UNIVERSIDADE FEDERAL DO PARANÁ

MARIA EDUARDA ALVES DOS SANTOS

Diversity and distribution patterns of the order Zoantharia (Cnidaria: Anthozoa)

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Dissertação apresentada como requisito parcial para obtenção do grau de Mestre em Sistemas Costeiros e Oceânicos. Curso de Pós-Graduação em Sistemas Costeiros e Oceânicos, Centro de Estudos do Mar, Setor de Ciências da Terra, Universidade Federal do Paraná.

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"Diversity and distribution patterns of the order Zoantharia (Cnidaria: Anthozoa)"

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Maria Eduarda Alves dos Santos

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RESUMO

Os padrões de diversidade nos oceanos são foco de teorias ecológicas e evolutivas. Estudos correlacionando dados filogenéticos e da distribuição de organismos permitem corroborar essas teorias que explicam os processos geradores da diversidade marinha. A compreensão desses processos também auxilia prever as consequências de eventos tais como alterações climáticas e bioinvasões

Os animais da ordem Zoantharia (Cnidaria: Anthozoa) são encontrados da zona entremarés até profundidades abissais em todos oceanos. Zoantários formam extensas colônias, as quais servem de proteção e alimento para outros organismos. Apesar de serem um grupo de animais abundantes e cosmopolitas, dados sobre a distribuição das espécies ainda são escassos em diversos locais. Por exemplo, até a realização desta dissertação não haviam estudos investigando as espécies de Zoantharia da costa brasileira e sua distribuição nessa província. A falta de investigações sobre a diversidade do grupo é causada principalmente pela dificuldade de identificação das espécies, as quais apresentam uma grande plasticidade morfológica. Para solucionar essa dificuldade, estudos acoplando dados moleculares e taxonômicos mostram ser uma excelente forma de identificar esses animais. Dados moleculares também acresceram no conhecimento sobre a história evolutiva da ordem Zoantharia, um dos grupos que divergiram mais basais em Metazoa. Até o momento não foram estimadas relações filogenéticas do grupo utilizando todos as famílias/genêros com dados disponíveis em bases de dados tais como o GenBank. Em adição, dados moleculares indicam um relacionamento estreito entre espécies de zoantário dos oceanos Atlântico e Pacífico/Índico, entretanto nenhum trabalho investigou quais são são esses pares de espécie e que processos podem ter gerado esse padrão.

O objetivo desta dissertação é contribuir na elucidação de padrões evolutivos nos oceanos, utilizando como modelo os zoantários. Nós analisamos as relações filogenéticas da ordem Zoantharia em dois aspectos complementares: 1) grandes clados e 2) espécies geneticamente próximas. Adicionalmente, nós

relacionamos esses dados com ecologia e zoogeografia do grupo. No primeiro capítulo, a relação filogenética entre todas as famílias de Zoantharia é analisada em conjunto com as principais características ecológicas de cada clado. O segundo capítulo examina a diversidade e distribuição de zoantários no Atlântico sudeste, preenchendo a lacuna de conhecimento sobre o grupo na costa brasileira. Os resultados desse capítulo são utilizados também na investigação das espécies próximas de zoantários entre as duas bacias oceânicas (Oceano Atlântico e oceanos Pacífico e Índico) no terceiro capítulo.

ABSTRACT

Diversity patterns in the oceans are focus of ecological and evolutionary theories. Studies correlating phylogenetic and distribution data of species allow support these theories which explain the processes generators of marine diversity. Undestand these processes also allow predict the consequences of events such as climate changes and bioinvasons.

Animals of the order Zoantharia (Cnidaria: Anthozoa) occur from intertidal to abissal zones in all oceans. Species of the group are able to form extensive colonies that serve as shelter and food resource to other organisms. Althought zoantharian are abundant and cosmopolitan, distribution species data are still scass in several localities. For example, until the present research, there were no studies on Zoantharia species in brazilian coast. The lack of investigation of the group diversity is mostly due to the difficulties in species identification, which present a high interspecific morphological variability. In order to overcome this problem, studies using both morphological and molecular data have proven to be an excellent way to identify species. Molecular data have also provide a better knowledge on the evolution history of the group, however, there is no estimarion of phylogenetic relationships between all the genera with data avaialable in data bases such as GenBank. Moreover, molecular data indicated a close-related relationship between species on Atlantic Ocean and Pacific/Indian oceans, but no study have investigation which are these species.

The goal of this dissertation is contribute in the elucidation of evolutionary patterns in the oceans, using as a model the zoantharians. We analyze phylogenetic relationships of the order Zoantharia in two complementary aspects: 1) large clades 2) close-related species. Furthemore, we linked these data with ecology and zoogeograpy of the group. In the first chapter, phylogenetic relationships between all Zoantharia families is analyzed along with their ecological traits. The second chapter examine diversity and distribution of zoantharians in southwest Atlantic, filling the gap of Zoantharia diversity in Brazil. Data of this study is also used in the investifation of close-

related species between the two ocean basins (Atlantic Ocean and Pacific/Indian oceans) on the third chapter.

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CAPÍTULO 1

Ecological traits and phylogeny of Zoantharia (Cnidaria: Hexacorallia)

Características ecológicas e filogenia dos zoantários

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Key words: Phylogenetic relationships, traits, benthos, Cnidaria

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Resumo

Análises filogenéticas acopladas com características ecológicas spara a compreensão da evolução de grupos marinhos. Zoantários (Cnidaria: Anthozoa) são animais basais que desempenham um importante papel ecológico e possuem distribuição cosmopolita. Portanto, eles são um excelente sistema para esse tipo de investigação. Nós examinamos relações filogenéticas das famílias da ordem Zoantharia usando os marcadores mitocondriais 16S rDNA e COI, juntamente com dados das características ecológicas das espécies. Os resultados da filogenia das nove famílias de zoantários suporta a divisão entre as subordens Brachycnemina e Macrocnemina. A maioria das características avaliadas tem origem em diferentes familías, tais como presença em oceano profundo, incorporação de sedimento no tecido e simbiose com zooxantela. O sucesso do grupo em ocupar diferentes ambientes provavelmente está relacionado à habilidade de adquirir/perder o status das características em diferentes clados. A elucidação de características adicionais, como o tipo de larva e metabólitos secundários produzidos, irá aprimorar nosso conhecimento da evolução de Zoantharia.

Ecological traits and phylogeny of Zoantharia (Cnidaria: Hexacorallia)

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Key words: Phylogenetic relationships, traits, benthos, Cnidaria

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Abstract

Phylogenetic analysis coupled with ecological traits are key to understand evolution in marine groups. Zoantharians (Cnidaria: Anthozoa) are early metazoans that play an important ecological role and occur in all oceans. Thus, they are an excellent system to perform these kind of investigation. We here examined phylogenetic relationship between families of order Zoantharia using mitochondrial markers 16S rDNA and COI, coupling this data with species ecological traits. Results of phylogenetic relationships among the nine zoantharian families support the division of suborders Brachycnemina and Macrocnemina. Mostly traits evaluated have origins in different families, such as

invasion of deep sea, incorporation of sediment in the tissue and symbiosis with zooxanthellae. The success of the group in occupy different environments is probably related to the ability to gain/lose these traits among different clades. Further elucidation of additional characteristics, such as larvae type and secondary metabolites production, will improve our knowledge on Zoantharia evolution.

Introduction

Investigations on phylogenetic relationships coupled with ecological data have enlightened our understanding of evolutionary processes in many marine taxa. For example, research on reef fishes have shown that lineage diversification may have been influenced by the ecological opportunity of preying upon different benthos [1]. Likewise, phylogenetic analyses of scleractinian corals suggest that deep-water solitary species are the most basal extant coral in many lineages of the group [2]. Molecular investigation of other common benthic anthozoans, such as sea anemones and corallimorpharians, have also increased our knowledge of their evolutionary history [3,4]. As an ancient metazoan form of life [5], a better understanding of the evolutionary history of cnidarians will not only help clarify animal evolution, but also improve our knowledge on many important questions regarding the phylum.

Zoantharians (Cnidaria: Anthozoa: Zoantharia) are found in all oceans and represent an important benthic group. Species of the order inhabit different marine systems such as coral reefs, sand patches, rubble zones, rodolith beds and artificial substrates, from the intertidal to abyssal zones [6–10]. They are the dominant taxa in some localities and can serve as shelter or food source to other organisms [11–13]. Moreover, bioprospecting surveys of zoantharians have demonstrated promising results in the field of pharmacognosy [14–16]. In a broader view, the widest evolutionary reconstruction focusing on Zoantharia was in a study by Fujii and Reimer [17]. These authors investigated all nine families of the order, using sixteen out of the twenty-six genera, with one species from each genus. Another comprehensive phylogeny of zoantharians by Swain [18] investigated sphincter muscle evolution, using eighteen genera from seven families. Thus, there has been no phylogeny of the order Zoantharia that has examined all genera with molecular sequences available in data bases such as GenBank.

Here, we investigated the phylogenetic relationships between twenty-three genera from all Zoantharia families, undertaking the most comprehensive phylogeny of the group up to now. In addition, we coupled analyses with their main ecological traits as available in the literature. Our study will contribute in the understanding of both the order Zoantharia and basal metazoan evolutionary history.

Results

Phylogenetic relationships of all nine families in the Zoantharia, along with their ecological traits, are summarized below and in Figure 1 (GenBank accession numbers used are indicated in S1).

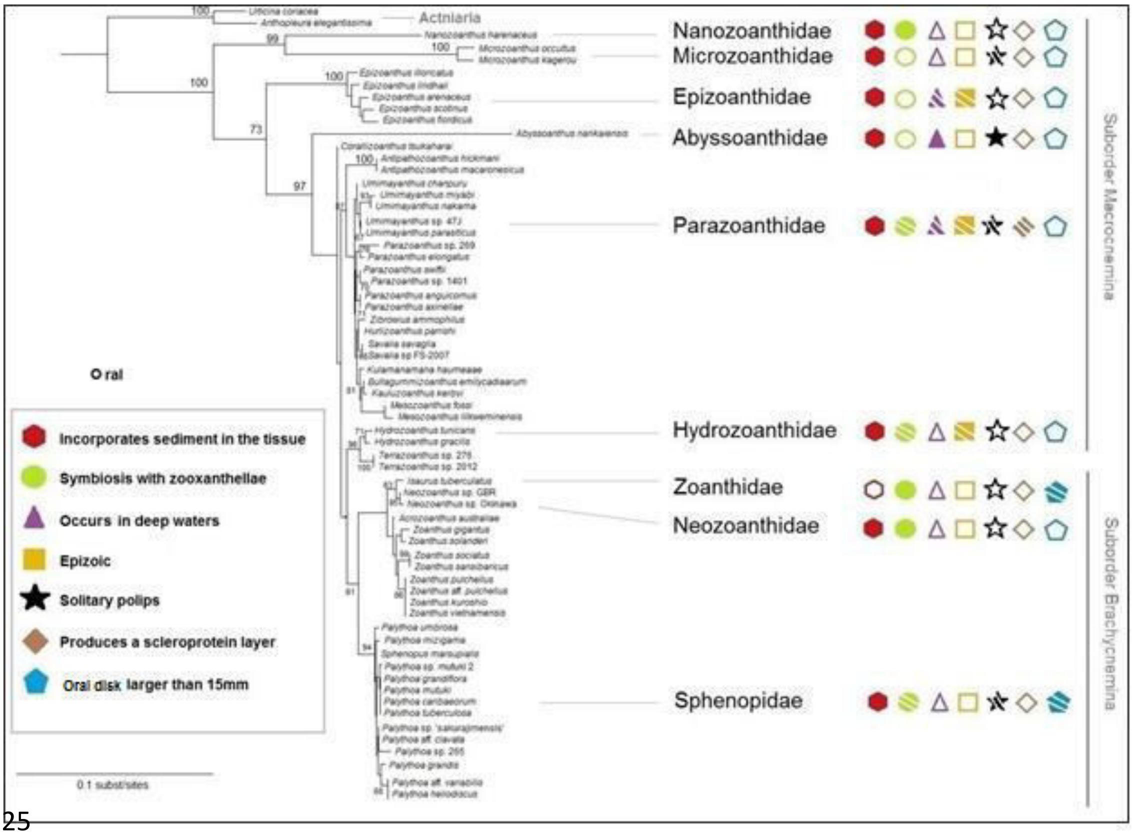

Figure 1: Estimated ML phylogenetic tree of order Zoantharia with mitochondrial markers 16S rDNA and COI. Sequences from representative of all nine zoantharians families were included: Nanozoanthidae, Microzoanthidae, Epizoanthidae, Parazoanthidae and Hydrozoanthidae of the suborder Macrocnemina; Sphenopidae, Neozoanthidae and Zoanthidae of the suborder Brachycnemina, and Abyssoanthidae (incertae sedis). Unfilled symbol: trait not present in family; symbols with patterned lines = trait present in at least one species of the family; filled symbols = trait present in all of species of family.

In our analyses, suborder Brachycnemina was monophyletic, as well as families Sphenopidae and Neozoanthidae of this suborder, while Zoanthidae was paraphyletic. In contrast, the suborder Macrocnemina was paraphyletic, although all families from this suborder were monophyletic. All zoantharians incorporate sediment in the tissue in some degree to the exception of species in the family Zoanthidae. All representatives of families Abyssoanthidae, Epizoanthidae, and Microzoanthidae are azooxanthellate, while those of families Zoanthidae, Neozoanthidae, and Nanozoanthidae are zooxanthellate. Others families have both zooxanthellate and azooxanthellate species. Epizoic relationships occurs in three Macrocnemina families (Epizoanthidae, Parazoanthidae, Hydrozoanthidae) and one family of Brachycnemina (Zoanthidae), while production of a scleroprotein layer is an exclusive trait of the Parazoanthidae genera Savalia, Kulamanamana, and potentially Antipathozoanthus [19]. With exception of Nanozoanthidae, Microzoanthidae and Hrydrozoanthidae, all families from suborder Macrocnemina have species reported to deep-waters, while zoantharians from suborder Brachycnemina are exclusively found in shallow environments. In addition, family Abyssoanthidae is exclusively found in the deep sea. Species with large orals disk are observed only in Brachycnemina.

Analyses of maximum likelihood (ML) ancestral trait reconstruction suggests that incorporation of sediment in tissue is an ancestral state (proportional likelihood = 99; Figure S2), while symbioses with zooxanthellae, presence in deep sea, epizoic relationship, non-colonial polyps, production of scleroprotein layer, and large oral disk (>15mm) are derived tratis (proportional likelihoods = 95, 69, 96, 99, 99, 97, respectively; Figures S3-8). Sediment-free status was evolved in the family Zoanthidae, yet, the ability of incorporate sediment was re-gained in a close-related clade (family Neozoanthidae; Figure S2). Symbioses with zooxanthellae evolved independently at least three times (family Nanozoanthidae, family Parazoanthidae and the ancestor of family Hydrozoanthidae and suborder Brachycnemina), although some clades recovered the azooxanthellate status latter in evolution (species within family Sphenopidae; Figure S3). The occurrence in deep-water have different origins, and it is a trait restrict to suborder Macrocnemina (Figure S5). In addition, production of a scleroprotein axis has only been reported in the macrocnemic family Parazoanthidae (Figure S7). All epizoic extant zoantharians likely have the same common ancestor (proportional likelihood = 83; Figure S4). The change from colonial state to solitary polyps has occurred at least twice (families Abyssoanthidae and Sphenopidae; Figure S6), as well as the development of polyps with a large oral disk (families Zoanthidae and Sphenopidae; Figure S8).

Discussion

The phylogenetic relationships of families within the order Zoantharia in figure 1 corroborate relationships observed in previous studies [17,18,20]. Sequences of the families Abyssoanthidae, Nanozoanthidae and Microzoanthidae are highly divergent from other zoantharians, even with mitochondrial markers, which are conserved in most anthozoan orders [21]. ML ancestral reconstruction traits indicated that the common ancestor of extant zoantharians was a colonial, azooxanthellate, shallow-water species with small polyps and sediment in the tissue that was not epizoic and did not produce a scleroprotein layer. This is different from what is known for other benthic cnidarians such as scleractinian corals, which likely had a solitary polyp ancestor from the deep sea [2], and stylasterid hydrozoans that also evidently evolved from a deep-water ancestor [22]. In our analyses, ancestral trait results were greatly influenced by the traits of families Microzoanthidae and Nanozoanthidae, two taxa that have recently been described [7,17] with records only from less than 50 meters depths. Thus, it is possible that species from both families may also be present in the deep-sea, but this is not yet known. Clearly, understanding the full breadth of these two families' distributions and traits is critical to understanding the past evolution of the order as a whole.

The different ecological traits of species, along with the high morphological variability reported to zoantharians [6,23], must be the result of use of distinct resources, related for example to nutrition or habitat occupation. These traits have synergetic effects on species ecology. For example, a larger polyp diameter in anthozoans has been related to production of a higher volume of egg and testes [24] and with the capacity to ingest bigger prey items [25]. We discuss the ecological traits of zoantharians according to probable functions, although it must be highlighted that these characteristics may interact in a complex way and their outcome/function is often still unknown. Detailed research with a focus on these interactions is needed to clarify the function and importance of many of these traits, including polyp size and epizoic relationship.

