



**Evolution and phylogeny of glass-sponge-associated
zoantharians, with description of two new genera and three
new species**

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Abstract:	<p>Hexactinellid sponges are important members of deep-sea benthic ecosystems as they provide available hard substrate habitats for filter feeding invertebrates. However, symbioses between hexactinellid sponges and their epibiont symbionts are poorly known. Zoantharians associated with hexactinellid sponges have been reported widely from deep sea marine ecosystems, either on the bodies or stalks of hexactinellid sponges, but there has been a lack of research on their diversity and phylogenetic relationships. In this study, 20 specimens associated with amphidiscophoran and hexasterophoran sponges were collected from the waters of Australia and Japan in the Pacific, and from Curaçao in the southern Caribbean, and in addition we examined previously collected museum specimens. Based on our molecular phylogenetic analyses and morphological observations, we formally describe two new genera and three new species of Zoantharia as well as newly report several previously described species. Our results demonstrate that the diversity of glass-sponge-associated zoantharians is much higher than has been previously thought. The results suggest at least two independent origins for the symbioses between hexactinellid sponges and zoantharians. The new taxa described in this work further reconfirm that the deep sea harbors high levels of undescribed zoantharian diversity.</p>

1 ABSTRACT

2 Hexactinellid sponges are important members of deep-sea benthic ecosystems as
3 they provide available hard substrate habitats for filter feeding invertebrates. However,
4 symbioses between hexactinellid sponges and their epibiont symbionts are poorly known.
5 Zoantharians associated with hexactinellid sponges have been reported widely from deep sea
6 marine ecosystems, either on the bodies or stalks of hexactinellid sponges, but there has been
7 a lack of research on their diversity and phylogenetic relationships. In this study, 20
8 specimens associated with amphidiscophoran and hexasterophoran sponges were collected
9 from the waters of Australia and Japan in the Pacific, and from Curaçao in the southern
10 Caribbean, and in addition we examined previously collected museum specimens. Based on
11 our molecular phylogenetic analyses and morphological observations, we formally describe
12 two new genera and three new species of Zoantharia as well as newly report several
13 previously described species. Our results demonstrate that the diversity of glass-sponge-
14 associated zoantharians is much higher than has been previously thought. The results suggest
15 at least two independent origins for the symbioses between hexactinellid sponges and
16 zoantharians. The new taxa described in this work further reconfirm that the deep sea harbors
17 high levels of undescribed zoantharian diversity.

18
19 **Keywords:** Epizoanthidae, hexactinellid sponge, deep sea, museum collections,
20 Parazoanthidae, symbiosis, Hexasterophora

26 INTRODUCTION

27 Class Hexactinellida Schmidt, 1870 (Porifera) consists of sponges forming
28 siliceous structures, and its species are found exclusively in the deep-sea (Dohrmann *et al.*
29 2008). Hexactinellid sponges are important in deep-sea benthic ecosystems as they provide
30 available hard substrate habitats for filter feeding invertebrates such as cnidarians, tunicates,
31 ophiuroids, bryozoans, and other sponges in the muddy environments of the deep-sea
32 seafloor (Beaulieu 2001; Leys *et al.* 2007). Although many hexactinellid sponge-associated
33 invertebrates have been observed (Beaulieu 2001; Kahn *et al.* 2020), symbioses between
34 hexactinellid sponges and symbionts are poorly described. Within the subclass Hexacorallia
35 Haeckel, 1896, some members of the orders Actiniaria Hertwig, 1882 and Zoantharia
36 Rafinesque, 1815 are known to have symbioses with hexactinellid sponges. *Spongiactis*
37 *japonica* Sanamyan, Sanamyan & Tabachnick, 2012, reported from Japanese waters, is
38 currently the only known actiniarian species to associate with hexactinellid sponges
39 (Sanamyan *et al.* 2012). On the other hand, many hexactinellid-sponge-associated
40 zoantharian species have been described; species of the genera *Epizoanthus* Gray, 1867 and
41 *Thoracactis* Gravier, 1918 within the family Epizoanthidae Delage & Hérouard, 1901, and of
42 *Isozoanthus* Carlgren in Chun, 1903 within Parazoanthidae Delage & Hérouard, 1901.
43 Hexactinellid sponge-zoantharian associations are distributed widely across the globe. For
44 instance, several species associated with stalks of *Hyalonema* such as *E. stellaris* Hertwig,
45 1888 and *E. fatuus* (Schultze, 1860) have been reported from the Indo-Pacific Ocean as well
46 as from the Atlantic Ocean (Fautin 2013; Bigatti 2015; Hajdu *et al.* 2017; Dueñas & Puentes
47 2018; NIWA 2018; Kahn *et al.* 2020). *Epizoanthus stellaris* is the most common epibiont on
48 stalks of *Hyalonema* Gray, 1832 in the deep-sea of the Northeast Pacific (Beaulieu 2001).
49 Thus, such associations may be widespread and common in the deep-sea.

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3 50 However, the taxonomy of hexactinellid zoantharians still faces many problems.
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5 51 Most importantly, several stalked hexactinellid-sponge-associated *Epizoanthus* and
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7 52 *Isozoanthus* species have not been examined since their original descriptions, or have been
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9 53 observed only a very few times. As well, the genus *Isozoanthus* is in need of taxonomic
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11 54 reexamination as previous studies using molecular phylogenetic analyses have cast doubt on
12
13 55 the validity of this genus (Sinniger *et al.* 2010, 2013). Thus, although some previous studies
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15 56 have reported on several stalked hexactinellid sponge-associated Parazoanthidae specimens,
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17 57 the formal taxonomic position of many of these specimens remains uncertain (Sinniger *et al.*
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19 58 2010).

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24 59 Furthermore, while many zoantharian species have been reported on the stalks of
25
26 60 hexactinellid sponges, there is little information on zoantharian species that are found
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28 61 growing on or in the bodies of hexactinellid sponges. Two species have been described with
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30 62 such growth forms; *Palythoa oligomyaria* (Wassilief, 1908) and *Thoracactis topsenti*
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32 63 Gravier, 1918. *Palythoa oligomyaria* was originally placed within the genus *Gemmaria*
33
34 64 Duchassaing de Fonbressin & Michelotti, 1860, and this genus group is currently a junior
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36 65 subjective synonym of *Palythoa* Lamouroux, 1816 (see Low *et al.* 2016). *Palythoa*
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38 66 *oligomyaria* was distinguished from other *Gemmaria* (= *Palythoa*) species by its marginal
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40 67 musculature as “the few known species of the genus *Gemmaria* have well-developed
41
42 68 sphincters. On the other hand, *P. oligomyaria* stands out because of the extraordinarily weak
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44 69 development of the sphincter” (p. 47 translated from Wassilief 1908). However, the
45
46 70 taxonomic position of *P. oligomyaria* is questionable for several reasons; as the original
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48 71 description is brief, subsequent studies have not been performed since its original description
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50 72 by Wassilief (1908), and no further specimens have been collected. Additionally, the genus
51
52 73 *Palythoa* generally contains zooxanthellate species living on rocks and coral reefs in
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54 74 subtropical and tropical shallow waters (Duerden 1903; Burnett 2002; Reimer *et al.* 2011),
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3 75 while *P. oligomyaria* is an azooxanthellate epibiont from the deep-sea; clearly the generic
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5 76 placement of this species is doubtful.
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8 77 As well, the taxonomic position of the monospecific genus *Thoracactis* (type species:
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10 78 *Thoracactis topsenti*) is uncertain; Gravier (1918) identified this species as an actinarian
11
12 79 based on the lack of zooxanthellae, channels, gaps, or cell islets. Subsequently, Reimer *et al.*
13
14 80 (2010) suggested that *Thoracactis* may be within the family Parazoanthidae and not in the
15
16 81 family Epizoanthidae based on its bright yellow color, which is commonly observed in some
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18 82 Parazoanthidae species (e.g., West, 1979). However, no formal phylogenetic and taxonomic
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20 83 reassessment of the position of this genus have been conducted.
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24 84 Recently, unidentified zoantharians have been reported living on main bodies of
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26 85 *Cyrtaulon sigsbeeii* (Schmidt, 1880), *Verrucocoeloidea liberatorii* Reiswig & Dohrmann,
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28 86 2014, *Heterorete pulchrum* Dendy, 1916, *Psilocalyx wilsoni* Ijima, 1927, and *Aspidoscopulia*
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30 87 *australia* Dohrmann, Göcke & Janussen, 2011 (Reiswig & Wheeler 2002a; Dohrmann *et al.*
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32 88 2011; Reiswig & Dohrmann 2014; van Soest *et al.* 2014; Montenegro *et al.* 2020). These
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34 89 observations suggest that the diversity of zoantharians found on bodies of hexactinellid
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36 90 sponges is higher than has been previously thought. However, phylogenetic studies focused
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38 91 specifically on zoantharians found on bodies and stalks of hexactinellid sponges have not yet
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40 92 been performed.
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44 93 Thus, further studies utilizing a combination of molecular phylogenetic and
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46 94 morphological analyses supplemented with ecological information are needed to better
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48 95 understand the diversity of hexactinellid sponge-associated zoantharians. In this study, we
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50 96 examined zoantharian specimens found on bodies and stalks of hexactinellid sponges
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52 97 collected from the Indo-Pacific and Atlantic Oceans as well as specimens in the collection of
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54 98 Naturalis Biodiversity Center. The results of our molecular phylogenetic analyses combined
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56 99 with data from morphological observations clarify the phylogenetic relationships and
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3 100 taxonomic positions of several hexactinellid sponge-associated zoantharian species, and we
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5 101 formally describe two new genera and three new species.
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10 103 **MATERIALS AND METHODS**

12 104 **Specimen collection**

14 105 Hexactinellid-sponge-associated zoantharian specimens were collected by several
16 106 methods; beam trawls, baskets, dredging, and utilizing remotely operated submersibles
18 107 (ROV), from the waters of Australia, Curaçao (southern Caribbean), and Japan. In addition,
20 108 we examined relevant specimens in the Coelenterata and Porifera collections (RMNH and
22 109 ZMA) at Naturalis Biodiversity Center, Leiden, the Netherlands, and a specimen in the
24 110 collection of the Department of Invertebrate Zoology of the Natural Museum of Natural
26 111 History, Washington, United States of America (Fig. 1, Table 1).
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33 113 **DNA extraction, PCR amplification and sequencing**

35 114 Total genomic DNA was extracted from tissue preserved in 70–99.5% ethanol
37 115 either by following a guanidine extraction protocol (Sinniger *et al.* 2010) or by using a spin-
39 116 column DNeasy Blood and Tissue Extraction kit following the manufacturer's instructions
41 117 (Qiagen, Hilden, Germany). PCR amplification using the Hot Star Taq Plus Master Mix kit
43 118 (Qiagen, Hilden, Germany) was performed for each of the genetic markers; COI
45 119 (mitochondrial cytochrome oxidase subunit I), mt 12S-rDNA (mitochondrial 12S ribosomal
47 120 DNA), mt 16S-rDNA (mitochondrial 16S ribosomal DNA), 18S-rDNA (nuclear 18S
49 121 ribosomal DNA), ITS-rDNA (nuclear internal transcribed spacer region of ribosomal DNA),
51 122 and 28S-rDNA (nuclear 28S ribosomal DNA) using published primers and protocols (Medlin
53 123 *et al.* 1988; Folmer *et al.* 1994; Apakupakul 1999; Chen *et al.* 2002; Sinniger *et al.* 2005,
55 124 2013; Swain 2009a, 2010; Fujii & Reimer 2011: Table S1). All PCR products were purified
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3 125 with 1 U of shrimp alkaline phosphatase (SAP) and 5 U of Exonuclease I (Takara Bio Inc.,
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5 126 Shiga, Japan) at 37°C for 40 min followed by 80°C for 20 min. Cleaned PCR products were
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7 127 sequenced in both directions on an ABI 3730Xl (Fasmac, Kanagawa, Japan). Additionally,
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10 128 some cleaned PCR products were sequenced on an ABI 3930 Genetic Analyzer (Applied
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12 129 Biosystems, Thermofisher) at the Genomic Unit, Scientific and Technological Support
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14 130 Center for Research (CACTI), University of Vigo (Spain). Obtained sequences in this study
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16 131 were deposited in GenBank under accession numbers XXXXX – XXXXX (Table 1).
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21 133 **Molecular and phylogenetic analyses**

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24 134 Molecular sequences were individually aligned in Geneious v10.2.3 (Kearse *et al.*
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26 135 2012) using the global alignment tool with free-end gaps and default settings. All output
27
28 136 alignments were visually inspected and manually curated. The resulting alignments were
29
30 137 subsequently concatenated with no overlapping positions.
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33 138 Publicly available sequences from families Parazoanthidae and Epizoanthidae,
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35 139 and two sequences from the genus *Microzoanthus* were downloaded from GenBank for each
36
37 140 marker and included into the alignments generated above; 43 sequences were added to COI,
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39 141 33 for mt 12S-rDNA, 64 for mt 16S-rDNA, 32 for 18S-rDNA, 26 for 28S-rDNA, and 55 for
40
41 142 ITS-rDNA (Table S2). Each region was individually aligned using MAFFT (Katoh &
42
43 143 Standley 2013) with the “auto” algorithm and default settings. Thereafter all alignments were
44
45 144 manually trimmed, curated and realigned as before. The resultant alignments were 396 bp in
46
47 145 length for COI, 829 bp for mt 12S-rDNA, 592 bp for mt 16S-rDNA, 1696 bp for 18S-rDNA,
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49 146 842 bp for 28S-rDNA, and 759 bp for ITS. For the 18S-rDNA region samples 23K, 52J,
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51 147 HPD1323, OK1, and ZMA10224 were missing one of the three amplified regions, and
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53 148 therefore missing positions were replaced by “N”s, as were all missing positions and gaps
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55 149 across markers. These alignments were subsequently concatenated to obtain a final dataset of
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3 150 5,114bp for 85 OTUs. All aligned datasets are available from the Dryad repository

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5 151 (<http://datadryad.org>, XXXXXXXX).

