

# Zooxanthellate zoantharians (Anthozoa: Hexacorallia: Zoantharia: Brachycnemina) in the northern Red Sea

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**Abstract** The Red Sea was one of the first areas of the Indo-Pacific to be investigated by marine taxonomists, and the literature on suborder Brachycnemina (Anthozoa: Hexacorallia: Zoantharia) for this region dates from the early nineteenth century. However, in the last 100 years there has been only one focused study on this group in the Red Sea. In the present study, specimens collected from the Saudi Arabian coast of the northern half of the Red Sea were phylogenetically analyzed by sequencing nuclear internal transcribed spacer regions of ribosomal DNA (ITS-rDNA), mitochondrial cytochrome oxidase subunit I (COI), and 16S ribosomal DNA (16S-rDNA). The new results were compared with historical data in the literature and recent results from the Persian Gulf and the southeastern coast of Africa. Results show the presence of six to seven potential Brachycnemina species in the

Red Sea; five to six *Palythoa* species (*Palythoa mutuki*, *P. tuberculosa*, *P. cf. heliodiscus*, *P. aff. heliodiscus*, and one to two species within the *P. sp.* “sakurajimensis” group) together with *Zoanthus sansibaricus*. While *P. mutuki*, *P. tuberculosa*, and *Z. sansibaricus* are known to be widely distributed in the Indo-Pacific, *P. cf. heliodiscus* and *P. sp.* “sakurajimensis” have not been reported from the Persian Gulf or the southeastern coast of Africa, and the current results represent large range extensions for these two species. Only one of the observed species, *P. aff. heliodiscus*, is potentially endemic to the Red Sea, further demonstrating the generally wide distributions of most zooxanthellate Brachycnemina species.

**Keywords** Saudi Arabia · Taxonomy · Zoogeography · Cnidaria · Species diversity

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## Introduction

Biogeographical data are key to understanding both the origins of diversity and evolution in the marine realm (Ekman 1953; Vermeij 1978; Briggs 2003; Rocha et al. 2008). Although recent studies have provided insights of global biogeographical provinces (see for example Briggs and Bowen 2012, 2013), distribution data is still scarce for many taxonomic groups.

One such group is the order Zoantharia Gray 1832, which are anthozoans found in a wide variety of marine ecosystems. In shallow subtropical and tropical regions, species of the suborder Brachycnemina Haddon and Shackleton 1891a often form a major component of benthic communities (Goreau 1959; Karlson 1983; Irei et al. 2011). Brachycnemina contains three families: Neozoanthidae Herberts 1972, Sphenopidae Hertwig 1882, and Zoanthidae Rafinesque 1815. In particular, species of the genera *Palythoa* Lamouroux 1816 (Sphenopidae) and *Zoanthus* Lamarck, 1801 (Zoanthidae) are common in

coral reef habitats (Karlson 1983; Bastidas and Bone 1996; Oigman-Pszczol et al. 2004; Irei et al. 2011) and thus have been the focus of both ecological (Reimer 1971; Sebens 1977; Longo et al. 2012; Ong et al. 2013; Santana et al. 2015) and marine natural product studies (Kelecom and Solé-Cava 1982; Miyashita et al. 2004; Soares et al. 2006; Chimetto et al. 2009), as well as being implicated in phase shifts (Cruz et al. 2015; Cruz et al. 2016) and outbreaks due to decreases in environmental health of coastal marine ecosystems (Costa et al. 2008).

However, despite their obvious importance in subtropical and tropical areas, our knowledge of *Brachynemina* species' distribution patterns remains patchy, with many marine regions lacking even information such as species lists or records of occurrence (Khushali and Pradeep 2013; Reimer et al. 2014, 2015; Santos et al. 2016). Therefore, information covering a wider portion of *Brachynemina* species' ranges is needed in order to undertake large-scale analyses to more completely understand their species diversity and ecology.

The Red Sea is one region where there is a paucity of data for zoantharians. The Red Sea Province is located in the Tropical Indo-West Pacific Region, neighboring the Western Indian Ocean Province on the west (Briggs and Bowen 2012). Despite being the first region of the Indo-Pacific to have its Zoantharia investigated (Savigny 1811; Audouin 1826; Gray 1828; Ehrenberg 1834; Milne Edwards 1857; Klunzinger 1877), since 1900 only two studies have examined Zoantharia of the Red Sea in detail (Carlgren 1900; Pax and Muller 1956) and none in the last 60 years. Even by the standards of zoantharian taxonomy in general, which has previously been described as "chaotic" (Burnett et al. 1997), the history of *Brachynemina* taxonomic studies in the Red Sea is particularly convoluted and problematic (Table S1).

However, recent research has explored the diversity and phylogeny of *Brachynemina* species in regions in the Indo-Pacific on either side of the Red Sea; both in the Persian Gulf (Koupaei et al. 2014, 2015) and along the southeast African coast (Risi and Macdonald 2015, 2016). In the present study, via field surveys and phylogenetic analyses, the species diversity of *Brachynemina* in the northern Red Sea is explored and compared with data in historical taxonomic literature and recent phylogenetic results from these neighboring regions. In addition, suggestions are given for future large-scale analyses of *Brachynemina* in the Red Sea and at larger, oceanic basin to global scales.

## Materials and methods

### Specimen collection

Specimens of *Brachynemina* were collected by SCUBA or snorkeling along the Saudi Arabian coast of the northern and central Red Sea in September and October 2013 at 11 sites