Some genera in order Zoantharia present polyps without sediment incorporation, or sediment incorporation in differing levels into the tissue, or may even secrete a scleroprotein layer. These three kind of polyp structures are able to occupy a major percentage of the substrate, such as large colonies of the sediment-free genus *Zoanthus* [26], “mats” of sediment aggregator *Palythoa* species [27], or colonies that produce a scleroprotein axis and create three-dimensional structures similar to scleractinian corals as seen in genus *Savalia* [28]. The ability to produce a scleroprotein layer is exclusive to the family Parazoanthidae, and has evolved in at least three genera. Species with this ability can be epizoic of antipatharians and octocorals. Investigations of epizoism and symbiosis with zooxanthellae have previously been conducted in the order Zoantharia by Swain [29]. He investigated families Parazoanthidae, Epizoanthidae and Hydrozoanthidae of the suborder Macrocnemina, which have both zooxanthellate and azoxanthellate species and can be epizoic, while all the species analyzed in the suborder Brachycnemina are zooxanthellate. We incorporate data available for the families described in the suborder Macrocnemina which do not have epizoic relationship (Microzoanthidae and Nanozoanthidae, as well as family Abyssoanthidae that is likely macrocnemic). Also, genus *Palythoa* from suborder Brachycnemina was found to present zooxanthellate-free species. This confirms that epizoism is frequently observed in species of Macrocnemina, however, some macrocnemic clades do not present this trait. Also, even though species of suborder Brachycnemina are not present in the deep ocean, there are azooxanthellate brachycnemic zoantharians. These species are found in shaded habitats like caves [30]. As there are also zooxanthellate and zooxanthellate-free macrocnemic species, this shows that zoantharians evolved the ability to gain/lose the symbiont relatively quickly. Corals also had the evolution of symbiosis with zooxanthellae in different clades [2].

Polyps with larger oral disks have evolved only in Brachycnemina. Furthermore, suborder Macrocnemina includes families with diminutive polyps (diameter smaller than 1 millimeter) that have not been reported in any other group of zoantharians [7,17]. The function of polyp size is still unclear in Zoantharia, although it may be related to the ingestion of particles of suitable size. For example, *Zoanthus sociatus* consumes detritus and smaller zooplankton items than other sympatric *Zoanthus* and *Palythoa* species that have a larger polyp diameter [31].

There is an overall lack of data about life history traits for almost all families of order Zoantharia, although some general patterns have been speculated on. For example, it appears that species in the suborder Brachycnemina have different larvae types than in Macrocnemina [32]. Further research focusing on type of reproduction and larvae will improve the understanding of the group' evolution.

In addition, zoantharians are known to produce a high number of secondary metabolites, yet only a few species have been investigated. These metabolites can be incorporated in the food web by other animals that prey upon zoantharians, with reports even in top predators' tissues [33,34]. Some molecules classes can be found in several clades, such as palytoxin reported from genera *Palythoa*, *Zoanthus* and *Parazoanthus* [35,36], while other substances have been reported until now only for a particular taxa (e.g. parazoanthines in genus *Parazoanthus* [37] or palyosulfonocetamides in genus *Palythoa* [14]). How specific are the secondary metabolite production pathways between species/genera/families and the role of subsequent chemical traits in the evolution and ecology of Zoantharia are still unknown.

Moreover, investigations of species' functional traits have been used to predict and explain the structure of communities and their responses to environmental conditions [38]. For instance, the propensity of reef fish species to disperse by rafting is believed to be related to specific traits, such as the position in water column above substratum and schooling behavior [39]. Functional traits are relatively more difficult to be recognized in benthic animals such as zoantharians but identification and interpretation of these characters are essential to understanding evolution in marine systems.

Conclusions

Phylogenetic relationships among the nine large clades (=families) indicated the suborder Brachycnemina is monophyletic, while Macrocnemina is pparaphyletic. The success of the group in occupying various habitats is probably due the different ecological traits developed by the zoantharian clades. For instance, zoantharians can have solitary polyps or form large colonies, as well as can incorporate sediment in tissue or produce a scleroprotein layer. Also, clades of the group can gain/lose the traits relatively quickly. However, there are still many ecological traits to be investigated, such as those related to reproduction and the production of secondary metabolites. Such research is essential to a better understanding of how these traits evolved in early metazoans.

Material and methods

Phylogenetic analyses

Phylogenetic relationships of the order Zoantharia were estimated using a concatenated alignment of sequences from the molecular mitochondrial markers 16S ribosomal DNA (mt 16S rDNA) and cytochrome oxidase subunit 1 (CO1). These markers were selected as they have the largest number of data available in GenBank, as well as being reported to have a good phylogenetic signal for the group [40]. We analyzed as many genera as possible from all the nine families of the order, and following included actiniarian representatives as outgroup. In total, sixty-eight species from twenty-three out of the twenty-six genera of Zoantharia were examined (S1). Species of the genera *Paleozoanthus* and *Thoracactis* (family Epizoanthidae) could not be analyzed because there are no data in GenBank, while *Isozoanthus* (family Parazoanthidae) does not have species sequences for both mt 16S rDNA and COI. We generated a concatenated alignment (16S rDNA+COI) using Clustal Omega [41], in which 16S rDNA sequences were edited with Gblock [42]. Phylogenetic relationships were estimated via PhyML [43] for Maximum-Likelihood (ML) analyses with the Kimura-2-parameter model of nucleotide distribution and estimated parameters[44]. Robustness of the ML reconstruction was tested with 1,000 booststrap replicates.

Ancestral state reconstruction of traits

Zoantharia trait data was compiled from the literature for all nine families. We herein consider as “traits” any characteristic that may be related directly or indirectly to the ecology of the group. We only used traits that had data available on previous published literature for all families. The following traits were analyzed: production of scleroprotein layer; incorporation of sediment in the tissue; epizoic relationship; symbiotic relationship with zooxanthellae; occurrence in deep sea (<200 meters); polyp type (colonial or not); and oral disk size larger than 15mm [7–9,19,20,29,30,45–54]. We limited our discussion to aspects not in previous studies. Ancestral state estimation was performed using Mesquite 3.04 [55] with a single-parameter Markov model (Mk1).

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Supporting information captions

Table S1: GenBank accession numbers of DNA sequences used in phylogenetic reconstructions in this study along with status of traits used in Mesquite analysis

Suborder	**Family**	**Species**	**Ecological trait**							**GenBank acession number**	
			Incorporates sediment in the tissue	Symbiosis with zooxanthellae	Epizoic	Occurs in deep waters	Produces a scleroprotein layer	Disk oral larger than 15mm	Colonial polyps	COI	16S rDNA
Actiniaria		*Urticina coriacea*								U91615	U91752
		Anthopleura elegantissima								KM612198	U40292
Macrocnemina	Microzoanthidae	*Microzoanthus kagerou*	1	0	0	0	0	0	0	HQ912802	HQ912853
		Microzoanthus occultus	1	0	0	0	0	0	0	HQ912861	HQ912860
	Nanozoanthidae	*Nanozoanthus harenaceus*	1	1	0	0	0	0	0	KF499610	KF499602
-	**Abyssoanthidae**	*Abyssoanthus nankaiensis*	1	0	0	1	0	0	1	AB247364	AB247346
Macrocnemina	Epizoanthidae	*Epizoanthus illoricatus*	1	0		0	0	0	0	AB247349	AY995929
		Epizoanthus fiordicus	1	0	1	0	0	0	0	EF672675	EF687814
		Epizoanthus arenaceus	1	0		0	0	0	0	EF672672	EF687812
		Epizoanthus scotinus	1	0		0	0	0	0	HM042365	KC218440
		Epizoanthus lindhali	1	0			0	0	0	EF672677	ef687816
	Parazoanthidae	*Antipathozoanthus macaronesicus*	1		1	0		0	0	HM130496	HM130467
		Antipathozoanthus hickmani	1					0	0	EU333790	EU333757
		Mesozoanthus lilkweminensis	1				0	0	0	HM042383	HM042363
		Mesozoanthus fossii	1	0	1	0	0	0	0	EF672653	EF687822
		Terrazoanthus sp. JDR-2012	1		1		0	0	0	EF672653	JX119153
		Terrazoanthus sp. 276	1		1		0	0	0	HM130495	HM130473

		Kauluzoanthus kerbyi	1	0		1	0	0	0	KC218393	KC218437
		Zibrowius ammophilus	1	0		1	0	0	0	KC218395	KC218439
		Savalia sp. FS-2007	1	0			1	0	0	EF672658	EF687819
		Savalia savaglia	1	0	1		1	0	0	AB247356	AY995925
		Corallizoanthus tsukaharai	1	0	1	1	0	0	0	KC218396	EU035623
		Bullagummizoanthus emilyacadiaarum	1	0		1		0	0	KC218390	KC218434
		Kulamanamana haumeaae	1	0		1	1	0	0	KC218388	KC218432
		Hurlizoanthus parrishi	1	0		1		0	0	KC218389	KC218433
		Parazoanthus axinellae	1	0	1	0	0	0	0	EF672659	AY995935
		Parazoanthus anguicomus	1	0	1		0	0	0	EF672660	EF687827
		Parazoanthus elongatus	1	0	1	0	0	0	0	EF672662	EF687829
		Parazoanthus sp. 269	1		1	0	0	0	0	HM130497	HM130468
		Parazoanthus sp. 1401	1			0	0	0	0	HM130500	HM130480
		Parazoanthus swiftii	1	0	1	0	0	0	0	AB247350	KR092446
		Parazoanthus parasiticus	1	1	1	0	0	0	0	EF672663	AY995938
		Umimayanthus sp. 47J	1		1	0	0	0	0	KR092600	KR092462
		Umimayanthus chanpuru	1		1	0	0	0	0	KR092640	KR092507
		Umimayanthus nakama	1		1	0	0	0	0	KR092580	KR092458
		Umimayanthus miyabi	1		1	0	0	0	0	KR092571	KR092455
	Hydrozoanthidae	*Hydrozoanthus gracilis*	1	1	1		0	0	0	KF499722	KF499686
		Hydrozoanthus tunicans	1	1	1		0	0	0	EF672667	EU828760
Brachycnemina	Sphenopidae	*Palythoa* sp. 265	1	1	0	0	0	0	0	HM130481	HM130464
		Palythoa heliodiscus	1	1	0	0	0	0	0	AB219216	AB219224
		Palythoa aff. variabilis	1	1	0	0	0	0	0	JX119162	JX119137
		Sphenopus marsupialis	1	1	0	0	0	0	1	JQ323179	JQ323164
		Palythoa umbrosa	1	0	0	0	0	0	0	LC006910	LC007002
		Palythoa grandis	1	1	0	0	0	0	0	JX119164	JX119139

	Palythoa mizigama	1	0	0	0	0	0	0	LC006941	LC007035
	Palythoa aff. clavata	1	1	0	0	0	0	0	KJ794177	JX119141
	Palythoa sp. 'sakurajimensis'	1	1	0	0	0	0	0	AB219217	KF499685
	Palythoa tuberculosa	1	1	0	0	0	0	0	AB219207	AB219219
	Palythoa caribaeorum	1	1	0	0	0	0	0	AB219208	HM130470
	Palythoa sp. mutuki 2	1	1	0	0	0	0	0	AB219212	AB219221
	Palythoa mutuki	1	1	0	0	0	0	0	AB219217	AB219225
	Palythoa grandiflora	1	1	0	0	0	0	0	JX119165	JX119142
Neozoanthidae	*Neozoanthus* sp. GBR	1	1	0	0	0	0	0	HM991257	HM991229
	Neozoanthus sp. Okinawa	1	1	0	0	0	0	0	HM991246	HM991242
Zoanthidae	*Zoanthus kuroshio*	0	1	0	0	0	1	0	AB252668	AB235410
	Zoanthus aff. *pulchellus*	0	1	0	0	0	0	0	HM130492	JX119145
	Zoanthus vietnamensis	0	1	0	0	0	0	0	KF499749	AB235409
	Zoanthus pulchellus	0	1	0	0	0	1	0	JX119156	jx119147
	Zoanthus solanderi	0	1	0	0	0	1	0	JX119158	JX119151
	Zoanthus sansibaricus	0	1	0	0	0	0	0	AB214174	AB235412
	Zoanthus sociatus	0	1	0	0	0	0	0	JX119154	JX119149
	Zoanthus gigantus	0	1	0	0	0	1	0	AB252676	AB235411
	Isaurus tuberculatus	0	1	0	0	0	0	0	JX119159	EF452256
	Acrozoanthus australiae	0		1	0	0	0	0	HM171912	HM171922

Figure S2: Analysis of trait "incorporation of sediment in the tissue" performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

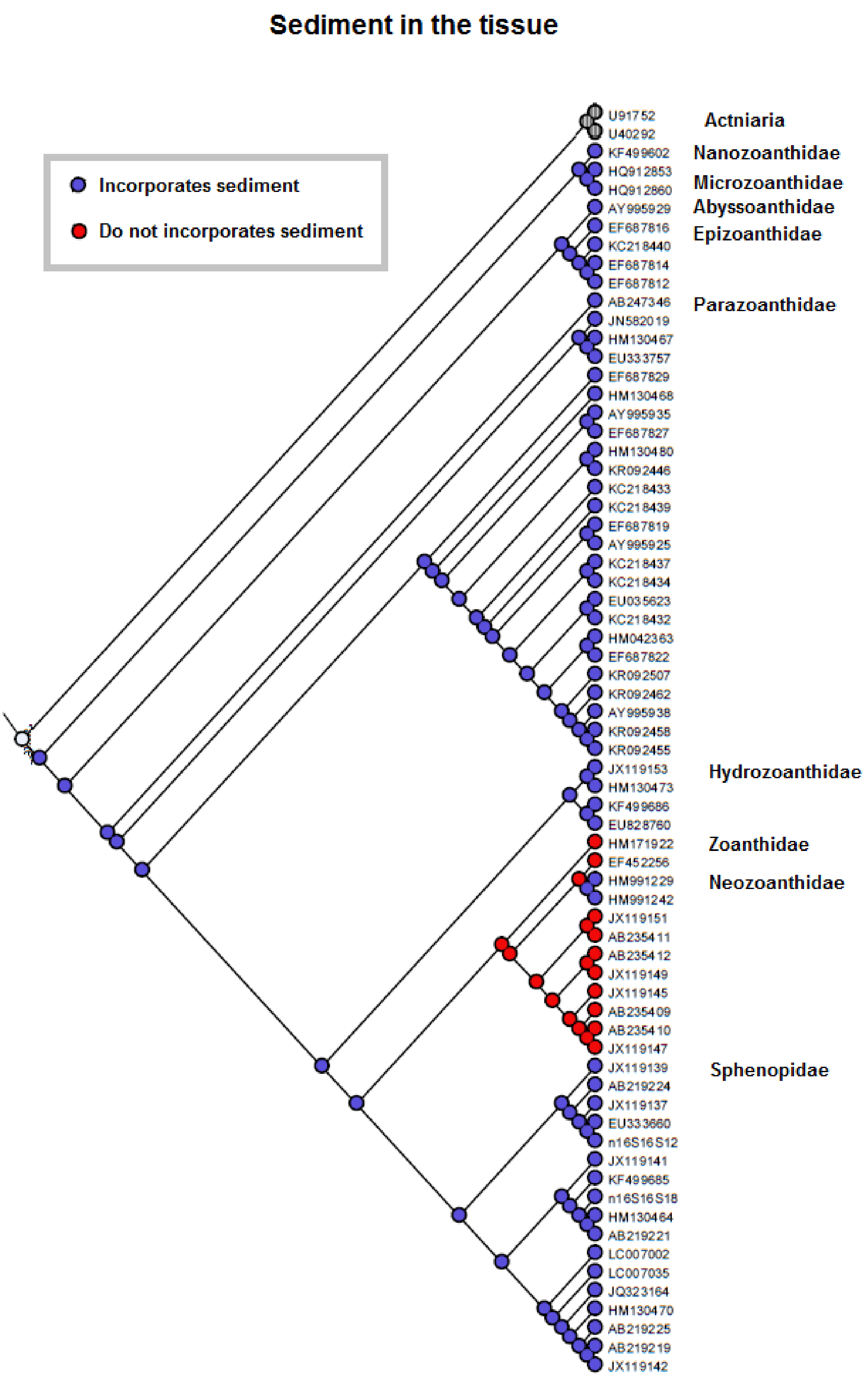


Figure S3: Analysis of trait "symbiosis with zooxanthellae" performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

