

University of Groningen

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

Kise, Hiroki; Montenegro, Javier; Santos, Maria E. A.; Hoeksema, Bert W.; Ekins, Merrick; Ise, Yuji; Higashiji, Takuo; Fernandez-Silva, Iria; Reimer, James D.

*Published in:*  
 Zoological journal of the linnean society

*DOI:*  
[10.1093/zoolinnea/zlab068](https://doi.org/10.1093/zoolinnea/zlab068)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
 Publisher's PDF, also known as Version of record

*Publication date:*  
 2022

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Kise, H., Montenegro, J., Santos, M. E. A., Hoeksema, B. W., Ekins, M., Ise, Y., Higashiji, T., Fernandez-Silva, I., & Reimer, J. D. (2022). Evolution and phylogeny of glass-sponge-associated zoantharians, with a description of two new genera and three new species. *Zoological journal of the linnean society*, 194(1), 323-347. <https://doi.org/10.1093/zoolinnea/zlab068>

### **Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

### **Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

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

HIROKI KISE<sup>1,2,✉</sup>, JAVIER MONTENEGRO<sup>3</sup>, MARIA E. A. SANTOS<sup>1,4</sup>,  
BERT W. HOEKSEMA<sup>5,6</sup>, MERRICK EKINS<sup>7–9</sup>, YUJI ISE<sup>10</sup>, TAKUO HIGASHIJI<sup>11</sup>,  
IRIA FERNANDEZ-SILVA<sup>12</sup> and JAMES D. REIMER<sup>1,3</sup>

<sup>1</sup>*Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan*

<sup>2</sup>*Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology, AIST Tsukuba Central 7, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8567, Japan*

<sup>3</sup>*Tropical Biosphere Research Center, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan*

<sup>4</sup>*Okinawa Institute of Science and Technology, Evolution, Cell Biology and Symbiosis Unit, Onna, Okinawa, 904-0495, Japan*

<sup>5</sup>*Taxonomy, Systematics, and Geodiversity Group, Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, The Netherlands*

<sup>6</sup>*Groningen Institute for Evolutionary Life Sciences, University of Groningen, P.O. Box 11103, 9700 CC Groningen, The Netherlands*

<sup>7</sup>*Queensland Museum, PO Box 3300, South Brisbane, 4101, Brisbane, Queensland, Australia*

<sup>8</sup>*Griffith Institute of Drug Discovery, Griffith University, 4111, Brisbane, Queensland, Australia*

<sup>9</sup>*School of Biological Sciences, University of Queensland, St Lucia, 4072, Queensland, Australia*

<sup>10</sup>*Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Motobu, Okinawa 905-0227, Japan*

<sup>11</sup>*Okinawa Churaumi Aquarium, Okinawa Churashima Foundation, 424 Ishikawa, Motobu, Okinawa 905-0206, Japan*

<sup>12</sup>*Department of Biochemistry, Genetics and Immunology (School of Biology), University of Vigo, 36310 Vigo, Spain*

Received 3 November 2020; revised 4 July 2021; accepted for publication 19 July 2021

Hexactinellid sponges are important members of deep-sea benthic ecosystems because they provide available hard substrate habitats for filter-feeding invertebrates. However, symbioses between hexactinellid sponges and their 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. Despite these records, 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 these were examined in addition to museum specimens. Based on molecular phylogenetic analyses and morphological observations, we formally describe two new genera and three new species of Zoantharia and report several previously described species. The results suggest at least two independent origins for the symbioses between hexactinellid sponges and zoantharians. Our results demonstrate that the diversity of hexactinellid sponge-associated zoantharians is much higher than has been previously thought. The

\*Corresponding author. E-mail: [hkm11sea@yahoo.co.jp](mailto:hkm11sea@yahoo.co.jp)

[Version of record, published online 29 September 2021;  
<http://zoobank.org/> urn:lsid:zoobank.org:pub:5CBBACDD-0A2D-4F22-A732-0CD2D5E3D9AD]

new taxa described in this work further reconfirm that the deep-sea harbours high levels of undescribed zoantharian diversity.

ADDITIONAL KEYWORDS: deep-sea – Epizoanthidae – hexactinellid sponge – Hexasterophora museum collections – Parazoanthidae – symbiosis.

