



Diversity of zoantharian species and their symbionts from the Macaronesian and Cape Verde ecoregions demonstrates their widespread distribution in the Atlantic Ocean

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Abstract Zooxanthellate zoantharians (Cnidaria: Anthozoa) are commonly found in tropical and subtropical marine regions around the world. However, due to the low genetic variability of commonly used DNA markers combined with high levels of intraspecific morphological variation, misidentifications and species synonyms are commonly found in the literature. In this study, zoantharians from the suborder Brachycnemina collected in the Macaronesia and Cape Verde ecoregions were studied combining morphological, molecular and ecological data, in order to comprehensively assess the species diversity of the region. Moreover, molecular analyses of endosymbiotic Symbiodiniaceae zooxanthellae were also performed to provide more information on each holobiont. Our integrative results demonstrate that Brachycnemina species diversity

increases as seawater temperature rises toward the tropics with a total of nine species recorded: one from waters around northern Madeira, five in the Canary Islands and seven in the southernmost Cape Verde Archipelago. All species were seen to host either Symbiodiniaceae of the genera *Symbiodinium* (former *Symbiodinium* ‘Clade A’) or *Cladocopium* (former *Symbiodinium* ‘Clade C’). Moreover, this study records for the first time the presence of *Palythoa grandis*, *P. aff. clavata*, *P. grandiflora*, an unknown *Zoanthus* species and *Z. pulchellus* in the East Atlantic Ocean. These results show no endemic zooxanthellate zoantharians in the East Atlantic, with all species shared with the West Atlantic.

Keywords Macaronesia · Molecular analyses · Morphological analyses · Zoantharian distribution · *Symbiodinium* · *Cladocopium*

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Introduction

Macaronesia is the name given to a group of five archipelagos in the east Atlantic Ocean; from north to south composed of the Azores, Madeira, the Selvagens Islands, the Canary Islands and Cape Verde (Fernández-Palacios et al. 2011). Although these islands share many characteristics, the more tropical climate and biota of Cape Verde place this archipelago into its own ecoregion (Spalding et al. 2007). The Macaronesian biogeographic region, that includes the Azores, Madeira, Selvagens Islands and the Canary Islands, is within the Lusitanian province of the Temperate Northern Atlantic realm, while Cape Verde is an ecoregion within the West African Transition province of the Tropical Atlantic realm (Spalding et al. 2007). In this sense, Macaronesia is a collection of 30 oceanic islands that differ in latitude, altitude, area and distance from the

Africa continent (Fernández-Palacios et al. 2011), and the group is located in the western branch of the Gulf Stream, the Canary Current (Barton et al. 1998). The biodiversity and unique marine fauna of the Macaronesian ecoregion, included within the Mediterranean biodiversity hot spot, have long been noted as one of the most important areas for conservation worldwide (Myers et al. 2000). Additionally, the Cape Verde Islands are often regarded as a ‘laboratory of evolution’ due to their high levels of endemism and as an important hot spot of tropical biodiversity (Roberts et al. 2002; Peters et al. 2016).

The total diversity of species of most marine taxa from the eastern Atlantic Islands is far from being known. Such is the case of the Zoantharia, an order of hexacorals (Cnidaria: Anthozoa: Hexacorallia), whose study is still somewhat limited in this region. Previous reports have noted the presence of some zoantharian species in these two ecoregions (i.e., Haddon and Duerden 1896; Brito 1984; Morri et al. 2000; Villar et al. 2003; Ocaña and Brito 2004; Ocaña et al. 2007; Monteiro et al. 2008; Reimer et al. 2010; Cen-Pacheco et al. 2014). However, only two recent studies have examined the shallow zooxanthellate zoantharians of the Cape Verde Islands utilizing molecular methods combined with morphological analyses, and these studies are somewhat limited in specimen numbers and scope (Reimer et al. 2010; López et al. 2018).

Zoantharians are commonly found in intertidal rocky platforms and from shallow waters to the deep sea. As with many other anthozoans, most shallow tropical and subtropical zoantharians live in symbioses with photosynthetic dinoflagellate zooxanthellae of the family Symbiodiniaceae (Burnett et al. 1995, 1997; Burnett 2002; Reimer et al. 2007a; Costa et al. 2013; LaJeunesse et al. 2018). Despite being very common benthic organisms, zoantharian species remain taxonomically problematic due to high levels of intraspecific variation, resulting from phenotypic plasticity in polyp shape, colony shape, size and oral disk color (Burnett et al. 1995, 1997; Ryland and Lancaster 2003; Ong et al. 2013). For these reasons, much recent research has combined morphological, molecular and ecological data to more successfully identify as well as revise zoantharian taxa (e.g., Reimer et al. 2006a; Sinniger et al. 2008; Koupaei et al. 2014; Irei et al. 2015). Additionally, characterizing the Symbiodiniaceae of zoantharians may aid in understanding the ecology and biogeography of holobionts (Reimer and Todd 2009). Given these difficulties, species diversity of zoantharians, particularly shallow-water *Zoanthus* and *Palythoa* spp., may be overestimated at the present time (Burnett et al. 1997; Reimer et al. 2004). Moreover, high larval dispersal abilities support this hypothesis (Ryland et al. 2000; Polak et al. 2011), despite recent examinations from insular and isolated oceanic

regions that have confirmed the presence of potentially endemic or rare zoantharian species (Irei et al. 2015).

Most recent knowledge about the molecular systematics of zoantharians is from Indo-Pacific species. However, some studies have also been performed in the Atlantic Ocean, such as in the Caribbean Sea (Swain and Wulff 2007; Reimer et al. 2012), Ascension Island (Reimer et al. 2017a), Brazil (Santos et al. 2016), the Azores (Carreiro-Silva et al. 2017), the Canary Islands (Ocaña and Brito 2004; Ocaña et al. 2007) and the Cape Verde Islands (Reimer et al. 2010; López et al. 2018), reporting potentially unknown species and increasing the distribution ranges of many others.

This study focuses on zoantharians from the suborder Brachycnemina, including the families Sphenopidae (*Palythoa*) and Zoanthidae (*Zoanthus*, *Isaurus*). Six zooxanthellate zoantharian species have previously been recorded from the two ecoregions examined in this study, two belonging to the genus *Palythoa*: *P. caribaeorum* Duchassaing and Michelotti, 1860 (Morri and Bianchi 1995; Morri et al. 2000; Monteiro et al. 2008; Reimer et al. 2010) and *P. canariensis* Haddon and Duerden, 1896 (Pax 1908; Brito 1984; Araújo and Freitas 2003); three to *Zoanthus*: *Z. aff. pulchellus sensu* Reimer et al. (2010), *Z. sociatus*, Ellis, 1768 and *Z. solanderi* LeSueur, 1818 *sensu* Karlson (1980) (López et al. 2018); and one to the genus *Isaurus*: *I. tuberculatus* Gray, 1828 (Reimer et al. 2010). However, these previous studies generally reported on small numbers of specimens from limited locations, and no ecoregion-wide assessment of species diversity has been performed as of yet. Additionally, no molecular phylogenetic assessment of *P. canariensis* has been performed.

Because of the general lack of information about brachycnemine zoantharians from the Canary Islands, our aim was to confirm the identity of specimens from this region, and to add information on additional new specimens and morphotypes recently collected from the Cape Verde and Madeira Islands. We combined morphological, ecological and molecular analyses for host species, and also examined their *Symbiodinium* flora, in order to better understand the biogeography of the ecologically important suborder Brachycnemina.