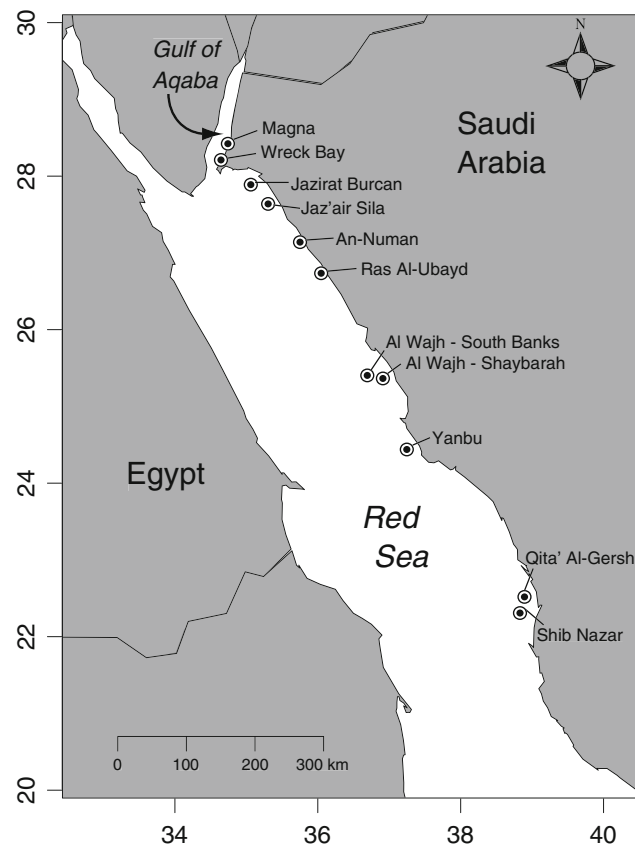
(Fig. 1, Table S2). A total of 72 *Brachynemina* specimens were collected from the intertidal zone to a depth of 45 m (Table S2, Fig. 2). Specimens were subsequently fixed in 95% ethanol and are currently housed at the Red Sea Research Center of the King Abdullah University of Science and Technology (KAUST; Thuwal, Saudi Arabia) under specimen numbers given in Table S2.

### Specimen identification and literature review

Specimens were identified based on a combination of DNA sequences and morphology. For morphological examinations, we generally followed the past works of Klunzinger (1877), Carlgren (1900), Pax and Muller (1956), and Ryland and Lancaster (2003), along with recent morphological and phylogenetic results of Reimer et al. (2007b) and Hibino et al. (2013). For details on morphology and identification rationale see Table S1, and for specimen identity conclusions see Table S2.

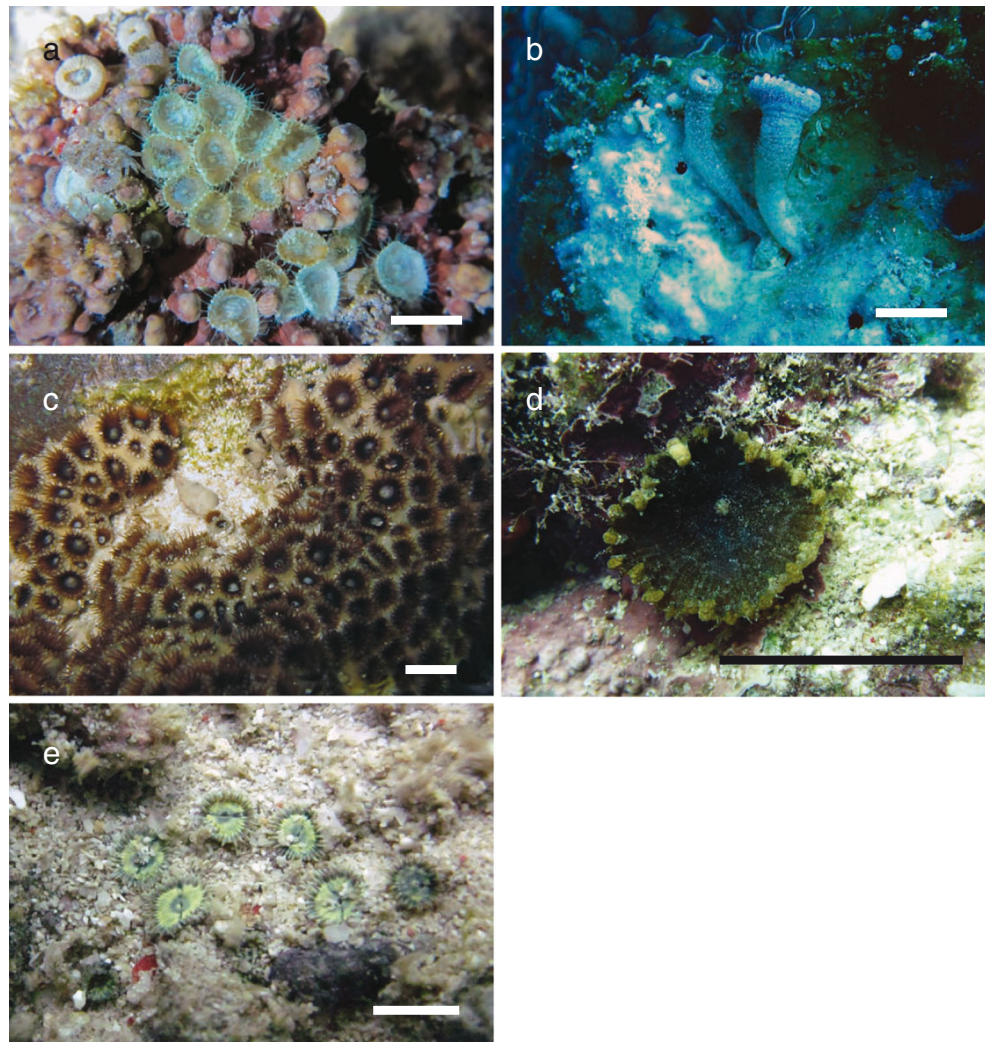
### DNA processing and amplification

DNA was extracted from specimens utilizing a Qiagen (Tokyo) Blood and Tissue Kit following the



**Fig. 1** Map of sampling sites in this study in the northern Red Sea along the coast of Saudi Arabia

**Fig. 2** Species of Brachycnemina observed in the northern Red Sea. a) *Palythoa* cf. *heliodiscus*, b) *Palythoa* aff. *heliodiscus*, c) *Palythoa tuberculosa*, d) *Palythoa* sp. “sakurajimensis”, and e) *Zoanthus sansibaricus*. Scale bars equal approximately 1 cm



manufacturer’s instructions. Direct PCR amplifications were performed for the nuclear internal transcribed spacer region of ribosomal DNA (ITS-rDNA), as well for two mitochondrial markers; cytochrome oxidase subunit I (COI) and 16S ribosomal DNA (16S-rDNA). The primer pairs ZoanF and ZoanR (both from Reimer et al. 2007c), COIZoanF (Reimer et al. 2007a) and HCO2198 (Folmer et al. 1994), and 16SbmoH and 16Sant1a (both from Sinniger et al. 2005) were used for each marker, respectively. All amplified products were then checked on a QIAxcel genetic analyzer (Qiagen), treated with ExoSTAR PCR clean-up (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and Sanger sequenced at the KAUST Bioscience Core Laboratory.