## INTRODUCTION

Members of Zoantharia Rafinesque, 1815, and particularly the suborder Macrocnemina Haddon & Shackleton, 1891a, are known to associate with a variety of host substrates, including octocorals, polychaetes, hydroids, echinoderms, molluscs and sponges (Haddon & Shackleton, 1891a; Sinniger *et al.*, 2005, 2010, 2013; Reimer *et al.*, 2008, 2019; Swain, 2010; Montenegro *et al.*, 2015; Kise & Reimer, 2016, 2019; Kise *et al.*, 2019). Zoantharian colonies use these substrates to better penetrate the water column and suspension feed. With a wide breadth of associations, research on this order can instruct and inform our understanding on how such associations evolve in marine invertebrates. Originally, zoantharian species were generally classified into a few families and genera based on these associations (Sinniger *et al.*, 2005), but systematic reconsiderations based on molecular phylogenetic analyses have shown that many of these groups have much more complex evolutionary histories than had been originally thought; the associations between zoantharians and their substrates have often evolved or switched multiples times across their evolution (Sinniger *et al.*, 2010; Swain, 2010). Thus, our understanding of the diversity and evolutionary history of Zoantharia has been increasingly refined in recent years. Among zoantharians, associations from the deep-sea, in particular, have been shown to be much more diverse and varied than previously was known (Sinniger *et al.*, 2013; Carreiro-Silva *et al.*, 2017; Reimer *et al.*, 2019). Examples include many different recently erected genera of octocoral-associated species zoantharians (Sinniger *et al.*, 2013) and the discovery of species associated with sea lily crinoids (Reimer *et al.*, 2019; Zapalski *et al.*, 2021). Other recent work from shallow tropical coral reefs has shown that sponge-associated zoantharians form several molecular clades (Montenegro *et al.*, 2015), but zoantharians associated with deep-sea sponges remain to be examined in detail.

Class Hexactinellida Schmidt, 1870 (Porifera) consists of sponges forming siliceous structures, and its species are found exclusively in the deep-sea (Dohrmann *et al.*, 2008). Hexactinellid sponges are important in deep-sea benthic ecosystems because they provide available hard substrate habitats for filter-feeding invertebrates, such as cnidarians, tunicates, ophiuroids, bryozoans and other sponges in the muddy

environments of the deep-sea seafloor (Beaulieu, 2001; Leys *et al.*, 2007). Although many hexactinellid sponge-associated invertebrates have been observed (Beaulieu, 2001; Kahn *et al.*, 2020), symbioses between hexactinellid sponges and symbionts are poorly described. Within the subclass Hexacorallia Haeckel, 1896, some members of the orders Actiniaria Hertwig, 1882 and Zoantharia are known to have symbioses with hexactinellid sponges. *Spongiactis japonica* Sanamyan *et al.*, 2012, reported from Japanese waters, is currently the only known actinarian species to associate with hexactinellid sponges (Sanamyan *et al.*, 2012). On the other hand, many hexactinellid sponge-associated zoantharian species have been described: species of the genera *Epizoanthus* Gray, 1867 and *Thoracactis* Gravier, 1918, within the family Epizoanthidae Delage & Hérouard, 1901, and of *Isozoanthus* Carlgren in Chun, 1903 within Parazoanthidae Delage & Hérouard, 1901. Hexactinellid sponge–zoantharian associations are distributed widely across the globe. For instance, several species associated with stalks of *Hyalonema*, such as *Epizoanthus stellaris* Hertwig, 1888 and *Epizoanthus fatuus* (Schultze, 1860), have been reported from both the Indo-Pacific and the Atlantic Oceans (Bigatti, 2015; Hajdu *et al.*, 2017; Dueñas & Puentes, 2018; NIWA, 2018; Kahn *et al.*, 2020; Reimer & Sinniger, 2021). *Epizoanthus stellaris* is the most common epibiont on stalks of *Hyalonema* Gray, 1832 in the deep-sea of the North-East Pacific (Beaulieu, 2001). Thus, such associations may be widespread and common in the deep-sea.