Materials and methods

Study sites and initial identification

Collection of specimens was carried out between 2014 and 2016 from 20 sites in the Canarian and Cape Verde Archipelagos, as well as from Madeira Island (Fig. 1). Zoantharians of the genus *Palythoa*, *Zoanthus* and *Isaurus* were collected on intertidal rocky platforms by walking or

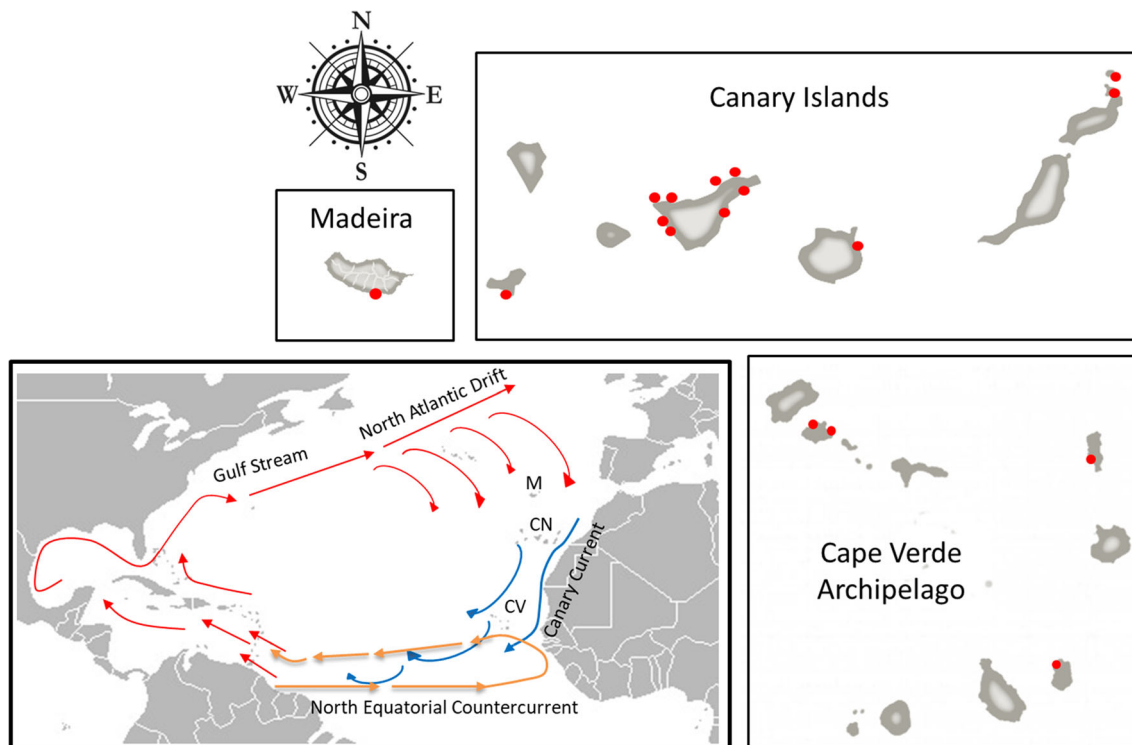


Fig. 1 Study sites around Macaronesian and Cape Verde Islands where zoantharian specimens were collected. Course of the main currents in the North Atlantic Ocean is shown

snorkeling, and in the subtidal zone by scuba diving. We collected all different morphotypes observed. A total of 67 specimens: 34 from the Canary Islands, 31 from Cape Verde and two from Madeira, were collected and stored in 99.5% ethanol for subsequent molecular analyses (ESM1). Specimens were given initials according to the geographic location where they were collected from (MA Madeira, CN Canary Islands and CV Cape Verde), followed by a sample number (ESM1).

Preliminary morphological analyses were performed utilizing *in situ* photographs. We examined the numbers of tentacles, polyp shape and colony size, following the published literature (see Reimer et al. 2012 for details).

Molecular analyses

DNA extraction, PCR amplification and sequencing

Fifty milligrams of tissue from polyps of each specimen were used to extract DNA following the procedures outlined in López et al. (2015). For mitochondrial DNA (mtDNA) analyses, fragments of the mitochondrial cytochrome oxidase subunit I (COI) gene and 16S ribosomal DNA (16S) were amplified by polymerase chain reaction (PCR) using the zoantharian-specific primers designed by López et al. (2018) and Sinniger et al. (2005), respectively.

Additionally, the 23S ribosomal DNA region (23S) of their Symbiodiniaceae was amplified using primers designed by Santos et al. (2002) for a subset of samples that were selected taking into account location and habitat. Moreover, because of its less conservative nature, the Internal Transcribed Spacer region of ribosomal DNA (ITS-rDNA) of both zoantharians and their symbiont were amplified. The ITS-rDNA of zoantharians was amplified using primers designed by Swain (2009) and by Reimer et al. (2007b). For Symbiodiniaceae, zooxanthellae-specific primers by Rowan and Powers (1992) and Hunter et al. (1997) were used.

PCR amplifications from template genomic DNA were performed using AmpONE Taq DNA polymerase (GeneAll Biotechnology, South Korea), following the manufacturer's instructions. 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 52 °C for 16s, 57 °C for COI, 48 °C for ITS (zoantharian), and 53 °C for 23S and 50 °C for ITS (Symbiodiniaceae) 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 Veriti 96-well thermocycler (Applied Biosystems, USA). Aliquots of amplified products were electrophoresed on 1.7% agarose gel to verify DNA amplification. Subsequently, PCR products were enzymatically purified with ExoSAP-IT kit (GE Healthcare, Illustra) according to the

manufacturer's instructions, and the samples sequenced at the Genomic Service (SEGAI) of the University of La Laguna.

Phylogenetic analyses

DNA sequences were edited and assembled using MEGA7: 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 MEGA7.

The best-fitting model of nucleotide substitution for each data set was selected in jModelTest (Darriba et al. 2012) according to the Bayesian Information Criterion (Schwarz 1978). Phylogenetic trees of zoantharians and their symbionts were inferred from each single gene and from the concatenated COI and 16S rDNA sequences (zoantharians) by maximum likelihood (ML) and Bayesian inference (BI), using the previously determined models of nucleotide evolution (K80, K80+I, TrN+G for COI, 16S and ITS of zoantharians, respectively, F81 and K80+G for 23S and ITS of Symbiodiniaceae, respectively).

All phylogenetic analyses were performed through the Mobyly SNAP Workbench (Monacell and Carbone 2014). ML analyses were conducted using RAxML software (Stamatakis 2014) with 1000 replicates of bootstrap. When concatenated sequences were analyzed, the GTR+CAT approximation was used to accommodate heterogeneity rate among partitions. For BI, we used MrBayes software (Huelsenbeck and Ronquist 2001). In this case, two independent runs were performed with defaults prior values, running 10 million generations and sampling each 100 generations. All parameters were unlinked across partitions when concatenated sequences were used. Convergence of all parameters in the two independent runs was assessed using Tracer 1.5 software (Rambaut and Drummond 2007). After removing 25% of samples as a burn-in, the remaining trees were used to obtain a majority consensus tree. Posterior probabilities higher than 0.95 were considered significant (Huelsenbeck et al. 2001). Finally, trees were visualized and edited with Figtree v1.4.3 (Rambaut 2016).