### Phylogenetic analyses

The nucleotide sequences of ITS-rDNA, 16S-rDNA, and COI were aligned separately using the Geneious v8.1 (Kearse et al. 2012; <http://www.geneious.com>) alignment tool with the option “global alignment with free end gap”

with 99 refinement iterations. For ITS-rDNA independent alignments were performed for each genus as sequences are highly divergent between the two groups (Reimer et al. 2007b, c), while for 16S-rDNA and COI the sequences of genus *Zoanthus* and *Palythoa* were aligned together. The terminal regions in the alignments were trimmed and shorter or poor quality sequences were eliminated. All alignments were refined with the MUSCLE (Edgar 2004) alignment tool implemented in Geneious, configured with a “maximum number of iterations: 100”; “maximum number of trees to build: 1000”; “optimization: anchor”; “terminal gaps: half penalty”. After MUSCLE refinement, all alignments were manually curated. In this manner, four aligned datasets were generated; 465 sites for 46 sequences for ITS-rDNA of *Zoanthus*, 519 sites for 135 sequences for ITS-rDNA of *Palythoa*, 452 sites of 253 sequences for 16S-rDNA, and 306 sites of 89 sequences for COI. Novel sequences obtained during this study were deposited in GenBank (accession numbers MF033907-MF034019, and MF036061-



MF036114). It should be noted that COI sequences generated from this study and deposited in GenBank are much longer (~560 bp) than the sequences utilized in the analyses. COI sequences were trimmed to 306 bp in the alignment in order to maximize comparisons with sequences previously reported from other locations.

Phylogenetic reconstruction was performed for each alignment using neighbor-joining (NJ) and maximum-likelihood (ML). NJ trees were constructed in Geneious with the Hasegawa-Kishino-Yano genetic distance model (HKY; Hasegawa et al. 1985); 1000 bootstrap replicate trees were constructed using the same method. The ML trees were reconstructed using PhyML (Guindon et al. 2010) with Smart Model Selection (ATGC: SMS), with the following model parameters: HKY85 + G + F for *Zoanthus* ITS-rDNA, *Palythoa* ITS-rDNA, and COI, and TN93 + G + F for 16S-rDNA; 1000 bootstrap replicate trees were constructed using the same methods.

Phylogenetic alignments and tree files are available as downloadable supplementary material.

## Results

### ITS-rDNA (Fig. 3)

The ITS-rDNA phylogenetic trees provided more resolution than the mtDNA trees, with some important differences from the mtDNA trees.

#### *Palythoa* ITS-rDNA (Fig. 3a)

The maximum-likelihood tree resulting from the *Palythoa* ITS-rDNA alignment showed several clades that corresponded to morphospecies. *Palythoa heliodiscus* and related species formed one clade, containing two subclades. One subclade included both current Red Sea specimens' sequences (9, 31, 32, 88, 130, 150, 171, 183, 185, 193) and previously reported sequences from the Pacific; this well-supported subclade (ML = 83%, NJ = 100%) was designated *Palythoa* cf. *heliodiscus*. The other very well-supported subclade (ML = 98%, NJ = 100%) contained sequences from three Red Sea specimens that were not similar to any other reported sequence, this subclade was designated *P. aff. heliodiscus*.

Sequences from almost all Red Sea *Palythoa tuberculosa* colonies formed a large polytomy at the base of the other large clade within the tree, clustering with previously reported *P. tuberculosa* sequences from across the Indo-Pacific. The only exceptions were three specimens' sequences (23, 98, 167) that formed a well-supported subclade (ML = 95%, NJ = 96%) close to *Palythoa mutuki* and related sequences.

Sequences from several specimens formed a well-supported subclade (ML = 92%, NJ = 72%) with previously

reported *P. mutuki* sequences from the Indo-Pacific and *P. nelliae* sequences from South Africa. This grouping was designated as *P. mutuki* in this study.

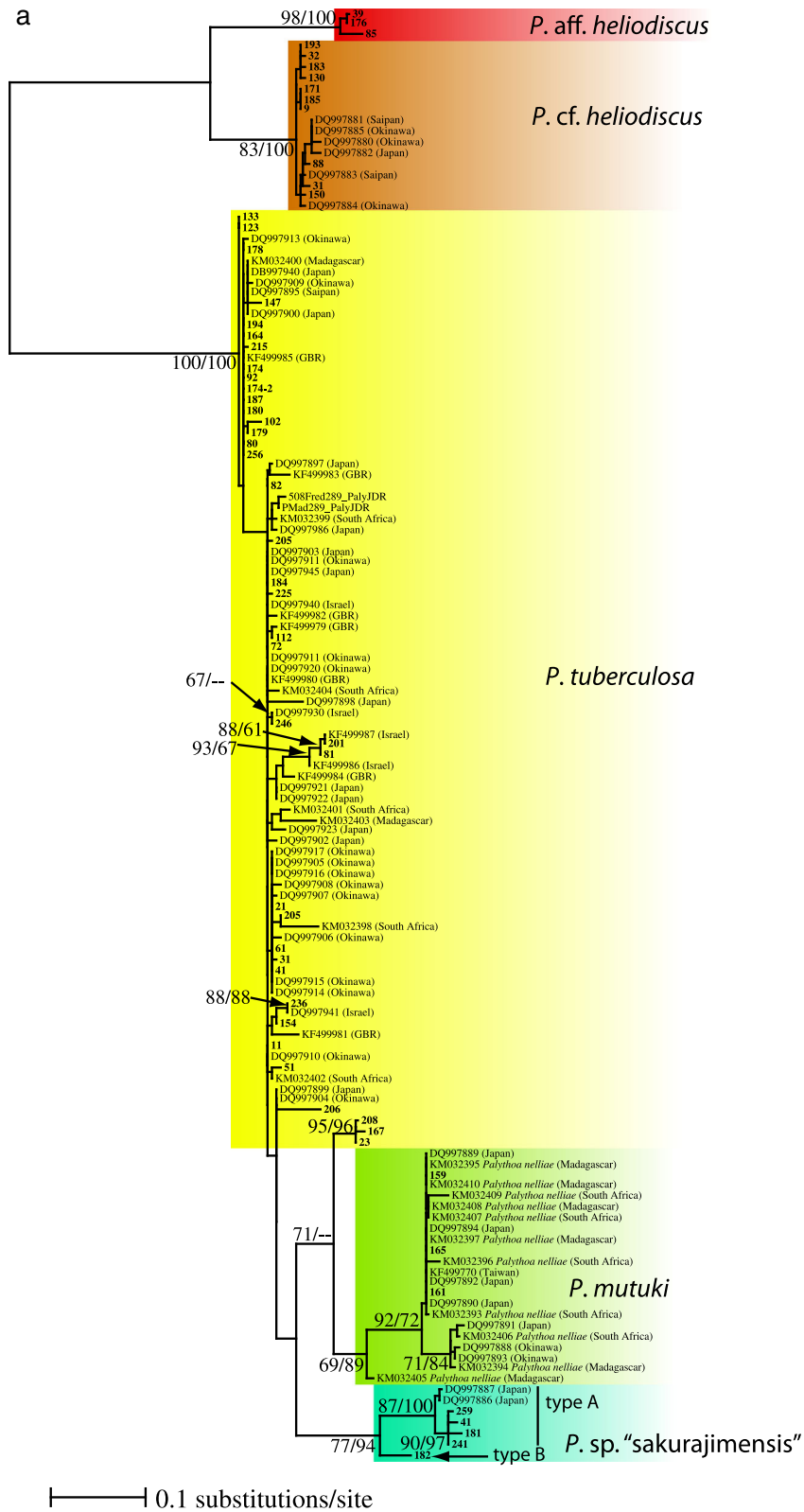
Finally, sequences from four specimens (41, 181, 182, 241) clustered with previously reported *P. sp.* "sakurajimensis" sequences from Japan and Taiwan in a moderately supported clade (ML = 77%, NJ = 94%). Of these four sequences, three formed a subclade (ML = 87%, NJ = 100%) with all previously reported sequences; this grouping was designated as *P. sp.* "sakurajimensis" group A. The remaining single sequence from specimen 182 was basal and divergent and was designated as *P. sp.* "sakurajimensis" group B.