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8 152 Phylogenetic reconstructions were performed over the concatenated alignment
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10 153 using Maximum-likelihood (ML) and Bayesian inference (BI). TOPALi v2.5 (Milne *et al.*
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12 154 2009) was used to select the best fitting model for each molecular marker and independently
13
14 155 for ML and BI analyses. The selected models for ML were K80+G for COI and mt 12S-
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16 156 rDNA, SYM+G for mt 16S-rDNA, K81uf+I+G for 18S-rDNA, TIM+G for 28S-rDNA, and
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18 157 TrN+I+G for ITS. The same models were selected for BI, except for K80+I for 18S-rDNA,
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20 158 GTR+G for 28S-rDNA, and HKY+I+G for ITS. All phylogenetic estimations were
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22 159 performed using the substitution models indicated above per partition in RAxML-NG v.0.9
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24 160 (Kozlov *et al.* 2019) standalone version for ML, and in the MrBayes v3.2.6 (Ronquist &
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26 161 Huelsenbeck, 2003) plugin version in Geneious for BI. RAxML-NG was configured to use
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28 162 12345 initial seeds, search for the best tree among 100 preliminary parsimony trees, branch
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30 163 length was scaled and automatically optimized per partition, and model parameters were also
31
32 164 optimised. MrBayes was configured to use the models and parameters above with the
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34 165 following settings; four MCMC heated chains were run for 5,000,000 generations with a
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36 166 temperature for the heated chain of 0.2. Chains were sampled every 200 generations. Burn-in
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38 167 was set to 25% at which point the average standard deviation of split frequency (ASDOSF)
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40 168 was steadily below 0.01. Sequences of genus *Microzoanthus* (family Microzoanthidae) were
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42 169 used as outgroup in ML and BI analyses.
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171 **Morphological observations**

172 We observed four categories of morphological characters; external morphology,
173 internal morphology, cnidae, and morphology of associated hexactinellid sponges. External
174 morphology was examined based on preserved specimens and photographs and included

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3 175 lengths and diameters of individual polyps, tentacle numbers, coloration of polyps and
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5 176 coenenchyme, and relative development of coenenchyme. Diameters of oral disks and polyp
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7 177 dimensions were measured when polyps were expanded. The internal morphology was
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9 178 observed based on histological sections of 8–10 μm thickness stained with hematoxylin and
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11 179 eosin after decalcification with Morse solution for 24–48 h (1:1 vol; 20% citric acid: 50%
12
13 180 formic acid). We additionally observed hand-cut polyps for marginal musculature position
14
15 181 and type, and mesenterial arrangement and number of mesenteries. Classification of marginal
16
17 182 musculature shapes followed Swain *et al.* (2015). Cnidae were observed in the tentacles,
18
19 183 column, actinopharynx, and mesenteries filaments under a Nikon Eclipse80i
20
21 184 stereomicroscope (Nikon, Tokyo). Cnidae sizes were measured using ImageJ ver. 1.45s
22
23 185 (Rasband 2012). Cnidae classification followed England (1991) and Ryland & Lancaster
24
25 186 (2004) exception for the treatment of basitrichs and microbasic b-mastigophores as
26
27 187 mentioned in Kise *et al.* (2019). Associated hexactinellid sponges were identified based on
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29 188 morphology (Reiswig & Wheeler 2002a; Reiswig & Wheeler 2002b).
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38 190 Abbreviations used

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40 191 NSMT: National Science Museum, Tsukuba, Ibaraki, Japan

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42 192 QM: Queensland Museum, Queensland, Australia

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44 193 RMNH: Rijksmuseum van Natuurlijke Historie (now at Naturalis Biodiversity Center),
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46 194 Leiden, the Netherlands

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48 195 RUMF: Ryukyu University Museum (Fujukan), University of the Ryukyus, Okinawa, Japan

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50 196 USNM: Natural Museum of Natural History, Washington, United States of America

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52 197 ZMA: Zoological Museum Amsterdam (now at Naturalis Biodiversity Center), Leiden, the
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3 200 **RESULTS**

4
5 201 Systematics

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8 202 Phylum Cnidaria Hatschek, 1888

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10 203 Class Anthozoa Ehrenberg, 1831

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12 204 Order Zoantharia Rafinesque, 1815

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17 206 Family Epizoanthidae Delage & Hérouard, 1901

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19 207 Genus *Epizoanthus* Gray, 1867

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21 208 Type species *Dysidea papillosa* Johnston, 1842, by monotypy (see also Opinion 1689, ICZN

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24 209 1992).

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28 211 **Diagnosis.** Macrocnemic zoantharians with simple mesogleal musculature, readily

29
30 212 distinguishable from *Palaeozoanthus* by the presence of non-fertile micromesenteries

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32 213 (Sinniger & Häussermann 2009).

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37 215 *Epizoanthus fatuus* (Schultze, 1860)

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39 216 Fig. 2A, B.

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41 217 Synonymy: *Palythoa fatua* Schultze 1860: 36, taf. 2, fig. 1–2; *Palythoa fatua* – Andres 1884:

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43 218 311; *Sidisia fatua* – Lwowsky 1913: 589–596, taf. 19, fig. 5–8; *Sidisia fatua* var. *alba* –

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45 219 Lwowsky 1913: 597.

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49 221 **Material examined.** MISE-HK33-2 (NSMT Co-XXXX), off Amakusa, Kumamoto, Japan,

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51 222 32°24'44.8"N 129°28'01.3"E (position approximate, exact location unknown), 1000 m depth,

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53 223 beat trawl, coll. D. Uyeno on the training vessel *Nagasaki-maru*, 2011, fixed in 99.5%

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55 224 ethanol. MISE-HK132 (NSMT Co-XXXX), Sagami Bay, Kanagawa, Japan, 35°08'27.5"N–

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225 35°08'33.5"N, 139°32'12.2"E–139°32'44.3"E, 133–274 m depth, dredging, coll. H. Kotsuka
226 on vessel *Rinkai-maru*, 12 Feb 2015, fixed in 99.5% ethanol.

227 **Description.** *External morphology.* Preserved colonies of examined specimens consist of 10–
228 50 cylindrical polyps connected by strongly developed dark brown and light beige
229 coenenchyme on stalks of hexactinellid sponges (*Hyalonema* sp.). Column of preserved
230 polyps dark brown and light beige in coloration. Colonies cover upper part of stalks, do not
231 cover around the spiculous anchor. Contracted preserved polyps 0.8–2.9 mm in height, 1.9–
232 3.6 mm in diameter. Capitulum swollen, and diameter of capitulum larger than scapus when
233 contracted. Capitulary ridges present and strongly pronounced when contracted, 14 in
234 number. The number of tentacles of each polyp in this study 28, and tentacles arranged in two
235 rows. Ectoderm and mesoglea of polyps and coenenchyme heavily encrusted with numerous
236 sand and silica particles.

237 *Internal morphology.* Zooxanthellae absent. Number of mesenteries 28, in macrocnemic
238 arrangement. Mesogleal thickness 0.1–0.3 mm and gradually wider in direction from
239 capitulum towards scapus. Mesoglea either as thick as or thinner than ectoderm. Reticulate
240 mesogleal musculature. Siphonoglyph distinct and V-shaped. Mesenterial filaments present.

241 **Habitat and distribution.** Indo-Pacific Ocean: near Indonesia (Carlgren 1923), East China
242 Sea (Pei 1998), the Bay of Bengal, India, and Japan (Lwowsky 1913).

243 **Associated host.** *Hyalonema* spp.

244 **Remarks.** This species has been reported in several studies, which indicate the presence of
245 intraspecific variation (Andres 1884; Lwowsky 1913, Carlgren 1923). In fact, we observed
246 several morphological differences such as coloration and polyp dimensions between the two
247 examined specimens (NSMT Co-XXXX and NSMT Co-XXXX). As well, some genetic
248 variation of *E. fatuus* was observed in ITS-rDNA sequences (8 bp), and thus the possibility
249 remains that *E. fatuus* contains cryptic species, as ITS-rDNA has been demonstrated to be

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3 250 among the most variable genetic regions currently utilized to delineate species within
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5 251 Zoantharia (e.g., Reimer et al. 2007, Montenegro et al. 2015). However, the sequences of the
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7 252 two examined specimens formed a strongly supported monophyletic clade and we therefore
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9 253 identify the examined specimens as a single species, *E. fatuus*, in this study. Additional
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11 254 specimens and fine-scale genetic analyses are required to better examine if there is any
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13 255 cryptic diversity within this group.
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19 257 *Epizoanthus* aff. *fatuus* (Schultze, 1860)

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21 258 Fig. 2C.
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26 260 **Material examined.** QM G337590. Hunter CMR, Australia, New South Wales,
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28 261 32°34'30.0"S–32°37'53.8"S, 153°08'31.2"E–153°09'42.1"E, 1006–1036 m depth, beam trawl,
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30 262 coll. M. Ekins on *RV Investigator*, Cruise IN2017_V03, 03 June 2017, fixed in 99.5% EtOH.
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33 263 **Description.** *External morphology.* Preserved colony of the examined specimen consists ca.
34
35 264 40 cylindrical polyps connected by strongly developed light beige coenenchyme on stalks of
36
37 265 hexactinellid sponges (*Hyalonema* sp.). Colony covers upper part of the stalks, but not
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39 266 around the spiculous anchor. Contracted preserved polyps 1.0–3.0 mm in height, 1.5–3.5 mm
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41 267 in diameter.
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46 269 **Remarks.** The capitulum of this specimen (QM G337590) were not swollen, while
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48 270 capitulum of the examined specimens of *Epizoanthus fatuus* (NSMT Co-XXXX, NSMT Co-
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50 271 XXXX) were swollen when contracted. On the other hand, the numbers of tentacles and
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52 272 mesenteries were the same between QM G337590 and the examined specimens of *E. fatuus*.
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54 273 Therefore, we here preliminarily identified the examined specimen as *Epizoanthus* aff.
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56 274 *fatuus*.
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5 276 *Epizoanthus stellaris* Hertwig, 18886
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8 277 Fig. 2D.9
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12 279 **Material examined.** QM G337585. Hunter CMR, New South Wales, Australia,13
14 280 32°28'44.4"S–32°30'25.2"S, 152°59'27.6"E–52°59'38.4"E, 1006–1036 m depth, beam trawl,15
16 281 coll. M. Ekins on *RV Investigator*, Cruise IN2017_V03, 03 June 2017, fixed in 99.5% EtOH.17
18 282 **Description.** *External morphology.* Preserved colony consists of ca. 40 nearly saucer-shaped19
20 283 polyps connected by strongly developed dark brownish coenenchyme on stalks of21
22 284 hexactinellid sponges (*Hyalonema* sp.). Colony covers the upper part of the stalks, but not23
24 285 around the spiculous anchor. Contracted preserved polyps only rise a little from the25
26 286 coenenchyme and very flat, 0.4–1.1 mm in height, 3.0–6.9 mm in diameter. Capitulary ridges27
28 287 present and well pronounced when contracted, approximately 14–18 in number. Ectoderm29
30 288 and mesoglea of polyps and coenenchyme heavily encrusted with numerous sand and silica31
32 289 particles.33
34 290 *Internal morphology.* Zooxanthellae absent. Number of mesenteries 28–36, in macrocnemic35
36 291 arrangement. Mesogleal thickness ca. 0.1–0.3 mm. Numerous and various size of pigment37
38 292 cells in the ectoderm and mesoglea. Mesoglea thicker than ectoderm and endoderm in39
40 293 column, actinopharynx and mesenteries. Reticulate mesogleal musculature short and poorly41
42 294 developed. Siphonoglyph distinct and V-shaped. Mesenterial filaments present.43
44 295 **Habitat and distribution.** Tasman Sea at depths of 1006–1036 m depth in this study. The45
46 296 type locality of this species is off Samboangan, Philippines at a depth of 150 m depth (8247
48 297 fathoms). This species has been recorded from the Indian Ocean (Fautin 2012), New Zealand49
50 298 (NIWA 2018), the Northeast and eastern Pacific Ocean (Beaulieu 2001; Fautin 2012), and the51
52 299 Caribbean Sea (Dueñas & Puentes 2018).
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3 300 **Associated host.** *Hyalonema* sp.
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5 301 **Remarks.** *Epizoanthus stellaris* can be distinguished from other *Epizoanthus* species found
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7 302 on stalks of hexactinellid sponges; the strongly lamellated polyps of *E. stellaris* are not
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9 303 observed in *E. fatuus*, *E. armatus*, or *E. longiceps* (Lwowsky, 1913) (2.0–10.0 mm in height:
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11 304 Carlgren 1923; Lwowsky, 1913). Lwowsky (1913) synonymized *E. stellaris* as *E. fatuus* on
12
13 305 account of high amounts of morphological variability that are commonly found within
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15 306 zoantharian species. However, the results of our molecular phylogenetic analyses support that
16
17 307 *E. stellaris* and *E. fatuus* are distinct species. Beaulieu (2001) observed *E. stellaris* frequently
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19 308 in the eastern Pacific Ocean. It should be noted that several species may be contained in the
20
21 309 *E. stellaris* observed by Beaulieu (2001), as similarities in external morphological features
22
23 310 with various *Epizoanthus* species found on stalks of hexactinellids were observed in their
24
25 311 study.
26
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32