Results

Morphological analyses

A preliminary identification by means of morphological analyses recognized two general types of zoantharian specimens (Fig. 2). Specimens with sand in their body wall were grouped into the family Sphenopidae (*Palythoa*), while specimens with no sand encrustation were members

of the family Zoanthidae (*Zoanthus*, *Isaurus*) (Ryland and Lancaster 2003; Reimer 2010).

Family Sphenopidae

Specimens of *Palythoa canariensis* were found in colonies formed by hundreds of 'liberae' polyps (Pax 1910), with a poorly developed coenenchyme. However, we also frequently observed smaller *P. canariensis* colonies with 10–20 polyps (Fig. 2c). Mean oral disk diameter of expanded polyps was 8.50 ± 0.18 mm ($n = 20$) with a maximum of 11.9 mm. Number of tentacles of its brown–green polyps ranged 44–66 with a mean value of 52 ± 5 mm ($n = 20$ polyps examined).

P. caribaeorum had embedded 'immersae' polyps (Pax 1910) with a well-developed coenenchyme (Fig. 2e, f, Table 1), forming colonies composed of thousands of polyps that covered huge extensions in the subtidal zone. In this species, mean oral disk diameter of expanded polyps was 6.00 ± 0.08 mm ($n = 20$) with a maximum of 9.3 mm (Table 1). All polyps had yellow–green appearance, showing fewer numbers of tentacles than *P. canariensis*, ranging 24–34 with a mean value of 29.24 ± 2 tentacles (Table 1; $n = 20$ polyps examined).

Although specimens CN30 and CN86–CN89 (ESM1) collected in Alcalá site (west Tenerife Island) were initially identified as *P. canariensis*, they had much larger polyps, reaching up to 21.97 mm for expanded oral disk diameter (mean value 14 ± 0.34 mm; $n = 20$ polyps examined) (Table 1). In addition, the overall colony sizes were also larger (maximum colony area found 2784.23 cm² compared to 603.27 cm² for *P. canariensis*). Although the ranges of number of tentacles greatly overlapped (42–70 for *Palythoa* specimens from Alcalá and 44–66 for *P. canariensis* specimens), mean numbers were also higher in Alcalá samples than in specimens from the other collection sites (54 ± 6). Therefore, we considered these specimens as a different *Palythoa* species (Table 1).

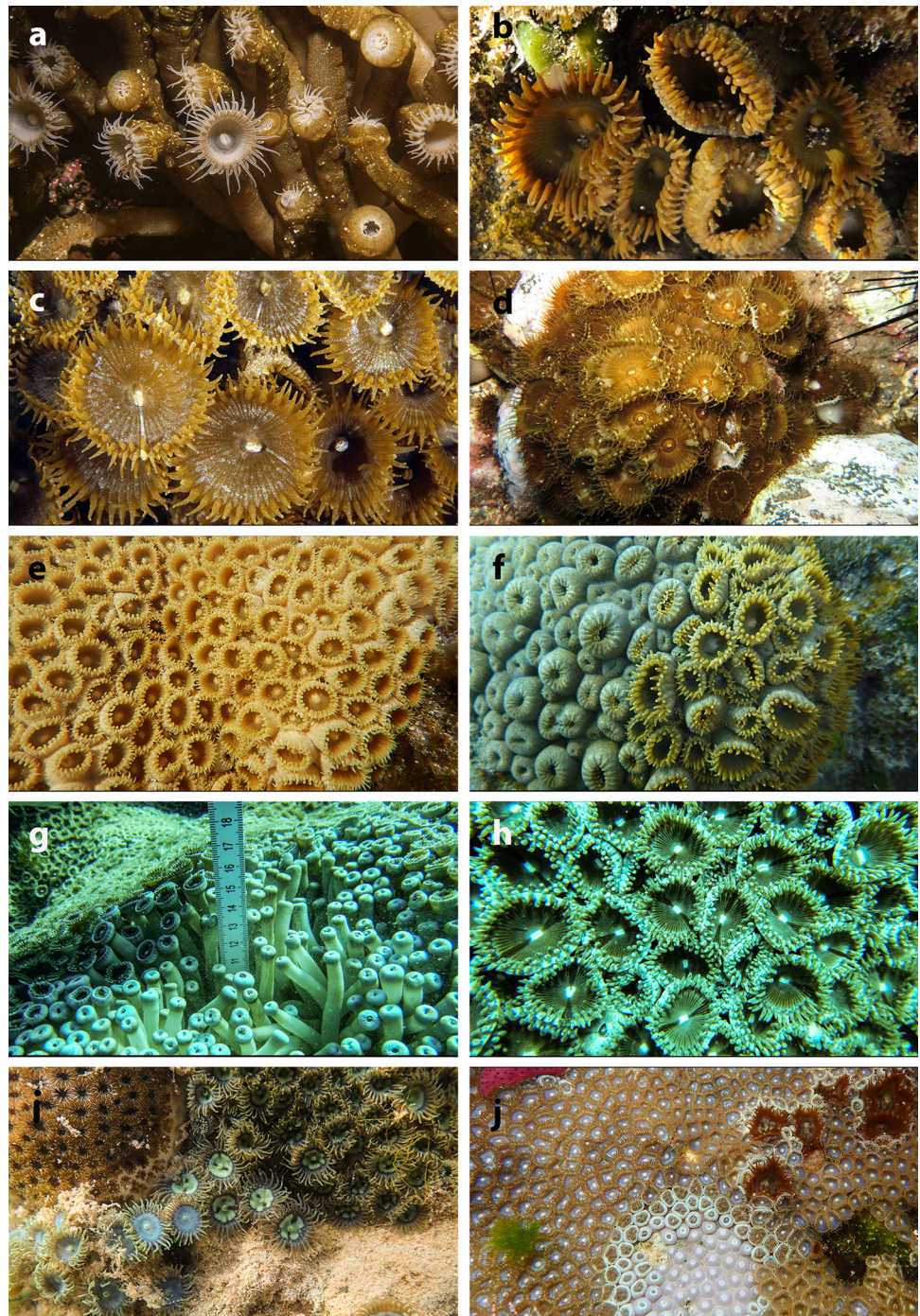
Finally, specimens CV18, MA95 and MA96 (ESM1) were initially identified as *P. canariensis* due to their similar morphology to *P. canariensis* from the Canaries.

Family Zoanthidae

Regarding family Zoanthidae, specimen CN131 was easily identified as *Isaurus tuberculatus* due to its large polyp size with external tubercles (Fig. 2a) (Muirhead and Ryland 1984; Reimer et al. 2008). All other non-sand-encrusted specimens were members of the genus *Zoanthus*.

On the other hand, three *Zoanthus* morphotypes were observed (Fig. 2). All colonies found in the intertidal zone of the Canary Islands and specimens CV15 and CV52 (ESM1) from the subtidal zone of Cape Verde were

Fig. 2 Zoantharian species examined in this study. **a** *Isaurus tuberculatus*, **b** *Palythoa grandiflora*, **c** *Palythoa* aff. *clavata*, **d** *Palythoa grandis*, **e**, **f** *Palythoa caribaeorum*, **g**, **h** *Zoanthus pulchellus*, **i** *Zoanthus sociatus* and **j** *Zoanthus* aff. *pulchellus*



classified as morphotype 1 (MP1), having a poorly developed coenenchyme (Pax 1910) with white–gray oral disk and tentacles (Table 1; Fig. 2g, h). We found a wide range of variation within the oral disk diameter of morphotype 1, from 0.3 to 1.4 cm (Table 1). However, these *Zoanthus* specimens could not be identified to species level by morphological characteristics alone as they did not exactly fit with any described species.