#### *Zoanthus* ITS-rDNA (Fig. 3b)

ITS-rDNA sequences from *Zoanthus* specimens in the Red Sea were all shown to cluster with previously reported sequences of *Zoanthus sansibaricus* from both Japan and South Africa and not with those of any other *Zoanthus* species. The sequence from specimen 177 clustered with sequences of the *Z. sansibaricus* "distant" ITS-rDNA allele (ML = 100%; NJ = 100%), while those from specimens 148, 197, 228, and 238 clustered with those from the "normal" *Z. sansibaricus* allele (ML = 66.7%; NJ = 100%) (for "distant" and "normal" ITS-rDNA allele explanation see Aguilar and Reimer 2010).

#### 16S-rDNA (Fig. S1)

The phylogenetic tree generated from the 16S-rDNA alignment showed all Red Sea *Zoanthus* specimens' sequences (196, 197, 199, 228, 238, 239, 243, 271) to be identical with previously reported *Zoanthus sansibaricus* sequences from Japan, South Africa and the Persian Gulf, within a clade (ML = 65.5%, NJ = 59.6%). For *Palythoa*, specimen 165 was identical to sequences of *P. mutuki* "2" from Japan (DQ997841), as well as numerous sequences from South Africa of *P. nelliae* and *P. mutuki* sequences from the Persian Gulf. Numerous Red Sea specimens morphologically identified as either *P. tuberculosa* (23, 80, 147, 167, 169, 178, 179, 200, 201, 210, 213) or *P. mutuki*-related (158, 181, 182, 241, 259) were all identical to previously reported sequences of *P. tuberculosa* from numerous locations, as well as to *P. sp.* "sakurajimensis" from Japan (DQ997842), *P. nelliae* from South Africa, *P. mutuki* "1" from Japan (DQ997875) and *P. aff. mutuki* from the Persian Gulf, within a large "mixed" clade with high support (ML = 99.9%; NJ = 99.9%). One sequence (174) was different by a single base pair substitution from this group. Finally, several specimens from the Red Sea (9, 27, 28, 31, 39, 85, 88, 130, 150, 157, 170, 171, 172, 176, 183, 185, 188, 189, 194, 195) had 16S-rDNA sequences identical to those reported for *P. heliodiscus* from Japan (DQ997844, AB219223) and from the aquarium trade (*P. cf.*



**Fig. 3** a) Maximum likelihood (ML) tree of internal transcribed spacer region (ITS-rDNA) sequences of *Palythoa*. b) Maximum likelihood (ML) tree of internal transcribed spacer region (ITS-rDNA) sequences of *Zoanthus*. Different colors represent major species groups. Sequences

from previous studies with GenBank accession numbers, location, and species name (if not same as name of each species clade). Sequences from specimens in this study in bold. Values at nodes represent ML and neighbor-joining (NJ) values

