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Zooxanthellate zoantharians (Anthozoa: Hexacorallia: Zoantharia: Brachycnemina) in the northern Red Sea --Manuscript Draft--

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Abstract:	<p>The Red Sea was one of the first areas of the Indo-Pacific to be investigated by marine taxonomists, and the literature of suborder Brachycnemina (Anthozoa: Hexacorallia: Zoantharia) for this region dates from the early 19th 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</p>	

	large range extensions for these two species. Only one of the observed species, <i>P. aff. heliodiscus</i> , is potentially endemic to the Red Sea, further demonstrating the generally wide distributions of most zooxanthellate <i>Brachycnemina</i> species.
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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 of suborder Brachycnemina (Anthozoa: Hexacorallia: Zoantharia) for this region dates from the early 19th 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 zooanthellate Brachycnemina species.

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Keywords: Saudi Arabia, taxonomy, zoogeography, Cnidaria, species diversity

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 & 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 Jr. et al. 2008).

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8 However, despite their obvious importance in subtropical and tropical areas,
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10 our knowledge of Brachycnemina species' distribution patterns remains patchy, with
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12 many marine regions lacking even information such as species lists or records of
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14 occurrence (Khushali and Pradeep 2013; Reimer et al. 2014, 2015; Santos et al. 2016).
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17 Therefore, information covering a wider portion of Brachycnemina species' ranges is
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19 needed in order to undertake large-scale analyses to more completely understand
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21 their species diversity and ecology.
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26 The Red Sea is one region where there is a paucity of data for zoantharians.
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28 The Red Sea Province is located in the Tropical Indo-West Pacific Region, neighboring
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30 the Western Indian Ocean Province on the west (Briggs and Bowen 2012). Despite
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32 being the first region of the Indo-Pacific to have its Zoantharia investigated (Savigny
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34 1811; Audouin 1826; Gray 1828; Ehrenberg 1834; Milne-Edwards 1857; Klunzinger
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36 1877), since 1900 only two studies have examined Zoantharia of the Red Sea in detail
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38 (Carlgren 1900; Pax & Muller 1956), and none in the last 60 years. Even by the
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40 standards of zoantharian taxonomy in general, which has previously been described
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42 as "chaotic" (Burnett et al. 1997), the history of Brachycnemina taxonomic studies in
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44 the Red Sea is particularly convoluted and problematic (Table S1).
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52 However, recent research has explored the diversity and phylogeny of
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54 Brachycnemina species in regions in the Indo-Pacific on either side of the Red Sea;
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56 both in the Persian Gulf (Koupaei et al. 2014, 2015) and along the southeastern
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7 African coast (Risi and Macdonald 2015, 2016). In the present study, via field surveys
8 and phylogenetic analyses, the species diversity of Brachycnemina in the northern Red
9 Sea is explored and compared with data in historical taxonomic literature and recent
10 phylogenetic results from these neighboring regions. In addition, suggestions are
11 given for future large-scale analyses of Brachycnemina in the Red Sea and at larger,
12 oceanic basin to global scales.
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25 **Materials and methods:**

26 Specimen collection

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31 Specimens of Brachycnemina were collected by SCUBA or snorkeling along
32 the Saudi Arabian coast of the northern and central Red Sea in September and October
33 2013 at 11 sites (Figure 1, Table S2). A total of 72 Brachycnemina specimens were
34 collected from the intertidal zone to a depth of 45 m (Table S2, Figure 2). Specimens
35 were subsequently fixed in 95% ethanol, and are currently housed at the Red Sea
36 Research Center of the King Abdullah University of Science and Technology (KAUST;
37 Thuwal, Saudi Arabia) under specimen numbers given in Table S2.
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50 Specimen identification and literature review

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52 Specimens were identified based on a combination of DNA sequences and
53 morphology. For morphological examinations, we generally followed the past works of
54 Klunzinger (1877), Carlgren (1900), Pax and Muller (1956), and Ryland and Lancaster
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(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 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 HC02198 (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 analyser (Qiagen), treated with ExoSTAR PCR clean-up (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) 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

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8 genus as sequences are highly divergent between the two groups (Reimer et al. 2007b,
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10 c), while for 16S-rDNA and COI the sequences of genus *Zoanthus* and *Palythoa* were
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12 aligned together. The terminal regions in the alignments were trimmed, and shorter or
13
14 poor quality sequences were eliminated. All alignments were refined with the MUSCLE
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16 (Edgar 2004) alignment tool implemented in Geneious, configured with a “maximum
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18 number of iterations: 100”; “maximum number of trees to build: 1000”; “optimization:
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20 anchor”; “terminal gaps: half penalty”. After MUSCLE refinement all alignments were
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22 manually curated. In this manner, four aligned datasets were generated; 465 sites for
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24 46 sequences for ITS-rDNA of *Zoanthus*, 519 sites for 135 sequences for ITS-rDNA of
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26 *Palythoa*, 452 sites of 253 sequences for 16S-rDNA and 306 sites of 89 sequences for
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28 COI. Novel sequences obtained during this study were deposited in GenBank
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30 (accession numbers XXXXX-XXXXX). It should be noted that COI sequences generated
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32 from this study and deposited in GenBank are much longer (~560 bp) than the
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34 sequences utilized in the analyses. COI sequences were trimmed to 306 bp in the
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36 alignment in order to maximize comparisons with sequences previously reported
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38 from other locations.
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50 Phylogenetic reconstruction was performed for each alignment using
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52 Neighbor-joining (NJ) and Maximum-likelihood (ML). NJ trees were constructed in
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54 Geneious with the Hasegawa-Kishino-Yano genetic distance model (HKY; Hasegawa et
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56 al. 1985); 1000 bootstrap replicate trees were constructed using the same method.
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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

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8 to cluster with previously reported sequences of *Zoanthus sansibaricus* from both
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10 Japan and South Africa, and not with those of any other *Zoanthus* species. The
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12 sequence from specimen 177 clustered with sequences of the *Z. sansibaricus* “distant”
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14 ITS-rDNA allele (ML=100%; NJ=100%), while those from specimens 148, 197, 228,
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16 and 238 clustered with those from the “normal” *Z. sansibaricus* allele (ML=66.7%;
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18 NJ=100%) (for “distant” and “normal” ITS-rDNA allele explanation see Aguilar and
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20 Reimer 2010).
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25 26 16S-rDNA (Fig. S1) 27