The other two morphotypes (MP2 and MP3) were found exclusively in Cape Verde and could be easily distinguished from each other by coenenchyme development. Specimens belonging to MP2 had a well-developed coenenchyme with ‘immersae’ polyps (Pax 1910) with an external greyish coloration, brown tentacles and blue or green oral disk. These characteristics match with *Z. aff. pulchellus* previously reported from Cape Verde (Reimer et al. 2010). It was not possible to measure the oral disk

Table 1 Distribution and morphological characteristics of the species analyzed in this study

	<i>Palythoa</i> aff. <i>clavata</i>	<i>Palythoa</i> <i>grandiflora</i>	<i>Palythoa</i> <i>caribaeorum</i>	<i>Zoanthus</i> aff. <i>pulchellus</i> (MP1)	<i>Zoanthus</i> <i>pulchellus</i> (M2)	<i>Zoanthus sociatus</i> (MP3)
Distribution	Canary Islands (13), Cape Verde (2)	Alcalá (5) (Tenerife, Canary Islands)	Canary Islands (10), Cape Verde (10)	Canary Islands (5), Cape Verde (2)	Cape Verde (9)	Cape Verde (5)
Deep (m)	Intertidal to 10 m	Intertidal to 2 m	Intertidal to 15 m	Low intertidal to 10 m	Intertidal	Intertidal to 3 m
External oral disk color	Brown–green	Brown–green	Yellow–green	White or grayish and green	Brown–green	Mint green, fluorescent green, yellow
Coenenchyme development	Not well developed	Not well developed	Well developed	Not well developed	Well developed	Not well developed
Polyp structure	Liberiae	Liberiae	Immersae	Liberiae	Immersae	Liberiae
Number of tentacles	44–66	42–70	24–34	40–56	44–54	40–50
Oral disk diameter (mm)	8.5 ± 0.18	14 ± 0.34	6 ± 0.08	5.5 ± 0.26	–	26.5 ± 0.53
Max oral disk diameter (mm)	11.9	19.7	9.3	14	–	3.54

Numbers in brackets are the number of samples analyzed of each location

diameter of MP2 as most of the polyps were partially closed and other polyps were so close one to the other that it was very difficult to distinguish between them with the tentacles expanded (Fig. 2f).

On the other hand, the characteristics of MP3 were similar to *Z. sociatus* found in Maio Island (Cape Verde) (López et al. 2018). Polyps were ‘liberae’ in form with a poorly developed coenenchyme (Pax 1910), and included a wide variation in oral disk colors; from yellow or green fluorescent in the middle to the entire oral disk bluish (Fig. 2i). Mean oral disk diameter (0.47 ± 0.1 mm) and number of tentacles (48–62) were higher in MP3 than the values recorded in Maio ($n = 20$ polyps) (Table 1).

Molecular analyses

New sequences obtained in this study were deposited in GenBank (accession numbers MH612382–MH612581) (ESM1).

Zoantharian phylogenetics analyses

A matrix of 962 nucleotide positions was obtained after concatenation of partial fragments of COI and 16S rDNA for 55 samples. Among the 962 nucleotide sites utilized for the phylogenetic analyses, 56 were variable and 55 were phylogenetically informative. Seventeen additional

sequences from GenBank corresponding to different zoantharian species were included to perform the phylogenetic analyses. One specimen of *Parazoanthus swiftii* from the suborder Macrocnemina was added to the analyses as outgroup.

Phylogenetic trees using BI and ML approaches from concatenated mitochondrial fragments recovered the same topology (Fig. 3) with two very strongly supported clades corresponding to the families Sphenopidae (BI posterior probability/ML bootstrap% = 1.0/100%) and Zoanthidae (1.0/100%), both clearly differentiated from outgroup *Parazoanthus* (Fig. 3). Identical topologies with lower supports were obtained for each fragment separately (data not shown).

Within the family Sphenopidae, four subclades were recovered. One moderately supported subclade included sequences from all specimens previously identified as *P. caribaeorum* (0.77/90%), and also included identical sequences to its Indo-Pacific sister species *P. tuberculosa* Esper, 1805 (Fig. 3). It has been shown that Zoantharia species from the Atlantic and their sister species from the Indian/Pacific Oceans have highly similar or identical mtDNA and ITS-rDNA sequences (Reimer et al. 2010, 2012). Sequences of the unidentified *Palythoa* sp. specimens from Alcalá (CN30, CN86, CN87 and CN89) formed a subclade derived from *P. caribaeorum* and closely related to *P. grandiflora* Verrill, 1900 from Brazil