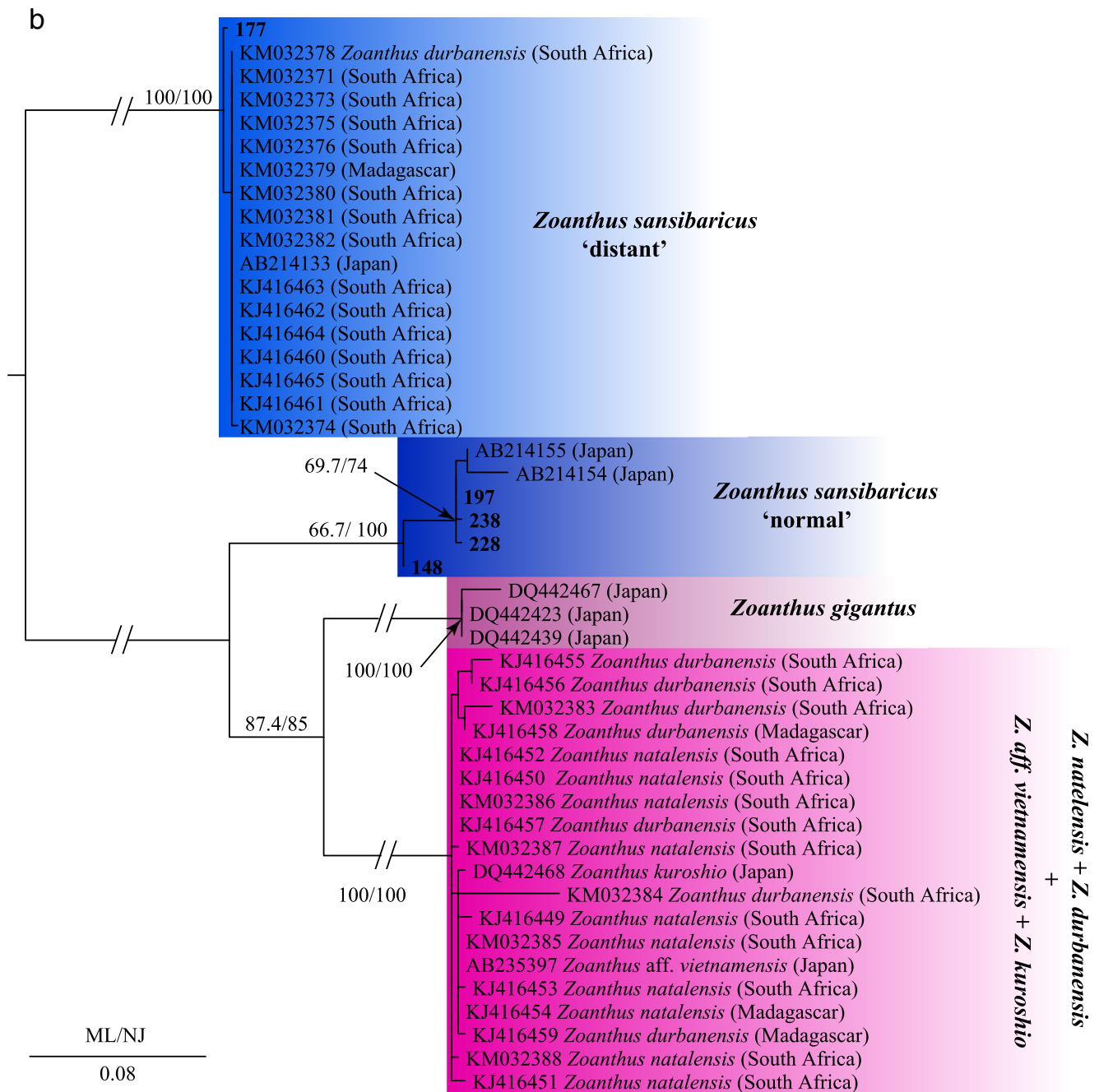


Fig. 3 (continued)

*helioidiscus*; HM754466) and formed a subclade within the large “mixed” clade (ML = 70.6%, NJ = 77.2%).

### COI (Fig. S2)

The COI tree showed results similar to those of the 16S-rDNA tree, with specimen 243 identical to previously reported *Z. sansibaricus* from the Persian Gulf (shorter fragment sequences also identical to *Z. sansibaricus*: 228, 238, 271); these sequences formed one clade. Within *Palythoa*, many

specimens identified morphologically as *P. tuberculosa* (40, 80, 81, 97, 116, 147, 167, 169, 174, 186, 198, 200, 201, 202, 205, 206, 207, 208, 210, 213) were identical to previously reported sequences of *P. tuberculosa*, *P. sp.* “sakurajimensis”, and *P. mutuki* “1”, forming a polytomy, with two *P. tuberculosa* specimens slightly derived from these sequences in a subclade (177, 187) (ML = 67%, NJ = 67%). Several other specimens (41, 90, 159, 160, 161, 165, 166, 181) were identical to *P. mutuki* “2” from Japan (AB291212), with one sequence (259) slightly derived from

these sequences. Sequences from two specimens (158, 182) were identical to previously reported sequences of *P. aff. mutuki* sensu Koupaei et al. (2015) (ML = 64%, NJ = 63%). Finally, many sequences (27, 28, 31, 39, 85, 88, 130, 150, 157, 170, 171, 172, 176, 183, 185, 189, 192, 193, 194, 195) were identical to previously reported *P. heliodiscus* from Japan (AB219216) (ML = 94%, NJ = 95%).

## Discussion

### Red Sea Brachynermina species diversity compared with neighboring provinces

Our results show that there are six to seven species of suborder Brachynermina in the northern Red Sea. Based on molecular comparisons, three of the same brachynerminid species can be found in the Persian Gulf (Koupaei et al. 2014, 2015) and in South Africa (Risi and Macdonald 2015, Risi and Macdonald 2016); *Zoanthus sansibaricus*, *Palythoa tuberculosa*, and *P. mutuki*-*P. nelliae*, with another species, *P. sp.* “sakurajimensis” group B, reported from the Persian Gulf (as *P. aff. mutuki*, Koupaei et al. 2015). *Zoanthus sansibaricus*, *Palythoa tuberculosa*, and *P. mutuki*-*P. nelliae* have also been reported from many localities across the Indian and Pacific oceans (Burnett et al. 1997; Hibino et al. 2013; Joseph et al. 2014; Reimer and Todd 2009; Reimer et al. 2008, 2011, 2014). Overall, this initial estimate of species diversity of Brachynermina in the northern Red Sea is higher than that of the Persian Gulf, where four to five species have been reported (depending on whether *P. mutuki*-related specimens are treated as one or two species; Koupaei et al. 2014, 2015). This is equal to reports from the southwest Indian Ocean, where six to seven species have been reported, depending on if *Z. natalensis* and *Z. durbanensis* are treated as separate species or not (Risi and Macdonald 2015, Risi and Macdonald 2016). It should be noted that recent surveys from both the Persian Gulf and the southwest Indian Ocean were limited to sites at three islands and to depths of  $\leq 3$  m (Koupaei et al. 2014, 2015). Therefore, more surveys from more locations in other areas of both the Persian Gulf and the southwest Indian Ocean including collection at deeper depths are clearly needed to facilitate more accurate comparisons of species diversity across these regions.

Although more surveys are needed to confirm total zoantharian diversity in the Red Sea, we found molecular evidence of one possible endemic brachynerminid zoantharian species in this study, *P. aff. heliodiscus*. However, based on mtDNA sequences, this group may be within *P. cf. heliodiscus*, and further research with more specimens (currently  $n = 3$ ) is needed to confirm the unique identity of this group.

In fact, the Red Sea Province is known as a region with high endemism levels of shallow water reef organisms, such as hard

corals, ascidians, crustaceans, and reef fishes (reviewed in DiBattista et al. 2016). The single potential endemic Red Sea species of suborder Brachynermina appears to be an exception to the rule, and suggests that the group may have a different evolutionary history, with widespread generalist species, unless capacity to detect species is limited. However, until now there has been no molecular indication of such a limitation (Sinniger et al. 2005, 2008; Fujii and Reimer 2013).

