

Article

Shallow-Water Species Diversity of Common Intertidal Zoantharians (Cnidaria: Hexacorallia: Zoantharia) along the Northeastern Coast of Trinidad, Southern Caribbean

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Abstract: Zoantharians are colonial cnidarians commonly found in shallow tropical Caribbean coral reefs, and are known to be globally distributed. Common species in genera *Zoanthus* and *Palythoa* occur at Toco, Trinidad, where they are more abundant than their Scleractinia counterparts relative to benthic coverage. In this study, distribution, morphological and molecular data were collected to determine species and symbiont identification to provide more insight on zoantharians. The Line Intercept Point (LIT) transect method recorded coverage at three sites: Salybia (SB), Pequelle (PB), and Grande L'Anse (GA) Bays along the northeastern coast. Variations in morphology, such as tentacle count, oral disk color and diameter were collected from colonies in situ. All specimens were zooxanthellate, and molecular and phylogenetic analyses were done by sequencing the cytochrome oxidase subunit I (COI) gene, and the internal transcribed spacer (ITS) region for species and symbiont identification, respectively. Results showed mean Zoantharia percentage cover was $32.4\% \pm 5.1$ ($X \pm SE$) at SB, $51.3\% \pm 6.5$ (PB), and $72.2\% \pm 6.1$ at GA. Zooxanthellate zoantharians were identified as *Palythoa caribaeorum*, *Palythoa grandiflora*, *Zoanthus pulchellus*, and *Zoanthus sociatus*. Symbiodiniaceae genera were identified as *Cladocopium* and *Symbiodinium* in *Palythoa* and *Zoanthus* spp., respectively. Although this is the first molecular examination of zoantharians, and their symbionts in Trinidad, more research is needed to identify and document species distribution and symbiont biodiversity to understand their ecology in these dynamic ecosystems.

Keywords: symbiodiniaceae; zoantharians; Trinidad coral reefs; zooxanthellate; biogeography



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1. Introduction

Zoantharians (Anthozoa: Hexacorallia: Zoantharia) make up a considerable benthic component of tropical and sub-tropical shallow-water reefs, similar to Scleractinia (hard corals) and Actiniaria (sea anemones) [1–4]. Zoantharians are sessile colonial anemone-like organisms with two rings of tentacles surrounding circular to polygonal disks, and forming colonies of polyps [5]. They grow in dense mats or small patches throughout the shallow intertidal rocky zones, or coral reef ecosystems, and play an important ecological role in their tropical and sub-tropical habitats [6–12].

Zoantharian coverage on coral reef ecosystems can be extensive [9,10]. For instance, Karlson (1981) noted that two species, *Zoanthus sociatus* (Ellis, 1768) and *Zoanthus solanderi* (LeSueur, 1818) had extensive coverage at a northern intertidal area in Jamaica, which he subsequently named the ‘*Zoanthus zone*’, as originally named by Tom Goreau in the 1950s [13]. Additionally, Lopez et al. (2018) reported a zoantharian zone located at Cabo Verde Islands, where molecular methods and morphological analysis confirmed the presence of two zoantharian species. Zoantharians, such as *Palythoa caribaeorum* (Duchassaing and Michelotti, 1860) and *Z. sociatus* cover large areas in subtidal and intertidal zones [7,9,10,12]. Additionally, many, but not all zoantharian species maintain symbiotic re-

relationships with Symbiodiniaceae (zooxanthellae), hence determining the identity of these holobionts adds more understanding of their ecology and physiological characteristics.

Molecular analyses of zoantharians assist in species identification, where morphological identification is difficult, or impossible. For example, phenotypic plasticity in zoantharians, specifically large variation in morphological characteristics, such as polyp shape, colony shape, size and oral disk color, may cause them to be overlooked in ecological surveys, even though their numbers may be abundant [5,8,14,15]. Given the difficulties in morphological and molecular examination of zoantharians, more priority should be given to determining their distribution and diversity in the Caribbean, especially since zooxanthellate zoantharians play key ecological roles in marine ecosystems [10]. Although success in identifying potential unidentified zoantharian species through morphological characteristics has been proven to be successful [16], the addition of molecular analyses has alleviated issues with morphological ambiguities [16,17]. Additionally, zoantharian species diversity including molecular analyses of zooxanthellate symbionts of family Symbiodiniaceae will continue to add more information about symbiont ecology in light of global climate change [11,18,19].

Although distributed world-wide in tropical and subtropical waters in the Atlantic and Indo-Pacific regions [8,20], zoantharians and Symbiodiniaceae genera identity at the southern-most part of the Caribbean, specifically Trinidad and Tobago, are limited. Two zoantharian genera, *Zoanthus* and *Palythoa* spp., are commonly observed occupying shallow waters, and in different intertidal zones along the northeastern coast of Trinidad [9,10,12,14], but relatively few studies have highlighted morphological and molecular details of these species in this part of the southern Caribbean region.

The purpose of this study is to quantitatively assess zoantharian benthic coverage, and use morphological and genetic analyses to identify zoantharians, and confirm Symbiodiniaceae genera identities. Molecular knowledge of these benthic organisms will be recorded here for the first time at this southernmost part of the Caribbean Sea. Consequently, all ecological aspects of identifying the extent of zoantharian benthic coverage provides more understanding of the dynamic coral reef ecosystem. In general, zoantharian distribution in the Caribbean needs to be continually monitored, and species identification confirmed, especially in the face of increased climate change and anthropogenic activities [9].

2. Materials and Methods

2.1. Study Site and Sampling

The northeastern coast of Trinidad has undefined patch reefs, and a fringing reef (see [12]). Beaches within close vicinity of these reefs are largely defined as sandy, stony, or rocky. The tropical climate has two distinct seasons, with a dry period from January to mid-June and a rainy period that extends the remainder of the year (June–December). Tides are semidiurnal with maximum high tides reaching 2 m (meters) in open water, and extreme low tides can reach 0.2 m.