28 The phylogenetic tree generated from the 16S-rDNA alignment showed all
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30 Red Sea *Zoanthus* specimens’ sequences (196, 197, 199, 228, 238, 239, 243, 271) to be
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32 identical with previously reported *Zoanthus sansibaricus* sequences from Japan, South
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34 Africa and the Persian Gulf, within a clade (ML=65.5%, NJ=59.6%). For *Palythoa*,
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36 specimen 165 was identical to sequences of *P. mutuki* ‘2’ from Japan (DQ997841), as
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38 well as numerous sequences from South Africa of *P. nelliae*, and *P. mutuki* sequences
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40 from the Persian Gulf. Numerous Red Sea specimens morphologically identified as
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42 either *P. tuberculosa* (23, 80, 147, 167, 169, 178, 179, 200, 201, 210, 213) or *P.*
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44 *mutuki*-related (158, 181, 182, 241, 259) were all identical with previously reported
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46 sequences of *P. tuberculosa* from numerous locations, as well as with *P. sp.*
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48 “sakurajimensis” from Japan (DQ997842), *P. nelliae* from South Africa, *P. mutuki* ‘1’
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50 from Japan (DQ997875), and *P. aff. mutuki* from the Persian Gulf, within a large ‘mixed’
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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. heliodiscus*; 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 Brachycnemina species diversity compared with neighboring provinces

Our results show that there are six to seven species of suborder Brachycnemina in the northern Red Sea. Based on molecular comparisons, three of the same brachycnemic species can be found in the Persian Gulf (Koupaei et al. 2014, 2015) and in South Africa (Risi and Macdonald 2015, 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 Brachycnemina 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), and equal to that reported from the southwest Indian Ocean, where six to seven species have been reported,

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7 depending on if *Z. natalensis* and *Z. durbanensis* are treated as separate species or not
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9 (Risi and Macdonald 2015, 2016). It should noted that recent surveys from both the
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11 Persian Gulf and the southwest Indian Ocean were limited to sites at three islands and
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13 to depths of ≤ 3 m (Koupeai et al. 2014, 2015). Therefore, more surveys from more
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15 locations in other areas of both the Persian Gulf and the southwest Indian Ocean
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17 including collection at deeper depths are clearly needed to facilitate more accurate
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19 comparisons of species diversity across these regions.
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26 Although more surveys are needed to confirm total zoantharian diversity in
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28 the Red Sea, we found molecular evidence of one possible endemic brachycnemic
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30 zoantharian species in this study, *P. aff. heliodiscus*. However, based on mtDNA
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32 sequences, this group may be within *P. cf. heliodiscus*, and further research with more
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34 specimens (currently n=3) is needed to confirm the unique identity of this group.
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39 In fact, the Red Sea Province is known as a region with high endemism levels
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41 of shallow water reef organisms, such as hard corals, ascidians, crustaceans, and reef
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43 fishes (reviewed in DiBattista et al. 2016). The single potential endemic Red Sea
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45 species of suborder Brachycnemina appears to be an exception to the rule, and
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47 suggests that the group may have a different evolutionary history, with widespread
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49 generalist species, unless capacity to detect species is limited. However, until now
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51 there has been no molecular indication of such a limitation (Sinniger et al. 2005, 2008;
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53 Fujii and Reimer 2013).
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8 Accordingly, the wide distribution of Brachycnemina species across the
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10 Indo-Pacific Ocean and limited local speciation in the Red Sea is likely at least partially
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12 due to the exceptional high dispersal capacity of brachycnemic zoantharians. For
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14 example, *Palythoa tuberculosa* is reported to have planktonic larvae living up to more
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16 than five months (Polak et al. 2011). Although there is a lack of sexual
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18 reproduction/larval data for many species, there have also been reports of several
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20 forms of asexual reproduction in both *Palythoa* and *Zoanthus* spp. (Karlson 1983;
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22 Acosta 2001; Acosta et al. 2005; Acosta and González 2007; Stampar et al. 2007). In
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24 addition, some species of brachycnemic zoantharians appear to have evolved a
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26 generalist strategy and, thus, the ability to adapt to different environments.
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28 Corroboration of this hypothesis is the ability of various species to tolerate a large
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30 range of temperatures (Koupaei et al. 2015, Reimer et al. 2016a) and shading levels
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32 (Karlson 1983; Ong et al. 2013). Moreover, some *Palythoa* and *Zoanthus* species have
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34 flexible associations with *Symbiodinium* (Burnett 2002; Kamezaki et al. 2013; Reimer
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36 et al. 2016b), which may confer flexible physiological characteristics. Still, it is obvious
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38 that more detailed investigations of biological and ecological characteristics of
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40 additional Brachycnemina species are required in order to determine the processes
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42 responsible for their zoogeographic patterns. Furthermore, surveys combined with
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44 molecular analyses of specimens from the understudied Indian Ocean are essential to
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46 improve the knowledge of zoantharian diversity and distribution.
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Identities of Brachycnemina 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 genera, as well as relatively brief original species descriptions (Burnett et al. 1995, 1997). *Zoanthus* currently has approximately 42 species in the literature, and *Palythoa* 131 species (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, 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 & Muller 1956) (summarized in Table S1), we propose the following identities for Brachycnemina 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.*