However, the taxonomy of hexactinellid zoantharians still faces many problems. Most importantly, several stalked hexactinellid sponge-associated *Epizoanthus* and *Isozoanthus* species have not been examined since their original descriptions, or have been observed only a few times. Also, the genus *Isozoanthus* is in need of taxonomic re-examination, as previous studies using molecular phylogenetic analyses have cast doubt on the validity of this genus (Sinniger *et al.*, 2010, 2013). Thus, although some previous studies have reported on several stalked hexactinellid sponge-associated Parazoanthidae specimens, the formal taxonomic position of many of these specimens remains uncertain (Sinniger *et al.*, 2010).

Furthermore, while many zoantharian species have been reported on the stalks of hexactinellid sponges, there is little information on zoantharian species that

are found growing on, or in, the bodies of hexactinellid sponges. Two species have been described with such growth forms: *Palythoa oligomyaria* (Wassilieff, 1908) and *Thoracactis topsenti* Gravier, 1918. *Palythoa oligomyaria* was originally placed within the genus *Gemmaria* Duchassaing & Michelotti, 1860, and this genus group is currently a junior subjective synonym of *Palythoa* Lamouroux, 1816 (see: Low *et al.*, 2016). *Palythoa oligomyaria* was distinguished from other *Gemmaria* (= *Palythoa*) species by its marginal musculature as ‘the few known species of the genus *Gemmaria* have well-developed sphincters. On the other hand, *P. oligomyaria* stands out because of the extraordinarily weak development of the sphincter’ (translated from: Wassilieff, 1908: 47). However, the taxonomic position of *P. oligomyaria* is questionable for several reasons: as the original description is brief, subsequent studies have not been performed since its original description by Wassilieff (1908), and no further specimens have been collected. Additionally, the genus *Palythoa* generally contains zooxanthellate species living on rocks and coral reefs in subtropical and tropical shallow waters (Duerden, 1903; Burnett, 2002; Reimer *et al.*, 2011), while *P. oligomyaria* is an azooxanthellate epibiont from the deep-sea; clearly the generic placement of this species is doubtful.

The taxonomic position of the monospecific genus *Thoracactis* (type species: *Thoracactis topsenti*) is also uncertain. Gravier (1918) identified this species as an actinarian based on the lack of zooxanthellae, channels, gaps or cell islets. Subsequently, Reimer *et al.* (2010) suggested that *Thoracactis* may be in the family Parazoanthidae and not in the family Epizoanthidae, based on its bright yellow colour, which is commonly observed in some Parazoanthidae species (e.g. West, 1979). However, no formal phylogenetic and taxonomic reassessments of the position of this genus have been conducted.

Recently, unidentified zoantharians have been reported living on the main bodies of *Cyrtaulon sigsbeeii* (Schmidt, 1880), *Verrucocoeloidea liberatorii* Reiswig & Dohrmann, 2014, *Heterorete pulchrum* Dendy, 1916, *Psilocalyx wilsoni* Ijima, 1927 and *Aspidoscopulia australia* Dohrmann *et al.*, 2011 (Reiswig & Wheeler, 2002a; Dohrmann *et al.*, 2011; Reiswig & Dohrmann, 2014; Van Soest *et al.*, 2014; Montenegro *et al.*, 2020). These observations suggest that the diversity of zoantharians found on bodies of hexactinellid sponges is higher than has been previously thought. However, phylogenetic studies focused specifically on zoantharians found on bodies and stalks of hexactinellid sponges have not yet been performed.

Thus, further studies utilizing a combination of molecular phylogenetic and morphological analyses, supplemented with ecological information, are needed

to better understand the diversity of hexactinellid sponge-associated zoantharians. In this study, we examined zoantharian specimens found on bodies and stalks of hexactinellid sponges collected from the Indo-Pacific, plus specimens in the collection of Naturalis Biodiversity Center (Leiden, the Netherlands). Much of the Zoantharia collection housed at Naturalis is based on specimens collected from several surveys in West Africa during the CANCAP-VII expedition (1986) and from recent (2013–14) marine biodiversity expeditions in Curaçao and Bonaire (Caribbean Netherlands).