33 313 *Epizoanthus* aff. *armatus* Carlgren, 1923

34 314 Fig. 2E.
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39

40 316 **Material examined.** NSMT Co-XXXX (MISE-HPD1323), Kuroshima Island, Kagoshima,
41
42 317 Japan, 24°13'36.1"N, 124°06'18.0", 468 m depth, ROV, coll. J. D. Reimer on RV
43
44 318 *Natsushima*, 19 Sep 2011, fixed in 90% ethanol.
45
46

47 319 **Description.** *External morphology.* Preserved colony consists of ca. 80 cylindrical polyps
48
49 320 connected by strongly developed light brownish coenenchyme on stalks of hexactinellid
50
51 321 sponges (*Hyalonema* sp.). Colony covers the upper part of the stalks, but not around the
52
53 322 spiculous anchor. Contracted preserved polyps well developed and 0.5–4.9 mm in height,
54
55 323 2.5–7.8 mm in diameter. Capitulary ridges present but weakly pronounced when contracted,
56
57 324 ca. 14–16 in number. The numbers of tentacles of each polyp ca. 28–32, and tentacles
58
59
60

1
2
3 325 arranged in two rows. The morphological characters and dimensions observed in the
4
5 326 examined specimen agree well with the original description by Carlgren (1923).

7 327 *Internal morphology.* Zooxanthellae absent. Number of mesenteries 28–32, in macrocnemic
9
10 328 arrangement. Reticulate mesogleal musculature .

12 329 **Habitat and distribution.** Off Kuroshima, Okinawa, in the Ryukyu Archipelago, Japan at a
14
15 330 depth of 468 m. *Epizoanthus armatus* has previously been reported from off Somalia in the
16
17 331 Indian Ocean (Carlgren 1923).

19 332 **Associated host.** *Hyalonema* sp.

21 333 **Remarks.** Polyp dimensions of the examined specimen are larger than those of *E. fatuus* and
23
24 334 *E. stellaris*, and this specimen resembles *E. armatus* as described by Carlgren (1923).

26 335 *Epizoanthus armatus* was originally described from eastern Africa (Carlgren 1923). Kise *et*

28 336 *al.* (2018) reported the existence of *E. planus* Carlgren, 1923 in Japanese waters, and this

30 337 species was also originally described from eastern Africa. Therefore, it is possible that *E.*

32 338 *armatus* may also be distributed in Japanese waters. However, the collected depths of the

34 339 examined specimen in this study and the specimens Carlgren (1923) examined are different

36 340 (468 m vs 741–1362 m depth). As well, we could not observe internal morphology of the

38 341 examined specimen due to its poor preserved condition. Therefore, we here preliminarily

40 342 identified the examined specimen as *Epizoanthus* aff. *armatus*. Examination of additional

42 343 specimens combined with molecular analyses should help confirm the identity of this taxon.

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45
46
47 344

49 345 Family Parazoanthidae Delage & Hérouard, 1901

51 346 **Genus *Churabana* gen. nov.**

53 347 Fig. 3A-H.

55 348 ZooBank ID (LSID): XXXX

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1
2
3 350 **Type species.** *Churabana kuroshioae* sp. nov. by original designation.
4

5 351 **Diagnosis.** Parazoanthidae with obligate symbiotic relationship with massive
6
7 352 hexasterophoran sponges. Preserved polyps 3.0–4.0 mm in height, 2.8–4.0 mm in diameter.
8

9
10 353 Azooxanthellate. Cteniform endodermal marginal musculature.
11

12 354 **Remarks.** *Churabana* gen. nov. and other already described sponge-associated zoantharian
13
14 355 genera can be easily distinguished from each other by their host sponges (Hexactinellida
15
16 356 sponges vs Demospongiae sponge), and depths; the former can be found at >140 m, while the
17
18 357 latter are found in shallow coral reefs. Although *Churabana* gen. nov. and several species
19
20 358 within *Isozoanthus* and *Epizoanthus* are associated with Hexactinellida sponges, these three
21
22 359 genera can be distinguished from each other by hosts; the latter two genera are associated
23
24 360 with species within the subclass Amphidiscophora, while species of *Churabana* gen. n. are
25
26 361 associated with sponge species within the subclass Hexasterophora. *Churabana* gen. n. has a
27
28 362 unique deletion of 15 bp (from position 168–182 in our alignment) in its 16S-rDNA region.
29
30
31
32

33 363

34
35 364 **Etymology.** The generic name is derived from the Ryukyuan language words *chura* (=
36
37 365 beautiful) and *bana* (=flower) referring to the appearance of this species. Gender feminine.
38
39

40 366

41
42 367 ***Churabana kuroshioae* sp. nov.**
43

44
45 368 Synonymy: Parazoanthidae sp. 1 – Reimer *et al.* 2019: 7, fig. 2A.
46

47 369 Fig. 3A-H.
48

49 370 ZooBank ID (LSID): XXXX
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51 371
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53
54 372 **Material examined.** Holotype: RUMF-ZG-04447, near Iejima Island, Motobu, Okinawa,
55
56 373 Japan, 26°54'53.6"N, 127°37'50.9"E, 600–650 m, baskets, coll. T. Higashiji on vessel *Daini-*
57
58
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60

374 *kuroshio-maru*, 02 March 2018, divided into two pieces, one portion fixed in 5–10%

375 saltwater formalin, and other in 99.5% ethanol.

376 Paratype: RUMF-ZG-04448, near Iejima Island, Motobu, Okinawa, Japan, 26°54'53.6"N,

377 127°37'50.9"E, 600–650 m, baskets, coll. T. Higashiji on vessel *Daini-kuroshio-maru*, 02

378 March 2018, fixed in 99.5% ethanol. Voucher number (MISE-JMG51J), Nanpo Trough,

379 Kikaijima Island, Kagoshima, Japan, 28°20'21.64"N, 129°57'14.56"E, 520 m depth, ROV,

380 coll. Javier Montenegro on RV *Natsushima*, 14 Oct 2011, fixed in 99.5% ethanol.

381 **Etymology.** The species is named after the *Daini-kuroshio-maru*, as the type specimens were
382 collected by this vessel.

383 **Description.** *External morphology.* Preserved specimens consist of four truncated cone shape

384 or cylindrical polyps. The polyp bases embedded within the sponge *Pararete* Ijima, 1927.

385 Solitary polyps arise irregularly from *Pararete* species. The living polyps cream pink or

386 beige, and tentacles cream or whiteish transparent in coloration. Preserved polyps beige and

387 partially red. Surface of column rough, and ectoderm continuous. Ectoderm and mesoglea of

388 polyps encrusted with numerous and various sizes of sand and silica particles. The living

389 expanded oral disks ca. 1.5–2.0 mm in diameter, expanded polyps ca. up to 10 mm in height,

390 4.0–5.0 mm in diameter. Preserved contracted preserved polyps 3.0–4.0 mm in height, 2.8–

391 4.0 mm in diameter. Capitulary ridges discernible, 15–16 in number when contracted. 30–32

392 tentacles in number.

393 *Internal morphology.* Zooxanthellae absent. Cyclically transitional marginal musculature.

394 Encircling sinus present and basal canals of mesenteries absent. Mesenteries thin, 30–32 in

395 macrocnemic arrangement. Mesoglea thickness 0.5–1.6 mm. Mesoglea thicker than ectoderm

396 and endoderm. Siphonoglyph distinct and U-shaped. Mesenterial filaments present.

1
2
3 397 *Cnidae*. Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs,
4
5 398 and spirocysts (Fig. 4A, Table 2).
6
7

8 399

9
10 400 **Distribution and habitats.** Ryukyu Archipelago, Japan: Near Iejima Island, Okinawa and
11
12 401 Nanpo Trough, Kikaijima Island, Kagoshima, Japan at depths of 520–650 m.
13
14

15 402

16
17 403 **Associated host.** *Pararete* sp. 1 and *Pararete* sp. 2
18

19 404 **Remarks.** *Churabana kuroshioae* sp. nov. resembles *Vitrumanthus* gen. nov. species.
20

21 405 However, *C. kuroshioae* sp. nov. and species within *Vitrumanthus* gen. nov. can be easily
22

23 406 separated by differences in their marginal musculatures (see also Remarks of *Vitrumanthus*
24

25 407 gen. nov.).
26
27

28 408

29
30 409 **Genus *Vitrumanthus* gen. nov.**
31

32
33 410 **Type species.** *Vitrumanthus schrieri* sp. nov. by original designation.
34

35 411 Figs. 5-7.
36

37 412 ZooBank ID (LSID): XXXX
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40 413

41
42 414 **Diagnosis.** Parazoanthidae with obligate symbiotic relationship with massive
43

44 415 hexasterophoran sponges. Preserved polyps 0.3–3.1 mm in length, 0.8–3.4 mm in diameter.
45

46 416 Azooxanthellate. Cyclically transitional marginal musculature.
47

48 417 **Remarks.** *Vitrumanthus* gen. nov. is distinguished genus from the Hexasterophora sponge-
49

50 418 associated *Churabana* gen. nov by its marginal musculature: in *Vitrumanthus* gen. nov.
51

52 419 cyclically transitional marginal musculature with several mesogleal lacunae was observed,
53

54 420 while in *Churabana* gen. nov. cteniform endodermal marginal musculature with comb-like
55

56 421 mesogleal pleats was observed. Molecular phylogenetic analyses clearly support the
57
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59
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1
2
3 422 distinctiveness between *Churabana* gen. nov. and *Vitrumanthus* gen. nov. In the 16S-rDNA
4
5 423 region, *Vitrumanthus* gen. nov. has a unique deletion of 15 bp (positions 136–150 in our
6
7 424 alignment).
8
9

10 425

11
12 426 **Etymology.** The generic name is derived from the Latin word *vitrum* (= glass) and Greek
13
14 427 word *anthos* (=flower) referring to this genus' appearance. Gender masculine.
15
16

17 428

18
19 429 ***Vitrumanthus schrieri* sp. nov.**

20
21 430 Synonymy: Parazoanthidae sp. – Montenegro *et al.* 2020: 7-8, fig. 2; "zoanthid symbionts",
22
23 431 "zoanthids" - Reiswig & Dohrmann 2014: 241–242, fig. 4A,B; "white zoanthids" - Van Soest
24
25 432 *et al.* 2014: 438.

26
27 433 Fig. 5A-F.
28
29

30 434 ZooBank ID (LSID): XXXX
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34
35 436 **Material examined.** Holotype: RMNH.COEL.42429, SubStation, Curaçao, 12°14'01"N,
36
37 437 68°53'32"W, depth range 161–243 m depth, manned submarine *Curasub*, coll. B. W.