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8 *mutuki* at only one site in this study, it would be premature to say that this species is
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10 rare in the Red Sea. This species has also been reported from the Persian Gulf
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12 (Koupeai et al. 2014, 2015). Additionally, based on molecular results (Fig. 3), it
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14 appears *P. nelliae* Pax, 1935 from the southwest Indian Ocean, is likely to be the same
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16 species as *P. mutuki* (Risi and Macdonald 2016).
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20 At least two nominate species previously described from the Red Sea appear to
21
22 potentially fit well with *P. mutuki* (as redescribed by Ryland and Lancaster (2003)); *P.*
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24 *fuliginosa* (Ehrenberg, 1834) and *Hughea hemprichii* Ehrenberg, 1834. However, both
25
26 species were synonymized with *P. lesueuri* Audouin, 1826 by Klunzinger (1877) (Table
27
28 S1), and as their type specimens are lacking, there is no way to confidently assign
29
30 either species name to this current specimen. Thus, based on phylogenetic results and
31
32 the work of Ryland and Lancaster (2003) we therefore assign the specimen to *P.*
33
34 *mutuki*. Similarly, other ‘liberae’ (=polyps well-clear of coenenchyme; Pax 1910) Red
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36 Sea *Palythoa* specimens that we have placed in *P. cf. heliodiscus*, *P. aff. heliodiscus*, and
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38 *P. sp. “sakurajimensis”* also could potentially match with the ambiguous descriptions
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40 of *P. fuliginosa* and *H. hemprichii*, but again following Klunzinger (1877) we do not
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42 consider either species-name group available.
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52 The confirmed specimens of *P. mutuki* from this study were found deep within
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54 cracks on the reef crest at Magna, and no detailed in situ images are available. Future
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56 work in the Red Sea focused on zoantharians should include more work in the
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8 intertidal zone, an area where many Brachycnemina species are known to be
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10 particularly common (Goreau 1959; Villaça and Pitombo 1997; Irei et al. 2011).
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13 2. *Palythoa* cf. *heliodiscus* (Ryland & Lancaster, 2003)
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15 Figure 2a
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18 Unlike *P. mutuki*, represented by specimens from a single site, numerous
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20 specimens of a species that is phylogenetically very closely related or identical to *P.*
21
22 *heliodiscus* were collected during this survey (Fig. 3a). These Red Sea specimens were
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24 collected over a wide range of depths (3 to 35 m; Table S2) and across a wide range of
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26 sampling locations (eight sites; Table S2), and thus this species group appears to be
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28 common in the Red Sea, and found across a wide range of depths. Colonies of this
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30 species were generally small, consisting of less than 20 polyps (Fig. 2a).
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35 However, the final identity of this species group remains somewhat ambiguous
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37 although we have identified specimens in this study as *P. cf. heliodiscus*. The species
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39 *Palythoa leseuri* Audouin, 1826 was described from the Red Sea based on Savigny's
40
41 (1811) images, but unfortunately the original description of the species lacks many
42
43 details; while *P. heliodiscus* was recently described in detail from the Great Barrier
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45 Reef (Ryland and Lancaster 2003). Red Sea specimens in this study were
46
47 morphologically very similar to *P. heliodiscus*, having large oral disks (up to 1 cm in
48
49 diameter), but also had some differences, namely various purple and/or green
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51 patterns on their oral disk (Fig. 2a) as opposed to the plain tan/brown of *P. heliodiscus*,
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7 and with tentacles proportionally longer than as reported in *P. heliodiscus* in Australia
8 (Ryland and Lancaster 2003) (see Fig. 2a). Purple and green oral disk coloration is
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10 more similar to *P. cf. heliodiscus* sensu Reimer et al. (Figure 8d in Reimer et al. 2014)
11
12 reported from the Central Indo-Pacific. *Palythoa cf. heliodiscus* was not found in
13
14 surveys in the Persian Gulf (Koupaei et al. 2014, 2015) or the southwest Indian Ocean
15
16 (Risi and Macdonald 2015, 2016). Polyps lack the broad and conspicuous pale-colored
17
18 knob-like capitular ridge marking the directive endocoels as seen in closed *P. mutuki*
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20 (Ryland and Lancaster 2003) or *P. sp. "sakurajimensis"* polyps (Fig. 2b).
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24 However, as with *P. mutuki* above, due to a lack of original type specimens of *P.*
25
26 *lesueuri*, and sparse details in the description, we cannot say with any certainty exactly
27
28 what *P. lesueuri* truly is, and thus for now we identify these Red Sea specimens as *P. cf.*
29
30 *heliodiscus*; these specimens are likely *P. heliodiscus* but reserve final judgement.
31
32 Additionally, *P. heliodiscus* appears to be very similar to *P. vestitus* (Verrill, 1928) from
33
34 Hawaii (as in Walsh and Bowers 1971), which would be the senior synonym in such a
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36 case, and it is clear that this species-group needs a thorough morphological and
37
38 molecular examination followed by taxonomic revision if needed. Inclusion of
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40 specimens from the Red Sea would be a key part of such work.
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52 3. *Palythoa* aff. *heliodiscus*

53 Figure 2b

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55 In the ITS-rDNA tree, three specimens (39, 85, 176) formed a clade related to
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8 but separate from *P. cf. heliodiscus*. Although mtDNA shows these specimens to be part
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10 of *P. cf. heliodiscus*, their ITS-rDNA genetic distances (~17-20%) from *P. cf. heliodiscus*
11
12 suggest these three specimens potentially represent a species-level grouping. No clear
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14 field images of open polyps exist, specimens appear similar to *P. cf. heliodiscus*, with
15
16 polyps lacking the broad and conspicuous pale-colored knob-like capitular ridge
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18 marking the directive endocoels as seen in closed *P. mutuki* (Ryland and Lancaster
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20 2003) or *P. sp. "sakurajimensis"* polyps (Fig. 2b). Specimens were found at depths of
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22 4-30 m. Notably, all specimens had polyps deeply embedded within cracks of the
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24 carbonate substrate (Fig. 2b).
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31 It is possible this grouping represents a species endemic to the
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33 central-northern Red Sea, as no specimens were found either in the Gulf of Aqaba or in
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35 the more southern sites examined in this study, and no similar ITS-rDNA sequences
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37 have been reported to date. Additional specimens are needed to confirm the identity
38
39 and status of this putative endemic species. For now, this species is designated *P. aff.*
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41 *heliodiscus*, being closely related yet different from *P. heliodiscus* and *P. cf. heliodiscus*.
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47 *Palythoa* sp. "sakurajimensis" *sensu* Reimer et al. (2007b)

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50 Figure 2c

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52 A somewhat unexpected result from this study was the high phylogenetic
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54 similarity between a large number (n=8; Table S2) of Red Sea *Palythoa* specimens with
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56 a known but formally undescribed species from the northwestern Pacific; *Palythoa* sp.
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7 “sakurajimensis” sensu Reimer et al. (2007b).
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10 Similar to *P. cf. heliodiscus*, this species was always found in small colonies or single
11 polyps (Fig. 2c). In the Red Sea, this species has a large brown oral disk lacking the
12 patterns seen in *P. cf. heliodiscus*, with septae (=radii) either only faintly or not visible.
13
14 Polyps were close to 1 cm in diameter, and appeared larger and more robust than
15 those of *P. cf. heliodiscus*. Additionally, tentacles of this putative species appear to be
16 short and comparatively thicker than those of *P. cf. heliodiscus* (compare Figs. 2a & 2c).
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18 Polyps with the broad and conspicuous pale-colored knob-like capitular ridge marking
19 the directive endocoels as seen in closed *P. mutuki* (Ryland and Lancaster 2003; Fig.
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21 2b).
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33 Our ITS-rDNA phylogenetic results showed the presence of two species-level
34 groups, which we designated as type A (Fig. 2c, Fig. 3a) and type B (Fig. 3a). In
35 addition, type B specimen 182 was identical to specimen 158 (for which not ITS-rDNA
36 sequence was acquired) in the COI tree, different from other *Palythoa* sp.
37 “sakurajimensis” sequences acquired in this study (Fig. S2). COI sequences for
38 specimens 158 and 182 were identical to previously reported *P. aff. mutuki* sequences
39 from the Persian Gulf (Koupaei et al. 2015). Although there are no ITS-rDNA
40 sequences available from specimen 158 or from Persian Gulf specimens, and all in situ
41 images from the Red Sea are of polyps that are partially or completely closed, based on
42 COI sequences these specimens may be identical *P. aff. mutuki* sensu Koupaei et al.
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(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)