The results of our molecular phylogenetic analyses, combined with data from morphological observations, clarify the phylogenetic relationships and taxonomic positions of several hexactinellid sponge-associated zoantharian species, and we formally describe two new genera and three new species (authored by Kise, Montenegro & Reimer).

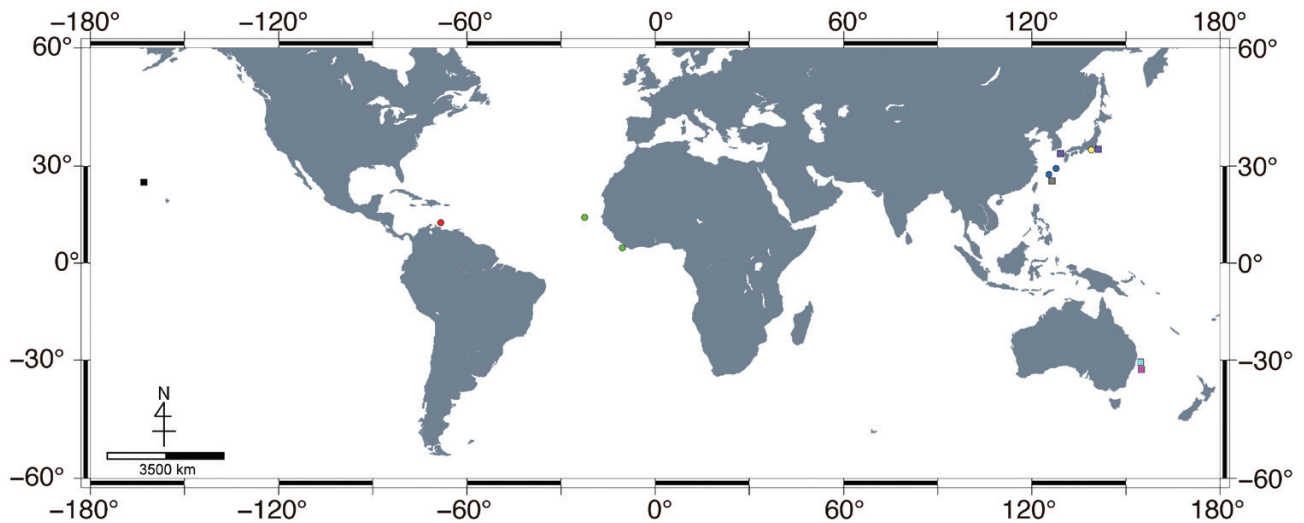
## MATERIAL AND METHODS

### SPECIMEN COLLECTION

Hexactinellid sponge-associated zoantharian specimens were collected by several methods: beam trawls, baskets, dredging and using remotely operated submersibles (ROV), from the waters of Australia, Curaçao and Japan. In addition, we examined relevant specimens in the Coelenterata and Porifera collections (RMNH and ZMA) at Naturalis Biodiversity Center, Leiden, the Netherlands, and a specimen in the collection of the Department of Invertebrate Zoology of the National Museum of Natural History, Washington, DC, USA (Fig. 1; Table 1).

### DNA EXTRACTION, POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION AND SEQUENCING

Total genomic DNA was extracted from tissue preserved in 70.0–99.5% ethanol either by following a guanidine extraction protocol (Sinniger *et al.*, 2010) or by using a spin-column DNeasy Blood and Tissue Extraction kit following the manufacturer’s instructions (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification, using the Hot Star Taq Plus Master Mix kit (Qiagen, Hilden, Germany), was performed for each of the genetic markers: *COI* (mitochondrial cytochrome *c* oxidase subunit I), mt 12S-rDNA (mitochondrial 12S ribosomal DNA), mt 16S-rDNA (mitochondrial 16S ribosomal DNA), 18S-rDNA (nuclear 18S ribosomal DNA), ITS-rDNA (nuclear internal transcribed spacer region of ribosomal DNA) and 28S-rDNA (nuclear 28S ribosomal DNA), using published primers and protocols (Medlin *et al.*, 1988; Folmer *et al.*, 1994; Apakupakul, 1999; Chen *et al.*, 2002; Sinniger *et al.*, 2005, 2013;