Accordingly, the wide distribution of Brachynermina species across the Indo-Pacific Ocean and limited local speciation in the Red Sea is likely at least partially due to the exceptional high dispersal capacity of brachynerminid zoantharians. For example, *Palythoa tuberculosa* is reported to have planktonic larvae living up to more than five months (Polak et al. 2011). Although there is a lack of sexual reproduction/larval data for many species, there have also been reports of several forms of asexual reproduction in both *Palythoa* and *Zoanthus* spp. (Karlson 1983; Acosta 2001; Acosta et al. 2005; Acosta and González 2007; Stampar et al. 2007). In addition, some species of brachynerminid zoantharians appear to have evolved a generalist strategy, and thus the ability to adapt to different environments. Corroboration of this hypothesis is the ability of various species to tolerate a large range of temperatures (Koupaei et al. 2015, Reimer et al. 2016a) and shading levels (Karlson 1983; Ong et al. 2013). Moreover, some *Palythoa* and *Zoanthus* species have flexible associations with *Symbiodinium* (Burnett 2002; Kamezaki et al. 2013; Reimer et al. 2016b), which may confer flexible physiological characteristics. Still, it is obvious that more detailed investigations of biological and ecological characteristics of additional Brachynermina species are required in order to determine the processes responsible for their zoogeographic patterns. Furthermore, surveys combined with molecular analyses of specimens from the understudied Indian Ocean are essential to improve the knowledge of zoantharian diversity and distribution.

### Identities of Brachynermina of the Red Sea

Zoantharian taxonomy, particularly that of the genera *Zoanthus* and *Palythoa*, has long been plagued by uncertainty due to both the large numbers of species described within each genus, as well as relatively brief original species descriptions (Burnett et al. 1995, 1997). *Zoanthus* currently has approximately 42 species in the literature and 131 species of *Palythoa* (WoRMS 2016), and there are many more in the historical literature (see Biogeoinformatics of the Hexacorals; Fautin and Buddemeier 2008). Molecular evidence suggests these numbers to be inadvertently overinflated due to redescrptions of the same species from different localities (Burnett et al. 1994, 1995, 1997; Reimer et al. 2004; Ryland 2015). Additionally, many species of *Zoanthus* and *Palythoa* are morphologically plastic, resulting in potentially different morphotypes under different environmental conditions (Ong et al. 2013), which can further add to difficulty in accurate



identification. Genetic studies have been proposed as the most likely way to settle species identities across large geographic scales (Ryland and Lancaster 2003; Ryland 2015).

The comparison of our Red Sea zoantharian sequence data with information from other locations helps in the inference of possible species identities of specimens in this study. Our phylogenetic results indicate the presence of five to six species of *Palythoa* and one species of *Zoanthus* along the Saudi Arabian coast of the northern Red Sea. Comparing these phylogenetic results with previous molecular results from the Persian Gulf (Koupaei et al. 2014, 2015) and the southeast coasts of Africa and Madagascar (Risi and Macdonald 2015, Risi and Macdonald 2016; Ryland 2015), along with information in the historical literature (Savigny 1811; Audouin 1826; Gray 1828; Ehrenberg 1834; Milne Edwards 1857; Klunzinger 1877; Carlgren 1900; Pax and Muller 1956) (summarized in Table S1), we propose the following identities for Brachyneremina species in the Red Sea found in this study.

### 1. *Palythoa mutuki* (Haddon & Shackleton, 1891b)

Only five specimens from our collections were seen to be phylogenetically close to previously reported sequences of *P. mutuki* (Fig. 3; Table S2), and all of these specimens were found in the Gulf of Aqaba at Magna (Table S2). These results were somewhat unexpected given the apparent commonality of this species in other regions; multiple specimens of this species have been reported from the Persian Gulf (Fig. 3; Koupaei et al. 2014, 2015). The Red Sea specimens were found in <2 m of water (Table S2), and this species is known to be common in shallow or intertidal waters in Australia (Ryland and Lancaster 2003), Japan (Irei et al. 2011), and the South Pacific (Ryland and Lancaster 2003). It may be that the sampling methods in this study contributed to low numbers of *P. mutuki*; specimens were collected primarily by SCUBA diving and very little work was performed in shallow intertidal areas, particularly on the outer exposed edges of reefs, where *P. mutuki* has been seen to be common in other studies (Irei et al. 2011). Thus, despite finding specimens of *P. mutuki* at only one site in this study, it would be premature to say that this species is rare in the Red Sea. This species has also been reported from the Persian Gulf (Koupaei et al. 2014, 2015). Additionally, based on molecular results (Fig. 3), it appears *P. nelliae* Pax, 1935 from the southwest Indian Ocean, is likely to be the same species as *P. mutuki* (Risi and Macdonald 2016).

At least two nominal species previously described from the Red Sea appear to potentially fit well with *P. mutuki* (as redescribed by Ryland and Lancaster 2003); *P. fuliginosa* (Ehrenberg, 1834) and *Hughea hemprichii* Ehrenberg, 1834. However, both species were synonymized with *P. lesueuri* Audouin, 1826 by Klunzinger (1877) (Table S1), and as their type specimens are lacking, there is no way confidently to assign either species name to this current specimen. Thus,

based on phylogenetic results and the work of Ryland and Lancaster (2003) we, therefore, assign the specimen to *P. mutuki*. Similarly, other “liberae” (=polyps well-clear of coenenchyme; Pax 1910) Red Sea *Palythoa* specimens that we have placed in *P. cf. heliodiscus*, *P. aff. heliodiscus*, and *P. sp.* “sakurajimensis” also could potentially match with the ambiguous descriptions of *P. fuliginosa* and *H. hemprichii*, but again following Klunzinger (1877) we do not consider either species-name group valid.

The confirmed specimens of *P. mutuki* from this study were found deep within cracks on the reef crest at Magna, and no detailed in situ images are available. Future work in the Red Sea focused on zoantharians should include more work in the intertidal zone, an area where many Brachyneremina species are known to be particularly common (Goreau 1959; Villaça and Pitombo 1997; Irei et al. 2011).

### 2. *Palythoa cf. heliodiscus* (Ryland & Lancaster, 2003)

Unlike *P. mutuki*, represented by specimens from a single site, numerous specimens of a species that is phylogenetically very closely related or identical to *P. heliodiscus* were collected during this survey (Fig. 3a). These Red Sea specimens were collected over a wide range of depths (3 to 35 m; Table S2) and across a wide range of sampling locations (eight sites; Table S2), and thus this species group appears to be common in the Red Sea and is found across a wide range of depths. Colonies of this species were generally small, consisting of less than 20 polyps (Fig. 2a).