Figure 2d

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

Figure 2e

Only one species of *Zoanthus* was confirmed from our surveys, and this was

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8 phylogenetically placed within a clade with previously reported *Z. sansibaricus*
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10 sequences from Japan and southwest Africa (Fig. 3b). Similar to *P. tuberculosa* and *P.*
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12 *mutuki-P. nelliae*, *Z. sansibaricus* is widely distributed across the Indo-Pacific, and
13
14 based on molecular data has been recently reported from both the Persian Gulf
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18 (Koupaei et al. 2014, 2015) and southwest Africa (Risi and Macdonald 2015, 2016).
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21 It is interesting that one of the previously reported *Zoanthus* species from the Red
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23 Sea, *Z. calypso* Pax and Muller, 1956, was noted as “Doubtless *Zoanthus calypso* is a
24
25 close relative of *Zoanthus sansibaricus*” (Pax and Muller 1956). Similarly, Carlgren
26
27 (1900) noted that the Red Sea species *Z. bertholetti* Audouin, 1826 (see Table S1 for
28
29 taxonomic review) was likely closely related to *Z. sansibaricus*. Thus, there is a history
30
31 of reports of *Z. sansibaricus*-like specimens from the Red Sea, and based on our
32
33 molecular data, it appears that previous Red Sea *Zoanthus* may in fact be *Z.*
34
35 *sansibaricus*. Although *Z. bertholetti* would be the senior species-group name of these
36
37 three binomens, both Carlgren (1900) and Pax and Muller (1956) determined it was
38
39 impossible to clearly identify this species as the type specimen of *Z. bertholetti* was
40
41 lost and the original description brief; leading Pax and Muller (1956) to erect *Z.*
42
43 *calypso*. From our results, it appears that *Z. calypso* is a junior synonym of *Z.*
44
45 *sansibaricus*, although the type locality of *Z. calypso* is further south in the Red Sea
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47 than the area examined in this study, and specimens from the type locality are needed
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49 to confirm this hypothesis. In turn, it has been suggested that *Z. coppingeri* Haddon
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7 and Shackleton, 1891a from Australia may be a senior synonym of *Z. sansibaricus*, and
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9 molecular confirmation and type specimen examination is needed to confirm this
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11 hypothesis (Burnett and Ryland 1997).
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15 *Zoanthus sansibaricus* was not commonly encountered in this study, and colonies
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17 were generally small (e.g. <100 polyps). Additionally, although this species has been
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19 reported as common from shallow waters and the intertidal zone in Japan (Irei et al.
20
21 2011) and South Africa (Risi and Macdonald 2015, 2016), in the northern Red Sea it
22
23 was most commonly seen at depths of >10 m, reminiscent of deeper populations along
24
25 walls in Okinawa, Japan (Kamezaki et al. 2013).
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31 Clarifying the taxonomy of Brachycnemina in the Indo-Pacific

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34 From a taxonomic point of view, some of the most senior Brachycnemina
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36 species-group names in the Indo-Pacific are found in the Red Sea, and due to the
37
38 numerous problems facing the taxonomy of most species of Brachycnemina (e.g. Table
39
40 S1), inclusion of Red Sea specimens and their data are key to unraveling the identity of
41
42 many Indo-Pacific Brachycnemina species. In Zoantharia, the problem of reconciling
43
44 molecular data with confounding morphological and confused taxonomic information
45
46 has been known for over two decades (Burnett et al. 1994, 1995, 1997; Ryland 2015),
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48 and the acquisition of comprehensive multi-disciplinary data sets should allow linking
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50 current molecular results with past, morphologically-based taxonomic literature
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52 (Swain and Swain 2014). The need for morphological information can be further
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7 demonstrated by recent work on South African *Zoanthus* spp. by Ryland (2015), in
8 which polyp diameters showed the presence of three species groups (*Z. sansibaricus*, *Z.*
9 *natalensis* Carlgren, 1939, *Z. lawrencei* Carlgren, 1939), contradicting recent molecular
10 evidence of showing two species-level clades in the same region (*Z. sansibaricus*, *Z.*
11 *natalensis*-*Z. durbanensis* Carlgren, 1939; Risi and Macdonald 2016).
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21 Additionally, the molecular results of this study demonstrated problems
22 inherent with identifying zoantharians (and many anthozoans) with mitochondrial
23 DNA markers alone. Although mitochondrial DNA markers such as 16S-rDNA and COI
24 have been shown to often have utility in Zoantharia (Sinniger et al. 2008), they often
25 fail to properly delineate between closely related species due to slow sequence
26 evolution (=low mutation rates; Shearer et al. 2002; Huang et al. 2008). This has
27 previously been noted for some *Palythoa* species (Sinniger et al. 2008), and is clearly
28 evident from our 16S-rDNA and COI results here. While the results from these two
29 markers provided information that was helpful in sorting out some species
30 identifications, other groups were mixed together into large clades or polytomies (e.g.
31 *P. tuberculosa* + *P. mutuki* + *P. sp. "sakurajimensis"* with 16S-rDNA). As well, relatively
32 short sequences from specimens in studies in neighboring regions limited our ability
33 to effectively compare our results to these other marine areas (e.g. COI sequences of
34 ~300 bp). Additionally, in this study, only the combined *Zoanthus* and *Palythoa*
35 ITS-rDNA analyses (Fig. 3) were able to effectively delineate all six to seven observed
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8 species. Thus, we strongly recommend the acquisition of both 1) long mitochondrial
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10 sequences, in particular those of 16S-rDNA and COI as they are utilized widely in
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12 zoantharian phylogenies, and 2) the acquisition of ITS-rDNA sequences. Even though
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14 *Zoanthus* and *Palythoa* ITS-rDNA sequences are often very difficult to align (Reimer et
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16 al. 2007b, 2007c; Aguilar and Reimer 2010), at the very least they provide accurate
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18 species-levels information that can be further supported by mitochondrial sequences
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20 and morphological analyses.
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26 One logical route to take in untangling the complex taxonomy of
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28 Brachycnemina species in the Indo-Pacific would be to follow the taxonomic history of
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30 species in as closely as possible, working in a chronological manner starting from the
31
32 oldest (=senior binomens) literature. Acquiring both molecular (ITS-rDNA, 16S-rDNA,
33
34 COI) and morphological data in this manner should allow for a relatively straight
35
36 forward assessment of which species are valid and which are inadvertent synonyms of
37
38 previously described species. As mentioned in the first molecular studies of
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40 Brachycnemina (Burnett et al. 1994, 1995, 1997) and in more recent works (Reimer et
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42 al. 2011; Risi and McDonald 2016), molecular results have consistently shown the
43
44 reoccurring presence of many of the same species groups (e.g. *Palythoa tuberculosa*, *P.*
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46 *mutuki*, *Zoanthus sansibaricus*) in various marine provinces, such as the East Pacific
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48 Region (Reimer et al. 2008), Indo-Polynesian (Koupaei et al. 2014; Joseph et al. 2014;
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50 Reimer et al. 2004, 2006, 2007b, 2011, 2014), and the Western Indian Ocean (Risi and
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7 Macdonald 2016), suggesting these Brachycnemina species are widespread and that
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9 much taxonomic work remains (Ryland 2015). Formal taxonomic revision based on
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11 molecular data underpinned by a morphological framework to allow comparisons
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13 with historical literature (Swain and Swain 2014; Swain et al. 2015, 2016) will then
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15 allow comparative and population genetic studies to confidently move forward,
16
17 increasing our understanding of this important component of shallow subtropical and
18
19 tropical benthic communities.
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58 **References:**