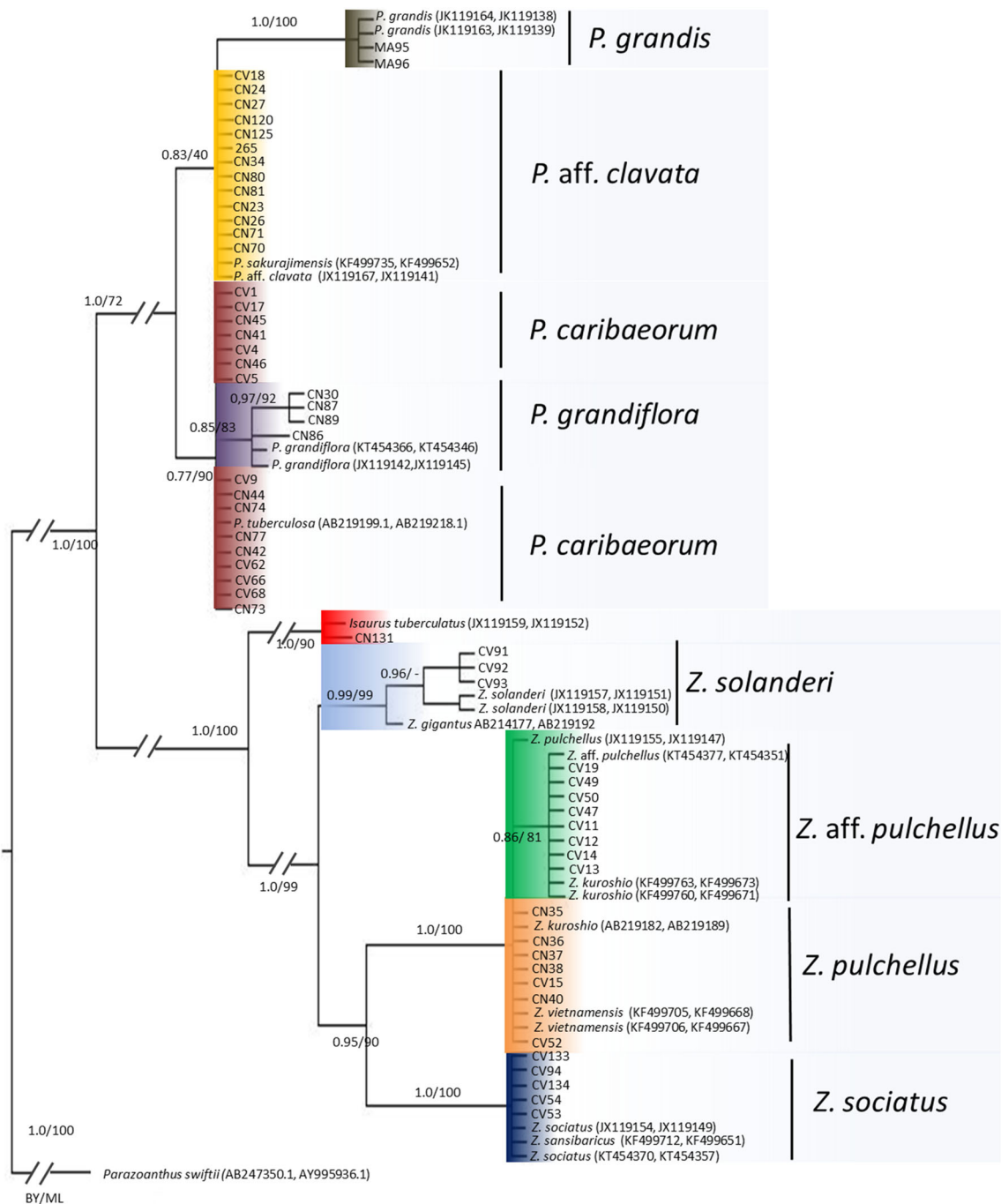


Fig. 3 Phylogenetic tree of concatenated mitochondrial COI and 16S-rDNA genes obtained by Bayesian inference (BI). Numbers above branches represent Bayesian posterior probabilities and

maximum likelihood bootstraps, respectively. Sequences with names and GenBank Accession Number from previous studies

(0.85/83%) (Fig. 3). Concatenated phylogenetic analyses confirmed that specimens MA95 and MA96 from Madeira belonged to a different clade, similar to previously reported sequences of *P. grandis* Verrill, 1900 from Florida and *P. cf. grandis* from Dominica. All sequences morphologically identified as *P. canariensis* had exactly the same sequences for both COI and 16S fragments (Fig. 3), including the

specimens from the Canary and Cape Verde Islands. All of these formed a well-supported subclade (1.0/100%) including the previously reported sequences from *P. aff. clavata sensu* Reimer et al. (2012) from Florida and its sister species in the Pacific Ocean, *P. sp. ‘sakurajimensis’ sensu* Reimer et al. (2007b).

Because the bootstrap supports of *Palythoa* species clades were low, a subset of samples representing all locations and habitats was selected to amplify ITS-rDNA due to its higher levels of divergence within Brachycnema (Reimer et al. 2007b), and its ability to more clearly delineate *Palythoa* spp. (Reimer et al. 2007c). The alignment included 24 sequences of 743 nucleotides with 242 positions variable and 223 phylogenetically informative. The relationships observed in the phylogenetic tree based on concatenated sequences of COI and 16S to *Palythoa* spp. were confirmed with the ITS-rDNA phylogenetic analyses. Four well-supported clades were obtained and differentiated from the outgroup *Zoanthus sansibaricus* Carlgren, 1900 (Fig. 4). Specimen MA95 matched with sequences of *P. cf. grandis* and *P. grandis* from Dominica and Florida, respectively (1.0/100%). All specimens identified as *P. caribaeorum* were similar to previously reported sequences of its sister species *P. tuberculosa* from Japan (0.99/96%). In the same way as in the concatenated tree, specimen CN86 from Alcalá formed a subclade derived from *P. caribaeorum* and had exactly the same sequences as *P. aff. mutuki sensu* Swain (2010) from the Caribbean (0.99/99%). All sequences that belonged to *P.*

canariensis formed a well-supported clade (1.0/97%) with previously reported sequences from *P. aff. clavata* from Florida.

Regarding the family Zoanthidae, the phylogenetic tree for concatenated sequences of COI and 16S-rDNA showed two clearly separated clades (Fig. 3). One well-supported clade was composed of the sequence from specimen CN131 and an *I. tuberculatus* sequence from Florida (1.0/90%), and the other clade included three well-supported subclades including the rest of the *Zoanthus* sp. sequences. One subclade included sequences of CV91, CV92 and CV93 along with sequences of *Z. solanderi* from Florida and its sister species *Z. gigantus* Reimer and Tsukahara, 2006 from Japan (0.99/99%). On the other hand, sequences from specimens CV53, CV54, CV94, 133 and 134 morphologically identified as MP3, clustered with sequences from *Z. sociatus* from Florida and Brazil, and together with sequences of sister species *Z. sansibaricus* from Taiwan, they formed a highly supported clade (1/100%). Finally, sequences of CV11–14, CV19, CV47, CV49 and CV50 were closely related to sequences from *Z. aff. pulchellus* from Florida and its sister species from the Pacific, *Z. kuroshio* Reimer and Ono, 2006. These sequences formed a

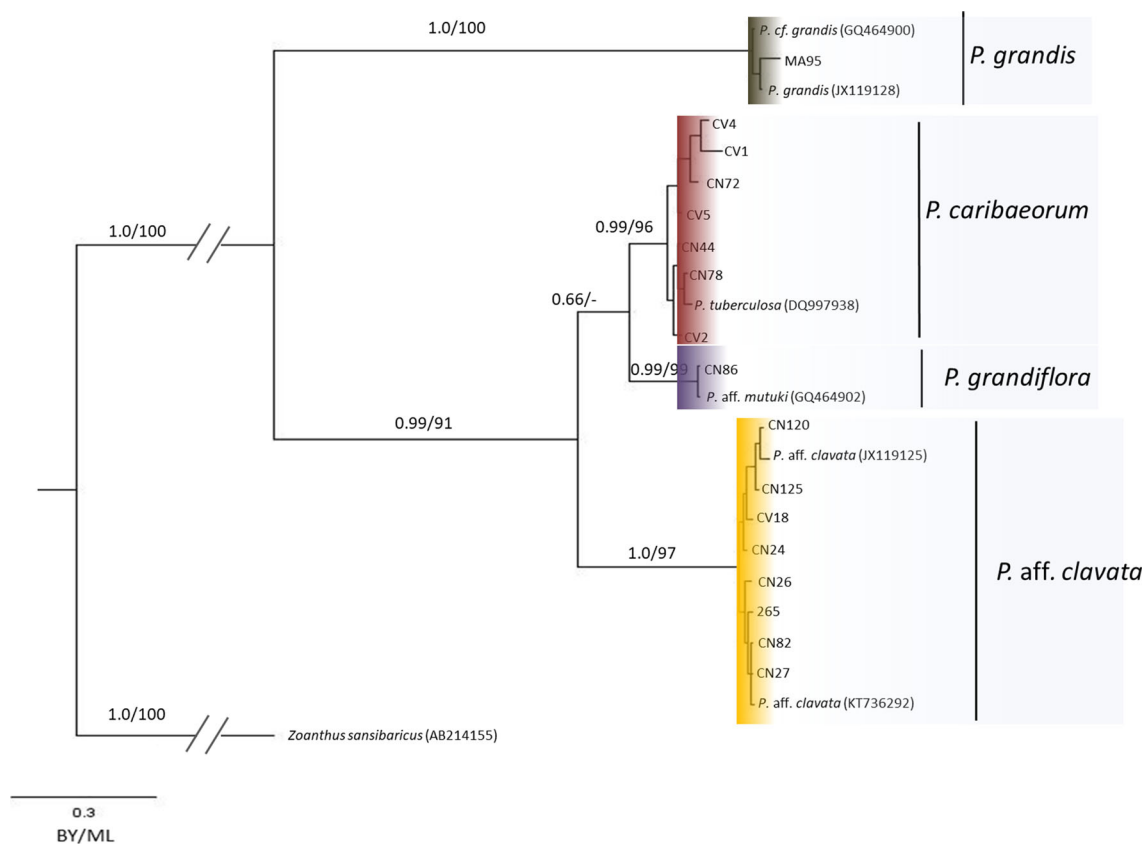


Fig. 4 Phylogenetic tree of the internal transcribed spacer of ribosomal DNA (ITS-rDNA) sequences for *Palythoa* species included in this study. Numbers above branches represent Bayesian posterior

probabilities and maximum likelihood bootstraps, respectively. Sequences with names and GenBank Accession Number from previous studies

moderately supported group (0.86/81%), which included all specimens in this study identified as MP2. Sequences from specimens identified as MP1 from the Canary Islands and Cape Verde were basal to the *Z. aff. pulchellus* and related sequences, and were identical to one previously reported sequence from *Z. kuroshio* from Japan and two sequences from the related species *Z. vietnamensis* Pax and Müller, 1957 from Taiwan.