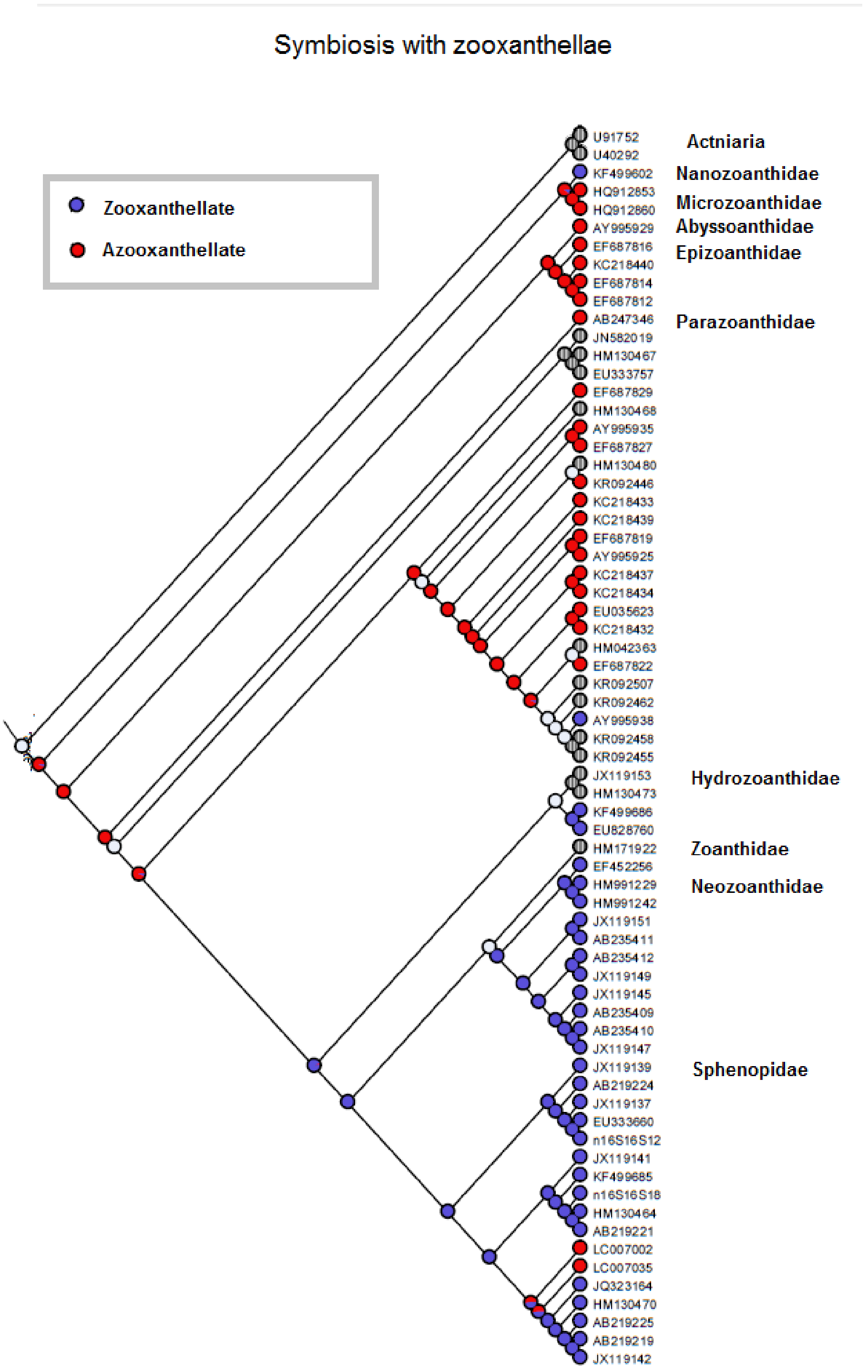


Figure S4: Analysis of trait "epizoism relationship" performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

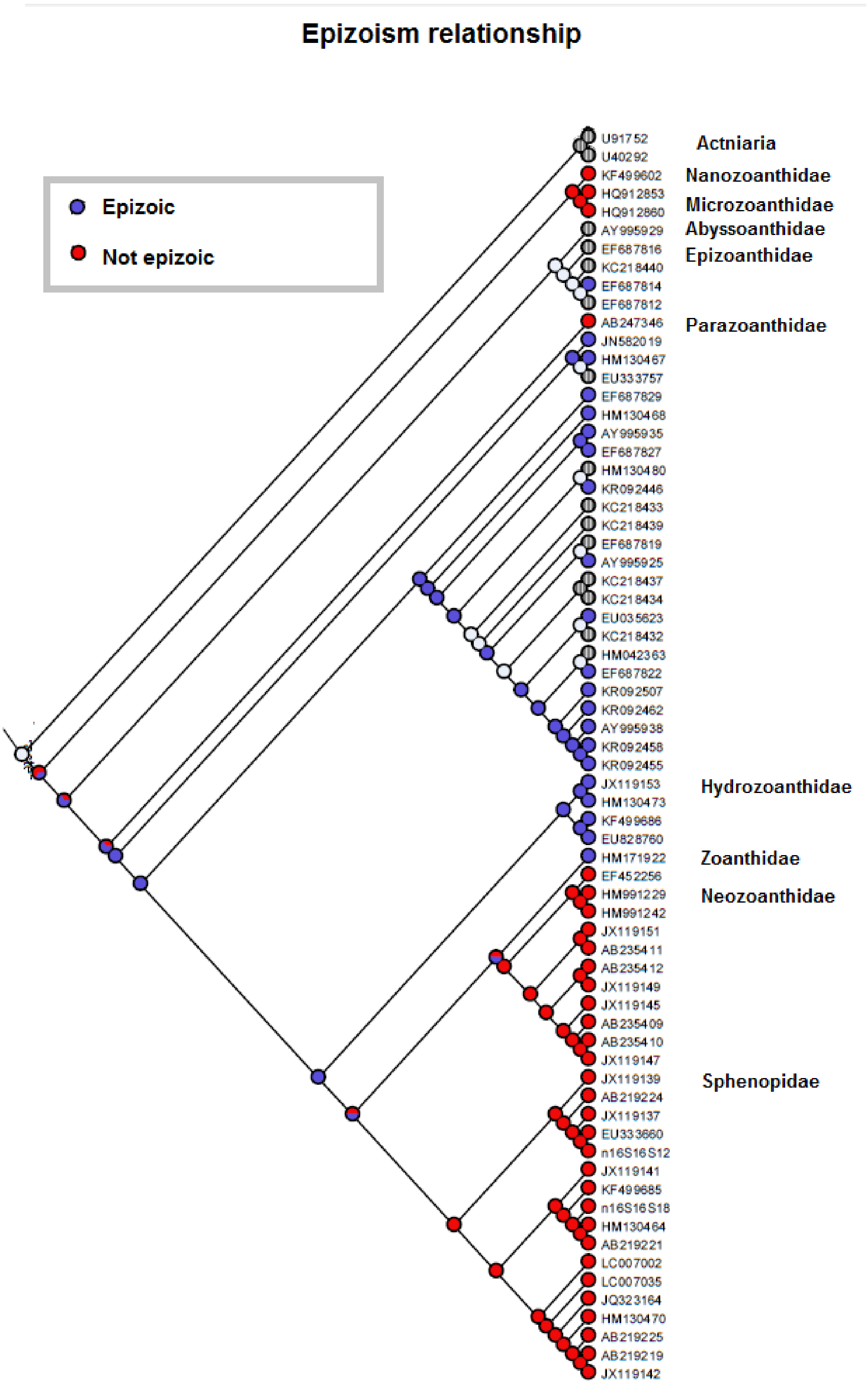


Figure S5: Analysis of trait "presence in deep waters" performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

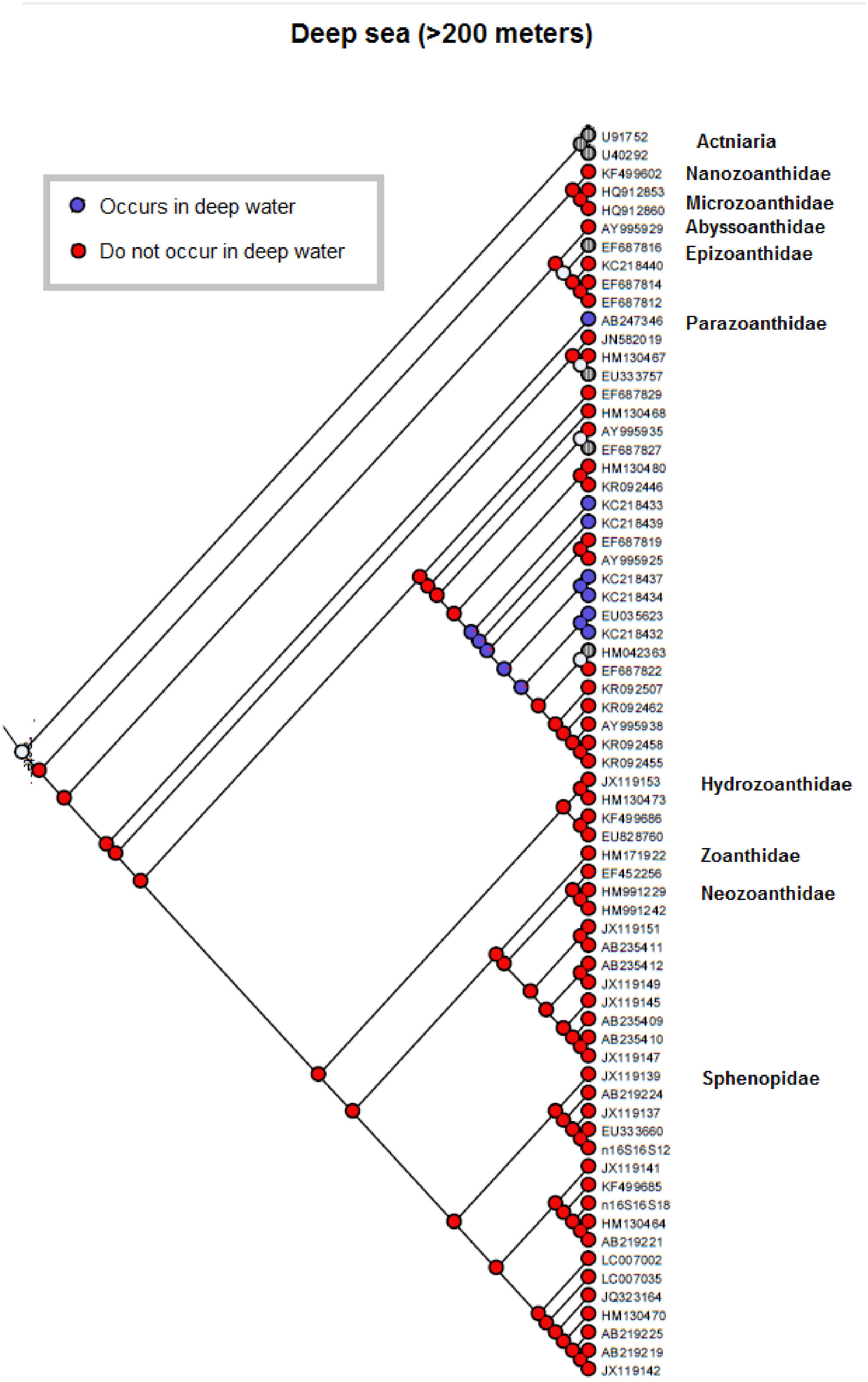


Figure S6: Analysis of trait “production of scleroprotein layer” performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

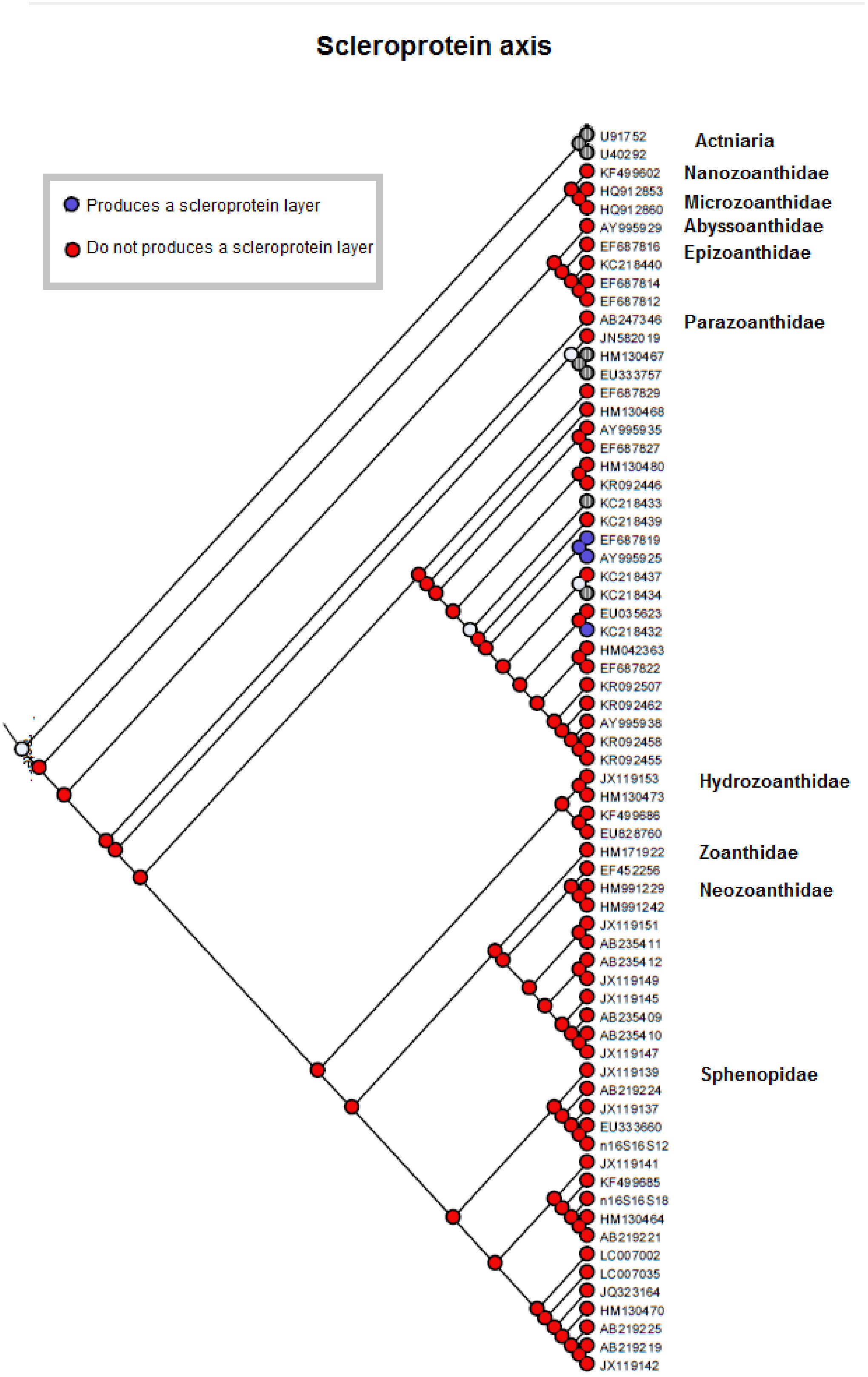


Figure S7: Analysis of trait “oral disk size” performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1