However, the final identity of this species group remains somewhat ambiguous although we have identified specimens in this study as *P. cf. heliodiscus*. The species *Palythoa leseuri* Audouin, 1826 was described from the Red Sea based on Savigny’s (1811) images, but unfortunately the original description of the species lacks many details, while *P. heliodiscus* was recently described in detail from the Great Barrier Reef (Ryland and Lancaster 2003). Red Sea specimens in this study were morphologically very similar to *P. heliodiscus*, having large oral disks (up to 1 cm in diameter), but also had some differences, namely various purple and/or green patterns on their oral disk (Fig. 2a) as opposed to the plain tan/brown of *P. heliodiscus*, and with tentacles proportionally longer than as reported in *P. heliodiscus* in Australia (Ryland and Lancaster 2003) (see Fig. 2a). Purple and green oral disk coloration is more similar to *P. cf. heliodiscus* sensu Reimer et al. (Fig. 8d in Reimer et al. 2014) reported from the Central Indo-Pacific. *Palythoa cf. heliodiscus* was not found in surveys in the Persian Gulf (Koupaei et al. 2014, 2015) or the southwest Indian Ocean (Risi and Macdonald 2015, 2016). Polyps lack the broad and conspicuous pale-colored knob-like capitular ridge marking the directive endocoels as seen in closed *P. mutuki* (Ryland and Lancaster 2003) or *P. sp.* “sakurajimensis” polyps (Fig. 2b).



However, as with *P. mutuki* above, due to a lack of original type specimens of *P. lesueuri*, and sparse details in the description, we cannot say with any certainty exactly what *P. lesueuri* truly is, and thus for now we identify these Red Sea specimens as *P. cf. heliodiscus*; these specimens are likely *P. heliodiscus*, but reserve final judgment. Additionally, *P. heliodiscus* appears to be very similar to *P. vestitus* (Verrill, 1928) from Hawaii (as in Walsh and Bowers 1971), which would be the senior synonym in such a case, and it is clear that this species-group needs a thorough morphological and molecular examination followed by taxonomic revision if needed. Inclusion of specimens from the Red Sea would be a key part of such work.

### 3. *Palythoa* aff. *heliodiscus*

In the ITS-rDNA tree, three specimens (39, 85, 176) formed a clade related to but separate from *P. cf. heliodiscus*. Although mtDNA shows these specimens to be part of *P. cf. heliodiscus*, their ITS-rDNA genetic distances (~17–20%) from *P. cf. heliodiscus* suggest these three specimens potentially represent a species-level grouping. No clear field images of open polyps exist, specimens appear similar to *P. cf. heliodiscus*, with polyps lacking the broad and conspicuous pale-colored knob-like capitular ridge marking the directive endocoels as seen in closed *P. mutuki* (Ryland and Lancaster 2003) or *P. sp. "sakurajimensis"* polyps (Fig. 2b). Specimens were found at depths of 4–30 m. Notably, all specimens had polyps deeply embedded within cracks of the carbonate substrate (Fig. 2b).

It is possible this grouping represents a species endemic to the central-northern Red Sea, as no specimens were found either in the Gulf of Aqaba or in the more southern sites examined in this study, and no similar ITS-rDNA sequences have been reported to date. Additional specimens are needed to confirm the identity and status of this putative endemic species. For now, this species is designated *P. aff. heliodiscus*, being closely related yet different from *P. heliodiscus* and *P. cf. heliodiscus*.

### 4. *Palythoa* sp. “sakurajimensis” sensu Reimer et al. (2007b)

A somewhat unexpected result from this study was the high phylogenetic similarity between a large number ( $n = 8$ ; Table S2) of Red Sea *Palythoa* specimens with a known, but formally undescribed species from the northwestern Pacific; *Palythoa* sp. “sakurajimensis” sensu Reimer et al. (2007b).

Similar to *P. cf. heliodiscus*, this species was always found in small colonies or single polyps (Fig. 2c). In the Red Sea, this species has a large brown oral disk lacking the patterns seen in *P. cf. heliodiscus*, with septae (=radial) either only faintly or not visible. Polyps were close to 1 cm in diameter and appeared larger and more robust than those of *P. cf. heliodiscus*. Additionally, tentacles of this putative species appear to be short and comparatively thicker than those of *P.*

*cf. heliodiscus* (compare Figs. 2a & c). Polyps with the broad and conspicuous pale-colored knob-like capitular ridge marking the directive endocoels as seen in closed *P. mutuki* (Ryland and Lancaster 2003; Fig. 2b).

Our ITS-rDNA phylogenetic results showed the presence of two species-level groups, which we designated as type A (Fig. 2c, Fig. 3a) and type B (Fig. 3a). In addition, type B specimen 182 was identical to specimen 158 (for which not ITS-rDNA sequence was acquired) in the COI tree, different from other *Palythoa* sp. “sakurajimensis” sequences acquired in this study (Fig. S2). COI sequences for specimens 158 and 182 were identical to previously reported *P. aff. mutuki* sequences from the Persian Gulf (Koupaei et al. 2015). Although there are no ITS-rDNA sequences available from specimen 158 or from Persian Gulf specimens, and all in situ images from the Red Sea are of polyps that are partially or completely closed, based on COI sequences these specimens may be identical *P. aff. mutuki* sensu Koupaei et al. (2015). More specimens and ITS-rDNA sequences are needed to confirm the final identity of this type B. No similar specimens have been reported from the southwest Indian Ocean (Risi and Macdonald 2015, 2016).

### 5. *Palythoa tuberculosa* (Esper, 1805)

Originally described from India (Esper 1805), this species was redescribed from specimens from the Red Sea by Klunzinger (1877). Recent phylogenetic examinations of ITS-rDNA sequences of specimens from the Gulf of Aqaba (Israel), Australia, and Japan, confirm the identity of this species as widespread throughout the Indo-Pacific (Hibino et al. 2013), and recent molecular studies have further confirmed the presence of this species in both the Persian Gulf (Koupaei et al. 2014, 2015) and the southwest Indian Ocean (Risi and Macdonald 2015, 2016).

In the Red Sea, this was the most commonly encountered species and was observed across the widest range of depths of all species (at eight sites here from <1 m to 45 m; Table S2, and also observed across the Saudi Arabian coast of the northern Red Sea in Reimer et al. 2016b). This may be perhaps due to its flexible association with endosymbiotic *Symbiodinium* spp. in this region (Reimer et al. 2016b).