Study sites in accessible areas along the northeastern coast are located at Salybia Bay (SB) (located between 10°50.097' N, 60°55.208' W and 10°50.100' N, 60°55.157' W), which is part of the only fringing reef in Trinidad. This system has been an important site for citizen/volunteer coral reef monitoring focused on cnidarian and invertebrate abundances and distributions, because it is very shallow during spring low tides (~0.2 m) and the intertidal zone extends some 200 m parallel to the shoreline. This fringing reef is affected by sediment discharge from local rivers throughout the year, specifically during the rainy period from June to December.

Pequelle Bay (PB), located to the east of SB (between 10°50.111' N, 60°55.129' W and 10°50.181' N, 60°54.954' W), has a mixture of rocks and tide pools along the intertidal zone. During spring low tides, this part of the reef becomes fully exposed for a 3-h period until tides return. Patchy undeveloped reefs are present at Grande L'Anse (GA), also known as Toco Bay (between 10°50.107' N, 60°56.772' W and 10°50.266' N, 60°56.674' W), which has a mixture of rocky outcrops. This study site has a mixture of sand, stony and rocky

beaches with village homes within close vicinity. Spring low tides revealed a rocky patch reef with scattered tide pools interspersed throughout the intertidal zone.

Volunteers marked the study area with GPS points, and quantified an area of 500 m² using a 50-m open reel fiberglass measuring tape during extreme low tides (<0.3 m). Each transect was placed within a 500 m² study area, and a total area of 1500 m² was examined at each site. The Line Intercept Transect (LIT) method [9] was used to examine only the lower intertidal areas at SB, PB, and GA. Transect positions within each 500 m² area were marked using a global positioning system (GPS). A 50 m open reel fiberglass measuring tape was placed parallel to the shoreline in each area, and benthic components were recorded at every 0.5 m interval on the measuring tape. Benthic components, such as reef-building corals, zoantharians, macroalgae, coral rubble, and other invertebrates, such as sea urchins, fireworms, and sea cucumbers were recorded if they touched the 0.5 m intervals along the measuring tape. This was repeated three times within each 500 m² study area, and for a total of 3 study areas. Water temperature and salinity were measured using a YSI Pro 1030 probe at three random points along each 50 m LIT. Percentage cnidarian and benthic cover was calculated for benthic communities.

2.2. Morphological Analyses and Specimen Collection

Morphological data for zoantharian colonies ($n = 30$) were recorded at three field sites, SB, PB, and GA in June 2019 from the northeastern coast of Toco, Trinidad. A hand-held caliper was used to measure oral disk diameter of 3 opened polyps per colony. Physical characteristics of each polyp, such as tentacle count and color, oral disc color, and polyp form (immersae, intermediate, liberae, see 4, 21) were recorded, together with in situ photographs, which were used to assist in identification (see Table 1). Additional samples were also collected at three other sites between December 2019–February 2020, and were primarily collected for genetic analyses. Samples were collected in areas with high wave action (open habitat exposed to waves), and low wave action (rocky habitat protected from waves). A total of 13 specimens (3–5 polyps per specimen) were collected from SB, PB, and GA. Additionally, specimens were collected at inaccessible areas along the northeastern coast, such as Galera Point (GP) ($n = 1$ specimen) located just left of the Keshorn Walcott Toco Lighthouse, which is 1 km east of PB, and has a mixture of rocks and tide pools where zoantharians were observed to have extensive coverage. Additionally, samples were collected from Straight Bay (StB) ($n = 2$ specimens), 1 km west of SB, and from western SB (WSB) ($n = 5$ specimens), which is an accessible area, but less frequented by local visitors.

Table 1. Zoantharian specimens from the northeastern coast of Trinidad with collection information, morphological data, GenBank accession numbers, and identification conclusions based on COI sequences.

Sample #	Collection Site α	Disk Color	Tentacle Color	Tentacle Count (3 Polyps Per Colony)	Disk Diameter (mm)	COI I.D. (GenBank Accession #)
P1-br-GA	GA	Brown	Brown	26	11.0	<i>P. caribaeorum</i> (MZ150796)
P2-br-GA	GA	Brown	Brown	36	10.8	<i>P. caribaeorum</i> (MZ150797)
P3-br-SB	SB	Brown	Brown	37	10.5	<i>P. caribaeorum</i> (MZ150798)
P4-gr-GA	GA	Green	Brown	46	13.8	<i>P. grandiflora</i> (MZ150799)
P5-gr-GA	GA	Green	Brown	45	12.6	<i>P. grandiflora</i> (MZ150800)
P6-br-GA	GA	Brown	Brown	33	10.7	<i>P. caribaeorum</i> (MZ150801)
P7-br-WSB	WSB	Brown	Brown	26	11.0	<i>P. caribaeorum</i> (MZ147090)
P8-br-WSB	WSB	Brown	Brown	26	11.0	<i>P. caribaeorum</i> (MZ147091)

Table 1. Cont.

Sample #	Collection Site α	Disk Color	Tentacle Color	Tentacle Count (3 Polyps Per Colony)	Disk Diameter (mm)	COI I.D. (GenBank Accession #)
Z1-gr-StB	StB	Green/Blue	Green	41	10.2	<i>Z. sociatus</i> (MZ147096)
Z2-gr-StB	StB	Green/Blue	Green	42	10.3	<i>Z. sociatus</i> (MZ147097)
Z3-gr-WSB	WSB	Green/Blue	Green	42	10.3	<i>Z. sociatus</i> (MZ150806)
Z4-gr-WSB	WSB	Green/Blue	Green	42	10.3	<i>Z. sociatus</i> (MZ150802)
Z5-gr-SB	SB	Green	Green	42	7.5	<i>Z. pulchellus</i> (MZ150803)
Z6-gr-SB	SB	Green	Green	42	6.9	<i>Z. pulchellus</i> (MZ156026)
Z7-br-gr-SB	SB	Green/Blue	Green	42	7.6	<i>Z. sociatus</i> (MZ150807)
Z8-org-SB	SB	Green	Green	46	10.2	<i>Z. pulchellus</i> (MZ150805)
Z9-org-GA	GA	Orange	Brown	46	10.3	<i>Z. pulchellus</i> (MZ150804)
Z10-gr-TLH	TLH	Green	Green	42	10.3	<i>Z. pulchellus</i> (MZ147092)
Z13-grey-GA	SB	Grey	Green	44	5.2	<i>Z. pulchellus</i> (MZ147093)
Z14-org-GA	SB	Orange	Brown	45	10.2	<i>Z. pulchellus</i> (MZ147094)
Z15-blu-TLH	TLH	Green/Blue	Green	42	10.3	<i>Z. sociatus</i> (MZ147095)

Abbreviations: PB = Pequelle Bay, SB = Salybia Bay, TLH = Toco Lighthouse, GA = Grande L'Anse, StB = Straight Bay, WSB = Western Salybia Bay. All samples (# represents number) were collected during extreme low tide (~0.31 m depth).