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1. Acosta A (2001) Asexual reproduction in a zoanthid by fragmentation: the role of exogenous factors. Bull Mar Sci 68:363–381
2. Acosta A, González AM (2007) Fission in the Zoantharia *Palythoa caribaeorum* (Duchassaing and Michelotii, 1860) populations: a latitudinal comparison. Bol Invest Mar Cost 36:151–166
3. Acosta A, Sammarco PW, Duarte LF (2005) New fission processes in the zoanthid *Palythoa caribaeorum*: description and quantitative aspects. Bull Mar Sci 76:1–26
4. Aguilar C, Reimer JD (2010) Molecular phylogenetic hypotheses of *Zoanthus* species (Anthozoa: Hexacorallia) using RNA secondary structure of the internal transcribed spacer 2 (ITS2). Mar Biodiv 40:195-204
5. ATGC: SMS. [(accessed on 15 July 2015)]. Available online: <http://www.atgc-montpellier.fr/sms/>
6. Audouin JV (1826) Explication sommaire des planches de polypes de l'Égypte et de la Syrie, publiées par Jules-César Savigny, membre de l'Institut; offrant un exposé des caractères naturels des genres, avec la distinction des espèces. pp. 225–244. In: Description de l'Égypte ou Recueil des observations et des recherches qui ont été faites en Égypte pendant l'expédition de l'armée française, publié par les ordres de Sa Majesté l'empereur Napoléon le Grand. Histoire naturelle. Tome premier. Quatrième partie [Text]. Imprimerie Impériale, Paris

7. Bastidas C, Bone D (1996) Competitive strategies between *Palythoa caribaeorum* and *Zoanthus sociatus* (Cnidaria: Anthozoa) at a reef flat environment in Venezuela. Bull Mar Sci 59:543–555
8. Briggs JC (2003) Marine centres of origin as evolutionary engines. J Biogeogr 30:1–18
9. Briggs JC, Bowen BW (2012) A realignment of marine biogeographic provinces with particular reference to fish distributions. J Biogeogr 39:12–30
10. Briggs JC, Bowen BW (2013) Marine shelf habitat: biogeography and evolution. J Biogeogr 40:1023–1035
11. Burnett WJ (2002) Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. Mar Ecol Prog Ser 234:105–109
12. Burnett WJ, Ryland JS (1997) Order Zoanthidea. In: Richmond MD (ed) A guide to the seashores of eastern Africa and the Western Indian Ocean Islands. Sida/Department for Research Cooperation, SAREC, Stockholm, pp 138–139
13. Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS (1994) High genetic variability and patchiness in a common Great Barrier Reef zoanthid (*Palythoa caesia*). Mar Biol 121:153–160
14. Burnett WJ, Benzie JAH, Beardmore J, Ryland JS (1995) Patterns of genetic subdivision in populations of a clonal cnidarian, *Zoanthus coppingeri*, from the Great Barrier Reef. Mar Biol 122:665–673

15. Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS (1997) Zoanthids (Anthozoa, Hexacorallia) from the Great Barrier Reef and Torres Strait, Australia: systematics, evolution and a key to species. *Coral Reefs* 16:55–68
16. Carlgren O (1900) Ostafrikanische Actinien, gesammelt von Herrn Dr. F. Stuhlmann 1898 und 1899. *Mitteilungen aus dem Naturhistorischen Museum in Hamburg*, 17 (Supplement 2):21–144, pls. 1–7
17. Carlgren O (1939) South African Actiniaria and Zoantharia. *Kungliga Svenska Vetenskapsakademiens Handlingar*, ser. 3, 17(3):1–148, pls. 1–3
18. Castresana J (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 17:540–552
19. Chimetto LA, Brocchi M, Gondo M, Thompson CC, Gomez-Gil B, Thompson FL (2009) Genomic diversity of vibrios associated with the Brazilian coral *Mussismilia hispida* and its sympatric zoanthids (*Palythoa caribaeorum*, *Palythoa variabilis* and *Zoanthus solanderi*). *J Appl Microbiol* 106:1818–26
20. Costa Jr. OS, Nimmo M, Attrill MJ (2008) Coastal nutrification in Brazil: a review of the role of nutrient excess on coral reef demise. *J South Am Earth Sci* 25:257–270
21. Cruz ICS, Loiola M, Albuquerque T, Reis R, Nunes JACC, Reimer JD, Mizuyama M, Kikuchi RKP, Creed JC (2015) Effect of phase shift from corals to Zoantharia on reef fish assemblages. *PLoS ONE* 10:e0116944
22. Cruz ICS, Henschen VM, Kikuchi RK, Creed JC (2016) The role of competition in

the phase shift to dominance of the zoanthid *Palythoa* cf. *variabilis* on coral reefs.