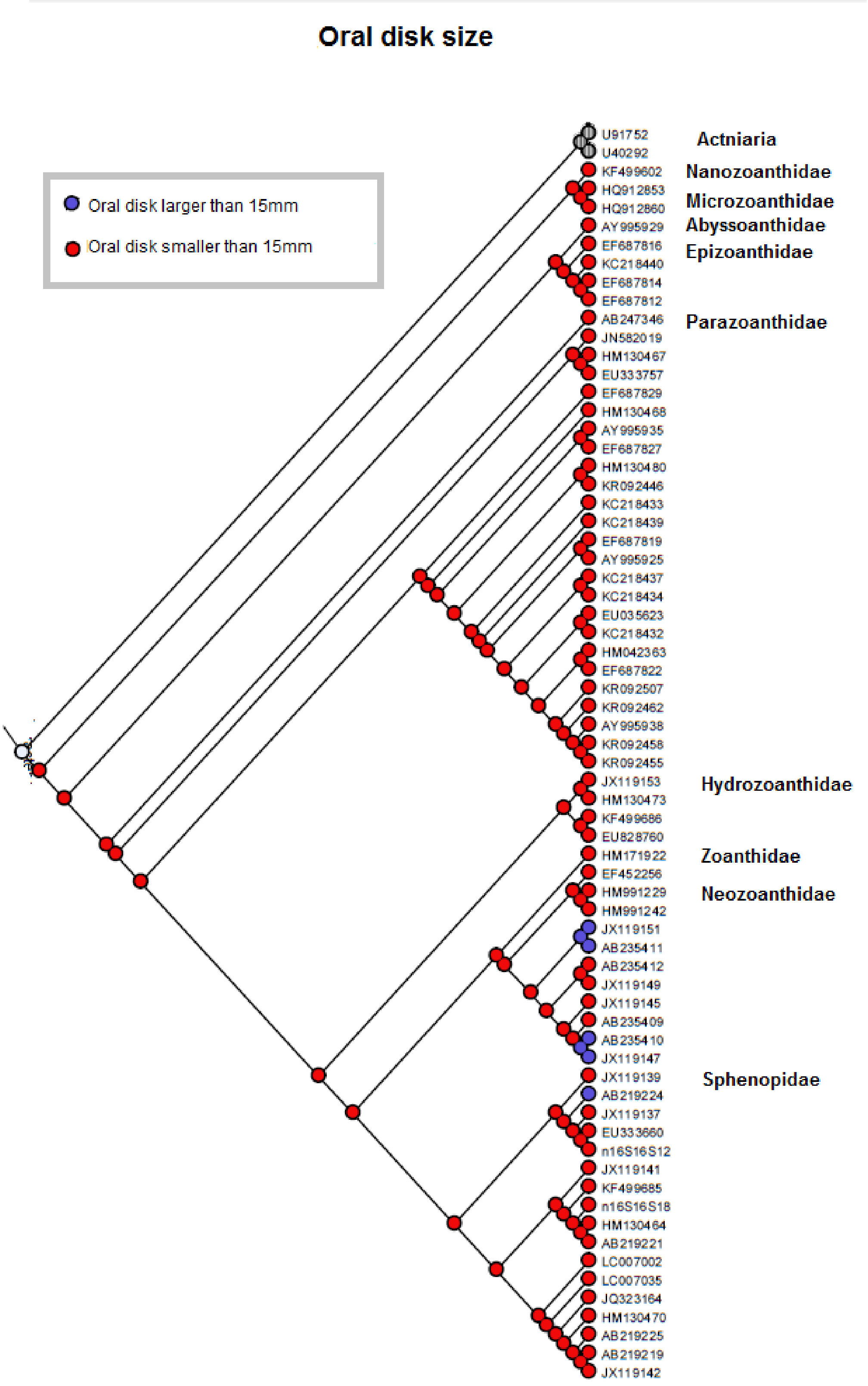
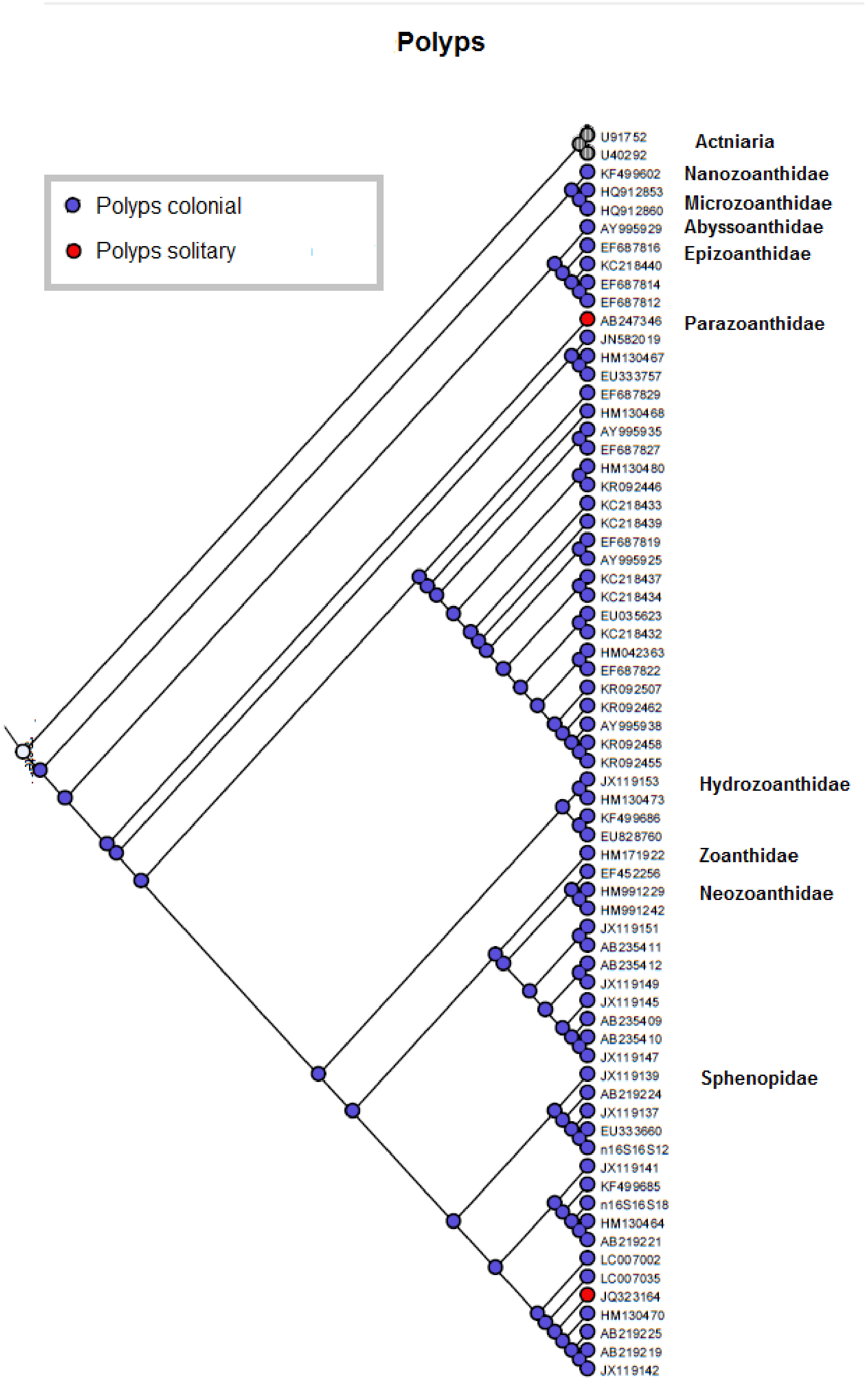


Figure S8: Analysis of trait “polyps type” performed in Mesquite. Species codes correspond to GenBank accession number of mitochondrial 16S rDNA sequences in table S1



CAPÍTULO 2

Overview of the order Zoantharia (Cnidaria: Anthozoa) in Brazil

Panorama da ordem Zoantharia (Cnidaria: Anthozoa) no Brasil

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Resumo

Zoantários (Hexacorallia: Zoantharia) são uma ordem de cnidários bentônicos que possuem distribuição cosmopolita e desempenham um importante papel em diversos ecossistemas marinhos. Embora sejam amplamente distribuídoss no Atlântico sudeste tropical, estudos sobre as espécies do Brasil e seus padrões de distribuição são escassos. Para preencher essa lacuna, nós realizamos coleta na costa e ilhas oceânicas em paralelo com uma extensiva revisão da literatura para sintetizar o conhecimento da ordem Zoantharia no Brasil. Adicionalmente, sequências dos marcadores mitocondriais 16S do DNA ribossomal (16S) e citocromo oxidase subunidade 1 (COI) dos espécimes foram determinados para confirmar sua identidade e posição na filogenia da ordem. Nosso resultados mostram a presença de pelo menos 13 espécies de Zoantharia nas águas brasileiras, incluindo novos registros de *Palythoa* aff. *clavata* e *Zoanthus* aff. *pulchellus*. Adicionalmente, ampliação de distribuição foi registrada para *Palythoa caribaeorum*, *P. grandiflora*, *P. variabilis*, *Zoanthus pulchellus* e *Parazoanthus swiftii.* Nós também apresentamos a primeira descrição dos padrões de distribuição para todas as espécies estudadas no Brasil. É esperado que o presente estudo sirva como base para futuras pesquisas no Atlântico sudeste tropical, onde a riqueza de Zoantharia ainda é pouca estudade e portando, provavelmente subestimada.

Keywords: Diversity; phylogeny; zoogeography; Zoantharia; Brazil; southwestern Atlantic

Palavras-chave: Diversidade, filogenia, zoogeografia, Zoantharia, Brasil, Atlântico sudeste

Mar Biodiv
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Overview of the order Zoantharia (Cnidaria: Anthozoa) in Brazil

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Abstract Zoantharia (Hexacorallia) is an order of benthic cnidarians, which are known to play an important ecological role in many marine ecosystems. Although the order has a cosmopolitan distribution, including the tropical southwestern Atlantic, research on Brazilian zoantharian species diversity and distribution patterns is lacking. In order to start addressing this gap, we conducted shallow-water coastal and oceanic island sampling in parallel with an extensive literature review to summarize the knowledge of the order Zoantharia from Brazil. Additionally, mitochondrial 16S ribosomal DNA and cytochrome oxidase subunit I (COI) sequences from specimens were determined to confirm their identity and phylogenetic position within the order. Our results show the presence of at least 13 zoantharian species in Brazilian waters, including first records of *Palythoa* aff. *clavata* and *Zoanthus* aff. *pulchellus*. Moreover, range extensions were confirmed for *Palythoa caribaeorum*, *P. grandiflora*, *P. variabilis*, *Zoanthus pulchellus,* and *Parazoanthus swiftii.* We also present the first report on the distribution patterns for all studied species in Brazil. The current study is expected to provide a baseline for further surveys in the tropical southwestern Atlantic, where Zoantharia species richness is still understudied and therefore very likely underestimated.

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Introduction

Reported from all oceans, zoantharians (Cnidaria: Anthozoa: Hexacorallia: Zoantharia) occur from the intertidal zone to depths greater than 5,000 m (Ryland et al. 2000; Reimer and Sinniger 2010). Most zoantharians do not produce a hard skeleton and are able to form extensive colonies, covering up to 50% of the substrate at some localities (Karlson 1983; Oigman-Pszczol et al. 2004). These colonies are commonly used for protection or as shelter by various invertebrates (Den Hartog and Holthuis 1984; Den Hartog and Türkay 1991; Messing et al. 1990; Pérez et al. 2005), and also as a food source for other marine animals, such as fishes, sea turtles, and sea stars (Gleibs and Mebs 1999; Stampar et al. 2007; Obuchi and Reimer 2011). Although zoantharians play a considerable ecological role in benthic ecosystems, species identification remains challenging due to their relatively simple body plan and high intraspecific plasticity (Burnett et al.

1997; Ryland and Lancaster 2003; Ong et al. 2013). As a result, they are usually neglected in marine surveys. However, studies combining morphological, ecological, and molecular data have proven to be an excellent way to identify these cnidarians (Reimer et al. 2004; Sinniger et al. 2008; Koupaei et al. 2014) and are also shedding light on their evolutionary relationships (Sinniger et al. 2005, 2010). The knowledge on the systematics of the order has increased with the description of new families, genera, and species, primarily in the Pacific and Indian Oceans (Sinniger et al. 2010; Fujii and Reimer 2011, 2013; Sinniger et al. 2013; Irei et al. 2015). Surveys undertaken at sites in the Atlantic Ocean, such as in the Caribbean (Swain and Wulff 2007; Reimer et al. 2012), Cape Verde islands (Reimer et al. 2010a), and Ascension Island (Reimer et al. 2014b) have reported potential new species and extended the distribution ranges for many others.

In Brazil, taxonomic and biogeographic research on the order Zoantharia is still in its infancy. The Brazilian coast from the mouth of the Amazon River, in the north, to the state of Santa Catarina, in the south, along with the oceanic islands (Rocas Atoll and Archipelagoes of Trindade and Martin Vaz, Fernando de Noronha and Saint Peter and Saint Paul), is recognized as an important biogeographic region, known as the Brazilian Province (Floeter et al. 2008; Briggs and Bowen 2012). Within this province that extends for more than 6, 000 km in the tropical southwestern Atlantic, the diversity of other common reef organisms has been relatively well studied (e.g., phylogenetic and distribution patterns of scleractinian corals, sea anemones and reef fishes [Laborel 1970; Russo et al. 1994; Zamponi et al. 1998; Castro and Pires 2001; Floeter et al. 2001, 2008; Nunes et al. 2008, 2011; Bender et al. 2013]). Although zoantharians are broadly distributed in Brazilian waters, data such as species lists and phylogenetic analyses are still lacking. Due to their important ecological role in Brazilian marine ecosystems (Mendonça-Neto et al. 2008; Cruz et al. 2015a), improvement on the knowledge of zoantharian species diversity is needed to provide a foundation for further studies. In addition, a better understanding of zoantharian species' distributions would help in the monitor biotic and abiotic events that affect marine community structures, such as pathogen outbreaks (Acosta 2001), introduction of invasive species (Mantelatto et al. 2011), climate change (Shinzato et al. 2011), and the potential for phase shifts (Cruz et al. 2015a, b). Therefore, the present study aimed to: (1) provide a list of Zoantharia species reported from Brazil along with the placement of species in the phylogeny of the group, and (2) draw the first picture of species distribution patterns in the tropical southwestern Atlantic. This study provides a baseline for future research on Brazilian zoantharians and contributes to the ongoing discussion of the species diversity and evolutionary patterns of the order Zoantharia.

Materials and methods

Bibliographic review

An extensive literature review was undertaken to summarize a list of Zoantharia species recorded from Brazilian waters. We included records from peer-reviewed papers and proceedings from the International Coral Reef Symposiums. In total, data from 69 scientific publications reporting zoantharians on the Brazilian coast and on oceanic islands were compiled. We followed recent revisions of the taxonomic nomenclature used in past literature in making our list of zoantharians from Brazil.

The genus *Protopalythoa*, previously thought to be separate from *Palythoa* (*see*, for example, Burnett et al. 1997, and Ryland and Lancaster 2003), has been considered as congeneric to *Palythoa* (Reimer et al. 2007b) and we therefore only use *Palythoa* in this study. Considering the anteriority rule, we have used *Savalia* Nardo, 1844 instead of its junior synonym *Gerardia* Lacazr-Duthiers, 1864 (Sinniger et al. 2005, 2007; Altuna et al. 2010). For the same reason, in this work we use *Zoanthus pulchellus* (Duchassaing and Michelotti, 1860) instead of *Z. nymphaeus* (Lesuer, 1817) following Duerden (1902) and Walsh (1967). *Palythoa brasiliensis* Heider, 1895, described from the Brazilian coast, has an incomplete original description (Kelecom and Solé-Cava 1982). In addition, it is most likely a synonym of *P. caribaeorum*, as junior synonyms of this latter species have also been reported from other locations in the Atlantic, such as *P. senegalensis* and *P. vicentina* in Reimer (2010). Furthermore, the holotype of *P. brasiliensis* is lost (Pax and Müller 1957). For these reasons, we have chosen not to include this species in the current study.

Specimen collection

Between 2010 and 2013, expeditions to collect zoantharians were carried out at ten sites along the Brazilian coast and on oceanic islands using SCUBA and snorkeling in depths ranging from 0 to 20 m (Fig. 1). Whenever possible, in situ images were taken prior to specimen sampling. In total, 31 specimens of zoantharians were collected, preserved in 95-100% ethanol and deposited in the Museum of Zoology, São Paulo University (MZUSP; collection numbers MZUSP 002655-002685). Two specimens previously deposited at the National Museum, Federal University of Rio de Janeiro (MNRJ), were also analyzed in the present study (MNRJ 1590 *Epizoanthus* sp. and MNRJ 1995 *Savalia* sp.) as they have been cited in previous literature (Migotto et al. 1999). Specimens were identified to the lowest taxonomic level possible and at least one sample from each morphotype was analyzed molecularly. Therefore, each species analyzed was identified based on the combination of morphological and molecular data, except *Epizoanthus*

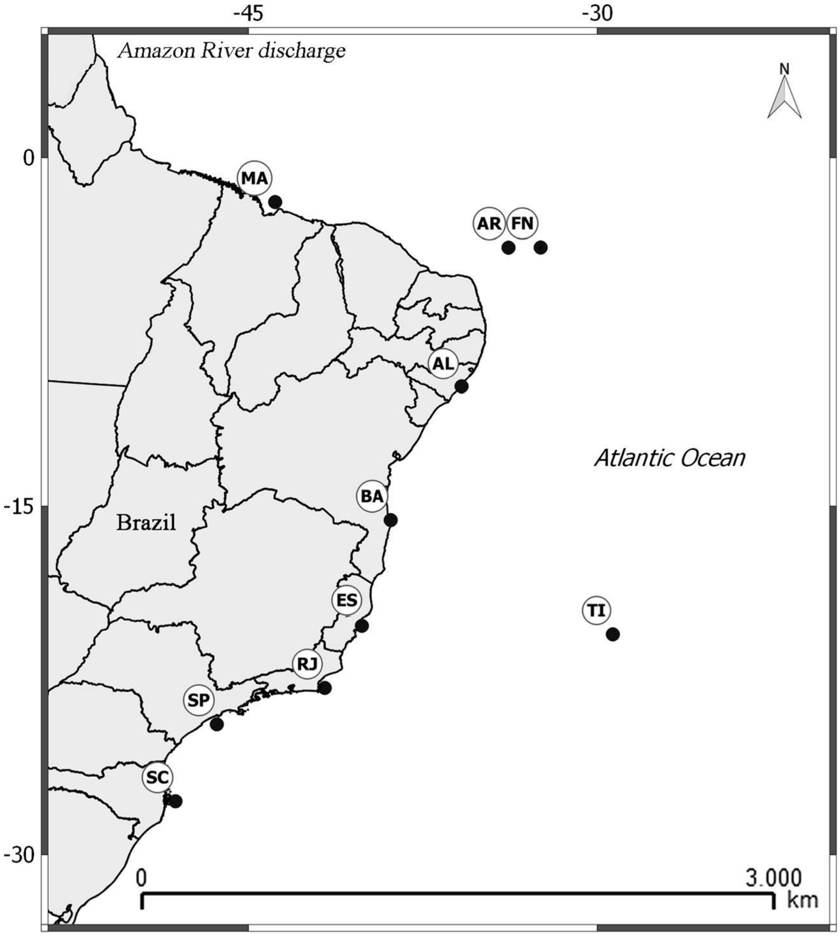

Fig. 1 Sampling sites along the Brazilian coast (*AL* Alagoas, *BA* Bahia, *ES* Espírito Santo, *MA* Maranhão, *RJ* Rio de Janeiro, *SC* Santa Catarina and *SP* São Paulo) and at oceanic islands (*AR* Rocas Attol, *FN* Fernando de Noronha Archipelago and *TI* Trindade and Martins Vaz Archipelago)

sp. and *Savalia* sp., as these samples were initially preserved in formalin and it was not possible to extract DNA. In addition, probably due to high sediment content in the tissue from sample BR1 (identified herein as Parazoanthidae), attempts to extract genomic DNA from this specimen were unsuccessful.

Morphological analyses

Species identification and external morphological examination followed Pax (1910), West (1979), Burnett et al. (1997), Reimer (2010), and Reimer et al. (2013a). For each specimen, the following characters were analyzed: polyp and oral disk color(s), polyp form, numbers of tentacles, and presence/absence of sand encrustation.

