Programa Mecenazgo Alumni of the University of La Laguna (2019–2020).

**Funding** Open Access funding provided thanks to the CRUE-CSIC agreement with Springer Nature.

#### Declarations

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

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