Mar Environ Res 115:28–35

23. DiBattista JD, Roberts MB, Bouwmeester J, Bowen BW, Coker DJ, Lozano-Cortes DF, Choat JH, Gaither MR, Hobbs JPA, Khalil MT, Kochzius M, Myers R, Paulay G, Robitzsch VSN, Saenz-Agudelo P, Salas E, Sinclair-Taylor TH, Toonen RJ, Westneat MW, Williams ST, Berumen ML (2016) A review of contemporary patterns of endemism for shallow water reef fauna in the Red Sea. J Biogeogr 43:423–439
24. Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl Acids Res 32:1792–1797
25. Ehrenberg CG (1834) Beiträge zur physiologischen Kenntniss der Corallenthiere im allgemeinen, und besonders des rother Meeres, nebst einem Versuche zur physiologischen Systematik derselbe. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin 1832 (1): 225–380, 1 table
26. Ekman S (1953) Zoogeography of the Sea. Sigwick and Jackson, London.
27. Esper EJC (1805) Die Pflanzenthiere in Abbildungen nach der Natur mit Farben erleuchtet nebst Beschreibungen. Raspe, Nürnberg. Theilen 1–3, Lieferungen 13
28. Fautin DG, Buddemeier RW (2008) Biogeoinformatics of the Hexacorals: <http://www.kgs.ku.edu/Hexacoral/>
29. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse

metazoan invertebrates. *Mol Mar Biol Biotech* 3:294–299

30. Fujii T, Reimer JD (2013) A new family of diminutive zooxanthellate zoanthids (Hexacorallia: Zoantharia). *Zool J Linn Soc* 169:509–522
31. Goreau T (1959) The ecology of Jamaican coral reefs I. Species composition and zonation. *Ecology* 40:67–90
32. Gray JE (1828) Radiata. In: *Spicilegia zoologica; or Original Figures and Short Systematic Descriptions of New and Unfigured Animals*. Treüttel, Würtz and Co., London, vol. 1, p. 8, pl. 6
33. Gray JE (1832) [T]he Animal Flowers, or Zoantharia, and the stellated corals. In: *Synopsis of the Contents of the British Museum. Twenty-Sixth Edition*. G. Woodfall and Son, London, pp. 94–106
34. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321. doi: 10.1093/sysbio/syq010.
35. Haddon AC, Shackleton AM (1891a) The Zoantheae. A revision of the British Actiniae. Part II. Reports on the zoological collections made in the Torres Straits by AC Haddon, 1888–1889. *Sci Trans R Dublin Soc ser. 2*, 4(12):609–672, pls. 58–60
36. Haddon AC, Shackleton AM (1891b) Reports on the zoological collections made in

Torres Straits by Professor A.C. Haddon, 1888–1889. Actiniae: I. Zoantheae. Sci Trans R Dublin Soc 4:673–701

37. Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174
38. Herberts C (1972) Etude systématique de quelques zoanthaires tempérés et tropicaux." Tethys Supplement 3: 69–156
39. Hertwig R (1882) Report on the Actiniaria dredged by H.M.S. *Challenger* during the years 1873–1876. In: Report of the Scientific Results of the Voyage of H.M.S. Challenger During the Years 1873–76, Under the Command of Captain George S. Nares, R.N., F.R.S. and the Late Captain Frank Tourle Thomson, R.N. Prepared Under the Superintendence of the Late Sir C. Wyville Thomson, Knt., F.R.S., &c. Regius Professor of Natural History in the University of Edinburgh Director of the Civilian Scientific Staff on Board and Now of John Murray, LL.D., Ph.D., &c. One of the Naturalists of the Expedition. Zoology—Vol. VI. Her Majesty's Government, Edinburgh. pp. 1–136, pls. 1–14
40. Hibino Y, Todd PA, Yang SY, Benayahu Y, Reimer JD (2013) Molecular and morphological evidence for conspecificity of two common Indo-Pacific species of *Palythoa* (Cnidaria: Anthozoa). Hydrobiologia 733:31–43
41. Joseph S, Poriya P, Kundu R (2014) Probing the phylogenetic relationships of a few newly recorded intertidal zoanthids of Gujarat coast (India) with mt DNA COI

1
2
3
4
5
6
7 sequences. Mitochondrial DNA 1736:1–7
8
9

- 10 42. Kamezaki M, Higa M, Hirose M, Suda S, Reimer JD (2013) Different zooxanthellae
11 types in populations of the zoanthid *Zoanthus sansibaricus* along depth gradients
12 in Okinawa, Japan. Mar Biodivers 43:61–70
13
14
15
16
17 43. Karlson RH (1983) Disturbance and monopolization of a spatial resource by
18 *Zoanthus sociatus* (Coelenterata, Anthozoa). Bull Mar Sci 13:118–131
19
20
21
22
23 44. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S,
24 Cooper A, Markowitz S, Duran C, Thierer T (2012) Geneious Basic: an integrated
25 and extendable desktop software platform for the organization and analysis of
26 sequence data. Bioinformatics 28:1647–1649
27
28
29
30
31
32
33 45. Kelecom A, Solé-Cava AM (1982) Comparative study of zoanthid sterols the genus
34 *Palythoa* (Hexacorallia, Zoanthidea). Comp Biochem Physiol 72B:677–682
35
36
37
38
39 46. Khushali PM, Pradeep MC (2013) Brachycnemic zooxanthellate zoanthids
40 (Cnidaria: Zoantharia) of Saurashtra coast: a preliminary survey. Res J Mar Sci
41 1:10–13
42
43
44
45
46
47 47. Klunzinger KB (1877) Die Korallthiere des Rothen Meeres. 1: Die Alcyonarien und
48 Malacodermen. Verlag der Gutmann'schen Buchhandlung (Otto Enslin), Berlin [in
49 German and Latin]
50
51
52
53
54 48. Koupaei AN, Mostafavi PG, Mehrabadi JF, Fatemi SMR (2014) Molecular diversity
55 of coral reef-associated zoanthids off Qeshm Island, northern Persian Gulf. Int
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 Aquat Res 6:1–10
8
9

- 10 49. Koupaei AN, Mostavi PG, Mehrabadi JF, Fatemi SMR, Dehghani H (2015) Diversity
11 of shallow water zoantharians in Hengam and Larak Islands, in the Persian Gulf. J
12 Mar Biol Assoc UK 1-11. doi:10.1017/S002531541500079X
13
14
15
16
17
18 50. Lamarck JBP (1801) Zoanthe. *Zoantha*. In: Système des animaux vertèbres, ou
19 Tableau général des classes, des ordres et des genres de ces animaux; Présentant
20 leurs caractères essentiels et leur distribution, d'après la considération de leurs
21 rapports naturels et leur organisation, et suivant l'arrangement établis dans les
22 galeries du Muséum d'Histoire Naturelle, parmi leur dépouilles conservées;
23 Précédé du discours d'ouverture du cours de zoologie, donné dans le Muséum
24 National d'Histoire Naturelle l'an 8 de la République. L'Auteur et Déterville, Paris,
25 p. 363
26
27
28
29
30
31
32
33
34
35
36
37
38
39 51. Lamouroux JVF (1816) Histoire des polypiers coralligènes flexibles, vulgairement
40 nommés zoophytes. F. Poisson, Caen, lxxxiv + [1] + 559 + [1] pp., pls. 1–19
41
42
43
44 52. Longo GO, Krajewski JP, Segal B, Floeter SR (2012) First record of predation on
45 reproductive *Palythoa caribaeorum* (Anthozoa: Sphenopidae): insights on the
46 trade-off between chemical defences and nutritional value. Mar Biodivers Rec
47 5:1–3
48
49
50
51
52
53
54
55 53. Milne Edwards H (1857) Histoire naturelle des coralliaires ou polypes
56 proprement dits. Tome premier. Roret, Paris, xxxiv + 326 pp. [5 Dec 1857
57
58
59
60
61
62
63
64
65