38
39 438 Hoeksema, 31 Mar 2014, fixed in 99.5% ethanol.
40
41

42 439 Paratype: RMNH.COEL.42430, SubStation, Curaçao, 12°05'04"N, 68°53'54"W, ca. 200 m
43
44 440 depth, manned submarine *Curasub*, coll. B. W. Hoeksema, 21 Apr 2014, fixed in 99.5%

45
46 441 ethanol.
47
48

49 442 Other material examined: RMNH.COEL.42620, Cargill Pier, Bonaire, Caribbean
50
51 443 Netherlands, 12°04'47.9"N, 68°17'37.7"W, 223 m depth, manned submarine *Curasub* based

52
53 444 on RV *Chapman*, coll. L. Becking and E. Meesters, 1 Jun 2013, fixed in 99.5% ethanol.
54
55 445 RMNH.COEL.42621, Cargill Pier, Bonaire, Caribbean Netherlands, 12°04'47.9"N,

56
57 446 68°17'37.7"W, 248 m depth, manned submarine *Curasub* based on RV *Chapman*, coll. L.
58
59
60

1
2
3 447 Becking and E. Meesters, 1 Jun 2013, fixed in 99.5% ethanol. RMNH.COEL.42622,
4
5 448 Kralendijk Pier, Bonaire, Caribbean Netherlands, 12°08'48.9"N, 68°16'55.6"W, 140 m depth,
6
7 449 manned submarine *Curasub* based on RV *Chapman*, coll. L. Becking and E. Meesters, 30
8
9 450 May 2013, fixed in 99.5% ethanol.
10
11

12 451

13
14 452 **Etymology.** The species *V. schrieri* is named after Adriaan “Dutch” Schrier, owner of the
15
16 453 manned submersible *Curasub* and RV *Chapman*, operated from Substation Curaçao, for his
17
18 454 generosity in allowing BWH to sample specimens.
19
20

21 455

22
23 456 **Description.** *External morphology.* Preserved specimen consists of ca. 50 cylindrical polyps.
24
25 457 Solitary or colonial polyps rise irregularly from the hexactinellid sponge *Verrucocoeloidea*
26
27 458 *liberatoriii* Reswig & Dohrmann, 2014. Living polyps white, and tentacle transparent in
28
29 459 coloration. Preserved polyps dark beige. Surface of column rough, and ectoderm continuous.
30
31 460 Ectoderm and mesoglea of polyps encrusted with numerous and various sizes of sand and
32
33 461 silica particles. The living expanded oral disks ca. up to 8.0 mm in diameter, expanded polyps
34
35 462 ca. up to 10 mm in height, 1.0–5.0 mm in diameter. Preserved contracted preserved polyps
36
37 463 0.3–2.1 mm in height, 0.8–2.8 mm in diameter. Capitulary ridges indiscernible. Tentacles 20–
38
39 464 24 in number.
40
41

42
43 465 *Internal morphology.* Zooxanthellae absent. Cyclically transitional marginal musculature.
44

45 466 Encircling sinus present and basal canals of mesenteries absent. Mesenteries thin. Mesoglea
46
47 467 thickness ca. 0.6 mm. Mesoglea thicker than ectoderm and endoderm. Siphonoglyph distinct
48
49 468 and V-shaped. Mesenterial filaments present.
50

51 469 *Cnidae.* Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs,
52
53 470 and spirocysts (Fig. 4B, Table 2).
54
55

56 471 **Distribution and habitats.** Dutch Caribbean: Curaçao and Bonaire at depths of 140–248 m.
57
58
59
60

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2
3 472 **Associated hosts.** *Verrucocoeloidea liberatorii* Reswig & Dohrmann, 2014, *Parahigginsia*
4
5 473 *strongylifera* van Soest, Meesters & Becking 2015, and *Cyrtaulon sigsbeeii* (Schmidt, 1880).
6
7

8 474 **Remarks.** In terms of host hexactinellid sponges, *Vitrumanthus schrieri* sp. nov.
9
10 475 can be distinguished from *V. vanderlandi* sp. nov. and *V. oligomyarius* comb. nov. as only *V.*
11
12 476 *schrieri* sp. nov. is associated with *Verrucocoeloidea* sponges. As well, *V. schrieri* sp. nov. is
13
14 477 not only associated with hexactinellid sponges but also with Demospongiae species in the
15
16 478 genus *Parahigginsia* (Van Soest *et al.* 2014). On the other hand, *V. vanderlandi* sp. nov. and
17
18 479 *V. oligomyarius* comb. nov. are associated with *Tretochone duplicata* (Topsent, 1928). In
19
20 480 addition, the column of *V. schrieri* sp. nov. is rough due to heavy encrustation, while columns
21
22 481 of *V. vanderlandi* sp. nov. and *V. oligomyarius* comb. nov. are smooth with almost no
23
24 482 encrustations. *Vitrumanthus* sp. (NSMT-XXXX) and *V. vanderlandi* sp. nov. are associated
25
26 483 with *Cyrtaulon* species.
27
28
29

30 484 Reiswig & Dohrmann (2014) reported zoantharians were found on
31
32 485 *Verrucocoeloidea liberatorii*, and we judge that these are likely *V. schrieri* sp. nov. based on
33
34 486 external morphology and the host hexactinellid sponge species.
35
36
37

38 487

39
40 488 ***Vitrumanthus vanderlandi* sp. nov.**

41
42 489 Fig. 6.

43
44 490 ZooBank ID (LSID): XXXX
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46

47 491

48
49 492 **Material examined.**

50
51 493 Holotype: RMNH.COEL.42623, CANCAP-VII Expedition Station CV 7.041, Cape Verde
52
53 494 Islands, SãoTiago, Ilheus Rombos, east of Cima, 14°56'59"N, 24°37'59"W, Agassiz trawl,
54
55 495 580 m depth coll. RV *HNIMS Tydeman*, 24 Aug 1986, fixed in 99.5% ethanol. Paratype:
56
57 496 RMNH.COEL.42624, CANCAP-VII Expedition Station CV 7.041, Cape Verde Islands,
58
59
60

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2
3 497 SãoTiago, Ilheus Rombos, east of Cima, 14°56'59"N, 24°37'59"W, Agassiz trawl, 580 m
4
5 498 depth, coll. RV *HNIMS Tydeman*, 24 Aug 1986.

6
7 499 Other material examined: RMNH.COEL.42625, Equalant II Expedition to the Gulf of
8
9 500 Guinea, as part of the ICITA Programme (International Cooperative Investigations of the
10
11 501 Tropical Atlantic), Sta. 18/8, 38 km off Liberia, 4°25'N, 8°29'W, trawl at a depth range of
12
13 502 700–800 m, on RV *Geronimo* Cruise 2, 31 Oct 1963, fixed in 99.5% ethanol.
14
15

16
17 503

18
19 504 **Etymology.** This species is named in honor of Dr. Jacob van der Land, expedition leader of
20
21 505 the CANCAP-VII expedition off the coast of West Africa (Van der Land 1987), during which
22
23 506 the type specimens of this species were collected.
24
25

26 507

27
28 508 **Description.** *External morphology* Preserved holotype specimen consists of 32 cylindrical
29
30 509 polyps. Base of polyps embedded in *Aphrocallistes* sponge. Solitary polyps arise irregularly
31
32 510 from host hexactinellid sponge. Preserved polyps cream white in coloration. Surface of
33
34 511 column very smooth, and ectoderm continuous. Ectoderm of polyps partially encrusted with
35
36 512 small size of sand and silica particles. Transparent capitulum contracted and rounded.
37
38 513 Contracted preserved polyps 0.3–2.5 mm in height, 0.8–3.0 mm in diameter. Capitulary
39
40 514 ridges indiscernible when contracted. Approximately 20 tentacles in number.
41
42
43

44 515 *Internal morphology.* Zooxanthellae absent. Cyclically transitional marginal musculature.

45
46 516 Mesoglea thickness 0.2–1.1 mm. Mesoglea thicker than ectoderm and endoderm.

47
48 517 Siphonoglyph distinct and V-shaped. Mesenterial filaments present.

49
50 518 *Cnidae.* Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, and
51
52 519 spirocysts (Fig. 4C, Table 2).
53

54
55 520 **Distribution and habitats.** Western coast of Africa: Guinea and Cape Verde at depths of
56
57 521 580–800 m.
58
59
60

1
2
3 522 **Associated host.** *Aphrocallistes beatrix* Gray, 1858, *Aphrocallistes* sp.
4

5 523 **Remarks.** *Vitrumanthus vanderlandi* sp. nov. is phylogenetically close to *V. oligomyarius*
6
7 524 comb. nov. However, these two species can be distinguished by dimensions of polyps and the
8
9 525 numbers of tentacles. As well, *V. vanderlandi* sp. nov. and *V. oligomyaria* comb. nov. are
10
11 526 associated with different host hexactinellid sponges (*Aphrocallistes* species vs. *Tretochone*
12
13 527 *duplicata*). Moreover, *V. vanderlandi* sp. nov. does not have any holotrich nematocysts in all
14
15 528 tissues we examined, while other species within *Vitrumanthus* gen. n. have holotrich
16
17 529 nematocysts present in some tissues.
18
19

20
21 530 Although the in situ polyp color of *V. vanderlandi* is unknown, Buhl-Mortensen *et al.* (2017)
22
23 531 have reported yellowed colored zoantharians that were observed on *A. beatrix* collected from
24
25 532 the Gulf of Guinea. Therefore, the coloration of polyps of this species may be yellow. Further
26
27 533 investigations with newly collected specimens are needed to confirm the in situ morphology
28
29 534 of *V. vanderlandi* sp. nov.
30
31

32
33 535

34
35 536 ***Vitrumanthus oligomyarius* comb. nov. (Wassilief, 1908)**

36
37 537 Synonym: *Gemmaria oligomyaria* Wassilief, 1908: 47, fig. 29; taf. I, fig. 29.

38
39 538 Fig. 7.
40
41

42
43 539

44
45 540 **Material examined.** CMNH ZG-4785, off Katsuura, Chiba, Japan, 34°50'N – 35°00'N,
46
47 541 140°20'W–140°30'W, 390, vertical long line fishing, coll. A. Tamura on vessel *Kiyo-maru*,
48
49 542 19 Jan 2006, divided into two pieces, one portion fixed in 5–10% saltwater formalin, and
50
51 543 other in 99.5% ethanol.
52

53
54 544 **Description.** External morphology. Preserved specimen consists of ca. >300 cylindrical
55
56 545 polyps that appear to be solitary on *Tretochone duplicata* (Topsent, 1928). Preserved polyps
57
58 546 dark yellow in coloration and trapezoid when opened. Polyps located all over the three-
59
60

1
2
3 547 dimensional structured hexactinellid sponge body. Surface of column very smooth, and
4
5 548 ectoderm continuous. Ectoderm of polyps partially encrusted with very small sized sand and
6
7 549 silica particles (ca. < 0.1 mm). The transparent capitulum contracted and rounded. Contracted
8
9 550 preserved polyps 0.5–3.1 mm in height, 1.2–3.4 mm in diameter. Capitulary ridges
10
11 551 indiscernible when contracted. 32–36 tentacles in number.
12
13

14 552 **Internal morphology.** Zooxanthellae absent. Cyclically transitional marginal musculature
15
16 553 from endoderm to mesogleal, and lacunae confined toward endoderm proximally. Encircling
17
18 554 sinus or mesogleal canal usually imperceptible and basal canals of mesenteries absent.
19
20 555 Mesenteries thin, 32–36 in number, in brachycnemic arrangement. Mesoglea thickness 0.7–
21
22 556 1.0 mm. Mesoglea thicker than endoderm but thinner than ectoderm in the body wall.
23
24 557 Siphonoglyph distinct and U-shaped. Mesenterial filaments present.
25
26

27
28 558 **Cnidae.** Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs,
29
30 559 and spirocysts (Fig. 4D, Table 2).
31
32

33 560 **Distribution and habitats.** Pacific coast of Japan: Suruga Bay, Shizuoka and off Katsuura,
34
35 561 Chiba at depths of < 390 m.
36

37 562 **Associated host.** *Tretochone duplicata* (Topsent, 1928).
38
39

40 563 **Remarks.** The morphological characteristics of *Vitrumanthus oligomyarius* comb. nov.
41
42 564 collected in this study are identical to those of the original description by Wassilief (1908).
43
44 565 The musculature of the sphincter of the examined specimen is in a cyclically transitional
45
46 566 arrangement containing several mesogleal lacunae; identical musculature was also mentioned
47
48 567 by Wassilief (1908). Moreover, surprisingly, the examined specimen by Wassilief (1908) and
49
50 568 this study both have brachycnemic mesenterial arrangements, with incomplete mesenteries in
51
52 569 the fifth mesenterial pair from the dorsal directive, a diagnostic characteristic of the suborder
53
54 570 Brachycnemina. This finding is unusual as *V. oligomyarius* is very clearly phylogenetically
55
56 571 located within the suborder Macrocnemina, which have complete fifth mesentery pairs from
57
58
59
60

1
2
3 572 the dorsal directive. On the other hand, the other morphological characteristics of *V.*
4
5 573 *oligomyarius* are similar to those of other macrocnemic species. It should be noted a similar
6
7
8 574 case has been reported in the past. *Parazoanthus douglasi* Haddon & Shackleton, 1891,
9
10 575 which likely belongs within the genus *Hydrozoanthus* Sinniger, Reimer & Pawlowski, 2010
11
12 576 (Reimer & Sinniger 2020), also has a brachycnemic mesenterial arrangement, while its other
13
14 577 characteristics such as being epizoic on hydroids and its marginal musculature are similar to
15
16 578 other macrocnemic species. *V. oligomyarius* comb. nov. can be easily distinguished from
17
18 579 other *Vitrumanthus* species by the numbers of tentacles and its unique mesenterial
19
20 580 arrangement.
21
22

23
24 581 This study reports on the existence of this species for the first time since its original
25
26 582 description.
27

28 583

29
30 584 Genus *Kauluzoanthus* Sinniger, Ocaña & Baco, 2013

31
32 585 **Type species.** *Kauluzoanthus kerbyi* Sinniger, Ocaña & Baco, 2013

33
34 586 **Diagnosis.** Polyps do not contract when fixed. Characteristic insertion/deletion pattern in the
35
36 587 16S V5 region sensu Sinniger *et al.* (2005) (Sinniger *et al.* 2013).
37

38 588

39
40 589 *Kauluzoanthus* sp.