Symbiodiniaceae alignments

A total of 620 nucleotide positions for chloroplast 23S RNAr gene and 717 for nuclear ITS-rDNA gene were analyzed for 41 and 24 samples of Symbiodiniaceae, respectively. Although 23S RNAr is more conservative than ITS-rDNA (16% and 30% variable sites, respectively), phylogenetic trees obtained with both ML and BI approaches showed similar topologies. Symbiodiniaceae sequences generated in this study belonged to two genera, *Symbiodinium* (former *Symbiodinium* 'Clade A') and *Cladocopium* (former *Symbiodinium* 'Clade C') (Fig. 5 and ESM2). All zoantharian specimens collected in the Canary

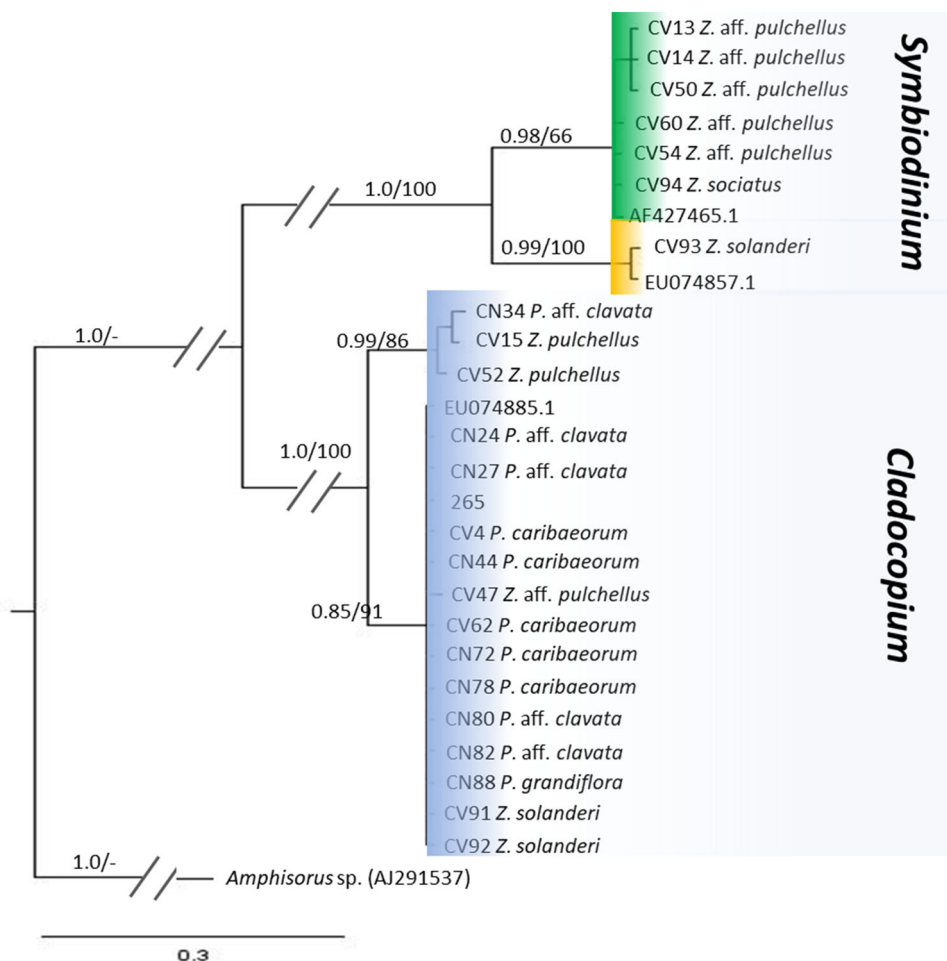
Islands hosted *Cladocopium*, forming a well-supported clade (1.0/100%) (Fig. 5 and ESM2). However, specimens from Cape Verde included both *Symbiodinium* and *Cladocopium* (Fig. 4 and ESM2).

All *Palythoa* species from Cape Verde hosted *Cladocopium* together with specimens of *Z. pulchellus*, *Z. aff. pulchellus* and *Z. solanderi* (Fig. 5). The other well-supported clade (1/100%) was composed of sequences belonging to *Symbiodinium* and included *Z. sociatus*, *Z. solanderi* and *Z. aff. pulchellus* hosts (Fig. 5).

Discussion

The results of this study highlight the amphi-Atlantic distribution of Brachycnemina. A total of nine species of shallow-water zoantharians were recorded within the Macaronesian and Cape Verde ecoregions, according to molecular, morphological and ecological data: four *Palythoa* spp, four *Zoanthus* spp. and one *Isaurus* sp. All specimens analyzed belonged to species commonly found in the West Atlantic, as well as in other archipelagos in the

Fig. 5 Bayesian inference tree of internal transcribed spacer ribosomal DNA from Symbiodiniaceae hosted by zoantharian specimens analyzed in this study. Numbers above branches represent Bayesian posterior probabilities and maximum likelihood bootstraps, respectively



open Atlantic Ocean such as Ascension Islands (Hartog and Türkay 1991; Reimer et al. 2012, 2017a; Santos et al. 2016). Long distances and oceanographic patterns are important biogeographic barriers between both East and West Atlantic Ocean (de Souza et al. 2017). However, these barriers may be permeable by natural or artificial ways depending on the ecology and life history of the organism involved (Nunes et al. 2011; Hoeksema et al. 2012, 2018; López et al. 2015; de Souza et al. 2017; Santos and Reimer 2018). Zoanthellae (Sphenopidae) and zoanthinae (Zoanthidae) larvae have long planktonic lifespan, up to 190 d (Polak et al. 2011), being commonly found around the Atlantic and explaining the amphi-Atlantic distribution of some Brachycnemina species whose larvae are able to cross the open ocean (Ryland et al. 2000). However, zoanthellae and zoanthinae larvae have not been found in the 20–24 °C water band of the Gulf Stream that flows easterly to the Azores (Ryland et al. 2000). The presence of zooxanthellate zoantharian species has not been recorded in this archipelago, but the larvae become

more frequent as temperature increases in the islands to the south, which are immersed in a descending branch of the Gulf Stream, the Canary Current (Figs. 1 and 6) (Barton et al. 1998). Supporting this theory, one Brachycnemina species was found in northern Madeira, five in the Canary Islands and seven in southern Cape Verde Archipelago.