Altogether specimens were collected at six sites by snorkeling along the northeast coast of Toco, Trinidad between June 2019 to February 2020. A total of 3–5 closed individual polyps were excised from colonies (specimen) using scalpel and tweezers, and placed in 1.5 mL collection vials with 95% ethanol (Carolina Biological, Burlington, NC, USA), then stored at -20°C . Specimens are housed at the Andrews Science Building, University of Tennessee-Southern (formerly Martin Methodist College), Pulaski, Tennessee, United States. Specimen catalog nos. are TRIN-2019-001 and TRIN-2020-001.

2.3. DNA Extraction, PCR and ITS 2

A total of 21 zoantharian polyps were analyzed. Deoxyribonucleic acid (DNA) was extracted from specimens (30–50 mg) of zoantharian tissue following the manufacturer's protocol of an E.Z.N.A. Tissue DNA Kit (Omega BIO-TEK, Model no. D3396-02 Norcross, GA, USA). Mitochondrial cytochrome oxidase subunit 1 (COI) was amplified using the following zoantharian-specific primers (LCOant 5'-TTTTCYACTAATCATAAAGATAT 3', COIant 5'-GCCCCACACAATAAAGCCCCAATAYYCCAAT 3') (see [21]). Polymerase chain reaction (PCR) amplifications from template DNA were carried out in a BIORAD 96-well thermocycler (Model No. MyCycler Thermal Cycler; Series No. 580BR 7657, Hercules, CA, USA) performed under the following conditions: initial set up at 95°C for 3 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 52°C at 1 min, extension at 72°C at 2 min, and final extension at 72°C for 5 min (see [22]). Aliquots from PCR amplification were checked by 1.7% agarose gel electrophoresis. Each PCR product was enzymatically purified with 1.8 μL Exonuclease I, and 3.6 μL Shrimp Alkaline Phosphatase (ExoSAP, ThermoFisher Scientific, Santa Clara, CA, USA), and incubated in the PCR thermocycler at 37°C for 30 min, followed by 95°C for 5 min (see [20]).

The internal transcribed spacer (ITS) 2 region of Symbiodiniaceae samples were amplified using the following primers: ITSintfor2, 5'-GAATTGCAGAACTCCGTG-3', ITS2 clamp, 5'-CGCCCCGCGCGCCCCGCGCCGTCGCGCCGCCCCCGCGGGGATCCATATGCTTA AGTTCAGCGGGT-3', with a "Touchdown" protocol [22–25]. Products from the PCR were electrophoresed overnight in gradient gels between 45–80% and stained with sybergreen. PCR-denaturation gradient gel electrophoresis (DGGE) gels were photographed, and distinct gel bands were excised using a scapula, then transferred to 1.5 mL Eppendorf tubes. Gel bands were excised to determine Symbiodiniaceae genera identities.

2.4. Phylogenetic Analysis

PCR products for COI gene were sequenced in both directions at Eurofins Genomics (Louisville, KY, USA). DNA sequences were initially inspected by eye and manually edited using Molecular Evolutionary Genetics Analysis (MEGA X, version 7.0 see [26]). Sequences were aligned using CLUSTAL W in MEGA X. Sequences were deposited in GenBank (accession numbers MZ147090-097, MZ150796-807) and were used to align with publicly accessible sequences for other zoantharians (GenBank accession JX119160, JX119164, JX119165, JX119167, JX119157, JX119156, JX119154, JX119159, JX119168, KT454365, AB214177, KF499705, KF499712). All alignments were inspected by eye using MEGA X and errors in nucleotide sequences that were low quality were trimmed prior to phylogenetic analysis. Analysis using maximum likelihood with bootstrap trees (1000 replicates) were incorporated according to the MEGA X protocols.

3. Results

3.1. Zoantharian Distribution

The lower intertidal zone measured an area of 12 km² at Salybia Bay (SB), which extends to Pequelle Bay (PB), and 7 km² at Grande L'Anse (GA/TB). SB showed benthic hard coral (Scleractinia) cover at 47.3% ± 4.5 (Mean% ± SE) and zoantharian cover at 32.4% ± 5.1 (Figure 1A), and specifically dominant concerning *Porites porites* (Pallas 1766) 47.2% ± 4.6, and zoantharians (*Palythoa* and *Zoanthus* spp. making up 31.4% ± 5.0 (Figure 1B). PB, which extends towards the eastern end of SB was dominated by zoantharians (51.3% ± 6.5, Figure 1C), with *Zoanthus* sp. and *Palythoa* sp. benthic cover as 34.1% ± 4.44 and 17.3% ± 8.8, respectively (Figure 1D). TB had a high zoantharian cover of 72.2% ± 6.0 (Figure 1E), with *Palythoa* sp. representing most of the cover at 62.6% ± 9.2, and *Zoanthus* sp. covering 8.4% ± 3.6 benthic cover (Figure 1F). Of the three sites in this study, benthic coverages were dominated by *Po. porites* and *Pa. caribaeorum*. However, there seemed to be mixed cover of *Po. porites* (24.0% ± 3.4), *Zoanthus* sp. (34.1% ± 4.1), and *Pa. caribaeorum* (17.3% ± 8.8) at PB.