41
42 590 Fig. 8.
43

44 591

45
46 592 **Material examined.** USNM 1424050, French Frigate Shoals, Hawaii (23°56.649' N,
47
48 593 166°02.187' W), 1225 m, ROV, coll. RV *Okeanos Explorer*, NOAA on 28 February 2016,
49
50 594 fixed in 99.5% EtOH.
51

52
53 595 **Description.** Non-encrusted azooxanthellate zoantharians. The examined specimen
54
55 596 associated with *Hyalonema* sp. Preserved polyps cylindrical and ca. 1.8–9.6 mm in height,
56
57
58
59
60

1
2
3 597 3.9–6.7 mm in diameter, with brown column in coloration. Thin coenenchyme completely
4
5 598 covering stalk of *Hyalonema* sp. Capitulary ridges discernible, 14–16 in number when
6
7 599 contracted. Tentacles relatively short, as long as expanded oral disk diameter. Numbers of
8
9
10 600 tentacles 28–32.

11
12 601 **Associated host.** *Hyalonema* sp.

13
14 602 **Distribution and habitats.** North Pacific Ocean: French Frigate Shoals, Hawaii at a depth of
15
16
17 603 1225 m.

18
19 604 **Remarks.** The genus *Kauluzoanthus* is known as associating with the gold coral
20
21 605 *Kulamanamana haumea* as well as with several octocoral species (Sinniger *et al.* 2013).
22
23 606 However, our examined specimen was associated with *Hyalonema* sponges, and was
24
25
26 607 genetically close to *Ka. kerbyi* based on the results in this study. This finding suggests that
27
28 608 *Kauluzoanthus* species are not host-specific to *Ku. haumea* or other octocorals. However,
29
30 609 we could not obtain any 16S-rDNA sequences, which contain the V5 region sensu Sinniger *et*
31
32 610 *al.* (2005). Therefore, further integrated studies including morphological and ecological
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35 611 studies are needed to describe this putative species.
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40 613 Molecular phylogeny

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42 614 All phylogenetic analyses (ML, BI) using the concatenated dataset show that the new genera
43
44 615 *Churabana* gen. nov. and *Vitrumanthus* gen. nov. are located within the family
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46
47 616 Parazoanthidae (Fig. 9). The basic topologies between ML and BI phylogenetic trees are
48
49 617 congruent, although there are a few differences. In the ML phylogenetic tree, *Vitrumanthus*
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51 618 gen. nov. is basal to *Churabana* gen. n. and a clade containing the Demospongiae sponge-
52
53 619 associated genera *Bergia* Duchassaing & Michelotti, 1860, *Parazoanthus* Haddon &
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55
56 620 Shackleton, 1891, and *Umimayanthus* Montenegro, Sinniger & Reimer, 2015 with weak
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58 621 nodal support (ML=40%), while in the BI phylogenetic tree *Churabana* gen. n. is basal to
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3 622 *Vitrumanthus* gen. nov. and the clade of Demosponge-associated zoantharian genera with
4
5 623 strong support (BI=0.99). Both *Churabana* gen. n. and *Vitrumanthus* gen. nov. are
6
7 624 monophyletic clades with strong nodal support (ML=100%, BI=1; ML=98%, BI=1).
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9 625 *Kauluzoanthus* sp. associated with *Hyalonema* sp. is sister to *Ka. kerbyi* with moderate
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11 626 support (ML=73%, BI=1).
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14 627 In Epizoanthidae, all hexactinellid sponge-associated species (*Epizoanthus fatuus*, *E.*
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16 628 *stellaris*, *E. aff. fatuus*, and *E. aff. armatus*) formed a monophyletic clade including *E. aff.*
17
18 629 *illoricatus* sensu Swain (2010) with moderate nodal support (ML=59%, BI=0.99), and this
19
20 630 clade was sister to a clade consisting of sequences of eunicid polychaete-associated species
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22 631 (*E. illoricatus* and *E. beriber*).
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31 634 **DISCUSSION**

32 33 635 **Phylogeny and taxonomy of hexactinellid sponge-associated zoantharians**

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35 636 Our phylogenetic analyses focused on hexactinellid sponge-associated
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37 637 zoantharians conducted for first time confirmed that the diversity of glass-sponge-associated
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39 638 zoantharians is higher than has been previously thought, as two new genera and three new
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41 639 species were formally described. Our study confirms that hexactinellid-sponge-associated
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43 640 zoantharians are located in both families Epizoanthidae and Parazoanthidae: zoantharians
44
45 641 found on stalks of Amphidiscophora sponges are found in both families, while zoantharians
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47 642 found on the bodies of Hexasterophora are unique to Parazoanthidae (Table S3).
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49
50 643 Amphidiscophora sponge-associated zoantharians have not been previously described,
51
52 644 although Sanamyan *et al.* (2012) described the Amphidiscophora sponge-associated
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54 645 actinarian *Spongiactis japonica* from the body of the Amphidiscophora sponge *Hyalonema*
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3 646 *siebold*. It should be noted that *S. japonica* was not found on stalks of spicules of the
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5 647 Amphidiscophora sponge.

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7 648 In Epizoanthidae, Amphidiscophora sponge-associated zoantharians were
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9 649 identified as *Epizoanthus* species, and the monophyly of this association was confirmed. The
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11 650 phylogenetic positions of *E. fatuus* and *E. stellaris* were established for first the time within
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13 651 Epizoanthidae, with additional information on their morphology provided by the present
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15 652 study.

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18 653 In our phylogenetic analyses, within Parazoanthidae, previously reported
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20 654 sequences of Amphidiscophora sponge-associated zoantharians Parazoanthid sp.
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22 655 [CORSAO], Parazoanthid sp. [EBISCO], Parazoanthid sp. [NC2], and Parazoanthid sp.
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24 656 [NC3] (Sinniger *et al.* 2010) formed a monophyletic clade, supporting previous studies
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26 657 (Sinniger *et al.* 2010; Swain 2010, 2018). These parazoanthid specimens may possibly
27
28 658 correspond to one or more of the *Isozoanthus* species described on the stalks of
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30 659 Amphidiscophora sponges: *I. africanus* Carlgren, 1923, *I. arenosus* Carlgren, 1923, and *I.*
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32 660 *valdiviae* Carlgren, 1923, described from East Africa and the Andaman Sea. Currently, no
33
34 661 DNA sequences exist for these *Isozoanthus* species. However, the taxonomic status of the
35
36 662 genus *Isozoanthus* is uncertain, and has been debated in several studies (e.g., Williams 2000;
37
38 663 Sinniger *et al.* 2010; Low *et al.* 2016). In fact, the species *Hydrozoanthus antumbrosus*
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40 664 (Swain, 2009b) and *Zibrowius primnoidus* (Carreiro-Silva *et al.* 2010) were both originally
41
42 665 described within the genus *Isozoanthus* based on available ecological and morphological
43
44 666 data. The type species of the genus is *Isozoanthus giganteus* Carlgren in Chun, 1903, and this
45
46 667 species is not associated with stalked glass sponges but is instead a large solitary species
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48 668 (Carlgren 1938). Moreover, the phylogenetic position of *I. giganteus* is closer to
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50 669 Epizoanthidae than to that of Parazoanthidae, although comparatively phylogenetically
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52 670 distant from all other zoantharians (Swain 2010, 2018). Thus, we consider that the
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3 671 phylogenetic clade of Parazoanthidae specimens found on stalks of hexactinellid sponges are
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5 672 likely distinct from *Isozoanthus* and represent an as-of-yet undescribed group. Further
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8 673 molecular and morphological analyses with specimens from this clade are needed to clarify
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10 674 the taxonomy of this group.

11
12 675 *Churabana* gen. nov. and *Vitrumanthus* gen. nov. are genera consisting of
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14 676 Hexasterophora sponge-associated zoantharian species. The phylogenetic results correspond
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16
17 677 with their marginal musculatures; the position of *Churabana* gen. nov. is relatively closer to
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19 678 demosponge-associated zoantharian genera (*Bergia*, *Parazoanthus* and *Umimayanthus*), and
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21 679 they share analogous marginal musculatures (cteniform endodermal arrangement), while
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24 680 *Vitrumanthus* gen. nov. has cyclically transitional marginal musculature as has been reported
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26 681 in specimens of *Corallizoanthus* and *Savalia* (Swain *et al.* 2015). Thus, these findings clearly
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28 682 support the importance of marginal musculature characters for use in the higher taxonomy of
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30 683 Zoantharia, as has been recently emphasized by Swain & Swain (2014) and Swain *et al.*
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32 684 (2015).

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35 685 Although the taxonomic position of *Thoracactis topsenti* is still not clear,
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37 686 *Thoracactis* and the two new genera in this study can be easily distinguished from each other
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39 687 by their external morphology. *Thoracactis topsenti* has an obligate association with the
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41 688 Hexasterophora sponge *Sarostegia oculata*, and polyps of *T. topsenti* are completely
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43
44 689 embedded in *S. oculata* and surrounded by sponge ectosomal formations (Topsent 1904;
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46
47 690 Gravier 1918). On the other hand, only the bases of polyps of *Churabana* gen. nov. and
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49 691 *Vitrumanthus* gen. nov. are embedded in host hexactinellid sponges. However, in this study,
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51 692 no *Thoracactis* specimens were available for examination, and thus further studies are
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53
54 693 necessary to determine the exact taxonomic position of *T. topsenti*.

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57
58 695 **The origin and evolution of hexactinellid sponge-associated zoantharians**
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3 696 Our results do not support a single origin for the symbioses between hexactinellid
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5 697 sponges and zoantharians. At least two independent origins are indicated based on the results
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7 698 of our phylogenetic analyses. Although Amphidiscophora sponge-associated *Epizoanthus* and
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10 699 Parazoanthidae can be easily distinguished from each other by their marginal musculatures,
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12 700 the appearance of the columns and mesoglea of both groups are similar (H. Kise, M. Ekins
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14 701 and J. D. Reimer unpubl. data). This similarity may reflect convergent adaptations associated to
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16 702 similar environmental constraints in the sponge-zoantharian associations of *Epizoanthus* and
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18 703 Parazoanthidae species. According to our analyses, Amphidiscophora sponge symbioses may
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20 704 represent a derived state from the Annelida symbiosis in Epizoanthidae, while
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22 705 Hexasterophora sponge symbioses appear to have been independently gained in
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24 706 Parazoanthidae (Fig. 9).

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26 707 The new taxa described in this work further reconfirm that the deep sea harbors
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28 708 high levels of undescribed zoantharian diversity as has been recently speculated (Sinniger *et*
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30 709 *al.* 2013; Carreiro-Silva *et al.* 2017; Reimer *et al.* 2019). Other recent work has clearly
31
32 710 highlighted the lack of information on deep-sea zoantharian data (Reimer *et al.* 2020), and
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34 711 even small numbers of new specimens may yet have a great influence on our understanding
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36 712 of our diversity of this early-diverging hexacorallian order. The present research also
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38 713 demonstrates that museum collections can contain important specimens of associated
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40 714 zoantharian species that awaiting discovery, and that a continued study of this material would
41
42 715 be a welcome addition to field sampling in order to obtain a more complete image of the
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44 716 zoantharian faunal composition in various regions around the world, in both shallow and in
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46 717 deep waters (Reimer *et al.* 2014, 2015; Montenegro *et al.* 2020).

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33 1024 **FIGURE LEGENDS**

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35 1025 Figure 1. Distribution of hexactinellid sponge-associated zoantharians examined in this study.

36
37 1026 Enclosed symbols indicate Hexasterophora sponge-associated zoantharians: *Churabana*

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39 1027 *kuroshioae* sp. nov. (dark blue), *Vitrumanthus schrieri* sp. nov. (red), *Vitrumanthus*

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41 1028 *vanderlandi* sp. nov. (green), *Vitrumanthus oligomyarius* comb. nov. (yellow). Boxes

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44 1029 indicate Amphidiscophora sponge-associated zoantharians: *Epizoanthus* aff. *armatus* (gray),

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46 1030 *Epizoanthus fatuus* (violet), *Epizoanthus stellaris* (light blue), *Epizoanthus* aff. *fatuus* (pink),

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49 1031 *Kauluzoanthus* sp. (black).