Sphenophidae species identification

All the specimens from the Canary and Cape Verde Islands identified by morphological characteristics as *P. caribaeorum* also were clustered together as one group in the molecular analyses (Fig. 4). *P. caribaeorum* is an amphi-Atlantic tropical species with the Canaries as its northern distribution limit in the East Atlantic Ocean. This species inhabit habitats ranging from intertidal rocky platforms to depths of at least 17 m in specific locations in the Canary Islands, especially in the western islands, where the seawater temperature is warmer than in the rest of the Archipelago (Barton et al. 1998, Fig. 6). In Cape Verde, *P. caribaeorum* is frequently found dominating rocky platforms and bedrocks at 4–10 m depth, especially around Sal Island, where it dominates the subtidal zone and overgrows, scleractinian coral species such as *Porites astreoides* and *Siderastrea radians* (Morri et al. 2000; Monteiro et al. 2008).

A few specimens previously cataloged as *P. canariensis* were initially misidentified due to difficulty distinguishing between this species and other ‘liberae’ *Palythoa* species in the field. Surprisingly, our molecular analyses showed the existence of three species groups within specimens initially identified as *P. canariensis*. Most specimens collected around the Canarian Archipelago and specimens CV18 and 265 from Cape Verde in Reimer et al. (2010) were closely related to *P. aff. clavata* from Florida (Reimer et al. 2012) in both phylogenetic trees, and the closest match in the taxonomic literature to this morphotype is *P. clavata* from St. Thomas (Duchassaing 1850). The original description is also identical to our morphological results with the exception of oral disk color, as our specimens were brown with green and white oral disks (Fig. 2c), while *P. clavata* was originally described as having violet or purple oral disk and tentacles (Duerden 1898). Some authors have suggested that color variation can be informative for distinguishing between species (West 1976; Swain 2009). However, intraspecific color variation is very common in both Macrocnemina (e.g., Herberts 1972) and Brachycnemina (e.g., Duerden 1898; Burnett et al. 1995, 1997; Reimer et al. 2004), and color variation occurs even within a single species, as is the case of *Z. sansibaricus*, which can change color depending on depth (Kamezaki et al. 2013). The results of the present study showed that in the case of *Palythoa* species, characters such as polyp color need to be

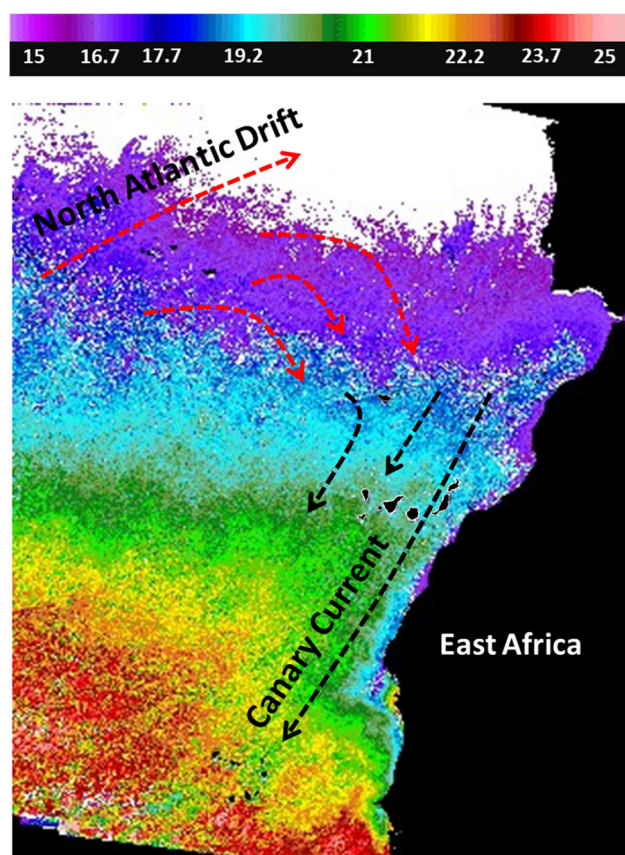


Fig. 6 Seawater temperature around Macaronesia and Cape Verde ecoregions corresponding to winter months (March average) (SAT Union System, ULPGC) and course of the Canary Current which branches south from the North Atlantic Drift and flows parallel to the east Africa coast to Senegal (red warm water current, black cool water current). Figure modified from Brito (Brito 2010)

taken with care as in some cases color may depend on the color of the sand/particles available at each location (Reimer et al. 2006b). For instance, specimens CN74 and CN73 of *P. caribaeorum* collected from Órzola (Lanzarote, Canary Islands), where the sand is composed of white shell fragments, are completely white in coloration (Fig. 2e, f) and in contrast to the usual yellow–green color of the species. *P. canariensis* frequently inhabits intertidal rocky shores around the Canary Islands and colonies can be found in the subtidal zone down to 10 m depths in well-lit areas. If *P. aff. clavata* and *P. canariensis* are in fact the same species, this case can demonstrate how zoantharian intraspecific morphological plasticity can cause taxonomic confusion. Clearly, thorough systematic research on *Zoanthus* and *Palythoa* spp. is needed to clarify the potentially very large numbers of synonyms in the groups (Burnett et al. 1997; Reimer et al. 2004, 2012, Low and Reimer 2016).

In this study, two species were recorded in the Macaronesian ecoregion for the first time, *P. grandiflora* and *P. grandis* (Fig. 2b, d, respectively) based on morphological and molecular analyses. Colonies of *P. grandiflora* were found in the low intertidal rocky platform up to 1 m depth in the subtidal of just one specific location, Alcalá, on the warmest side of Tenerife Island (Fig. 1; Brito 2010) and the colonies are found in an area where cropland irrigation waters flow directly into the sea. It is known that these colonies have inhabited this location since at least 1980 (A. Brito pers. com.); further research is needed to understand why this species inhabits just this one location in the region.

Our phylogenetic analyses showed that specimens MA95 and MA96 collected in Madeira belong to the *P. grandis*-*P. cf. grandis* complex. Until now, *P. canariensis* (= *P. aff. clavata*) was the only zoantharian species known from the southern coast of Madeira and around Porto Santo Island, representing the northernmost record of the species (Wirtz 1995; Araújo and Freitas 2003).

Zoanthidae species identification

Zoanthidae specimens in this study all belonged to either the *Isaurus* or *Zoanthus*. *I. tuberculatus* is a pan-tropical species easily identifiable due to its unique tubercular polyps (Muirhead and Ryland 1984). Our results confirmed its presence in the Canary Islands where this species reaches its northernmost distribution in the East Atlantic.