Other reef benthic components included coral rubble, sand, stone, rock, sea anemones, sea urchins, fireworms, and gorgonians. Cnidaria diversity at each site also showed the presence of *Siderastrea radians* (Pallas, 1766), various octocorals, *Millipora alcicornis* Linnaeus, 1758, *Stichodactyla helianthus* (Ellis, 1768) and *Epicystis crucifer* (Le Sueur, 1817), *Porites astreoides* Lamarck, 1816, and *Diploria clivosa* (Ellis and Solander, 1786). Simpson's Index of Diversity (1-D) was 0.57 (SB), 0.65 (PB), and 0.50 (TB) showing PB with the most cnidaria diversity. All three sites showed mean zoantharian cover between 37–72%, which highlighted a zone that was extensively covered by brown mats (*P. caribaeorum*), interspersed with green covered rocks (*Zoanthus* spp.) making this feature a common characteristic at all sites. In this zone, space for algae growth is almost negligible as seen by the low cover, <10% at all sites.

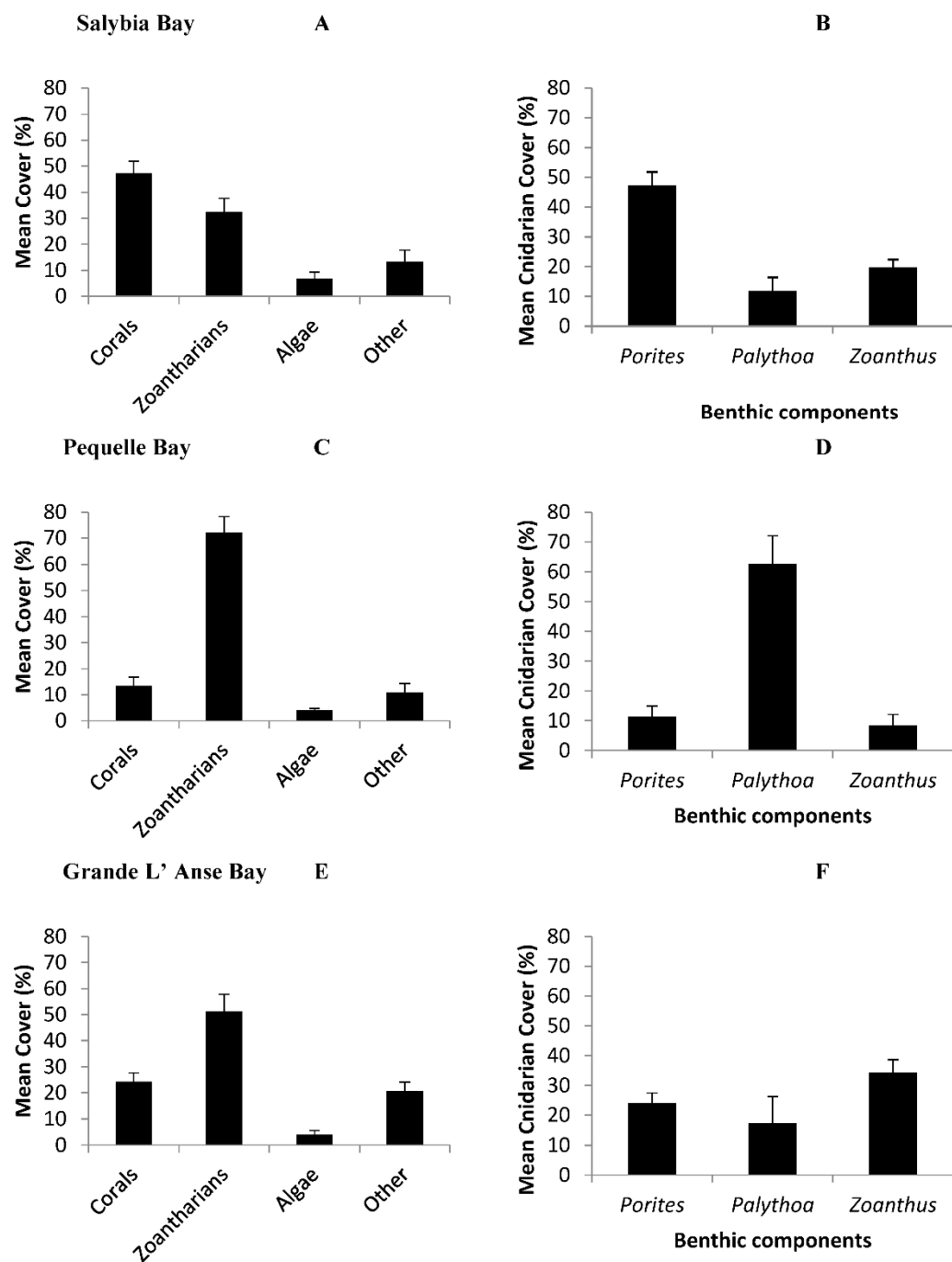


Figure 1. Mean benthic coverage (Mean% \pm SD) at three sites in the lower intertidal zones illustrating reef-building “corals”, zoantharians, algae represent green algae, and “Other” includes all other invertebrates, such as sea urchins, sea cucumbers (A,C,E), and mean benthic cover (Mean% \pm SD) of *Porites*, *Palythoa*, and *Zoanthus* spp. (B,D,E); (A,B) Salybia Bay (C,D) Pequelle Bay (E,F) Grande L’Anse Bay.

3.2. Specimen Morphological and Molecular Analyses

Morphological analyses and comparisons (see Table 1) showed specimens of *Palythoa caribaeorum* colonies (Figure 1A) with various levels of coenenchyme thickness (see description in [4,21]). *P. caribaeorum* oral disks and tentacle colors varied between brown and green, in comparison to that observed for *Zoanthus* spp. at all sites, where colors ranged from bright green, orange, grey, blue, and dark green. Maximum mean oral disk diameter for *P. caribaeorum* was $12.8 \text{ mm} \pm 0.96$ (Mean \pm SD, $n = 15$ polyps; 3 polyps per colony), which was larger than for *Zoanthus* spp. $10.2 \text{ mm} \pm 0.10$, $n = 15$ polyps (Table 1). Although *P. caribaeorum* and *P. grandiflora* (Figure 2B) could easily be identified through morphological analyses using maximum oral disk size, tentacle color, and tentacle numbers, analyses of *Zoanthus* spp. (Figure 2C–F) were challenging with the aforementioned characteristics. *Zoanthus* spp. colonies showed variation in coenenchyme thickness.