### 6. *Zoanthus sansibaricus* Carlgren, 1900

Only one species of *Zoanthus* was confirmed from our surveys, and this was phylogenetically placed within a clade with previously reported *Z. sansibaricus* sequences from Japan and southwest Africa (Fig. 3b). Similar to *P. tuberculosa* and *P. mutuki-P. nelliae*, *Z. sansibaricus* is widely distributed across the Indo-Pacific, and based on molecular data has been recently reported from both the Persian Gulf (Koupaei et al. 2014, 2015) and southwest Africa (Risi and Macdonald 2015, 2016).

It is interesting that one of the previously reported *Zoanthus* species from the Red Sea, *Z. calypso* Pax and Muller, 1956, was noted as “Doubtless *Zoanthus calypso* is a close relative of *Zoanthus sansibaricus*” (Pax and Muller 1956). Similarly, Carlgren (1900) noted that the Red Sea species *Z. bertholetti* Audouin, 1826 (see Table S1 for taxonomic review) was likely closely related to *Z. sansibaricus*. Thus, there is a history of reports of *Z. sansibaricus*-like specimens from the Red Sea, and based on our molecular data, it appears that previous Red Sea *Zoanthus* may in fact be *Z. sansibaricus*. Although *Z. bertholetti* would be the senior species-group name of these three binomens, both Carlgren (1900) and Pax and Muller (1956) determined it was impossible to clearly identify this species as the type specimen of *Z. bertholetti* was lost and the original description brief; leading Pax and Muller (1956) to erect *Z. calypso*. From our results, it appears that *Z. calypso* is a junior synonym of *Z. sansibaricus*, although the type locality of *Z. calypso* is further south in the Red Sea than the area examined in this study, and specimens from the type locality are needed to confirm this hypothesis. In turn, it has been suggested that *Z. coppingeri* Haddon and Shackleton, 1891a from Australia may be a senior synonym of *Z. sansibaricus*, and molecular confirmation and type specimen examination is needed to confirm this hypothesis (Burnett and Ryland 1997).

*Zoanthus sansibaricus* was not commonly encountered in this study, and colonies were generally small (e.g. <100 polyps). Additionally, although this species has been reported as common from shallow waters and the intertidal zone in Japan (Irei et al. 2011) and South Africa (Risi and Macdonald 2015, 2016), in the northern Red Sea it was most commonly seen at depths of >10 m, reminiscent of deeper populations along walls in Okinawa, Japan (Kamezaki et al. 2013).

### Clarifying the taxonomy of Brachyneremina in the Indo-Pacific

From a taxonomic point of view, some of the most senior Brachyneremina species-group names in the Indo-Pacific are found in the Red Sea, and due to the numerous problems facing the taxonomy of most species of Brachyneremina (e.g. Table S1), inclusion of Red Sea specimens and their data are key to unraveling the identity of many Indo-Pacific Brachyneremina species. In Zoantharia, the problem of reconciling molecular data with confounding morphological and confused taxonomic information has been known for over two decades (Burnett et al. 1994, 1995, 1997; Ryland 2015), and the acquisition of comprehensive multi-disciplinary data sets should allow linking current molecular results with past, morphologically based taxonomic literature (Swain and Swain 2014). The need for morphological information can be further demonstrated by recent work on South African *Zoanthus* spp. by Ryland (2015), in which polyp diameters showed the

presence of three species groups (*Z. sansibaricus*, *Z. natalensis* Carlgren, 1939, *Z. lawrencei* Carlgren, 1939), contradicting recent molecular evidence of showing two species-level clades in the same region (*Z. sansibaricus*, *Z. natalensis*-*Z. durbanensis* Carlgren, 1939; Risi and Macdonald 2016).

Additionally, the molecular results of this study demonstrated problems inherent with identifying zoantharians (and many anthozoans) with mitochondrial DNA markers alone. Although mitochondrial DNA markers such as 16S-rDNA and COI have been shown often to have utility in Zoantharia (Sinniger et al. 2008), they often fail to properly delineate between closely related species due to slow sequence evolution (=low mutation rates; Shearer et al. 2002; Huang et al. 2008). This has previously been noted for some *Palythoa* species (Sinniger et al. 2008), and is clearly evident from our 16S-rDNA and COI results here. While the results from these two markers provided information that was helpful in sorting out some species identifications, other groups were mixed together into large clades or polytomies (e.g. *P. tuberculosa* + *P. mutuki* + *P. sp.* “sakurajimensis” with 16S-rDNA). As well, relatively short sequences from specimens in studies in neighboring regions limited our ability to effectively compare our results to these other marine areas (e.g. COI sequences of ~300 bp). Additionally, in this study, only the combined *Zoanthus* and *Palythoa* ITS-rDNA analyses (Fig. 3) were able to effectively delineate all six to seven observed species. Thus, we strongly recommend the acquisition of both 1) long mitochondrial sequences, in particular those of 16S-rDNA and COI as they are utilized widely in zoantharian phylogenies, and 2) the acquisition of ITS-rDNA sequences. Even though *Zoanthus* and *Palythoa* ITS-rDNA sequences are often very difficult to align (Reimer et al., 2007b, c; Aguilar and Reimer 2010), at the very least they provide accurate species-levels information that can be further supported by mitochondrial sequences and morphological analyses.

One logical route to take in untangling the complex taxonomy of Brachyneremina species in the Indo-Pacific would be to follow the taxonomic history of species as closely as possible, working in a chronological manner starting from the oldest (=senior binomens) literature. Acquiring both molecular (ITS-rDNA, 16S-rDNA, COI) and morphological data in this manner should allow for a relatively straightforward assessment of which species are valid and which are inadvertent synonyms of previously described species. As mentioned in the first molecular studies of Brachyneremina (Burnett et al. 1994, 1995, 1997) and in more recent works (Reimer et al. 2011; Risi and Macdonald 2016), molecular results have consistently shown the reoccurring presence of many of the same species groups (e.g. *Palythoa tuberculosa*, *P. mutuki*, *Zoanthus sansibaricus*) in various marine provinces, such as the East Pacific Region (Reimer et al. 2008), Indo-Polynesian

(Koupaei et al. 2014; Joseph et al. 2014; Reimer et al. 2004, 2006, 2007b, 2011, 2014), and the Western Indian Ocean (Risi and Macdonald 2016), suggesting these Brachycnemina species are widespread and that much taxonomic work remains (Ryland 2015). Formal taxonomic revision based on molecular data underpinned by a morphological framework to allow comparisons with historical literature (Swain and Swain 2014; Swain et al. 2015, 2016) will then allow comparative and population genetic studies to move forward confidently, increasing our understanding of this important component of shallow subtropical and tropical benthic communities.

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