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54 1033 Figure 2. Images of preserved Amphidiscophora sponges-associated zoantharians. (A, B)

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56 1034 *Epizoanthus fatuus* collected from Japan, (C) *Epizoanthus* aff. *fatuus* collected from

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3 1035 Australia, (D) *Epizoanthus stellaris* collected from Australia, (E) *Epizoanthus* aff. *armatus*
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5 1036 collected from Japan. Scales: 10 mm.
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10 1038 Figure 3. Images of external and internal morphology of *Churabana kuroshioae* sp. nov. (A:
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12 1039 paratype: NSMT Co-XXXX; B-I: holotype: RUMF-ZG-04447). (A) living polyps on
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14 1040 *Pararete* sp.1 in situ at, (B) living polyps on *Pararete* sp.2 in an aquarium at Okinawa
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16 1041 Churaumi Aquarium, Motobu, Japan, (C) close-up image of preserved polyp, (D-F)
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18 1042 longitudinal section of polyp, (G) cross-section of polyp, (H) close-up image of cyclically
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20 1043 transitional marginal musculature, (I) drawing of cteniform endodermal marginal
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22 1044 musculature. Abbreviations: A=actinopharynx, Dd=dorsal directives, Cemm=cteniform
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24 1045 endodermal marginal musculature, Ec=ectoderm, En=endoderm, M=mesoglea,
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26 1046 S=siphonoglyph, O=oral disk, 5th=5th mesentery from dorsal directives. Scales: 5 mm (A,B),
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28 1047 2 mm (C-H).
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35 1049 Figure 4. Cnidae in the tentacles, column, actinopharynx, and mesenterial filaments of
36
37 1050 holotypes of new species in this study. (A) cnidae of *Churabana kuroshioae* sp. nov., (B)
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39 1051 cnidae of *Vitrumanthus schrieri* sp. nov., (C) cnidae of *Vitrumanthus vanderlandi* sp. nov.,
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41 1052 (D) cnidae of *Vitrumanthus oligomyarius* comb. nov. Abbreviations: Hl=holotrichs large,
42
43 1053 Hm=holotrichs medium, Hs=holotrich small, O=basitrichs and microbasic b-mastigophores,
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45 1054 Pm=microbasic p-mastigophores, S=spirocysts.
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51 1056 Figure 5. Images of external and internal morphology of *Vitrumanthus schrieri* sp. nov. (B-F:
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53 1057 holotype: RMNH.COEL.42429). (A) living polyps on *Verrucocoeloidea liberatorii* in situ,
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55 1058 (B) preserved polyps, (C) close-up image of preserved polyp, (D) close-up image of tentacle
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57 1059 musculature, (E) close-up image of cyclically transitional marginal musculature, (F) drawing
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3 1060 of cyclically transitional marginal musculature. Abbreviations: A=actinopharynx,
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5 1061 Tem=tentacle musculature, Ctmm=cyclically transitional marginal musculature. Scale bars:
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8 1062 10 mm (A), 25 mm (B), 5 mm (C), 0.5 mm (D,E).
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12 1064 Figure 6. Images of external and internal morphology of *Vitrumanthus vanderlandi* sp. nov.
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14 1065 (holotype: RMNH.COEL.42623). (A) preserved polyps on *Aphrocallistes beatrix*, (B,C)
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16 1066 close-up image of polyp, (D) close-up image of tentacle musculature, (E) longitudinal section
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18 1067 of polyp, (F) drawing of cyclically transitional marginal musculature. Abbreviations:
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20 1068 A=actinopharynx, Te=tentacle, Tem= tentacle musculature, Ctmm=cyclically transitional
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22 1069 marginal musculature. Scale bars: 3 mm (A), 1.5 mm (B,C,E), mm (C), 0.3 mm (D).
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28 1071 Figure 7. Images of external and internal morphology of *Vitrumanthus oligomyarius* comb.
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30 1072 nov. (CMNH ZG-4785). (A) preserved polyps on *Tretochone duplicata*, (B,C) cross section
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32 1073 of polyp. (D) longitudinal section of polyp, (E,F) close-up images of cyclically transitional
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34 1074 marginal musculature, (G) close-up image of tentacle musculature. Abbreviations:
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36 1075 A=actinopharynx, Dd=dorsal directives, Ctmm=cyclically transitional marginal musculature,
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38 1076 Ec=ectoderm, M=mesoglea, Mm=marginal musculature, S=siphonoglyph, O=oral disk,
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40 1077 5th=5th mesentery from dorsal directives. Scale bars: 5 mm (A), 1.5 mm (B,C), 0.5 mm
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42 1078 (D,E), 0.1 mm (F), 0.25 mm (G).
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49 1080 Figure 8. Images of *Kauluzoanthus* sp. (A) in situ image of *Kauluzoanthus* sp. on stalks of
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51 1081 *Hyalonema* sp., (B) preserved colony. Scale: 10 mm (A). Images credits: Okeanos/NOAA.
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56 1083 Figure 9. Maximum likelihood tree based on combined dataset of COI, 12S-rDNA, 16S-
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58 1084 rDNA, 18S-rDNA, 28S-rDNA, and ITS-rDNA. Green colored box indicates Hexasterophora
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3 1085 sponge-associated zoantharians, and blue colored boxed indicate Amphidiscophora sponge-
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5 1086 associated zoantharians. Number at nodes represent ML bootstrap values (> 50% are shown).
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8 1087 Black circles on nodes indicate high support of Bayesian posterior probabilities (>0.95).
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For Review Only

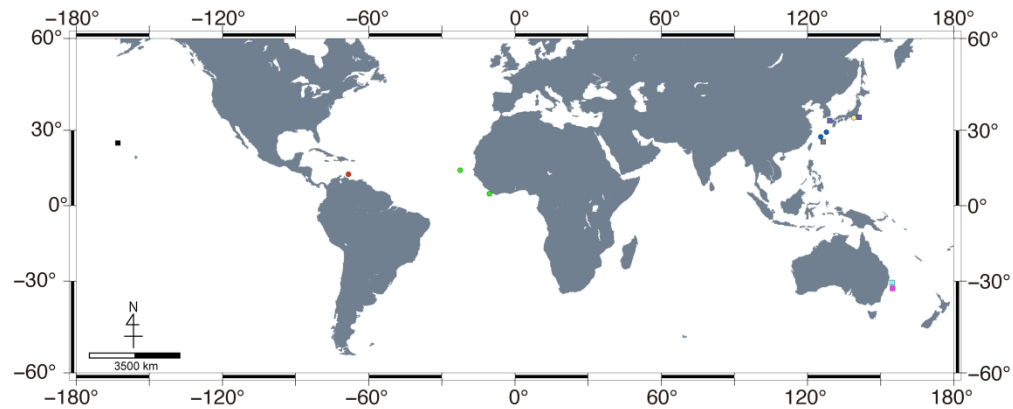


Figure 1. Distribution of hexactinellid sponge-associated zoantharians examined in this study. Enclosed symbols indicate Hexasterophora sponge-associated zoantharians: *Churabana kuroshioae* sp. nov. (dark blue), *Vitrumanthus schrieri* sp. nov. (red), *Vitrumanthus vanderlandi* sp. nov. (green), *Vitrumanthus oligomyarius* comb. nov. (yellow). Boxes indicate Amphidiscophora sponge-associated zoantharians: *Epizoanthus* aff. *armatus* (gray), *Epizoanthus fatuus* (violet), *Epizoanthus stellaris* (light blue), *Epizoanthus* aff. *fatuus* (pink), *Kauluzoanthus* sp. (black).

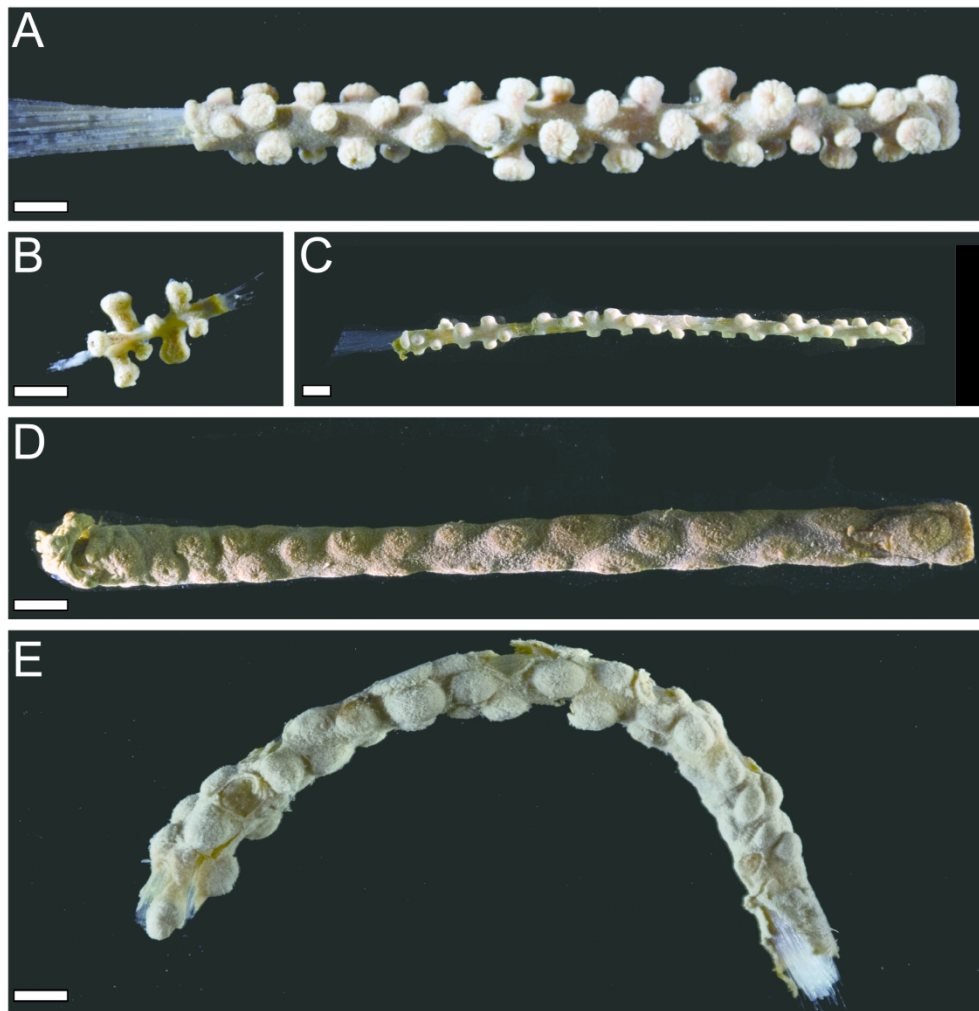


Figure 2. Images of preserved Amphidiscophora sponges-associated zoantharians. (A, B) *Epizoanthus fatuus* collected from Japan, (C) *Epizoanthus* aff. *fatuus* collected from Australia, (D) *Epizoanthus stellaris* collected from Australia, (E) *Epizoanthus* aff. *armatus* collected from Japan. Scales: 10 mm.

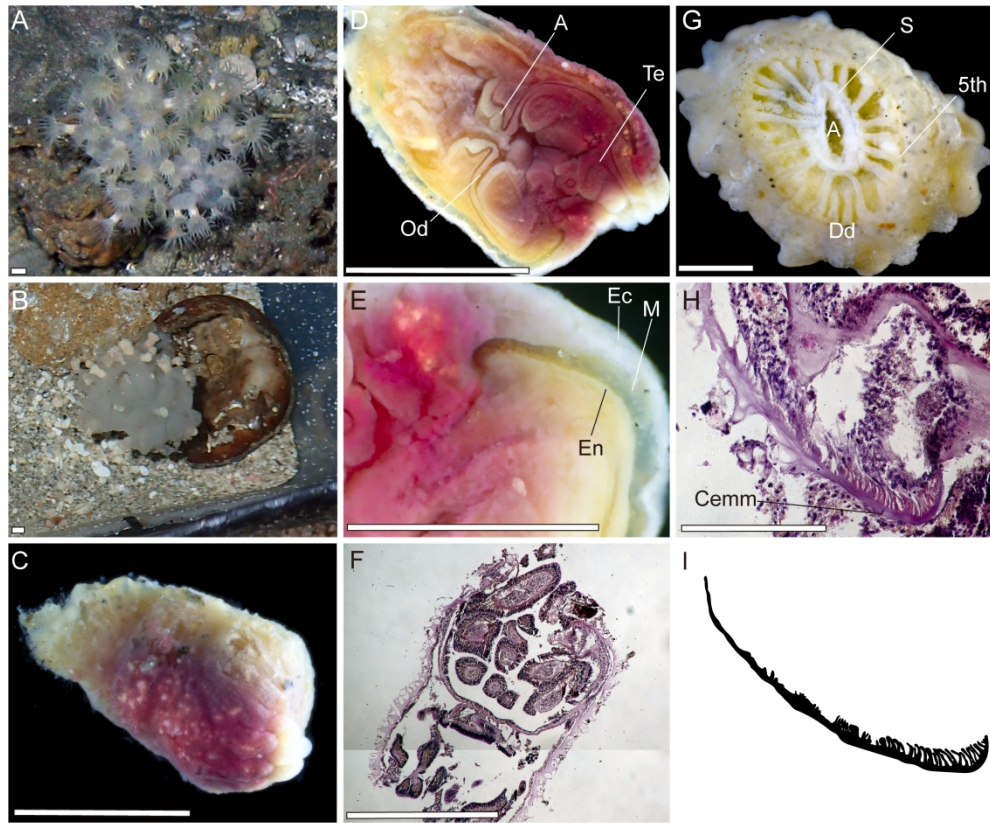
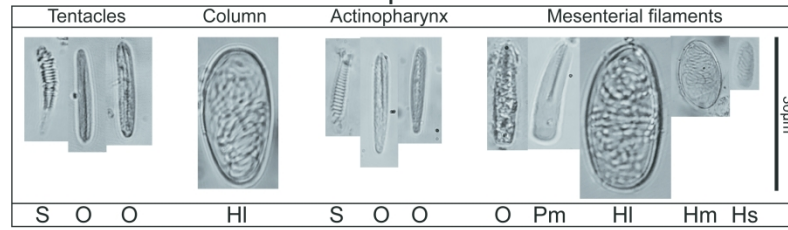
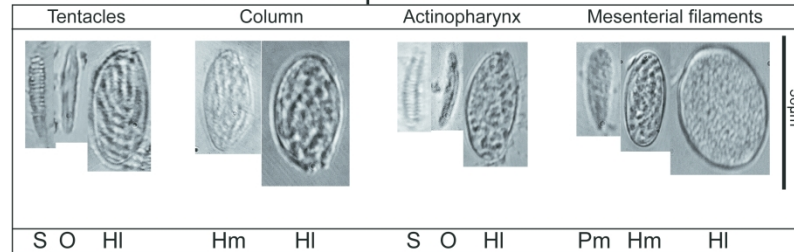


Figure 3. Images of external and internal morphology of *Churabana kuroshioae* sp. nov. (A: paratype: NSMT Co-1754; B-I: holotype: RUMF-ZG-04447). (A) living polyps on *Pararete* sp.1 in situ at, (B) living polyps on *Pararete* sp.2 in an aquarium at Okinawa Churaumi Aquarium, Motobu, Japan, (C) close-up image of preserved polyp, (D-F) longitudinal section of polyp, (G) cross-section of polyp, (H) close-up image of cyclically transitional marginal musculature, (I) drawing of cteniform endodermal marginal musculature. Abbreviations: A=actinopharynx, Dd=dorsal directives, Cemm=cteniform endodermal marginal musculature, Ec=ectoderm, En=endoderm, M=mesoglea, S=siphonoglyph, O=oral disk, 5th=5th mesentery from dorsal directives. Scales: 5 mm (A,B), 2 mm (C-H).