DNA was successfully amplified for 21 COI amplicons, which were approximately 780 bp in length. Phylogenetic analysis of the mitochondrial cytochrome oxidase subunit I (COI) gene identified zoantharians as *Zoanthus pulchellus*, and *Zoanthus sociatus* (see Figure 3) and harboring *Symbiodinium* sp. (formerly *Symbiodinium* clade A), whereas *Palythoa caribaeorum* and *Palythoa grandiflora* (Verrill, 1900) (see Figure 3) harbored *Cladocopium* sp. (formerly *Symbiodinium* clade C) in the symbiont family Symbiodiniaceae. The COI tree (Figure 3) distinguished *Palythoa* and *Zoanthus* spp., which belong to families Sphenopidae and Zoanthidae, respectively. Blasted specimens aligned with sequences from Florida (GenBank accession numbers JX119156, JX119154, JX119165) and Brazil (GenBank accession number KT454365). Other Atlantic Ocean sequences (GenBank accession numbers JX119160, JX119164, JX119167, JX119157, JX119159, JX119168 AB214177 KF499705, KF499712) aligned with closely related zoantharian species, such as *P. caribaeorum* and *P. grandiflora*; *Z. pulchellus* and *Z. vietnamensis* (Pax and Müller, 1957); *Z. sociatus* and *Z. sansibaricus* (Carlgren, 1900). For instance, *Palythoa* sp. (MZ150797-801, MZ147090-91) aligned with *Palythoa caribaeorum* (KT454365) and *Palythoa grandiflora* (JX119165). Additionally, *Zoanthus pulchellus* (JX119156) aligned with MZ150802-805, MZ147092-094, MZ156026, and was distinguished from *Zoanthus sociatus* (JX119154) aligned with MZ147095-097 and MZ150806-807, and were well supported by phylogenetic analyses (ML bootstrap% > 60%) (Figure 3).

Results for specimens in family Sphenopidae revealed moderate support for subclade *P. caribaeorum* (KT454365) and *P. grandiflora* (JX119165) (63%). Results for specimens in family Zoanthidae revealed moderate support for *Z. pulchellus* (JX119156) (69%), and high support for *Z. sociatus* (JX119154) (94%) (Figure 3). *Zoanthus pulchellus* specimens (MZ147092-094, MZ150802-805, MZ156026) also matched sequences from *Z. vietnamensis*, which is widely distributed across Indo-Pacific coral reefs, and *Zoanthus sociatus* specimens (MZ147095-097, MZ150806-807) sequences matched *Z. sansibaricus*, which is also widely distributed across Indo-Pacific coral reefs.

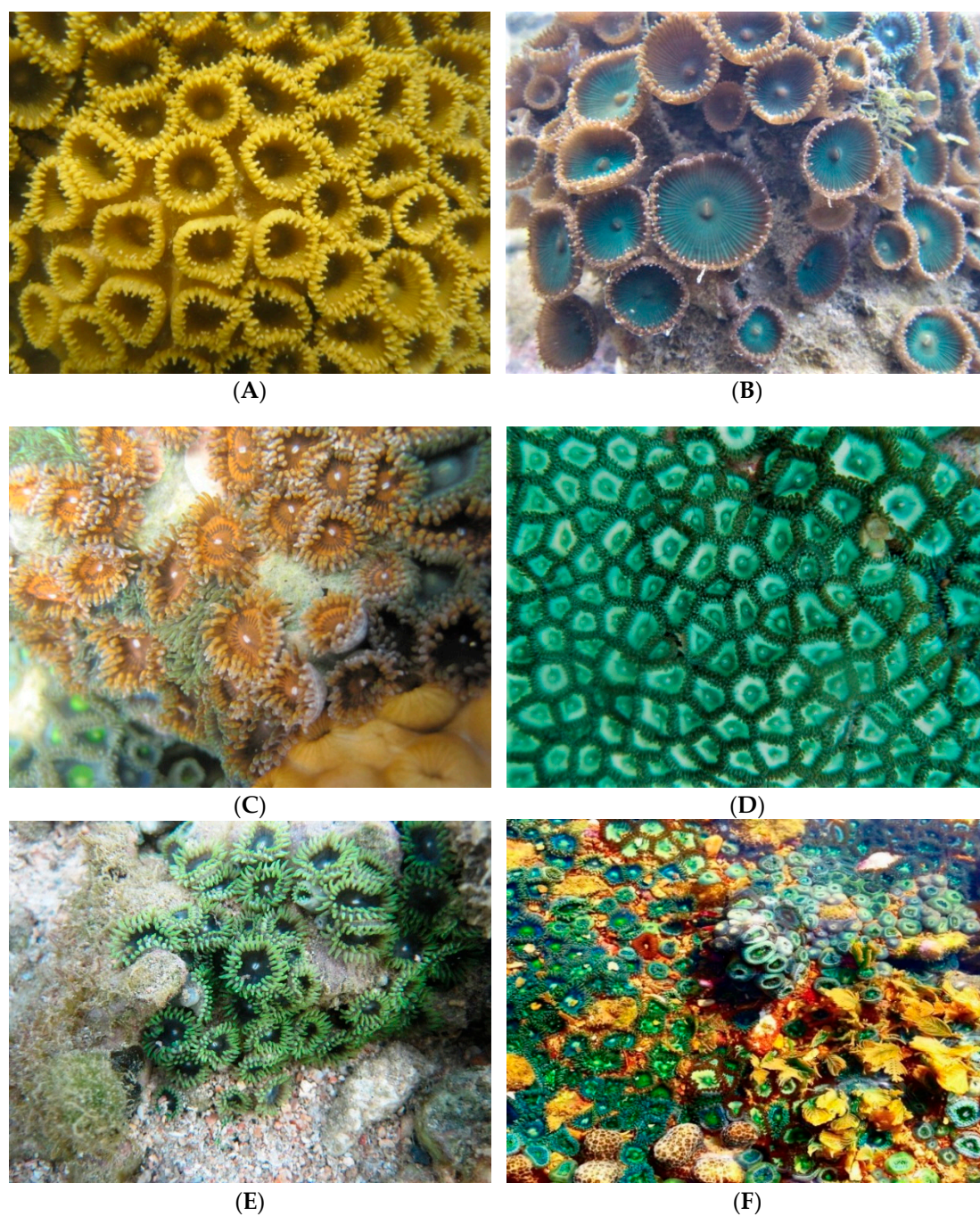


Figure 2. Zoantharian colonies along the northeastern coast of Toco, Trinidad (A) brown color morphotype of *Palythoa caribaeorum* specimen P3-br-SB, (B) green color morphotype of *Palythoa grandiflora* specimen P4-gr-GA, (C–E) color morphotype variation in *Zoanthus pulchellus* specimen Z9-org-GA, Z5-gr-SB, Z6-gr-SB, respectively (F) color morphotype in *Zoanthus sociatus* specimen Z15-blu-GA.