Although there are large amounts of phenotypic plasticity found in *Zoanthus* spp., particularly concerning oral disk color and polyp height (Fig. 2; Ong et al. 2013), the morphological characteristics used in the current study were successful in separating species. Moreover, our morphological species delimitations are in agreement with

the phylogenetic results obtained with the concatenated COI and 16S rRNA sequences, as has previously been reported for other members of the genus *Zoanthus* (Sininger et al. 2008). Specimens CV15, CV52 as well as all *Zoanthus* specimens collected in the Canaries had COI and 16S-rDNA sequences identical to those previously reported from *Z. kuroshio* and *Z. vietnamensis* in the Pacific, two species phylogenetically closely related to each other (Reimer et al. 2013), and to *Z. pulchellus* from Florida (Reimer et al. 2012). We identified these specimens as *Z. pulchellus* (Duerden 1898; Pax 1910) since morphological analyses fit well with the description given to this species. It has been shown that polyp height can be misleading in identifying *Zoanthus* species, as sites with low hydrodynamic energy may have colonies with taller polyps than those from the same species inhabiting sites with stronger currents and wave action (Reimer et al. 2006a; Ong et al. 2013). We also found a great variety of polyp height within *Z. pulchellus* colonies depending on the hydrodynamics of the study location. In rocky platforms of northern Tenerife (Canary Islands), characterized by high hydrodynamic levels (Yanes et al. 2006) such as the Punta del Hidalgo and Los Silos sites, colonies had polyps which reached only a few millimeters in height. However, in sheltered sites, for example, inside the fishing port in La Restinga (El Hierro, Canary Islands), a large colony with polyps up to 13.5 cm in height (Fig. 2g) was found. This study recorded *Z. pulchellus* for the first time in the East Atlantic, inhabiting from intertidal rocky platforms to up to 20 m deep in the sublittoral of the western islands of the Canarian Archipelago. From its wide distribution in this study, it appears that *Z. pulchellus* has the highest thermal tolerance within the zoantharian species analyzed.

Cape Verde specimens CV11–14 and CV19 from Sal and CV47, CV49 and CV50 from São Vicente matched molecularly and morphologically with *Z. aff. pulchellus* previously reported from the Archipelago (Reimer et al. 2010), and also with *Z. kuroshio* from Japan. Although phylogenetically closely related, *Z. pulchellus* and *Z. aff. pulchellus* are morphologically well distinguished from each other, similar to what has been seen with their sister species *Z. kuroshio* and *Z. vietnamensis* from the Indo-Pacific Ocean (Reimer et al. 2006a). *Z. pulchellus* has polyps free of the coenenchyme with a great variety of polyp heights and pale greenish–yellowish colors in the oral disk and tentacles (Fig. 2g, h), while *Z. aff. pulchellus* has polyps immersed in the coenenchyme, limiting polyp height to a few mm, and a general whitish appearance with a characteristic white ring around dark brown tentacles (Fig. 2f). Therefore, and despite their close molecular relationships, it seems appropriate to consider *Z. aff. pulchellus* as a unique *Zoanthus* species, as in the case of their sister species *Z. kuroshio* and *Z. vietnamensis* in the

Pacific (Reimer et al. 2006a). Our results show that the distribution range of this species reaches to Sal Island in Cape Verde, indicating that it is widely distributed around the Archipelago as it has previously been reported from Santiago and São Vicente Islands (Reimer et al. 2010). Most specimens were found in the rocky intertidal zone, including some of them completely exposed to the open air at low tides, showing the species' tolerance to desiccation.

Morphotype 3 was identified as *Z. sociatus*, previously known from the western tropical Atlantic and also reported from Maio Island, as it matched with the morphological and molecular analyses of the samples described in the 'Zoanthus zone' found in Maio Island (López et al. 2018). This species is able to co-inhabit with *Z. solanderi*, and can cover huge areas in the subtidal zone as has been previously observed in Jamaica (Karlson 1981; López et al. 2018). Specimens of *Z. sociatus* (Fig. 2i) collected in São Vicente extend the distribution of the species within the Cape Verde archipelago, where it can inhabit intertidal rocky platforms, forming small colonies.

Symbiodiniaceae

The zoantharian species recorded in this study were collected across several archipelagos that have great differences in sea surface temperatures (Fig. 6). From Madeira (32°N) to Cape Verde (14°N), specimens of shallow-water zoantharians were collected from tide pools, in which seawater can experience both relatively extreme low and high temperatures and salinity values (Legrand et al. 2018), and also from depths to 10 m or more, where seawater conditions are more stable. Mean sea surface temperatures in Madeira, Canary and Cape Verde Islands during winter months (March) are approximately 18 °C, 20 °C and 22 °C, respectively, while in late summer (October), they reach 22.5 °C, 24 °C and 26 °C, respectively, showing a significant latitudinal gradient (Fig. 6, Brito 2010). Moreover, a pronounced longitudinal temperature gradient exists due to the Saharan upwelling influence (Barton et al. 1998; Vélez et al. 2015). Therefore, there is a decreasing gradient of seawater temperature from the warmer western islands to the eastern islands of the Canaries, with temperatures 3 °C lower in the eastern islands, and more tropical species inhabit the western islands (Fig. 6; Brito et al. 2005). However, in this study, we did not notice any specific Symbiodiniaceae shifts due to temperature as has been seen in some recent studies on zoantharians (Reimer et al. 2017b).

All *Palythoa* specimens examined from the Canarian and Cape Verde Archipelagos hosted *Cladocopium*, regardless of site or depth, while most *Zoanthus* specimens hosted *Symbiodinium* in Cape Verde. Many *Symbiodinium* are speculated to be adapted to high levels of UV light and

usually appears in hosts that inhabit the shallowest zones in tropical areas (LaJeunesse 2002; Finney et al. 2010) as seen in our specimens from Cape Verde. The fact that all *Palythoa* spp. hosted *Cladocopium*, even in the samples collected in the intertidal zone of tropical regions of Cape Verde, may be related to the occurrence of sand and detritus incrustations in their body wall, which can reach the 65% of wet tissue weight (Mueller and Haywick 1995), which would give symbionts some protection against UV light. The fact that one colony of *Z. solanderi* from Maio (Cape Verde) had *Symbiodinium* (CV93) and the others *Cladocopium* (CV91 and CV92) (Fig. 5) in the same environment may be due to the fact that many host species commonly host several types of Symbiodiniaceae within the same colony, such as the case of *Millepora alcicornis* in the Caribbean (Grajales and Sanchez 2016). It is expected that host species at shallower depths exhibit a marked shift in symbiont type due to higher light intensities and radiation that could exclude some symbiont types (LaJeunesse 2002; Finney et al. 2010). However, our results are based on ITS sequences, and there it is likely that there are different species present within these specimens, based on results from higher resolution markers (LaJeunesse and Thornhill 2011) in *Palythoa* in the Pacific Ocean (Noda et al. 2017) and the Red Sea (Reimer et al. 2017b).

In conclusion, our study showed an increase in the number of Brachycnemina zoantharian species as seawater temperature rises toward the tropics; at least one species inhabits the waters of Madeira (*Palythoa grandis*), five in the Canary Islands (*P. caribaeorum*, *P. aff. clavata*, *P. grandiflora*, *I. tuberculatus*, *Zoanthus pulchellus*) and seven in the Cape Verde Archipelago (*P. caribaeorum*, *P. aff. clavata*, *Z. pulchellus*, *Z. sp.*, *Z. sociatus*, *Z. solanderi*, *I. tuberculatus*). Although acceptable for discerning most species (Sinniger et al. 2008), the standard DNA markers for Zoantharia remain problematic in detecting closely related species (Mizuyama et al. 2018). Future application of next-generation sequencing data and analytical methods (e.g., Toonen et al. 2013) may help better solve such issues, and provide higher phylogenetic support for relationships currently less well supported. Our results also confirm that in the case of the Macaronesian region and Cape Verde Islands, zoantharians host two Symbiodiniaceae genera: *Symbiodinium* and *Cladocopium*. Under global warming, formerly low ocean temperatures may no longer act as effective barriers in the distribution of shallow-water zooxanthellate zoantharians, and these already widespread species may extend their distribution ranges, causing shifts in high latitudes ecosystems (Reimer et al. 2018).

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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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