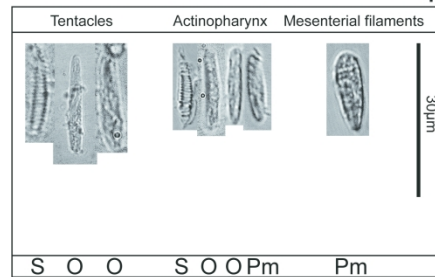
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7 **A. *Churabana kuroshioae* sp. nov.**



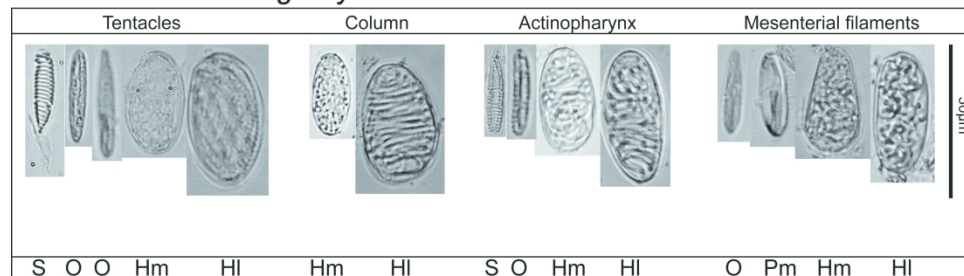
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15 **B. *Vitrumanthus schrieri* sp. nov.**



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24 **C. *Vitrumanthus vanderlandi* sp. nov.**



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34 **D. *Vitrumanthus oligomyarius* comb. nov.**



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45 Figure 4. Cnidae in the tentacles, column, actinopharynx, and mesenterial filaments of holotypes of new
46 species in this study. (A) cnidae of *Churabana kuroshioae* sp. nov., (B) cnidae of *Vitrumanthus schrieri* sp.
47 nov., (C) cnidae of *Vitrumanthus vanderlandi* sp. nov., (D) cnidae of *Vitrumanthus oligomyarius* comb. nov.

48 Abbreviations: HI=holotrichs large, Hm=holotrichs medium, Hs=holotrichs small, O=basitrichs and
49 microbasic b-mastigophores, Pm=microbasic p-mastigophores, S=spirocysts.

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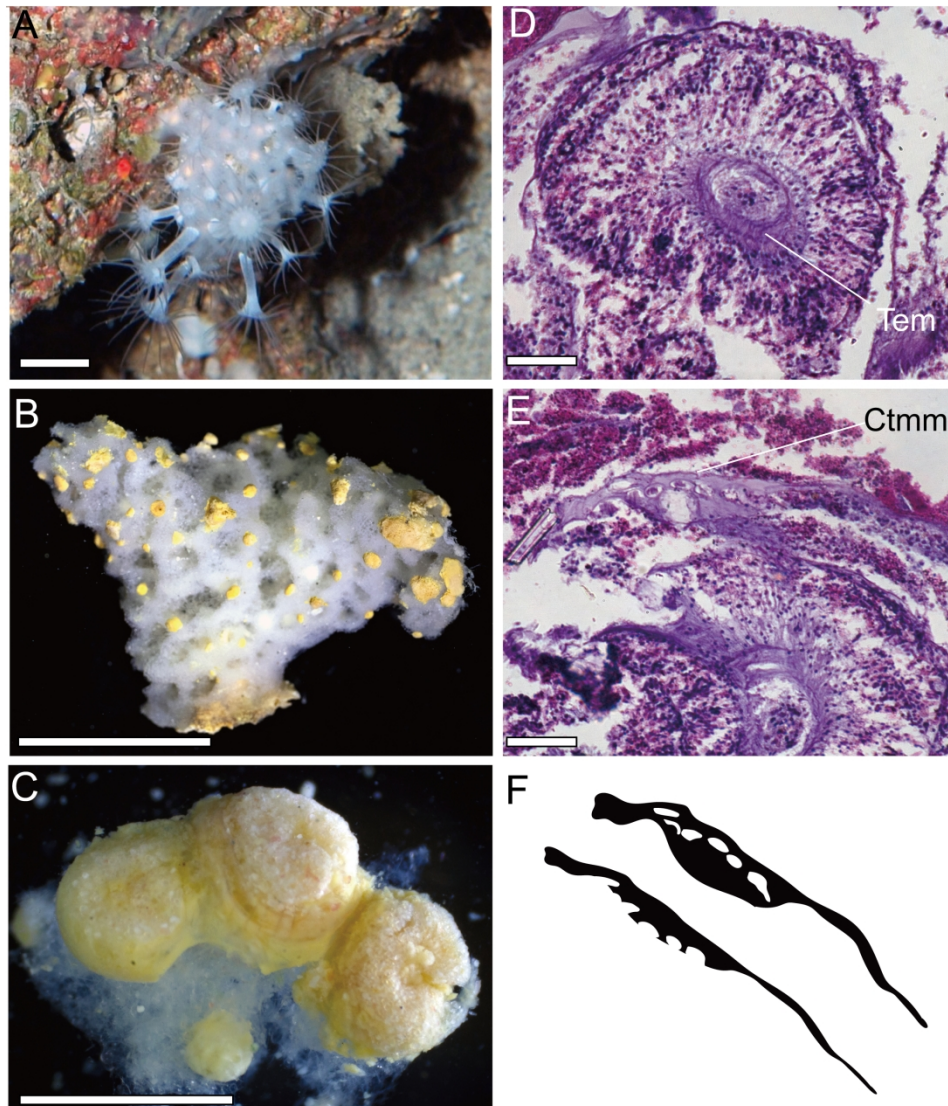


Figure 5. Images of external and internal morphology of *Vitrumanthus schrieri* sp. nov. (B-F: holotype: RMNH.COEL.42429). (A) living polyps on *Verrucocoeloidea liberatorii* in situ, (B) preserved polyps, (C) close-up image of preserved polyp, (D) close-up image of tentacle musculature, (E) close-up image of cyclically transitional marginal musculature, (F) drawing of cyclically transitional marginal musculature. Abbreviations: A=actinopharynx, Tem=tentacle musculature, Ctmm=cyclically transitional marginal musculature. Scale bars: 10 mm (A), 25 mm (B), 5 mm (C), 0.5 mm (D,E).

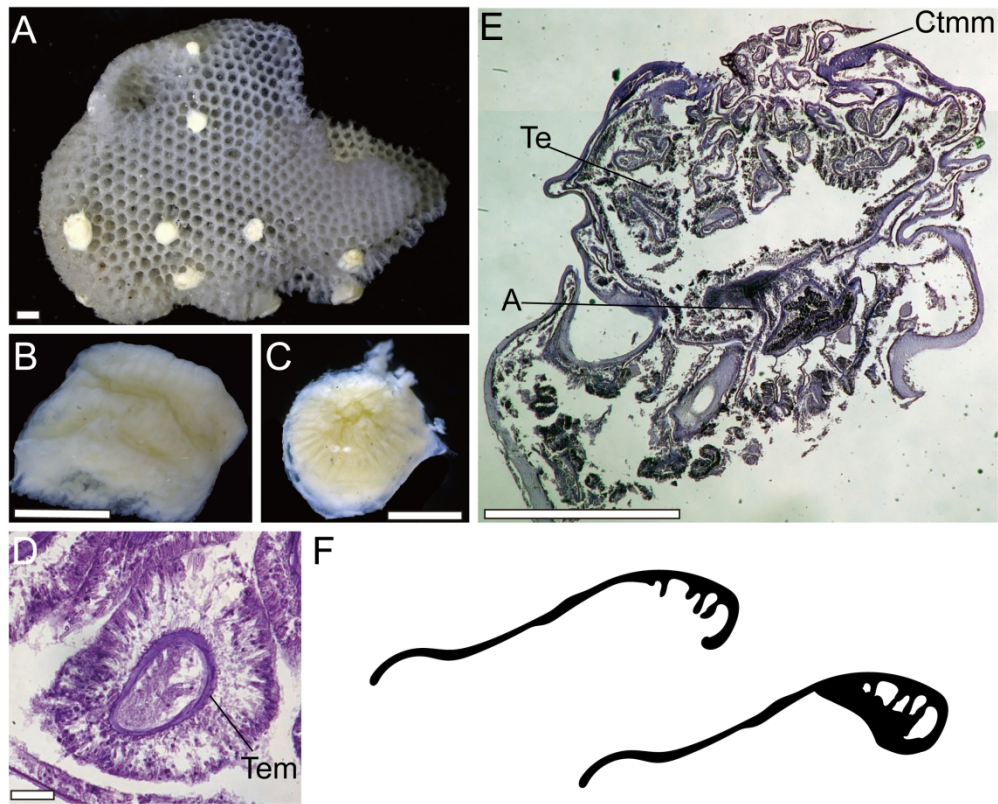


Figure 6. Images of external and internal morphology of *Vitrumanthus vanderlandi* sp. nov. (holotype: RMNH.COEL.42623). (A) preserved polyps on *Aphrocallistes beatrix*, (B,C) close-up image of polyp, (D) close-up image of tentacle musculature, (E) longitudinal section of polyp, (F) drawing of cyclically transitional marginal musculature. Abbreviations: A=actinopharynx, Te=tentacle, Tem= tentacle musculature, Ctmm=cyclically transitional marginal musculature. Scale bars: 3 mm (A), 1.5 mm (B,C,E), mm (C), 0.3 mm (D).

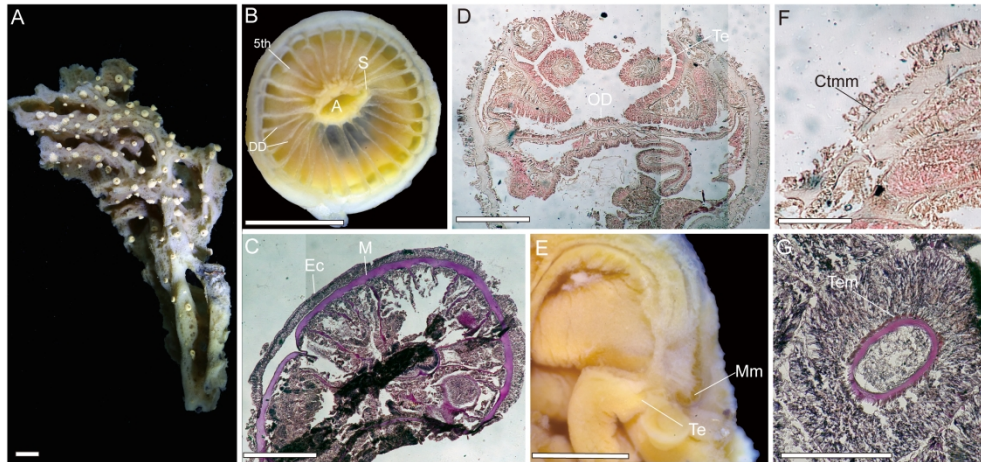


Figure 7. Images of external and internal morphology of *Vitrumanthus oligomyarius* comb. nov. (CMNH ZG-4785). (A) preserved polyps on *Tretochone duplicata*, (B,C) cross section of polyp. (D) longitudinal section of polyp, (E,F) close-up images of cyclically transitional marginal musculature, (G) close-up image of tentacle musculature. Abbreviations: A=actinopharynx, Dd=dorsal directives, Ctmm=cyclically transitional marginal musculature, Ec=ectoderm, M=mesoglea, Mm=marginal musculature, S=siphonoglyph, O=oral disk, 5th=5th mesentery from dorsal directives. Scale bars: 5 mm (A), 1.5 mm (B,C), 0.5 mm (D,E), 0.1 mm (F), 0.25 mm (G).