DNA extraction, polymerase chain reaction (PCR) and sequencing

DNA was extracted from approximately 25 mg of tissue from each specimen using a DNeasy Blood and Tissue kit (Qiagen, Seoul, Korea), following the manufacturer's animal tissue protocol. PCR amplification was performed using HotStarTaq DNA polymerase (Qiagen, Seoul, Korea) according to the manufacturer's instructions. Partial fragments of the mitochondrial markers 16S ribosomal DNA (mt 16S rDNA) and cytochrome oxidase subunit I (COI) were amplified using primers and procedures outlined in Sinniger et al. (2005) and Reimer et al. (2007a), respectively. Amplified products were visualized by 1% agarose gel electrophoresis and submitted to direct sequencing by Macrogen (Korea).

Phylogenetic analyses

Nucleotide sequences of COI and mt 16S rDNA were aligned and concatened using Clustal Omega (Sievers et al. 2011). We amplified sequences of both COI and mt 16S rDNA markers for 19 specimens. These sequences were aligned with previously published sequences deposited in GenBank of Parazoanthidae, Sphenopidae, and Zoanthidae species from the Atlantic Ocean, using sequences from Epizoanthidae as an outgroup (Sinniger et al. 2005, 2008; Reimer et al. 2010a, b, 2012, 2013b), once the monophyly of this family and the sister-group relationship with other Zoantharia families was demonstrated (Sinniger et al. 2005). The concatenated (mt 16S rDNA+COI) alignment of Brazilian specimens and respective closely-related species' sequences from GenBank contained 36 sequences with 1,223 sites, of which 497 sites were from COI and 726 sites were from mt 16S rDNA.

The alignment was inspected by eye and ambiguous positions were manually edited using MEGA6 (Tamura et al. 2013). Phylogenetic analyses were performed using PhyML (Guindon and Gascuel 2003) for Maximum-Likelihood (ML) with a Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) of nucleotide distribution and estimated parameters. Robustness of the ML reconstruction was tested with 1,000 bootstrap replicates. Bayesian inference was performed with MrBayes 3.0 (Ronquist and Huelsenbeck 2003) under the general time-reversible model (Rodriguez et al. 1990) of nucleotide substitution matrix with eight categories. Markov chains Monte Carlo simulations were run for 1,000,000 generations, with log-likelihoods and topologies saved at 100-generations intervals. The first 1,000 topologies were discarded as burn-in, and trees generated from the remaining 9,000 trees. In addition, sequences obtained from specimens BR10 and BR18 were shorter than other sequences analyzed in this study and therefore were only compared for similarity following Bo et al. (2012), with no additional phylogenetic analyses. These sequences were compared with previously reported Zoantharia sequences using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (NCBI-BLAST) search. New sequences determined in the present study were deposited in GenBank (accession numbers KT454341-KT454380).

Results

A total of 11 Zoantharia species has been recorded from Brazil in previous literature (Table 1). Sequences obtained from specimens BR10 and BR18 (COI from BR10, mt 16S rDNA from BR18) were only compared for similarity using BLAST. The COI sequence from BR10 had a length of 722 bp and BLAST comparison indicated it was identical (identity=100%, query coverage=91%) to previously reported sequences of *Palythoa tuberculosa* (KF840072). *P. tuberculosa* is from the Indian/Pacific Oceans and sibling to Atlantic *P. caribaeorum* (Reimer et al. 2012). The mt 16S rDNA sequence from BR18 had a length of 906 bp and BLAST search indicated it was identical (identity=100%, query coverage=83%) to previously reported sequences of *Palythoa* sp. sakurajimensis from the Pacific Ocean (DQ997842). Sister zoantharian species from the Atlantic and Indian/Pacific Oceans have high similarity or identical mtDNA sequences (Reimer et al. 2010a, 2012).

Four large clades corresponding to the families Epizoanthidae, Parazoanthidae, Sphenopidae, and Zoanthidae were retrieved in the resulting mt 16S rDNA + COI phylogenetic tree (Fig. 2). Family-level clades of Parazoanthidae, Sphenopidae, and Zoanthidae were generally well supported in phylogenetic analyses (ML/Bayesian=55/1.0, 89/0.99, 94/1.0, respectively). Sequences from Brazilian specimens BR09 and BR12 grouped within the Sphenopidae and corresponded to the genus *Palythoa*. Sequences from specimen BR09 were identical to previously reported sequences of *P. grandiflora* from the Caribbean (ML/Bayesian=72/0.97), while sequences from BR12 were identical to *P.* aff. *variabilis* from Caribbean (ML/Bayesian=96/1.0).

Sequences from 13 specimens (BR03, BR05, BR11, BR13-17, BR19-20, BR27-29) grouped within the Zoanthidae clade, and corresponded to species from the genus *Zoanthus*. Specimens BR14, BR16-17, BR19-20 and BR28 had identical sequences and formed a clade with previously reported *Z. sociatus* sequences from the Caribbean (ML/Bayesian=96/0.98). Sequences from BR29 were identical to previously reported *Z. pulchellus* from the Caribbean (ML/Bayesian = 61/59). Sequences from BR03, BR05, BR11, BR13, BR15 and BR27 were identical and formed a clade with previously reported sequences of *Z.* aff. *pulchellus* from Cape Verde (64/100). Within the Parazoanthidae clade, sequences BR02, BR06, BR07 and BR23 were identical to each other and to previously reported sequences from *Parazoanthus swiftii* from the Caribbean.

A list of 31 specimens identified from Brazil on the basis of molecular data and general external morphological characters is provided (ESM1). Results of these analyses and the literature review confirmed the presence of at least 13 Zoantharia species in Brazil (*Palythoa caribaeorum, P. variabilis, P. grandiflora, P.* aff. *clavata, Zoanthus sociatus, Z. solanderi, Z. pulchellus, Z.* aff. *pulchellus, Isaurus tuberculatus, Parazoanthus swiftii, P. catenularis, Savalia* sp. and *Epizoanthus* sp.; Figs. 3 and 4).

In Brazil, the suborder Brachycnemina is represented by families Sphenopidae and Zoanthidae, whereas the suborder Macrocnemina is represented by the families Parazoanthidae and Epizoanthidae. Reports of *Isaurus tuberculatus* was shown to be limited to the northeastern of Brazil, while *Zoanthus* and *Epizoanthus* have São Paulo State as the southernmost distribution limit (Fig. 3). The genus *Palythoa* and the family Parazoanthidae are reported all the way to Santa Catarina State in Brazil (Fig. 3).

Within *Palythoa*, *P. caribaeorum, P. grandiflora* and *P. variabilis* are widespread species, occurring from Santa Catarina State to the northeastern region (Fig. 3). However, their congener *P.* aff. *clavata* is reported herein only from Trindade Island and represents the first record in Brazil for this species. Within the genus *Zoanthus*, *Z. sociatus, Z. solanderi,* and *Z. pulchellus* were reported from several sites along the coast as well as from the oceanic islands. Finally, reports of the potentially undescribed species *Z.* aff. *pulchellus,* from São Paulo and Rio de Janeiro states as well as from Trindade and Martins Vaz Archipelago are new records for the southwestern Atlantic.

Table 1 Literature review of zoantharian species records from Brazil. Symbols indicate range extensions resulting from this study

Species name	Previous sites recorded[a]	References[b]	Site records from this study
Epizoanthus sp.	BA, ES, RJ, SP	1-2	
Isaurus tuberculatus	BA, CE, FN, RN	1, 3-7	
Parazoanthidae			SC+
Parazoanthus sp.	AS, BA, ES, MA, RJ, SC	1, 8-11	
Parazoanthus swiftii	RJ	12	RJ, SC+, SP+
Parazoanthus catenularis	MA	12	
Palythoa aff. *clavata*			TI+
Palythoa caribaeorum	AL, AR, AS, BA, CE, FN, PE, RJ, RN, SC, SP	1-2, 7-9, 11, 13-47	AR, BA, ES, FN, RJ, SC, SP, TI+
Palythoa grandiflora	RJ	21	AL+, RJ, SC+, SP+
Palythoa variabilis	BA[c], CE-SP, FN, SC	1-2, 7, 11, 13-14, 18, 23, 33-34, 38, 42, 46, 48, 50-54, 55c, 56c, 57	TI+
Palythoa sp.	AR, BA, ES, FN, RJ, RN, SP, TI	1, 8, 14-15	
Palythoa spp.	BA, RJ	17, 59	
Savalia sp.	SP	1-2	
Zoanthus pulchellus	AS, BA, ES, RJ	1, 9, 60	SP+
Zoanthus aff. *pulchellus*			ES+, RJ+,SP+, TI+
Zoanthus sociatus	AL, AS, AR, BA, CE, ES, FN, RJ, RN, PB, PE, SC[d],SP	1-4, 7-9, 13, 15-16, 18, 21, 29, 33, 36, 45, 61-62, 63[d], 64	AR, SP
Zoanthus solanderi	AL, BA, ES, FN, PE, RJ, RN, SP	1, 18, 28, 34	
Zoanthus spp.	BA	17, 38, 39	
Zoanthidea	BA, NE, RJ, RN, NE	35, 43, 65-69	

[a] Abbreviations used for sites along the Brazilian coast: *AL* Alagoas, *BA* Bahia, *CE* Ceará, *ES* Espírito Santo, *MA* Maranhão, *NE* Northeast region, *PB* Paraíba, *PE* Pernambuco, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *SC* Santa Catarina, and *SP* São Paulo. Those for oceanic islands are: *AR* Rocas Attol, *AS* Saint Peter and Saint Paul Archipelago, *FN* Fernando de Noronha Archipelago, and *TI* Trindade and Martins Vaz Archipelago

[b] The references are numbered according to their order of citation and correspond to: *1* Migotto et al. 1999, *2* Da Silveira and Morandini 2011, *3* Laborel 1970, *4* Pires et al. 1992, *5* Grohman and Peixinho 1995, *6* Rabelo and Matthews-Cascon 2007, *7* Soares et al. 2011, *8* Castro et al. 1999, *9* Amaral et al. 2000, *10* Campos et al. 2005, *11* Bouzon et al. 2012, *12* Swain 2009, *13* Sebens 1977, *14* Kelecom and Solé-Cava 1982, *15* Echeverría et al. 1997, *16* Villaça and Pitombo 1997, *17* De Barros et al. 2000, *18* Acosta 2001, *19* Acosta et al. 2001, *20* MacCord and Duarte 2002, *21* Oigman-Pszczol et al. 2004, *22* Acosta et al. 2005A, *23* Boscolo and Silveira 2005, *24* Pérez et al. 2005, *25* Soares et al. 2006, *26* Acosta and González 2007, *27* Stampar et al. 2007, *28* Chimetto et al 2008, *29* Correia and Sovierzoski 2008, *30* Mendonça-Neto et al 2008, *31* Mendonça-Neto and Gama 2008, *32* Souza et al. 2008, *33* Amaral et al. 2009, *34* Chimetto et al. 2010, *35* Francini-Filho and Moura 2010, *36* Francini-Filho et al. 2010, *37* Azevedo et al. 2011, *38* Chimetto et al. 2011, *39* Segal and Castro 2011, *40* Castro et al. 2012, *41* Costa et al. 2011, *42* Almeida et al. 2012, *43* Longo et al. 2012, *44* Martinez et al. 2012, *45* Melo et al. 2012, *46* Rabelo et al. 2013, *47* De Santana et al. 2014, *48* Rabelo et al. 2014, *48* Longo et al. 2000, *50* Kelmo et al. 2003, *51* Metri and Rocha 2008, *52* Wilke et al. 2008, *53* Wilke et al. 2009, *54* Costa et al. 2014, *55* Cruz et al. 2015A, *56* Cruz et al. 2015B, *57* Soares and Souza 2013, *58* Gasparini and Floeter 2001, *59* Teixeira et al. 2008, *60* Villar et al. 2003, *61* Rohlfs and Belém 1994, *62* Sarmento and Correia 2002, *63* Gherardi 2004, *64* Soares and Sousa 2011, *65* Coelho and Ramos-Porto 1980, *66* Costa Jr. et al. 2000, *67* Ferreira et al. 2001, *68* Costa Jr. et al. 2002, *69* Floeter et al. 2007

[c] Recorded to BA as *Palythoa* cf. *variabilis*

[d] Recorded to SC as *Zoanthus sociatus*; however specimens are *Palythoa grandiflora* (analyses of unpublished images, pers. comm. D. Gherardi)

Within the family Parazoanthidae, *Parazoanthus swiftii* was recorded from Santa Catarina, São Paulo, and Rio de Janeiro states, while *P. catenularis* was reported only from Maranhão state. The literature review also revealed an unidentified *Parazoanthus* sp. from Espírito Santo and Bahia states, as well as from Saint Peter and Saint Paulo Archipelago. The genus *Savalia* was recorded only from São Paulo state, and an unidentified specimen belonging to Parazoanthidae (BR01) was found off Santa Catarina state. Lastly, *Epizoanthus* sp. (family Epizoanthidae) was recorded from São Paulo, Rio de Janeiro, Espírito Santo and Bahia states.

Discussion

The literature review and sampling confirmed the presence of at least 13 zoantharian species in Brazilian waters (Fig. 3). Despite the relatively large number of past studies mentioning Zoantharia from Brazil, only one paper has specifically focused on the taxonomy of the group (Rohlfs and Belém 1994). The paucity and plasticity of diagnostic characters utilized in species identification have been a stumbling block in the understanding of both the diversity and biogeography of Zoantharia. In many cases, these problems have led to inexact higher-level taxonomic identification (i.e., order/genera level)

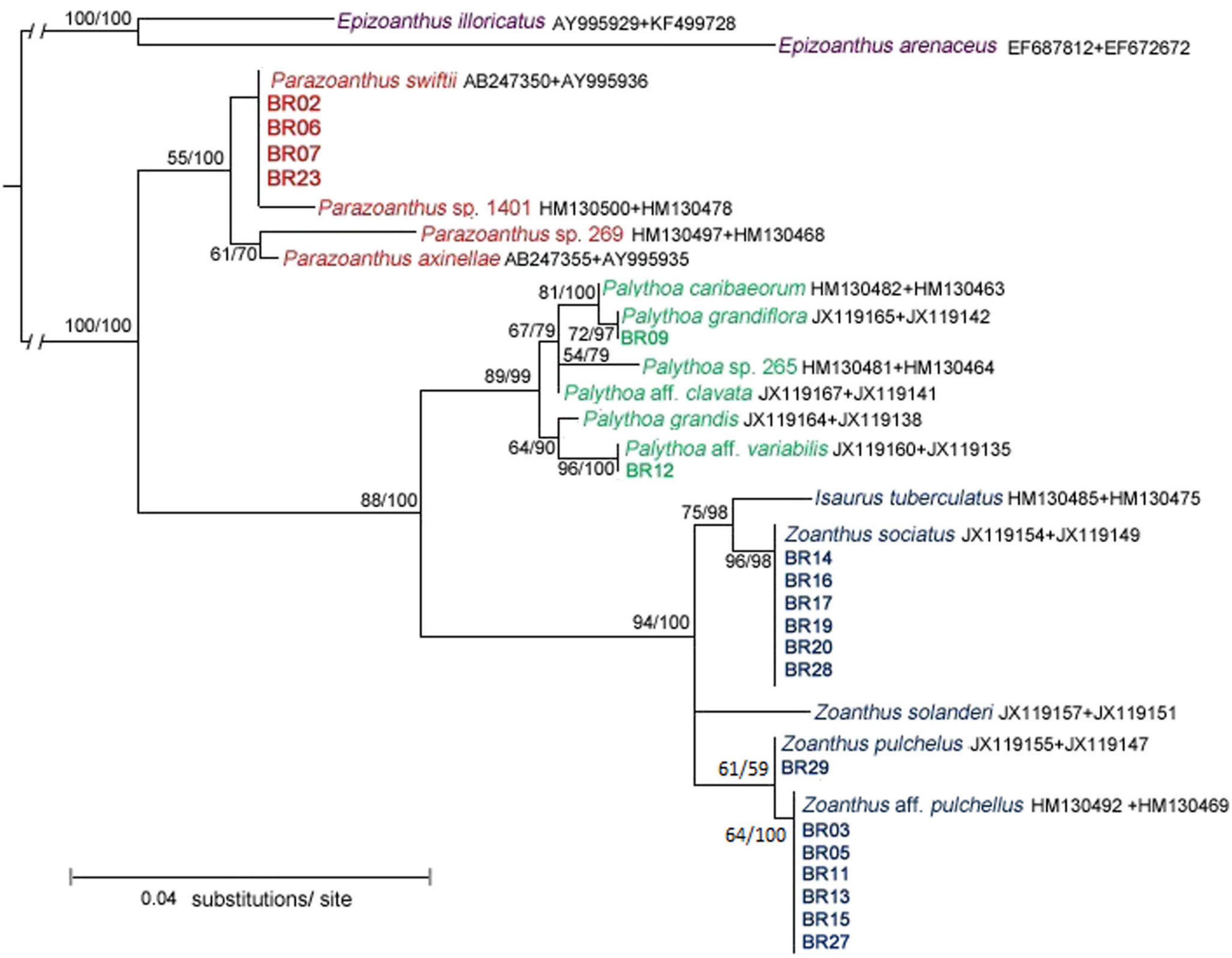

Fig. 2 Maximum likelihood (ML) tree obtained from alignments of mt 16S rDNA+COI sequences. Values at branches represent ML bootstraps and Bayesian posterior probabilities. Sequences from GenBank are indicated by accession numbers following species name, while sequences of specimens from Brazil collected in this study are shown with same codes as in ESM1. Sequences of Epizoanthus spp. were used as outgroup

or to mistaken identification (e.g., *Epizoanthus gabrielli* in Cruz et al. 2015b is actually a species of the genus *Palythoa* [Cruz et al. 2015a]). Similarly, *Zoanthus pacificus* has been reported from Espírito Santo state (Rohlfs, unpublished), but this record is likely to be *Z. sociatus*. This is because *Z. pacificus* is described from the Pacific Ocean and is most probably a junior synonym of *Z. sansibaricus* (Reimer et al. 2004). In addition, *Z. sansibaricus* is the sister species of *Z. sociatus* (Reimer et al. 2012), which occurs in Atlantic Ocean. Because of such problems, unconfirmed zoantharian distribution data based solely on past literature may be prone to error and should be interpreted carefully.