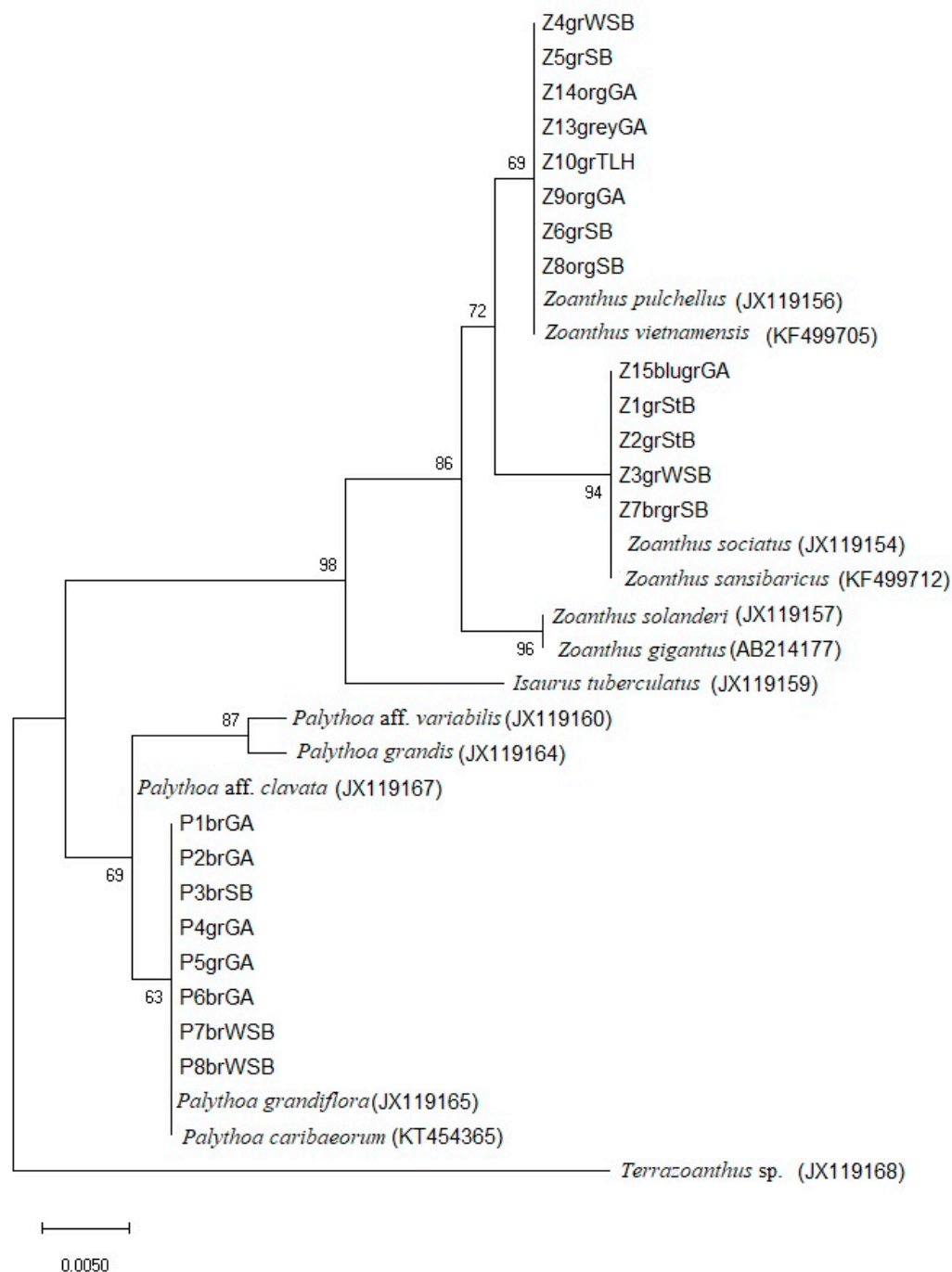


Figure 3. Phylogenetic maximum likelihood tree generated from the mitochondrial COI sequence alignment. GenBank accession numbers from other studies of species shown in parentheses. Numbers above branches represent maximum likelihood probabilities.

4. Discussion

Results showed the lower intertidal zones at Toco, Trinidad, to be dominated by zoantharians, specifically *Palythoa* and *Zoanthus* spp., similarly reported by Rabelo et al. [27] further south from Trinidad and Tobago on flat sandstone reefs in northeastern Brazil. As well, zoantharians were common in shallow-water habitats, and *Palythoa* and *Zoanthus* spp. were most common in shallow waters (<5 m), as reported at sites along the west coast of Curaçao [11]. In fact, Belford and Phillip [9,10,12,28] highlighted zoantharians being more abundant than their Scleractinia counterparts at this study's main sites. Lopez et al. [29] ob-

served extensive zoantharian coverage for species *Zoanthus solanderi* and *Zoanthus sociatus* at a “zoanthid zone” located at Cabo Verde Islands, central eastern Atlantic. Additionally, Karlson [6] observed similar extensive *Zoanthus* spp. coverage at Discovery Bay, Jamaica. This study reports a similar presence of zoantharians covering the majority of the benthic lower intertidal zone, dominated by *Palythoa* and *Zoanthus* spp. in the southern-most part of the Caribbean.