(Anonymous, 1857: 634)]

54. Miyashita M, Sasaki M, Hattori I, Sakai M, Tanino K (2004) Total synthesis of norzoanthamine. *Science* 305:495–499
55. Oigman-Pszczol SS, Figueiredo MADO, Creed JC (2004) Distribution of benthic communities on the tropical rocky subtidal of Armação dos Búzios, Southeastern Brazil. *Mar Ecol* 25:173–190
56. Ong CW, Reimer JD, Todd PA (2013) Morphologically plastic responses to shading in the zoanthids *Zoanthus sansibaricus* and *Palythoa tuberculosa*. *Mar Biol* 160:1053–1064
57. Pax F (1910) Studien an westindischen Actinien. Habilitationsschrift, welche nebst den beigefügten Thesen mit Genehmigung der hohen philosophischen Fakultät der Königlichen Universität Breslau zur Erlangung der Venia legendi. Montag, den 17. Oktober 1910, um 12 Uhr, im Musiksaale der Universität öffentlich verteidigt wird. Gustav Fischer, Jena, 166 + [2] pp., 10 pls
58. Pax F, Muller I (1956) Zoanthaires de la Mer Rouge. *Annales de l'Institut Océanographique* 32:1-77
59. Polak O, Loya Y, Brickner I (2011) The widely-distributed Indo-Pacific zoanthid *Palythoa tuberculosa*: a sexually conservative strategist. *Bull Mar Sci* 87:605–621
60. Rafinesque CS (1815) Analyse de la nature ou tableau de l'univers et des corps organisés. [The Author], Palerme [= Palermo]

61. Reimer AA (1971) Feeding behavior in the Hawaiian zoanthids *Palythoa* and *Zoanthus*. Pac Sci 25:512–520
62. Reimer JD, Todd PA (2009) Preliminary molecular examination of zooxanthellate zoanthids (Hexacorallia: Zoantharia) and associated zooxanthellae (*Symbiodinium* spp.) diversity in Singapore. Raffles Bull Zool 22:103-120
63. Reimer JD, Ono S, Fujiwara Y, Takishita K, Tsukahara J (2004) Reconsidering *Zoanthus* spp. diversity: molecular evidence of conspecificity within four previously presumed species. Zool Sci 21:517–525
64. Reimer JD, Ono S, Takishita K, Tsukahara J, Maruyama T (2006) Molecular evidence suggesting species in the zoanthid genera *Palythoa* and *Protopalythoa* (Anthozoa: Hexacorallia) are congeneric. Zool Sci 23:87–94
65. Reimer JD, Hirano S, Fujiwara Y, Sinniger F, Maruyama T (2007a). Morphological and molecular characterization of *Abyssanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a northwest Pacific methane cold seep. Invert System 21 (3): 255-262
66. Reimer JD, Takishita K, Ono S, Maruyama T (2007b) Diversity and evolution in the zoanthid genus *Palythoa* (Cnidaria: Hexacorallia) based on nuclear ITS-rDNA. Coral Reefs 399–410
67. Reimer JD, Takishita K, Ono S, Tsukahara J, Maruyama T (2007c). Molecular evidence suggesting intraspecific hybridization in *Zoanthus* (Anthozoa:

Hexacorallia). Zool Sci 24:346-359

68. Reimer JD, Sinniger F, Hickman CP (2008) Zoanthid diversity (Anthozoa: Hexacorallia) in the Galapagos Islands: a molecular examination. Coral Reefs 27:641–654
69. Reimer JD, Obuchi M, Irei Y, Fujii T, Nozawa Y (2011) Shallow-water brachycnemic zoanthids (Cnidaria : Hexacorallia) from Taiwan: a preliminary survey. Zool Stud 50:363–371
70. Reimer JD, Poliseno A, Hoeksema BW (2014) Shallow-water zoantharians (Cnidaria, Hexacorallia) from the Central Indo-Pacific. ZooKeys 57:1–57
71. Reimer JD, Wee HB, Put Jr. A, Hoeksema BW (2015) Zoantharia (Cnidaria: Anthozoa: Hexacorallia) of the South China Sea and Gulf of Thailand: species list based on past reports and new photographic records. Raffles Bull Zool 63:334–356
72. Reimer JD, Kim S, Arai S, Keshavmurthy S, Choi K (2016a) First records of zooxanthellae *Zoanthus* (Anthozoa: Hexacorallia: Zoantharia) from Korea and Japan (East) Sea. Mar Biodivers. doi:10.1007/s12526-016-0567-1
73. Reimer JD, Herrera M, Gatins R, Roberts MB, Parkinson JE, Berumen ML (2016b) Latitudinal variation in the symbiotic dinoflagellate *Symbiodinium* of the common reef zoantharian *Palythoa tuberculosa* on the Saudi Arabian coast of the Red Sea. J Biogeogr. doi:10.1111/jbi.12795