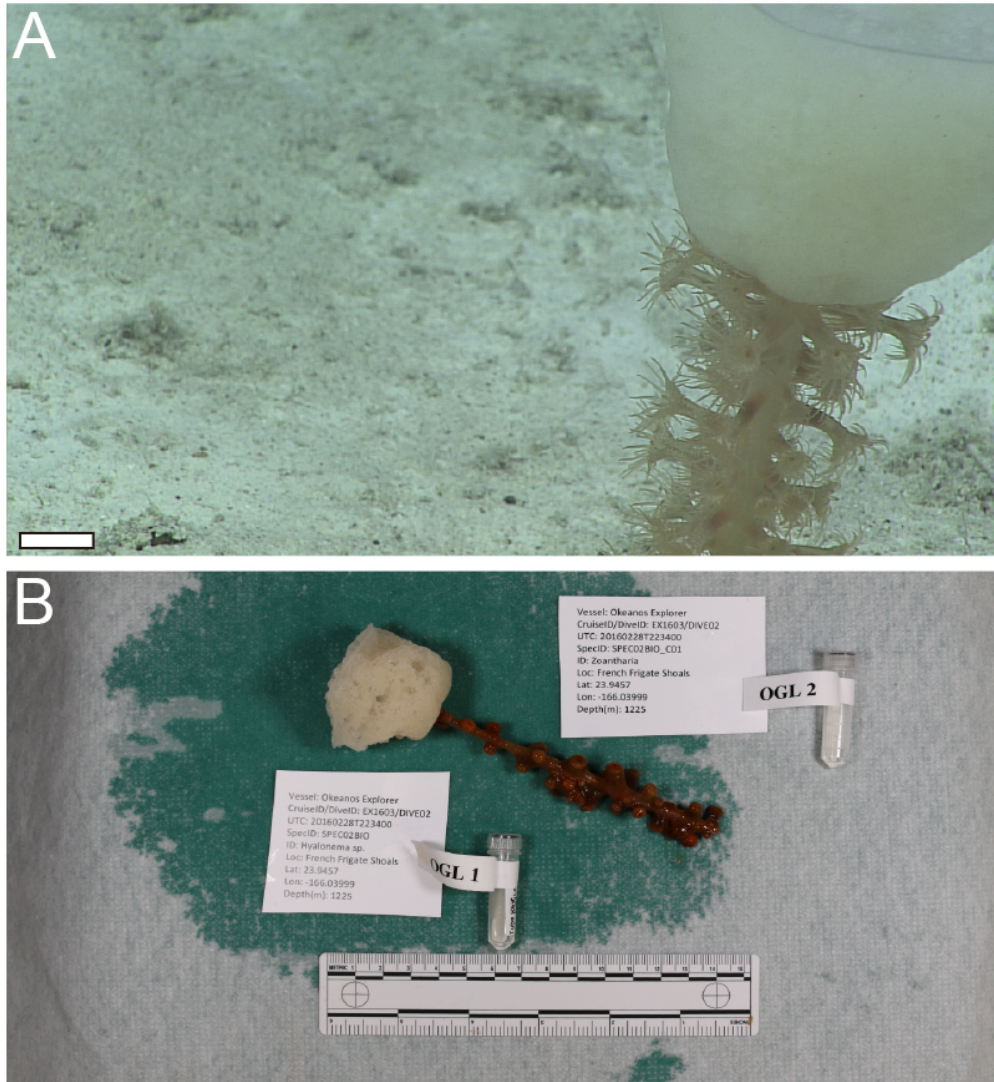


Figure 8. Images of *Kauluzoanthus* sp. (A) in situ image of *Kauluzoanthus* sp. on stalks of *Hyalonema* sp., (B) preserved colony. Scale: 10 mm (A). Images credits: Okeanos/NOAA.



Parazoanthidae

Epizoanthidae

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Table 1 The specimens examined in this study, with GenBank accession numbers of combined dataset of COI, 12S-rDNA, 16S-rDNA, 18S-rDNA, 28S-rDNA, and ITS-rDNA.

Voucher number	Specimen ID	Family	species	Collection locality	Coordinates		Date	Depth (m)	Collector	COI	12S	16S-rDNA	18S-rDNA	ITS-rDNA	28S-rDNA
					Latitude	Longitude									
RMNH.COEL.42620	2K	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Cargill pier, Bonaire, The Netherlands	12°04'47.9"N	68°17'37.7"W	June 1, 2013	223	L. Becking & E. Meesters	---	---	---	---	Accession numb	---
RMNH.COEL.42621	24I	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Cargill pier, Bonaire, The Netherlands	12°04'47.9"N	68°17'37.7"W	June 1, 2013	248	L. Becking & E. Meesters	---	---	---	---	Accession numb	---
RMNH.COEL.42622	10I	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Kralendijk pier, Bonaire, The Netherlands	12°08'48.9"N	68°16'55.6"W	May 30, 2013	140	L. Becking & E. Meesters	---	---	---	---	Accession numb	---
RMNH.COEL.42624	16A	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Cape Verde Islands, SãoTiago, Ilheus Rombos, E of Cima	14°56'59"N	NA24°37'59"W	August 24, 1986	580	R/V HNIMS Tydeman	---	---	---	---	Accession numb	---
RMNH.COEL.42623	4L	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Cape Verde Islands, SãoTiago, Ilheus Rombos, E of Cima	14°56'59"N	NA24°37'59"W	August 24, 1986	700–800	R/V HNIMS Tydeman	Accession numb	---	Accession numb	Accession numb	Accession numb	Accession numb
RMNH.COEL.42625	3L	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Gulf of Guinea, Guinea	4°25'N	8°29'W	October 31, 1963	380–510	ICITA	---	---	---	---	Accession numb	---
CMNH.ZG.4785	JDR307	Parazoanthidae	<i>Vitrumanthus oligomyariis</i> comb. nov.	off Katsura, Chiba, Japan	34°50'N-35°00'N	140°20'W-140°30'W	January 19, 2006	390	A. Tamura	Accession numb	---	Accession numb	Accession numb	Accession numb	Accession numb
NSMT Co-XXXX	50J	Parazoanthidae	Parazoanthidae sp.	Nanpo Trough, Kikaujima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E	October 14, 2011	450	J. Montenegro	Accession numb	Accession numb	---	Accession numb	Accession numb	Accession numb
NSMT Co-XXXX	51J	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Nanpo Trough, Kikaujima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E	October 14, 2011	520	J. Montenegro	Accession numb	---	Accession numb	Accession numb	Accession numb	---
NSMT Co-XXXX	52J	Parazoanthidae	<i>Vitrumanthus</i> sp.	Nanpo Trough, Kikaujima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E	October 14, 2011	520	J. Montenegro	Accession numb	---	Accession numb	Accession numb	Accession numb	---
RMNH.COEL.42430	6BH	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	SubStation, Curacao	12°05'04"N	68°53'54"W	April 21, 2014	200	B.W. Hoeksema	---	---	---	---	Accession numb	---
RMNH.COEL.42429	5BH	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	SubStation, Curacao	12°14'01"N	68°53'32"W	March 31, 2014	161–243	B.W. Hoeksema	---	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
RUMF-ZG-04447	MZ1	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Near Iejima, Okinawa, Japan	26°54'53.6"N	127°37'50.9"E	March 2, 2018	600–650	T. Higashiji	Accession numb	Accession numb	---	Accession numb	Accession numb	Accession numb
RUMF-ZG-04448	MZ3	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Near Iejima, Okinawa, Japan	26°54'53.6"N	127°37'50.9"E	March 2, 2018	600–650	T. Higashiji	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
NSMT Co-XXXX	HPD1323	Epizoanthidae	<i>Epizoanthus aff. armatus</i>	Kuroshima Island, Kagoshima, Japan	24°13'36.1"N	124°06'18.0"E	September 19, 2011	484	J.D. Reimer	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
NSMT Co-XXXX	HK132	Epizoanthidae	<i>Epizoanthus fatuus</i>	Sagami Bay, Kanagawa, Japan	35°08'27.5"N-35°08'33.5"N	139°32'12.2"E-139°32'44.3"E	February 12, 2015	133–274	H. Kotsuka	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
NSMT Co-XXXX	HK33-2	Epizoanthidae	<i>Epizoanthus fatuus</i>	Amakusa, Kumamoto, Japan	32°24'44.8"N	129°28'01.3"E	2011	1000	D. Uyeno	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
QM G337590	G337590	Epizoanthidae	<i>Epizoanthus aff. fatuus</i>	Hunter CMR, Australia	32°34'30.0"S- 32°37'53.8"S	153°08'31.2"E-153°09'42.1"E	June 3, 2017	2595–2474	M. Ekins	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
QM G337585	G337585	Epizoanthidae	<i>Epizoanthus stellaris</i>	Hunter CMR, Australia	32°28'44.4"S-32°30'25.2"S	152°59'27.6"E-152°59'38.4"E	June 3, 2017	1006–1036	M. Ekins	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb	Accession numb
USNM 1424050	OK1	Parazoanthidae	<i>Kaulozaanthus</i> sp.	Honohu Moana/EX1603	23°56.649' N	166°02.187' W	February 28, 2016	1227	Okeanos Explorer expedition, NOAA	Accession numb	---	---	---	Accession numb	Accession numb

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Table 3. Cnidae types and sizes observed in this study. Frequency: relative abundance of cnidae type in decreasing order; numerous, common, occasional, rare. n = number of cnidae measured.

Tissue	Type of cnidae	<i>Churabana kurashioae</i> sp. nov.				<i>Vitrumanthus schrieri</i> sp. nov.				<i>Vitrumanthus vanderlandi</i> sp. nov.				<i>Vitrumanthus oligomyrius</i> comb. nov.			
		Length (min-max, mean)	Width (min-max, mean)	Frequency	n	Length (min-max, mean)	Width (min-max, mean)	Frequency	n	Length (min-max, mean)	Width (min-max, mean)	Frequency	n	Length (min-max, mean)	Width (min-max, mean)	Frequency	n
Tentacle	Spirocysts	13.0-26.0, 20.2	2.0-4.0, 3.0	Numerous	58	12.0-21.0, 16.0	2.0-4.0, 2.7	Numerous	51	11.0-22.0, 16.1	1.0-4.0, 2.6	Numerous	75	11.0-24.0, 17.7	2.0-5.0, 3.3	Numerous	155
	Basitrichs and microbasic b-mastigophores	20.0-30.0, 25.3	2.0-5.0, 4.0	Numerous	105	13.0-18.0, 15.1	2.0-4.0, 2.8	Common	27	11.0-29.0, 22.9	2.0-4.0, 2.9	Numerous	54	13.0-20.0, 17.3	2.0-5.0, 3.3	Numerous	41
	Holotrichs (M)					24.0-26.0, 25.3	9.0-11.0, 10.3	Rare	3					18.0-20.0, 19.0	9.0-12.0, 10.5	Occasional	6
	Holotrichs (L)													29.0	14.0	Rare	4
Column	Microbasic p-mastigophores																
	Holotrichs (L)	33.0-34.0, 33.3	17.0-19.0, 17.7	Rare	3	22.0-26.0, 23.7	11.0-12.0, 11.3	Occasional	7					22.0-28.0, 25.5	12.0-14.0, 13.0	Rare	4
	Holotrich (M)					20.0	11.0	Rare	1					11.0-14.0, 12.5	2.0-4.0, 3.0	Rare	4
Actinopharynx	Spirocysts	13.0-17.0, 15.0	3.0	Rare	2	14.0-20.0, 16.5	2.0-3.0, 2.8	Rare	4	12.0-18.0, 16.1	2.0-4.0, 2.6	Occasional	7	15.0-19.0, 16.6	3.0-4.0, 3.1	Common	17
	Basitrichs and microbasic b-mastigophores	20.0-28.0, 22.7	2.0-4.0, 3.4	Common	18	15.0-17.0, 16.0	2.0-3.0, 2.6	Occasional	5	13.0-19.0, 15.3	2.0-4.0, 2.5	Common	15	13.0-19.0, 15.1	1.0-4.0, 2.5	Common	25
	Holotrichs (M)													17.0-18.0, 17.5	9.0-11.0, 10.0	Rare	2
	Holotrichs (L)					23.0	11.0	Rare	1					23.0-28.0, 25.6	11.0-15.0, 12.9	Occasional	11
	Microbasic p-mastigophores									13.0-14.0, 13.5	3.0-5.0, 4.0	Rare	2				
Mesenterial filaments	Basitrichs and microbasic b-mastigophores	21.0-27.0, 23.3	4.0-5.0, 4.3	Rare	4									10.0-20.0, 15.3	3.0-4.0, 3.7	Rare	3
	Microbasic p-mastigophores	18.0-27.0, 22.7	4.0-7.0, 5.4	Numerous	79	10.0-17.0, 15.0	3.0-5.0, 4.4	Occasional	11	13.0-17.0, 14.9	4.0-6.0, 4.7	Numerous	31	14.0-20.0, 17.1	4.0-6.0, 5.0	Common	23
	Holotrichs (S)	9.0-10.0, 9.6	2.0-3.0, 2.9	Occasional	9												
	Holotrichs (M)	11.0-12.0, 11.1	3.0	Occasional	6	20.0	9.0	Rare	1					20.0	11.0	Rare	2
	Holotrichs (L)	28.0-35.0, 32.6	14.0-21.0, 17.8	Common	20	21.0-25.0, 23.4	9.0-19.0, 11.3	Rare	11					23.0-28.0, 25.5	11.0-16.0, 13.1	Common	22

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