Although zoantharian coverage was extensive, identification of zoantharians was difficult, because color morphotypes varied among sites. Similar observations were mentioned by Reimer et al. [20] related to zoantharian identification in the field, and in other studies [30]. Phylogenetic and morphological analyses using the COI marker revealed *Palythoa* brown and green color morphs were specifically *Palythoa caribaeorum* and *Palythoa grandiflora*, respectively, while green, orange, grey, and blue-green were *Zoanthus pulchellus* and *Zoanthus sociatus*, respectively. However, it is worth mentioning that caution should be taken since only the COI marker was used in this study. For instance, Sinniger et al. [31] noted that although the COI marker is easily amplifiable with universal primers, and was hence used in this study, the addition of the mitochondrial 16S ribosomal DNA marker is useful for comparison and further species identification. In fact, the addition of 16S sequences adds distinct advantages as they are slightly more variable in zoantharians than COI, thereby adding useful phylogenetic information [31].

Closed polyps are often observed during extreme low tides, where desiccation plays a factor in distribution; however, Rabelo et al. [27] reported *Z. sociatus* resisting desiccation better than *P. caribaeorum*. However, in this study, the reverse was observed, where *P. caribaeorum* had a significantly higher benthic coverage than *Zoanthus* spp. at lower intertidal zones, nevertheless zoantharian distribution appears to be related to desiccation tolerance [27]. It is not precisely known why the reverse of [27] was observed in this study, however family Zoanthidae, such as *Z. pulchellus* and *Z. sociatus*, have generally been known to adapt to different environments [10,19]. Reimer et al. [20] reported variation in *Palythoa* sp. polyp form and color as a result of variable environments, such as degree of wave action, and benthic type, which were characteristics similarly observed at Toco, Trinidad [9,10,12,28].

Zoantharian color morphotypes were observed in both high and low wave action habitats, especially at the lower intertidal zones where *P. caribaeorum* carpeted much of the lower intertidal zones. However, *Z. pulchellus* mainly covered individual rocks, or crevasses, and displayed many colors throughout the intertidal zone. *Z. sociatus* also was found within crevasses, however it was not observed to be found in extensive coverage at sites in this study. It should be noted that extreme caution should be taken while surveying zoantharian coverage using color morphotypes to identify zoantharians since species may be conspecifics [8,19]. This study successfully used molecular analyses to assist with identification of *Zoanthus pulchellus* and *Zoanthus sociatus*. *Zoanthus* spp. display phenotypic plasticity in both oral disk color and polyp height [27]. Additionally, molecular analyses assisted in identifying *P. caribaeorum* and *P. grandiflora*; however it should be noted that these two species can be distinguished from morphological characteristics.

Further phylogenetic analyses of zoantharians confirmed the Symbiodiniaceae genera *Cladocopium* and *Symbiodinium* in *Palythoa* and *Zoanthus* spp., respectively. These results are consistent to past analyses of both species at different locations in the Caribbean Sea and Atlantic [31–33]. Similar results for both *Zoanthus* species mentioned in this study hosting *Cladocopium* and *Symbiodinium* were reported at Cape Verde Archipelagos [30]. As global climate will continue to affect oceanic water temperatures [34], identification and distribution of zooxanthellate zoantharians such as in this study will provide important baseline data for future analyses.

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Data Availability Statement: Photographs of zoantharian color morphotypes along the northeastern coast of Toco, Trinidad are available online at the Digital Public Library of America and Martin Methodist College Marine Biology Collection websites: <https://dp.la/search?q=martin+methodist+college> <https://www.artstor.org/2016/09/26/case-study-going-underwater-with-shared-shelf-commons/> (accessed on 9 June 2021).

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References

- Karlson, R.H. Alternate competitive strategies in a periodically disturbed habitat. *Bull. Mar. Sci.* **1980**, *30*, 894–900.
- Irei, Y.; Nozawa, Y.; Reimer, J.D. Distribution patterns of five zoanthid species in Okinawa Island, Japan. *Zool. Stud.* **2011**, *50*, 426–433.
- Irei, Y.; Sinniger, F.; Reimer, J.D. Description of two azooxanthellate *Palythoa* species (Subclass Hexacorallia, Order Zoantharia) from Ryukyu Archipelago, southern Japan. *Zookeys* **2015**, *478*, 1–26. [CrossRef] [PubMed]
- Reimer, J.D.; Shusuke, O.; Takishita, K.; Tsukahara, J.; Maruyama, T. Molecular evidence suggesting species in the zoanthid genera *Palythoa* and *Protopalpythoa* (Anthozoa: Hexacorallia) are congeneric. *Zool. Sci.* **2006**, *23*, 87–94. [CrossRef] [PubMed]
- Sinniger, F.; Montoya-Burgos, J.I.; Chevaldonné, P.; Pawlowski, J. Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. *Mar. Biol.* **2005**, *147*, 1121–1128. [CrossRef]
- Karlson, R.H. Reproduction patterns on *Zoanthus* spp. from Discovery Bay, Jamaica. Proceedings 4th International Coral Reef Symposium. *Manilla* **1981**, *2*, 699–704.
- Sebens, P.S. Intertidal distribution of zoanthids on the Caribbean coast of Panama: Effects of predation and desiccation. *Bull. Mar. Sci.* **1982**, *32*, 316–335.
- Reimer, J.D.; Ono, S.; Fujiwara, Y.; Takishita, K.; Tsukahara, J. Reconsidering *Zoanthus* spp. diversity: Molecular evidence of conspecificity within four previously presumed species. *Zool. Sci.* **2004**, *21*, 517–525. [CrossRef] [PubMed]
- Belford, S.G.; Phillip, D.A.T. Rapid assessment of a coral reef community in a marginal habitat in the southern Caribbean: S simple way to know what's out there. *Asian J. Biol. Sci.* **2011**, *4*, 520–531. [CrossRef]
- Belford, S.G.; Phillip, D.A.T. Intertidal distribution patterns of zoanthids compared to their scleractinian counterparts in the southern Caribbean. *Int. J. Oceanogr. Mar. Ecol. Syst.* **2012**, *3*, 67–75. [CrossRef]
- Reimer, J.D.; Wee, H.B.; García-Hernández, J.E.; Hoeksema, B.W. Zoantharia (Anthozoa: Hexacorallia) abundance and associations with Porifera and Hydrozoa across a depth gradient on the west coast of Curaçao. *Syst. Biodivers.* **2018**, *16*, 820–830. [CrossRef]
- Belford, S.G.; Phillip, D.A.T.; Rutherford, M.G.; Schmidt, R.S.; Duncan, E.J. Biodiversity of coral reef communities in marginal environments along the north-eastern coast of Trinidad, southern Caribbean. *Prog. Aqu. Farm. Mar. Biol.* **2019**, *2*, 180017.
- Goreau, T.F. The ecology of Jamaican coral reefs I. Species composition and zonation. *Ecology* **1959**, *40*, 67–90. [CrossRef]
- Burnett, W.J.; Benzie, J.A.H.; Beardmore, J.A.; Ryland, J.S. Zoanthids (Anthozoa, Hexacorallia) from the Great Barrier Reef and Torres Straights, Australia: Systematics, evolution and a key to species. *Coral Reefs* **1997**, *16*, 55–68. [CrossRef]
- Ryland, J.S.; Lancaster, J.E. Revision of methods for separating species of *Protopalpythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. *Invert. Syst.* **2003**, *17*, 407–428. [CrossRef]
- López, C.; Reimer, J.D.; Brito, A.; Simón, D.; Clemente, S.; Hernández, M. Diversity of zoantharian species and their symbionts from Macaronesian and Cape Verde ecoregions demonstrates their widespread distribution in the Atlantic Ocean. *Coral Reefs* **2019**, *38*, 269–283. [CrossRef]
- LaJeunesse, T.C.; Parkinson, J.E.; Gabrielson, P.W.; Jeong, H.J.; Reimer, J.D.; Voolstra, C.R.; Santos, S.S. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbiont. *Curr. Biol.* **2018**, *28*, 2570–2580. [CrossRef] [PubMed]
- Burnett, W.J. Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. *Mar. Ecol. Prog. Ser.* **2002**, *234*, 105–109. [CrossRef]