- 1
2
3
4
5
6
7
8 74. Risi MM, Macdonald AHH (2015) Possible synonymies of *Zoanthus* (Anthozoa:
9 Hexacorallia) species on the east coast of South Africa with Pacific congeners. Syst
10 Biodivers 13:93–103
11
12
13
14
15 75. Risi MM, McDonald AHH (2016) Molecular examination of rocky shore
16 brachycnemic zoantharians (Anthozoa: Hexacorallia) and their *Symbiodinium*
17 symbionts (Dinophyceae) in the southwest Indian Ocean. Mar Biodivers 46:113–
18 127
19
20
21
22
23
24
25 76. Rocha LA, Rocha CR, Robertson DR, Bowen BW (2008) Comparative
26 phylogeography of Atlantic reef fishes indicates both origin and accumulation of
27 diversity in the Caribbean. BMC Evol Biol 8:157
28
29
30
31
32
33 77. Ryland JS (2015) Size-defined morphotypes in *Zoanthus* (Hexacorallia:
34 Zoantharia) populations on shores in KwaZulu-Natal, South Africa. Zootaxa
35 3986:332–356
36
37
38
39
40
41 78. Ryland JS, Lancaster JE (2003) Revision of methods for separating species of
42 *Protopalythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. Invert Syst
43 17:407–428
44
45
46
47
48
49 79. Santana EFC, Alves AL, Santos ADM, Cunha MGGS, Perez CD, Gomes PB (2015)
50 Trophic ecology of the zoanthid *Palythoa caribaeorum* (Cnidaria: Anthozoa) on
51 tropical reefs. J Mar Biol Assoc UK 95:301–309
52
53
54
55
56
57 80. Santos MEA, Kitahara MV, Lindner A, Reimer JD (2016) Overview of the order
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7 Zoantharia (Cnidaria: Anthozoa) in Brazil. Mar Biodivers 46:547–559
8
9

- 10 81. Savigny J-C (1811) Description de l'Égypte ou Recueil des observations et des
11 recherches qui ont été faites en Égypte pendant l'expédition de l'armée française,
12 publié par les ordres de Sa Majesté l'empereur Napoléon le Grand. Histoire
13 naturelle. Tome deuxième [Atlas]. Imprimerie Impériale, Paris, pls. Polypes 1–14
14
15
16
17
18
19
20 82. Sebens K (1977) Autotrophic and heterotrophic nutrition of coral reef zoanthids.
21 Proc 3rd Int Coral Reef Symp 1:397–404
22
23
24
25
26 83. Sinniger F, Montoya-Burgos JI, Chevaldonné P, Pawlowski J (2005) Phylogeny of
27 the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial
28 ribosomal genes. Mar Biol 147:1121–1128
29
30
31
32
33
34 84. Sinniger F, Reimer JD, Pawlowski J (2008) Potential of DNA sequences to identify
35 zoanthids (Cnidaria: Zoantharia). Zool Sci 25:1253–1260
36
37
38
39 85. Soares C, Pérez C, Maia MBS, Silva RS, Melo LFA (2006) Avaliação da atividade
40 antiinflamatória e analgésica do extrato bruto hidroalcoólico do zoantídeo
41 *Palythoa caribaeorum* (Duchassaing & Michelotti, 1860). Braz J Pharmacogn
42 16:463–468
43
44
45
46
47
48
49 86. Stampar SN, Francisco P, Luiz Jr OJ (2007) Predation on the zoanthid *Palythoa*
50 *caribaeorum* (Anthozoa, Cnidaria) by a hawksbill turtle (*Eretmochelys imbricata*)
51 (Reptilia, Vertebrata) in southeastern Brazil. Mar Turtle Newsletter 117:3–5
52
53
54
55
56
57 87. Swain TD, Swain LM (2014) Molecular parataxonomy as taxon description:
58
59
60
61
62
63
64
65

examples from recently named Zoanthidea (Cnidaria: Anthozoa) with revision based on serial histology of microanatomy. Zootaxa 3796:81–107

88. Swain TD, Schellinger JL, Strimaitis AM, Reuter KE (2015) Evolution of anthozoan polyp retraction mechanisms: convergent functional morphology and evolutionary allometry of the marginal musculature in order Zoanthidea (Cnidaria: Anthozoa: Hexacorallia). BMC Evol Biol 15:123
89. Swain TD, Strimaitis AM, Reuter KE, Boudreau W (2016) Towards integrative systematics of Anthozoa (Cnidaria): evolution of form in the order Zoanthidea. Zool Scr 1-18. doi:10.1111/zsc.12195
90. Vermeij GJ (1978) Biogeography and adaptation: patterns of marine life. Harvard University Press, Cambridge, MA.
91. Verrill AE (1928) Hawaiian shallow water Anthozoa. Bull Bernice P Bishop Museum 49:1–30
92. Villaça R, Pitombo FB (1997) Benthic communities of shallow-water reefs of Abrolhos, Brazil. Rev Bras Oceanogr 45:35–43
93. Walsh GE, Bowers RL (1971) A review of the Hawaiian zoanthids with descriptions of three new species. Zool J Linn Soc 50:161–180
94. WoRMS Editorial Board (2016). World Register of Marine Species. Available from <http://www.marinespecies.org> at VLIZ. Accessed 2016-11-26. doi:10.14284/170

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7 **Figures and Tables:**
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10 Figure 1 – Map of sampling sites in this study in the northern Red Sea along the coast
11 of Saudi Arabia.
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18 Figure 2 – Species of Brachycnemina observed in the northern Red Sea. a) *Palythoa* cf.
19 *heliodiscus*, b) *Palythoa* aff. *heliodiscus*, c) *Palythoa* sp. “sakurajimensis”, d) *Palythoa*
20 *tuberculosa*, and e) *Zoanthus sansibaricus*. Scale bars equal approximately 1 cm.
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28 Figure 3 – a) Maximum likelihood (ML) tree of internal transcribed spacer region
29 (ITS-rDNA) sequences of *Palythoa*. b) Maximum likelihood (ML) tree of internal
30 transcribed spacer region (ITS-rDNA) sequences of *Zoanthus*. Different colors
31 represent major species groups. Sequences from previous studies with GenBank
32 accession numbers, location, and species name (if not same as name of each species
33 clade). Sequences from specimens in this study in bold. Values at nodes represent ML
34 and neighbor-joining (NJ) values.
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50 Supplementary Table S1 – Some taxonomic historical notes of Brachycnemina
51 species-group names in the Red Sea.
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58 Supplementary Table S2 – Brachycnemina specimens from the northern Red Sea
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collected in this study, their collection information, GenBank Accession Numbers, and identification.

Supplementary Figure S1 – Maximum likelihood (ML) tree of mitochondrial 16S ribosomal DNA (16S-rDNA) sequences of Brachygnemina. Sequences from previous studies with GenBank accession numbers, location, and species name. Sequences from specimens in this study in bold. Values at nodes represent ML and neighbor-joining (NJ) values.

Supplementary Figure S2 – Maximum likelihood (ML) tree of mitochondrial cytochrome oxidase subunit 1 (COI) sequences of Brachygnemina. Sequences from previous studies with GenBank accession numbers, location, and species name. Sequences from specimens in this study in bold. Values at nodes represent ML and neighbor-joining (NJ) values.

Figure 1

Figure 2

Figure 3a

0.1 substitutions/site

Figure 3b

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