Phenotypic plasticity has led to the description of numerous synonym species in Zoantharia (*see* Burnett et al. 1997; Reimer et al. 2004, 2012), as many morphological characteristics used for identification can have high intraspecific variation. For example, Kamezaki et al. (2013) showed that color variation occurs at different depths in *Zoanthus sansibaricus*. Likewise, sites with low hydrodynamic energy may lead to polyps with longer columns than those from the same species inhabiting sites with stronger currents and wave action (Reimer et al. 2006). Furthermore, polyp size and number of tentacles and mesenteries usually increase during ontogeny (Karlson 1988). Thus, DNA-based analyses are some of the most effective methods of zoantharian species identification as they are independent of intraspecific morphological variability (Shearer and Coffroth 2008). The high morphological plasticity of many zoantharians may be a way to facilitate colonization and adaptation to a wide range of habitats, and may also allow survivorship during environmental changes (Ong et al. 2013).

BLAST results and morphological analyses of specimen BR18 collected in Trindade Island, here identified as *Palythoa* aff. *clavata*, suggest a high similarity to *P.* aff. *clavata* previously reported from Florida (Reimer et al. 2012). Due to the vague and short original description of *P. clavata*

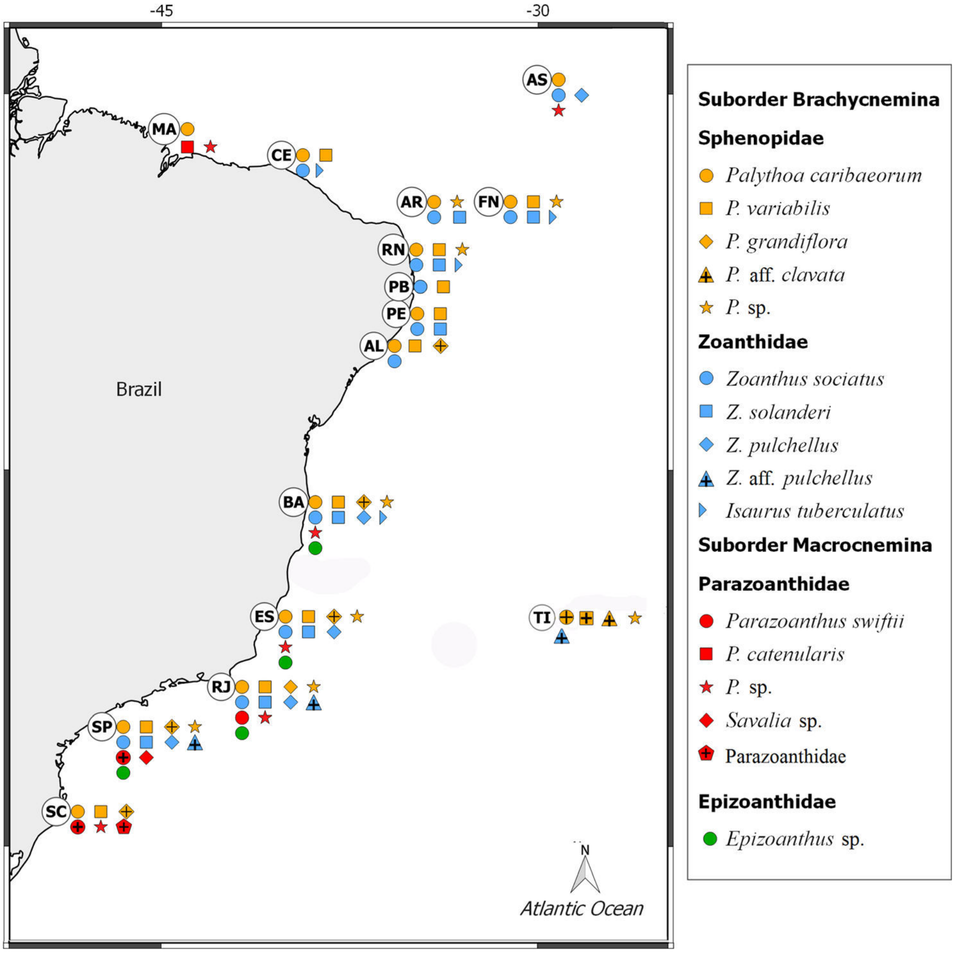

Fig. 3 Distribution of zoantharians in Brazil. *Symbols marked with a cross* correspond to range extensions presented in this study. The abbreviations used for sites along the Brazilian coast are: *AL* Alagoas, *BA* Bahia, *CE* Ceará, *ES* Espírito Santo, *MA* Maranhão, *PB* Paraíba, *PE* Pernambuco, *RJ* Rio de Janeiro, *RN* Rio Grande do Norte, *SC* Santa Catarina, *SP* São Paulo; those to oceanic islands are *AS* Saint Peter and Saint Paul Archipelago, *AR* Rocas Attol, *FN* Fernando de Noronha Archipelago, *TI* Trindade and Martins Vaz Archipelago

(Duchassaing 1850), the specimen in Reimer et al. (2012) as well as BR18 may represent an undescribed species. Although there have been no studies on the sexual reproduction of this putatively undescribed species, it is reasonable to expect that its larval dispersal is similar to other *Palythoa* spp., which can have planktonic larvae living up to 170 days (Polak et al.

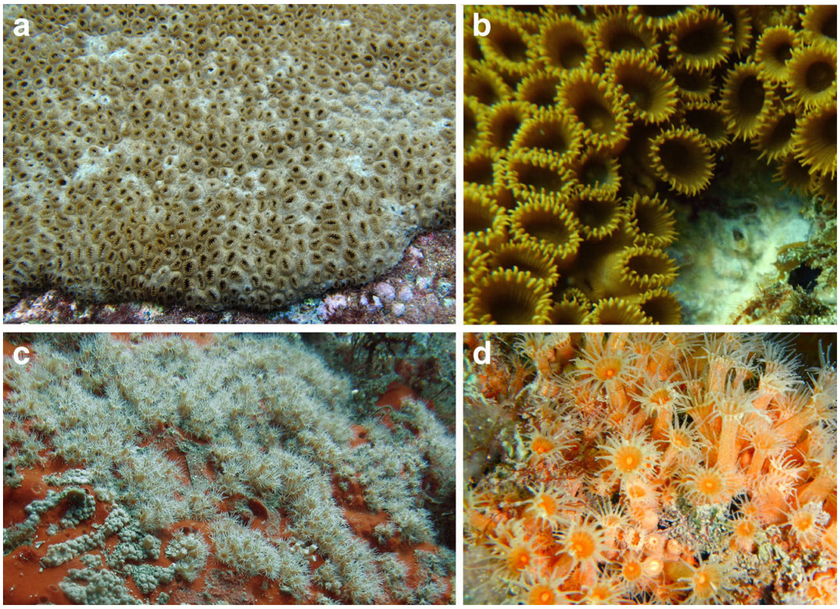

Fig. 4 Images of common zoantharian species in Brazil: *Palythoa caribaeorum* (**a** and **b**) and *Parazoanthus swiftii* (**c** and **d**)

2011). Thus, *Palythoa* aff. *clavata* may have a high dispersal ability, explaining how it has been recorded from Florida and the oceanic islands of Ascension (Reimer et al. 2014b) and Trindade (present study). Further surveys in Brazil should confirm if the distribution of this species is limited to Trindade Island. As well, molecular data from *P.* aff. *clavata* indicate that it is closely related to *P.* sp. sakurajimensis from the Pacific Ocean. There are similar records of sister zoantharian species from the Atlantic and Indian/Pacific Oceans with highly similar or identical mtDNA sequences (Reimer et al. 2010a, 2012). This is likely a result of the low mutation rate of mitochondrial DNA in most anthozoans (Shearer et al. 2002; Stampar et al. 2014), and thus only molecular markers with higher resolution can differentiate sister zoantharian species. Because of this, specimens need to have sampling location data included to help improve identification.

Specimen BR12 had identical mitochondrial sequences to *Palythoa* aff. *variabilis* from Florida. Unlike the Florida specimen, BR12 had morphological characters that matched the original description of *P. variabilis* and therefore we identified this specimen as the latter. Specimens of BR12 and BR18 only consisted of a few polyps each. For this reason, collection of more and larger specimens, followed by examination, should help us to understand the morphological variation within these species. Our results indicate the first record in the southwestern Atlantic of *Zoanthus* aff. *pulchellus* (BR03, BR05, BR11, BR13, BR15 and BR27), which was distributed along the southern coast of Brazil (São Paulo and Rio de Janeiro states) as well as Trindade Island. Similar specimens have previously been reported for the Caribbean (Reimer et al. 2012) and Cape Verde (Reimer et al. 2010a).

In Brazil, the suborder Macrocnemina is represented by at least four species belonging to families Parazoanthidae (three species) and Epizoanthidae (one species). It is likely that other macrocnemic zoantharians occur within the Brazilian province, especially considering that these species usually occur in deeper waters, and other understudied or cryptic habitats (e.g., family Microzoanthidae, Fujii and Reimer 2011). Polyps of macrocneminic specimens MNRJ 1590 (*Epizoanthus* sp.), MNRJ 1999 (*Savalia* sp.), and BR01 (Parazoanthidae) were contracted, and asides from a photograph of MNRJ 1999 (A. Migotto, personal communication), in situ images are not available for these specimens. These specimens could not be identified to species level due to the paucity of external morphological information and the failure to extract DNA from them. We have not included specimen BR01 in species list of zoantharians in Brazil, here identified as Parazoanthidae, as it has a scleroprotein layer, and thus may belong to same species as MNRJ 1999 (*Savalia* sp.). Histological examination of these specimens should help in confirming their identification. The macrocneminic species with the widest distribution along the Brazilian coast is *Parazoanthus swiftii*, which has also been reported from the Caribbean (Swain and Wulff 2007) and Ascension Island (Reimer et al. 2014b). Therefore, the ability to widely disperse appears to be present in both suborders of Zoantharia.

The distribution of zoantharians is at least partly due to their physiological and/or ecological traits, which often act as filters in the establishment of many marine species (Briggs 1974). For instance, environmental factors such as water temperature may act as a barrier in the distribution of zooxanthellate zoantharians (Ryland et al. 2000; Reimer et al. 2008a). In addition, it has been demonstrated that *Zoanthus* spp. are sensitive to variations in salinity (Soares and Sousa 2011). Therefore, the thermohaline properties of water masses in south Brazil (Piola et al. 2000) may be to some extent responsible for the southern distribution limits of *Zoanthus* and *Palythoa* spp. in this region. Moreover, most *Palythoa* species in this study have a wider distribution than *Isaurus tuberculatus*. For example, *Palythoa caribaeorum* is widespread along the Brazilian coast and oceanic islands, whereas *Isaurus tubercularus* is restricted to northeastern of Brazilian Province. It is conceivable that post-settlement difficulties, such as habitat availability or physiological requirements, may restrict the establishment of *I. tuberculatus*. However, as this species is relatively cryptic (Reimer et al. 2008b), further investigations are needed to confirm the distribution range of this species.

All zoantharian genera recorded in Brazilian waters have previously been reported from the Caribbean (Messing et al. 1990; Acosta et al. 2005b; Swain and Wulff 2007; Reimer et al. 2012), which in turn has a higher zoantharian diversity than Brazil. This pattern has also been observed in other groups of marine animals (Tittensor et al. 2010), and the Caribbean province may act as both a center of origin and a center of accumulation of species through geological time (Rocha et al. 2008; Briggs and Bowen 2013). In addition, the zoantharian diversity recently reported from Ascension Island is relatively low (four spp.; Reimer et al. 2014b), similar to neighboring Brazilian Trindade Island (four spp.). These data also indicate a related pattern of decreasing diversity in oceanic islands, as seen in other common reef animals, such as fishes (Floeter et al. 2008).

Conclusive species-level identifications of some specimens from Brazil in this and previous studies are still required (in Macrocnemina; *Savalia* sp., *Epizoanthus* sp., Parazoanthidae; and in Brachycnemina; *Palythoa* aff. *clavata* and *Zoanthus* aff. *pulchellus*), but for now no zoantharian species endemic to Brazil are known. This situation is different than in other hexacorallian orders—e.g., the endemic Brazilian sea anemone *Bunodosoma caissarum* Correa, 1964 (Russo and Solé-Cava 1991), and the scleractinian coral *Mussismilia brasiliensis* (Verrill, 1868) (Castro and Pires 2001). Similarly, endemism in Brazil is also shown by three species of the hydrozoan fire coral genus *Millepora* (Amaral et al. 2008). The lack of endemic zoantharian species may be due to exceptionally long-lived zoantharian larvae (Ryland et al. 2000; Polak et al. 2011) as well as their ability to reproduce asexually (Ryland 1997; Acosta et al. 2005a), both of which could lead to higher dispersal in the Atlantic Ocean. In addition, rafting may be an explanation for long-distance dispersal, which could be relevant for zoantharian species that live in shallow waters as the scleractinian coral *Favia fragum* (Hoeksema et al. 2012). Still, it is noteworthy that sympatric processes have also not led to the speciation of zoantharians in the Brazilian province, as has been reported in other animals with long living larvae, such as reef fishes (Rocha 2003). However, as the order Zoantharia has not yet been exhaustively studied in Brazil, more data are needed to make final conclusions on these observations.

Conclusions

Despite many zoantharian species having wide distribution ranges in Brazil, a paucity of morphological diagnostic characters combined with high intraspecific plasticity have historically hampered Zoantharia species identification and overall research on the order. For example, until this study there has only been one previously published record of *Palythoa caribaeorum* from Santa Catarina state (Bouzon et al. 2012), even though this species is widespread and abundant in shallow-water rocky shores, as well as easy to identify in the field. Overall, analyses of zoantharian distributions at a large geographic scale (e.g., thousands of kilometers) still suffer from scarce distribution records. However, recent zoantharian research has started to confirm species distributions in several regions around the globe (e.g., Reimer et al. 2008c in the Galapagos; Sinniger et al. 2013 on Hawaiian seamounts; Koupaei et al. 2014 in the Persian Gulf; Reimer et al. 2014a in the Central Indo-Pacific). It is predicted that additional Zoantharia species will be recorded from Brazil with the advance of research. As well, it will become necessary to study a wide range of biological traits, such as reproduction and interactions with other benthic organisms, in order to better understand species distribution and abundance patterns of the order Zoantharia.

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Supplementary material

Table S1: **Zoantharia specimens collected in this study. Sites abbreviations are the same as used in Fig. 1**

Sample	Site and date of collection	Depth (m)	mt 16S rDNA	COI	Species identification
BR01	SC-2010	~20	NA	NA	Parazoanthidae
BR02	SC-2013	~20	KT454342	KT454361	*Parazoanthus swiftii*
BR03	RJ-2013/6	<5	KT454349	KT454375	*Zoanthus* aff. *pulchellus*
BR04	SP-2012	<5	NA	NA	*Palythoa grandiflora*
BR05	ES-2012	<5	KT454350	KT454376	*Zoanthus* aff. *pulchellus*
BR06	SC-2013	~20	KT454345	KT454362	*Parazoanthus swiftii*
BR07	RJ-2013/6	<5	KT454343	KT454363	*Parazoanthus swiftii*
BR08	AL-2012/3	<5	NA	NA	*Palythoa grandiflora*
BR09	SP-2012	<5	KT454346	KT454366	*Palythoa grandiflora*
BR10	TI-2012/10	<5	NA	KT454365	*Palythoa caribaeorum*
BR11	TI-2013/6	<5	KT454351	KT454377	*Zoanthus* aff. *pulchellus*
BR12	TI-2013/4	~10	KT454341	KT454367	*Palythoa variabilis*
BR13	TI-2013/4	<5	KT454352	KT454378	*Zoanthus* aff. *pulchellus*
BR14	SP-2013/7	<5	KT454355	KT454368	*Zoanthus* aff. *pulchellus*
BR15	TI-2013/6	<5	KT454353	KT454379	*Zoanthus* aff. *pulchellus*
BR16	AR-2013	<5	KT454356	KT454369	*Zoanthus sociatus*
BR17	AR-2013	<5	KT454357	KT454370	*Zoanthus sociatus*
BR18	TI-2012/10	~10	KT454347	NA	*Palythoa* aff. *clavata*
BR19	AR-2013	<5	KT454358	KT454371	*Zoanthus sociatus*
BR20	SP-2013/7	<5	KT454359	KT454372	*Zoanthus sociatus*
BR21	SC-2013	<5	NA	NA	*Palythoa caribaeorum*
BR22	MA-2012	~10	NA	NA	*Palythoa caribaeorum*
BR23	SP-2013/2	NA	KT454344	KT454364	*Parazoanthus swiftii*
BR24	ES-2012	<5	NA	NA	*Palythoa caribaeorum*
BR25	BA-2012	<5	NA	NA	*Palythoa caribaeorum*
BR26	SP-2013/7	<5	NA	NA	*Palythoa caribaeorum*
BR27	SP-2013/7	<5	KT454354	KT454380	*Zoanthus* aff. *pulchellus*
BR28	SP-2013/7	<5	KT454360	KT454373	*Zoanthus sociatus*
BR29	SP-2013/8	<5	KT454348	KT454374	*Zoanthus pulchellus*
BR30	FN-2014	<5	NA	NA	*Zoanthus sociatus*
BR31	FN-2014	<5	NA	NA	*Palythoa caribaeorum*
BR32	SC-2013	~10	NA	NA	*Palythoa grandiflora*

CAPÍTULO 3

"I'd walk a thousand miles if I can just see you tonight": Isthmus of Panama closure as a species generator in Zoantharia (Cnidaria: Anthozoa)

"Eu andaria mil milhas se eu pudesse somente te ver essa noite": formação do Panamá como um gerador de espécies in Zoantharia (Cnidaria: Anthozoa)

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Keywords: Allopatric, biogeography, early metazoans, evolution, Panama Isthmus, sister species, Zoantharia

Palavras-chave: Alopatria, biogeografia, metazoans basais, Istmo do Panama, espécies, irmãs, Zoantharia

Resumo

O objetivo deste capítulo é investigar as espécies de zoantários entre os oceanos Atlântico e Pacífico/Índico que possuem um relacionamento filogenético extremamente próximo e como esse padrão foi gerado. Nós realizamos análises filogenéticas de marcadores mitocondriais e nucleares dos gêneros de Zoantharia que possuem espécies para ambas as bacias oceânicas. Adicionalmente, foram copilados dados de morfologia e distribuição para esses grupos. Os

nossos resultados mostram que existem ao menos nove espécies de zoantários com relação filogenética estreita, no qual uma espécie ocorre no Oceano Atlântico e outra nos oceanos Pacífico/Índico. Essas espécies apresentam sequencias de DNA mitocondrial idênticas e clados irmãos de marcadores nucleares. Além disso, as características morfológicas são bastante semelhantes. Por conta da distribuição dessas espécies e um relacionamento genético bastante próximo, o fechamento do Istmo do Panamá é indicado como o evento que gerou a especiação desses zoantários.