19. Reimer, J.D.; Shusuke, O.; Yasuo, F.; Junzo, T. Seasonal changes in morphological condition of symbiotic dinoflagellates (*Symbiodinium* spp.) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia) in Southern Japan. *South Pac. Stud.* **2007**, *27*, 2.
20. Reimer, J.D.; Foord, C.; Irei, Y. Species diversity of shallow water zoanthids (Cnidaria: Anthozoa: Hexacorallia) in Florida. *Hindawi Publ. Corp. J. Mar. Biol.* **2012**, *2012*, 856079. [[CrossRef](#)]
21. Pax, F. Studien an westindischen Actinien. In *Ergebnisse einer Zoologischen nach Westindien von Prof. W. Kukenthal und Dr. R. Hartmeyer im Jahre, 1907*; Spengel, J.W., Ed.; Zoologische Jahrbucher Supplement: Ann Arbor, MI, USA, 1910; Volume 11, pp. 157–330.
22. Sinniger, F.; Reimer, J.; Pawlowski, J. The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: Description of two new genera. *Mar. Biodivers.* **2010**, *40*, 57–70. [[CrossRef](#)]
23. LaJeunesse, T.C.; Trench, R. Biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal sea anemone *Anthopleura elegantissima* (Brandt). *Biol. Bull.* **2000**, *199*, 126–134. [[CrossRef](#)] [[PubMed](#)]
24. LaJeunesse, T.C. Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Mar. Biol.* **2002**, *141*, 387–400.
25. LaJeunesse, T.C.; Thornhill, D.J. Improved resolution of reef-coral endosymbiotic dinoflagellate (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. *PLoS ONE* **2011**, *6*, e29013. [[CrossRef](#)] [[PubMed](#)]
26. Kumar, S.; Strecher, G.; Li, M.; Knyaz, C.; Tamura, K. Molecular evolutionary genetic analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [[CrossRef](#)] [[PubMed](#)]
27. Rabelo, E.F.; Soares, M.D.; Bezerra, L.E.; Matthews-Cascon, H. Distribution patterns of zoanthids (Cnidaria: Zoantharia) on a tropical reef. *Mar. Biol. Res.* **2015**, *11*, 584–592. [[CrossRef](#)]
28. Belford, S.G. Spatial abundance and colour morphotype densities of the rock boring sea urchin (*Echinometra lucunter*) at two different habitats. *Thalassas* **2020**, *36*, 157–164. [[CrossRef](#)]
29. López, C.; Freitas, R.; Magileviciute, E.; Ratão, S.S.; Brehmer, P.; Reimer, J.D. Report of a *Zoanthus* zone from the Cabo Verde islands (Central eastern Atlantic). *Thalassas* **2018**, *34*, 409–413. [[CrossRef](#)]
30. Ong, C.W.; Reimer, J.D.; Todd, P.A. Morphological plastic responses to shading in the zoanthids *Zoanthus sansibaricus* and *Palythoa tuberculosa*. *Mar. Biol.* **2013**, *160*, 1053–1064. [[CrossRef](#)]
31. Sinniger, F.; Reimer, J.D.; Pawlowski, J. Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). *Zool. Sci.* **2008**, *25*, 1253–1260. [[CrossRef](#)] [[PubMed](#)]
32. LaJeunesse, T.C.; Loh, W.; Van Woesik, R.; Hoegh-Guldberg, O.; Schmidt, G.; Fitt, W. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Am. Soc. Limnol. Oceanogr.* **2003**, *48*, 2046–2054. [[CrossRef](#)]
33. Kumara, S.; Zacharia, P.U.; Sreenath, K.R.; Kripa, V.; George, G. GIS based mapping of zoanthids along Saurashtra coast, Gujarat, India. *J. Mar. Biol. Assoc. India* **2017**, *59*, 19–25.
34. Hughes, T.P.; Baird, A.H.; Bellwood, D.R.; Card, M.; Connolly, S.R.; Folke, C.; Grosberg, R.; Hoegh-Guldberg, O.; Jackson, J.B.C.; Kleypas, J.; et al. Climate change human impacts and the resilience of coral reefs. *Science* **2003**, *301*, 929–933. [[CrossRef](#)] [[PubMed](#)]