ORIGINAL ARTICLE

“I’d walk a thousand miles if I can just see you tonight”: Isthmus of Panama closure as species generator in Zoantharia (Cnidaria: Anthozoa)

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ABSTRACT

Aim Investigate close-related representatives of the order Zoantharia (Cnidaria: Anthozoa) that occur in the Atlantic and Pacific/Indian oceans, and discuss how this pattern was probably generated.

Methods We performed phylogenetic analysis of mitochondrial and nuclear markers of zoantharian genera that have species in both ocean basins. In addition, we compiled morphological and distributional data for the close-related species pairs.

Results There are at least nine species pairs of highly close-related zoantharian in which one species is reported to Atlantic Ocean and another to Pacific/Indian oceans, in four out of ten genera analyzed. These sister species present identical mitochondrial sequences and close-related nuclear sequences. In addition, morphological characters between sister species are very similar.

Main conclusions Our results indicated that Panama Isthmus was the vicariant event that drive to speciation of zoantharians between the ocean basins. Thus, the ancestor of these zoantharian species probably had a cosmopolitan distribution before the isthmus closure.

Keywords Allopatric, biogeography, early metazoans, evolution, Panama Isthmus, sister species, Zoantharia

INTRODUCTION

Understand the processes that lead to speciation in marine systems is one of the main questions in evolutionary biology. Parapatry have been pointed as one of the key speciation modes in marine realm, once most biogeographic barriers are soft and species with large larval dispersion can spread through them (Rocha et al., 2005). However, physical barriers cannot be crossed by marine taxa and therefore are relevant in species origination (Coyne, 1992). One main example of vicariance event in oceans was the formation of Panama Isthmus about 3 MYA (Lessios, 2008; Bacon et al., 2015).

Among benthic taxa, zoantharians (Cnidaria: Anthozoa) represent an important ecological vector as they can form large colonies used for other organisms as a food source (Obuchi & Reimer, 2011; Longo et al., 2012; Hart et al., 2013), as well as endosymbiont and epizoic partner (Den Hartog & Holthuis, 1984; Swain & Wulff, 2007; Montenegro et al., 2015). The order Zoantharia is cosmopolitan and can be found in several ecosystems from shallow to deep-waters (Ryland, 1997; Sinniger et al., 2005; Reimer & Sinniger, 2010), and has the ability to gain/lose contrasting ecological traits relatively fast (Santos et al. in prep.)

Furthermore, the group is an interesting model to investigate the relationships of close-related species, as there are reports of shallow-water species from different ocean basins (Atlantic Ocean and Pacific/Indian oceans) with highly similar molecular sequences (Reimer et al., 2010, 2012, 2014; Santos et al., 2015). However, no study has specifically investigated this pattern, and, mostly important, many of these close-related species pairs remain unknown. Although sister species pairs are observed in marine groups (see for example Knowlton, 1993), molecular data did not show this pattern for the close-related anthozoan group of scleractinian corals (Fukami et al., 2004). Herein we examined the close-related zoantharian species between Atlantic Ocean (AO) and Pacific/Indian oceans (PIO) using mitochondrial and nuclear markers, aiming to clarify the outcome of sister species inside order Zoantharia.

METHODS

Species characteristics and distribution

For all sister species pairs possible, we provide a list of macro morphological characters. The characteristics analyzed were: number of tentacles; range size of oral disk; color patterns and form of the colony (West, 1979; Burnett et al., 1997; Reimer, 2010; Reimer et al., 2013). In addition, the biogeographic provinces (Briggs & Bowen, 2012) where these species are reported are also provided.

Phylogenetic analysis

All Zoantharia genera with molecular sequences available for both ocean basins (AO and PIO) were investigated. For each group (families Parazoanthidae and Hydrozoanthidae; family Epizoanthidae; family Sphenopidae; family Zoanthidae) we created two alignments: a concatenated alignment of both mt 16S rDNA and COI and an alignment of nuclear marker ITS r-DNA (18S ribosomal RNA, internal transcribed spacer 1, 5.8S ribosomal RNA, internal transcribed spacer 2 and 28S ribosomal RNA). We used as outgroup genus *Epizoanthus,* members of the order Actiniaria and genus *Parazoanthus,* respectively. Sequences of ITS r-DNA were analyzed in a separated alignment because this marker has fewer sequences available in GenBank than the mitochondrial ones. For Epizoanthidae, we created only an alignment of mt 16S rDNA+COI, once most species do not have sequences for both mithocondrial and nuclear markers available yet. Although there are reports of *Abyssoanthus* species for both ocean basins, we have not included it in the analysis because there is no nuclear data available for this family. We generated a concatenated alignment of concatenated markers (16S rDNA+COI) using Clustal Omega (Sievers et al., 2014) and edited by Gblock (Castresana, 2000). Phylogenetic relationships were estimated via PhyML (Guindon & Gascuel, 2003) for Maximum-Likelihood (ML) analyses with the Kimura-2-parameter model of nucleotide distribution and estimated parameters (Kimura, 1980). Robustness of the ML reconstruction was tested with 1,000 booststrap replicates.

As in most anthozoans (Shearer et al., 2002, Stampar et al. 2012), mitochondrial markers are reported to have a slow mutation rate in Zoantharia (Sinniger et al. 2008), while nuclear markers have a better resolution for these animals (Reimer et al., 2010, 2012). Thus, we considered as “sister species” zoantharians groups that one species occurs in AO and another in PIO, but have a high close phylogenetic relationship (identical mitochondrial sequences; sister clades of nuclear marker ITS r-DNA, when available data of these markers).

RESULTS AND DISCUSSION

Sister species characteristics and distribution

There are at least nine sister species belonging to four genera from both suborders of order Zoantharia. These species are listed in table 1 along with macro morphological characters and distribution within biogeographic provinces, while phylogenetic relationships are show in figure 1.

Table 1: List of zoantharian sister species along with their morphological and distribution data

Suborder	Family	Species	Distribution		Oral disk diameter	Oral disk color	Colony form	Tentacle number
			Ocean	Provinces distribution				
Brachycnemina	Zoanthidae	*Zoanthus sansibaricus* Carlgren, 1900	PIO	Central-Pacific, Central Indo-Pacific, Sino-Japanese	2-12mm	orange, red, brown, green, purple, white, blue, yellow; purple, white, red, blue, green, brown, yellow, white; sometimes with elaborate petterns	liberae	54-56; 40-58
		Zoanthus sociatus (Ellis 1768)	AO	Brazilian, Caribbean	2-10mm	green, blue, yellow, sometimes with patterning; brown, patterning brown triangle	liberae	48-60

	Zoanthus kuroshio (Reimer and Ono, 2006)	PIO	Sino-Japanese	6 to 12mm	pink, light purple, green	intermediae, immersae	50-64
	Zoanthus aff. *pulchellus*	AO	Brazilian, Caribbean, Tropical Eastern Atlantic	10mm	green, pink	intermediae, immersae	50-64
	Zoanthus vietnamensis Pax and Muller, 1957	PIO	Sino-Japanese	3,95-5,55mm	pink, purple	liberae	-
	Zoanthus pulchellus (Duchassaing and Michelotti 1860)	AO	Brazilian, Caribbean	4-6mm	green, yellow, pink	-	50 to 60
	Palythoa tuberculosa (Esper, 1805)	PIO	Eastern Pacific , Central-Pacific, Central Indo-Pacific, Western Indian Ocean	2 to 8mm	pale, yellow, brown, green	immersae	38-52; 31-35

		Palythoa caribaeorum (Duchassaing and Michelotti, 1860)	AO	Ascension, Brazilian, Caribbean, Eastern Atlantic	~5mm	cream, brown, yellow, green, brown	immersae	28-34
	Sphenopidae							
		Palythoa mutuki (Haddon and Shackleton, 1891)	PIO	Palau, Indo-Polynesian, Galapagos	0.98-1.5cm; up to 2cm	brown, can have white line or greenish pattern	intermediae, liberae	52-56; 47-61
		Palythoa grandiflora (Verril, 1900)	AO	Brazilian, Caribbean	14 to 16mm	brown, oral disk may present greenish pattern or a bold white line in the ventral directive axis	-	52-56
		Palythoa heliodiscus (Ryland and Lancaster, 2003)	PIO	Indo-Polynesian, Sino-Japanese	up to 15mm; 2cm	brown, oral disk usually with fine pale radiating lines	libearae	>60; up to 80
		Palythoa variabilis (Duerden, 1898)	AO	Brazilian, Caribbean	~10mm	brown, greenish, white pale radiating lines on the oral disk	liberae	60-80

		Palythoa aff. *clavata*	PIO	Ascension, Brazilian, Caribbean	-	-	liberae	~60
		Palythoa sp. 'sakurajimensis'	AO	Sino-Japanese	-	-	-	-
Macrocnemina	Parazoanthidae							
		Antipathozoanthus hickmani Reimer and Fujji 2010	PIO	Galapagos	4-12mm	red, yellow, cream	-	~40
		Antipathozoanthus macaronesicus (Ocaña and Brito, 2004)	AO	Tropical Eastern Atlantic	4-6mm	yellowish or pinkwish, orange	-	~42
		Savalia sp. FS-2007	PIO	-	-	-	-	-
		Savalia savaglia (Bertoloni, 1819)	AO	Tropical Eastern Atlantic; Mediterranean; canary islands	-	yellow; orange	-	21-26;27

Suborder Brachycnemina have seven sister species pairs from two genera. Within the genus *Palythoa*, there are four sister species. Species *P. tuberculosa* and *P. caribaeorum* present the same coloration of polyps and an immersae form of colony. Number of tentacles and oral disk diameter (ODD) also overlaps. Nonetheless, *P. tuberculosa* seems to have slightly larger polyps and number of tentacles. Similarly, *P. mutuki* and *P. grandiflora* have overlapping ODD and number of tentacles, but with *P. mutuki* slightly larger ODD. Coloration and an intermdiae/liberae colony form of these species are also the identical, including oral disk pattern (white line or greenish color). Another species pair, *P. heliodiscus* and *P. variabilis* display overlapping number of tentacles, form of colony (liberae) and color patterns, and also similar ODD. *P.* sp. 'sakurajimensis' and *P.* aff. *clavata* are not formally described yet, but their genetic relationship places them as a pair of sister species. Similarly, in genus *Zoanthus*, there are three sister species. *Z. sansibaricus* and *Z. sociatus* have same ODD, a liberae colony and similar number of tentacles. In addition, the two species present a high variety of oral disk color and patterning. These species are also very similar to species *Z.* aff. *vietnamensis* (PIO) and *Z. pulchellus* (AO). However, the later pair can present a larger ODD. It is also possible that past literature may have confounded *Z. sociatus* and *Z. pulchellus*, which would explain some overlapping characters. In addition, sister species *Z. kuroshio* and *Z.* aff. *pulchellus* have similar ODD, tentacle number, colony form and most coloration patterns.

Likewise, suborder Macrocnemina has sister species in two genera. Within genus *Antipathozoanthus*, *A. hickmani* and *A. macaronesicus* have similar ODD, tentacle number and coloration. Additionally, the genus *Savalia* have a species pair report, however, there are no morphological information about species *Savalia* sp. FS-2007 available.

Zoantharians have a high morphological plasticity as is observed in table1. Some characteristics are similar among congeners, but the most characters relatedness is observed between sisters species. Ryland & Lancaster (2003) also indicated morphological similarities between *Palythoa* representatives reported from different ocean basins and discussed that these similar species probably have a close phylogenetic relationship (species *Palythoa grandiflora/P. mutuki* and *P. heliodiscus*/*P. variabilis*). We did not included internal morphology data once there is few information available about variability of these characters for most evaluated species.

There is an extensive lack of zoantharian record in several localities around the globe. Thus, so far, the group do not have enough data to perform biogeographic analysis in a large scale (Santos et al., 2015). This is particularly evidently for macrocnemic zoantharians, that can have cryptic habitats and diminutive size (Sinniger & Häussermann, 2009; Fujii & Reimer, 2013). Still, records of species distributions showed in table 1 confirm that most species are widely distributed in the ocean basin, and this data is for sure underestimated. Moreover, further surveys on poorly known groups, such as the recently described family Nanozoanthidae (Fujii & Reimer, 2013), will likely report sister species to other zoantharian clades.

Sister species phylogeny

Phylogenetic relationships are show in Figure 1-4. Although some of these genera have species reported to deep-waters, up to now there are only reports of shallow-water zoantharian sister species.

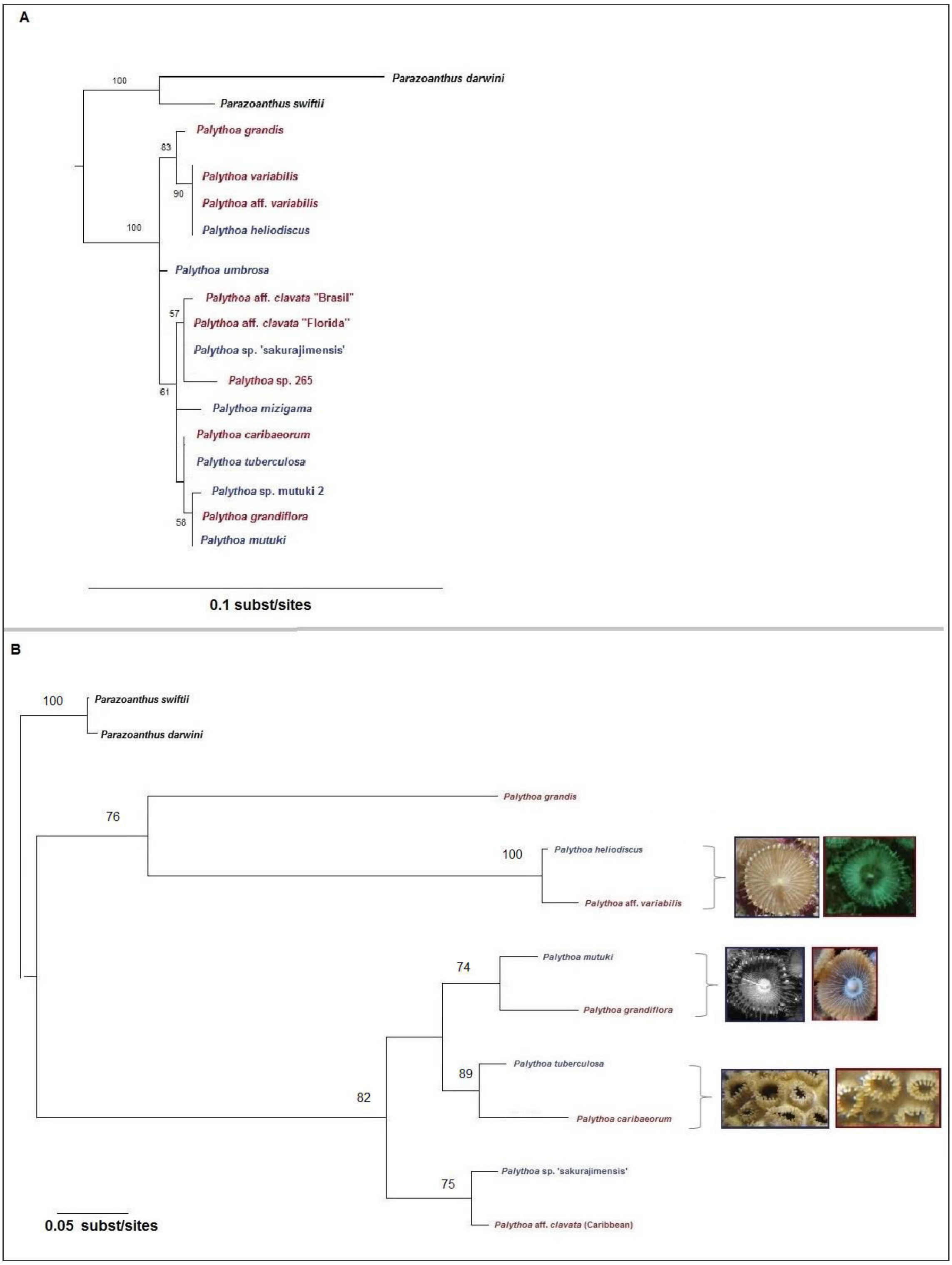

Figure 1: ML tree of family Sphenopidae obtained from alignments of mitochondrial 16S rDNA+COI (a) and nuclear (b) markers, using *Parazoanthus* representatives as outgroup. Values at the branches represent ML boostrap. Left images on figure b are the species from Pacific/Indian oceans, while images on the right are the species from Atlantic Ocean. Species in blue = sequences from Pacific/Indian oceans, whereas species in red = sequences from Atlantic Ocean.

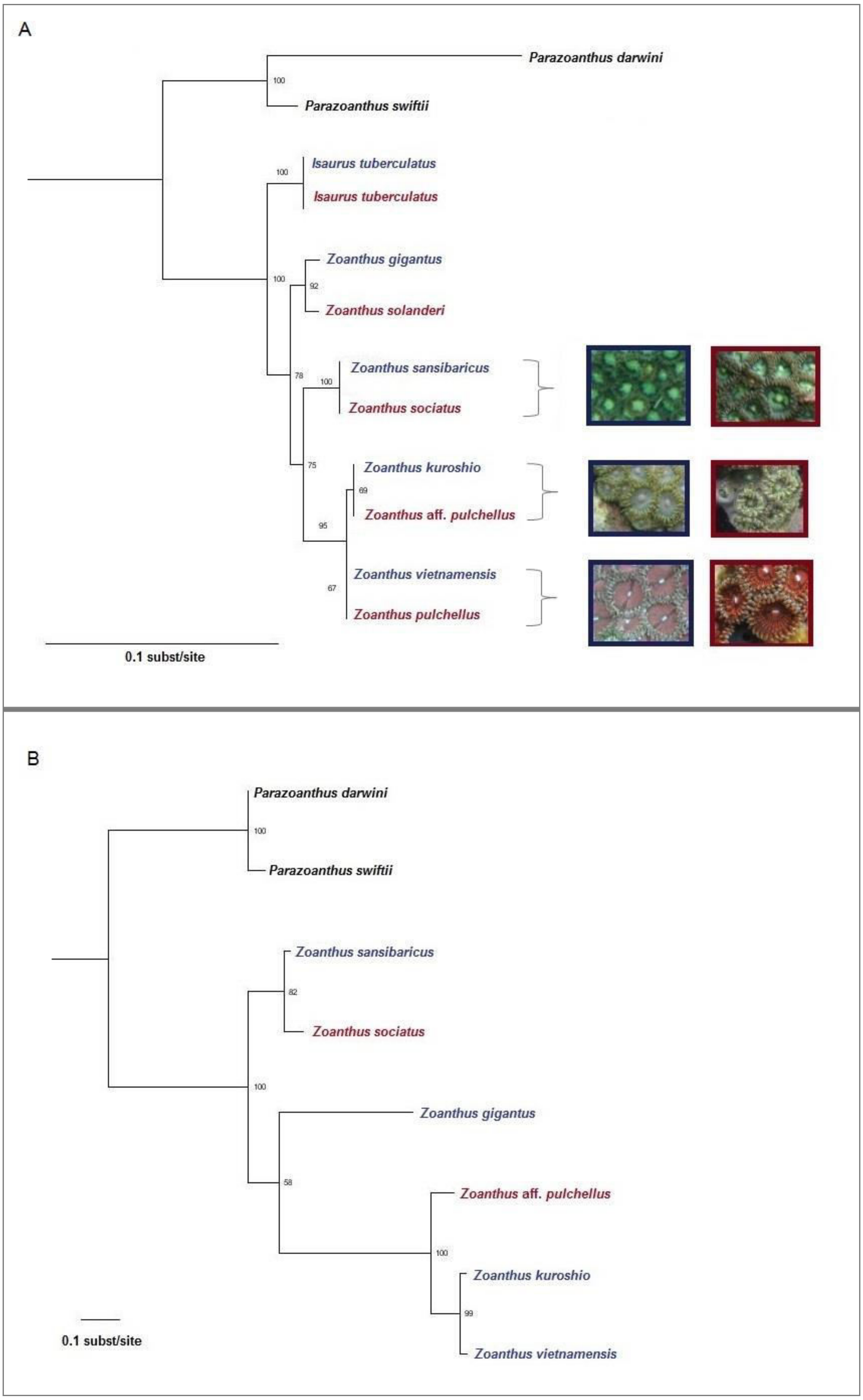

Figure 2: ML tree of family Zoanthidae obtained from alignments of mitochondrial 16S rDNA+COI (a) and nuclear (b) markers, using *Parazoanthus* representatives as outgroup. Values at the branches represent ML boostrap. Left images on figure b are the species from Pacific/Indian oceans, while images on the right

are the species from Atlantic Ocean. Species in blue = sequences from Pacific/Indian oceans, whereas species in red = sequences from Atlantic Ocean.

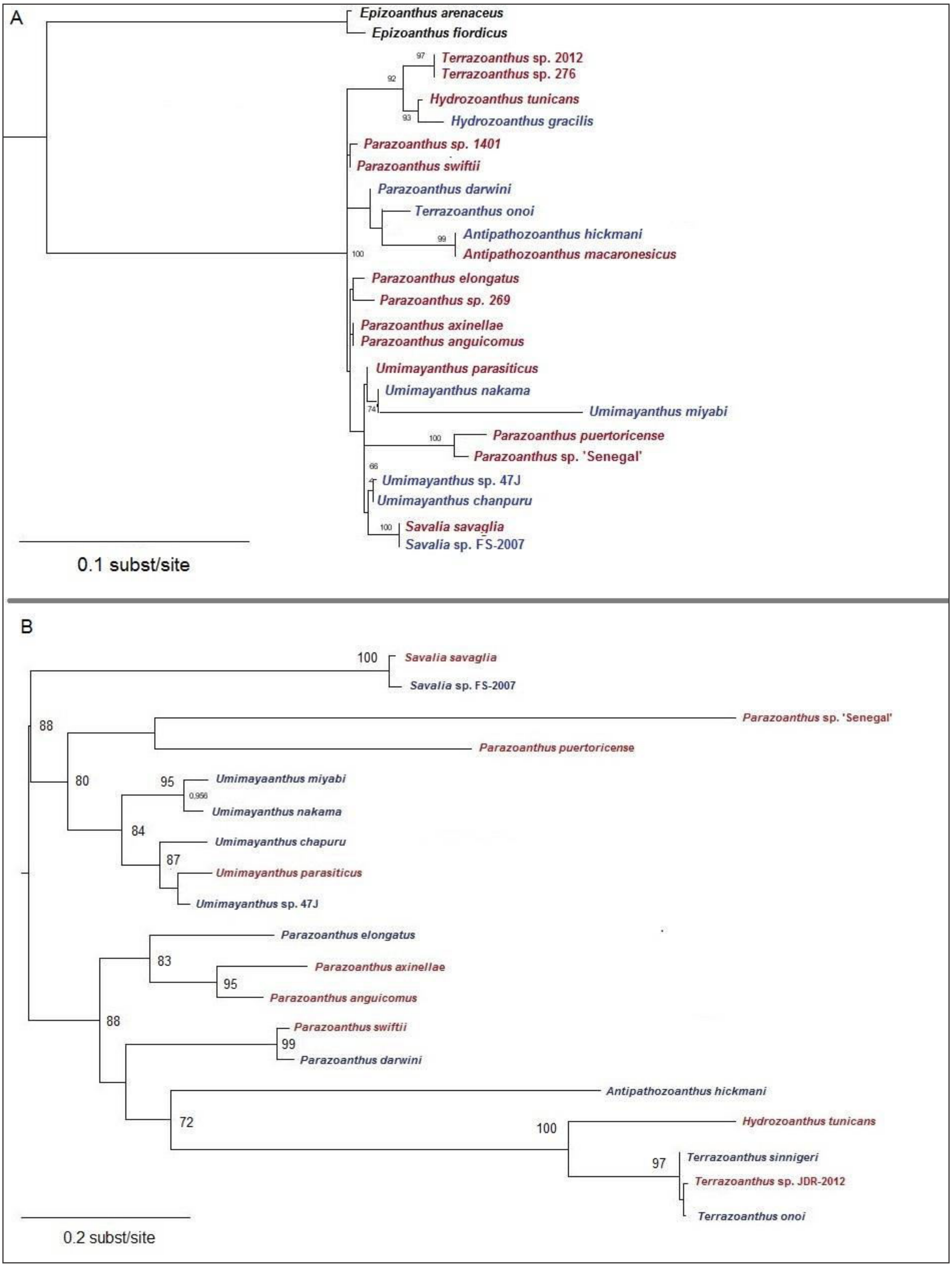

Figure 3: ML tree of family Parazoanthidae and Hydrozoanthidae obtained from alignments of mitochondrial 16S rDNA+COI (a) and nuclear (b) markers, using *Epizoanthus* representatives as outgroup.

Values at the branches represent ML bootstrap. Species in blue = sequences from Pacific/Indian oceans, whereas species in red = sequences from Atlantic Ocean.

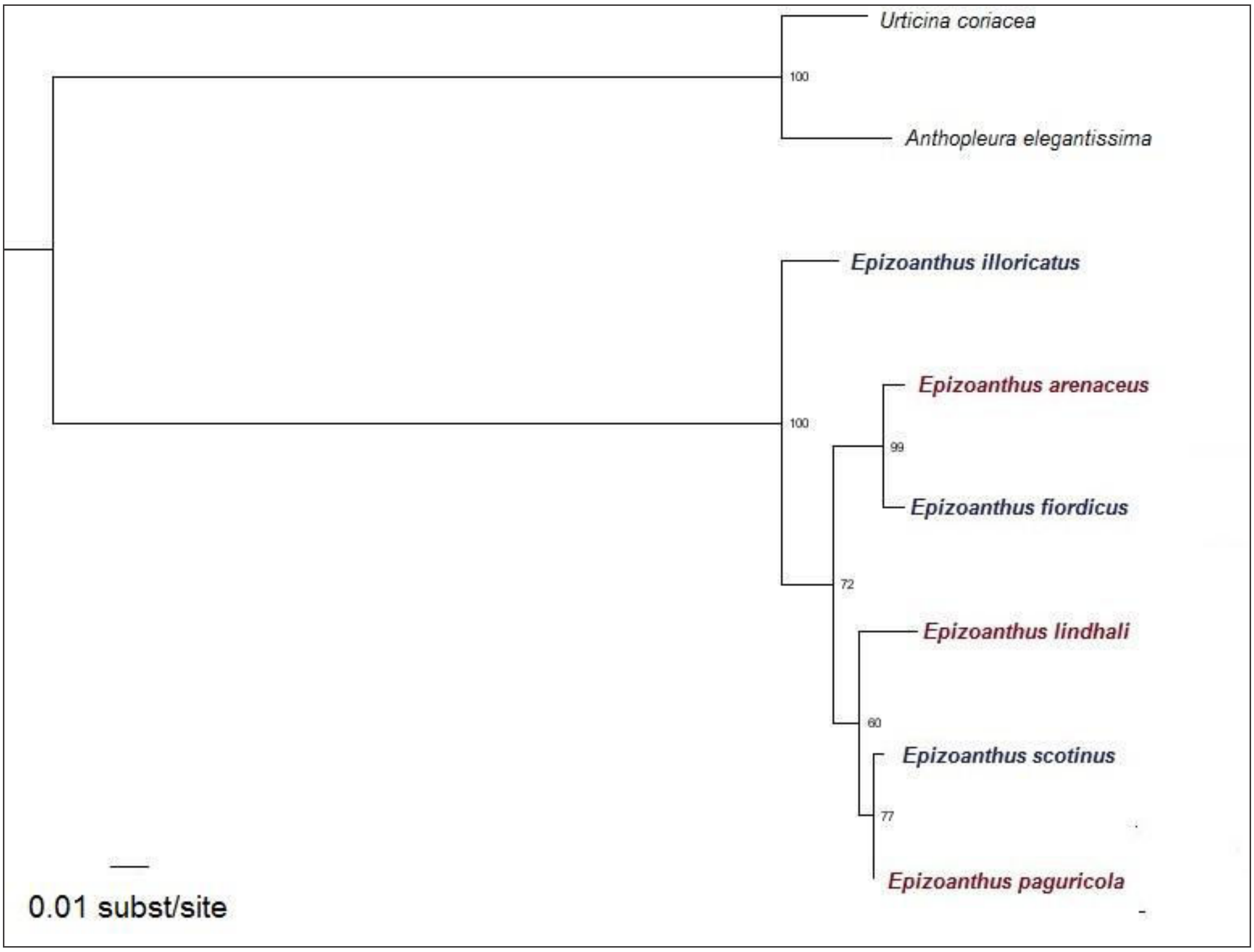

Figure 4: ML tree of family Epizoanthidae obtained from alignments of mitochondrial 16S rDNA+COI markers, using Actniaria representatives as outgroup. Values at the branches represent ML bootstrap. Species in blue = sequences from Pacific/Indian oceans, whereas species in red = sequences from Atlantic Ocean

Moreover, there are also close-related species groups from both ocean basins with highly similar but not identical sequences of mitochondrial markers, but not identical (suborder Macrocnemina: *Umimayanthus* parasiticus/U*.* 47J, *Parazoanthus swifti/ P.* sp. 1401 and *P. darwini, Hydrozoanthus gracilis*/*H. tunicans, Epizoanthus arenaceus/ E. fiordicus;* suborder Brachycnemina: *Zoanthus solanderii/Z. gigantus*)*.* The presence of close-related species between AO and PIO in different groups of the order indicated that it is not a monophyletic trait and an evolutionary pathway lead to this pattern in several clades of Zoantharia.

Isaurus tuberculatus is reported for both ocean basins, and there is no sequences of nuclear molecular markers for the specimen from AO. Likewise, there are additional shallow-water zoantharians species with records on both oceans (e.g. *Epizoanthus paguricola* was recorded from Atlantic Oceans and New Zealand), but no molecular data from specimens of the two ocean basins are yet available. These examples most probably consists of a species pair as is observed with other shallow-water zoantharians. Therefore, it is likely that these species might represent two or more related species.

How did zoantharia converged even thousands of kilometers apart?

It is remarkable the number of close-related species between the ocean basins observed in Zoantharia. There are molecular sequences available for species in both AO and PIO for ten out of twenty-six genera described, in which four genera show sister species, as well as highly close-related species in another three genera. Within scleractinian corals from AO and PIO, it is observed a deep molecular divergence between species that once were thought to be closely related regarding their morphological characters (Fukami et al. 2004). Therefore, the occurence of sister species between AO and PIO is an unusual pattern of what is known from other common corals. Reproduction studies with zoantharians indicated that species can have a planctonic larvae that lives up to 170 days (Ryland et al., 2000; Polak et al., 2011), as well as several forms of asexual reproductions modes (Acosta et al., 2005; Acosta & González, 2007). Still, there are several groups of Zoantharia with no reproduction data available in literature.

In a general way, processes in geological time scale are important historical events in allopatric evolution. For example, tectonic plates events have lead to the pattern of sister species in some groups of reef fishes (Bernardi et al. 2008; Floeter et al. 2008; Alva-Campbell et al. 2010). Due to a high genetic similarity between the zoantharian sister species and the widespread distribution of species within an ocean basin, the closure of Panama Isthmus is the most likely event that generate this pattern. Thus, it is likely that before the closure of Panama most zoantharian species have a cosmopolitan distribution. These molecular and morphological similarities observed within zoantharian sister species may also reflect a same resources use (e.g. niche position).

What could be if not the Panama Isthmus?

Another vicariance event that could have influenced speciation of Zoantharia was the closure of Thetis Sea. However, it is a much older event (12-20 MYA.; Dercourt et al., 1986) than the closure of Panama Isthmus. Thus, species would have time to accumulate more genetic differences. Another possible process is the speciation lead by ecological differences between the species. This is also insufficient plausible, once there are species widely distributed within an ocean basin in biogeographic provinces that have very distinct characteristics. Therefore, the closure of Panama Isthmus is the most likely processes that could have generated highly close-related zoantharian species between Atlantic and Pacific/Indian oceans.

CONCLUSIONS

This high dispersion capacity of zoantharians have lead to widespread species within an oceanic basin; moreover, vicariance events lead to the speciation into sister species among the oceans. Future investigation on connectivity and ecological aspects of sister species should provide a better understanding of this speciation process. Also, studies on poorly known groups are needed and it is likely that other species pairs will be reported to the order. Finally, surveys on zoantharians diversity are essential to fill gaps of species distribution and improve our knowledge on biogeographic patterns of order Zoantharia.

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