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

Swain, 2009a, 2010; Fujii & Reimer, 2011; Supporting Information, Table S1). All PCR products were purified with 1 U of shrimp alkaline phosphatase (SAP) and 5 U of Exonuclease I (Takara Bio Inc., Shiga, Japan) at 37 °C for 40 min followed by 80 °C for 20 min. Cleaned PCR products were sequenced in both directions on an ABI 3730XI (Fasmac, Kanagawa, Japan). Additionally, some cleaned PCR products were sequenced on an ABI 3930 Genetic Analyzer (Applied Biosystems, Thermofisher) at the Genomic Unit, Scientific and Technological Support Center for Research (CACTI), University of Vigo (Spain). Obtained sequences in this study were deposited in GenBank (see Table 1).

#### MOLECULAR AND PHYLOGENETIC ANALYSES

Molecular sequences were individually aligned in GENEIOUS v.10.2.3 (Kearse *et al.*, 2012), using the global alignment tool with free-end gaps and default settings. All output alignments were visually inspected and manually curated. The resulting alignments were subsequently concatenated with no overlapping positions.

Publicly available sequences from the families Parazoanthidae and Epizoanthidae, and two sequences from the genus *Microzoanthus* Fujii & Reimer, 2011, were downloaded from GenBank for each marker and included into the alignments generated above; 43 sequences were added to *COI*, 33 for mt 12S-rDNA, 64 for mt 16S-rDNA, 32 for 18S-rDNA, 26 for 28S-rDNA and 55 for ITS-rDNA (Supporting Information, Table S2). Each region was individually aligned using MAFFT (Katoh & Standley, 2013) with

the ‘auto’ algorithm and default settings. Thereafter, all alignments were manually trimmed, curated and realigned as before. The resultant alignments were 396 bp in length for *COI*, 829 bp for mt 12S-rDNA, 592 bp for mt 16S-rDNA, 1696 bp for 18S-rDNA, 842 bp for 28S-rDNA and 759 bp for ITS. For the 18S-rDNA region, samples RMNH.COEL.42429, NSMT-Co 1755, NSMT-Co 1759, USNM 1424050 and RMNH.COEL.42623 were missing one of the three amplified regions and, therefore, missing positions were replaced by N’s, as were all missing positions and gaps across markers. These alignments were subsequently concatenated to obtain a final dataset of 5114 bp for 85 OTUs. All aligned datasets are available from the first author and at the Figshare repository (<https://dx.doi.org/10.6084/m9.figshare.14616657>).

Phylogenetic reconstructions were performed over the concatenated alignment using maximum likelihood (ML) and Bayesian inference (BI). TOPALi v.2.5 (Milne *et al.*, 2009) was used to select the best-fitting model for each molecular marker and independently for ML and BI analyses. The selected models for ML were K80+G for *COI* and mt 12S-rDNA, SYM+G for mt 16S-rDNA, K81uf+I+G for 18S-rDNA, TIM+G for 28S-rDNA and TrN+I+G for ITS. The same models were selected for BI, except for K80+I for 18S-rDNA, GTR+G for 28S-rDNA and HKY+I+G for ITS. All phylogenetic estimations were performed using the substitution models indicated above per partition in RAxML-NG v.0.9 (Kozlov *et al.*, 2019) standalone version for ML and in the MrBayes v.3.2.6 (Ronquist & Huelsenbeck, 2003) plugin version in

GENEIOUS for BI. RAxML-NG was configured to use 12 345 initial seeds, search for the best tree among 100 preliminary parsimony trees, branch length was scaled and automatically optimized per partition, and model parameters were also optimized. MrBayes was configured to use the models and parameters above with the following settings: four MCMC heated chains were run for 5 000 000 generations with a temperature for the heated chain of 0.2. Chains were sampled every 200 generations. Burn-in was set to 25%, at which point the average standard deviation of split frequency (ASDOSF) was steadily below 0.01. Sequences of genus *Microzoanthus* (family Microzoanthidae) were used as outgroup in ML and BI analyses.

#### MORPHOLOGICAL OBSERVATIONS

We observed four categories of morphological characters: external morphology, internal morphology, cnidae and morphology of associated hexactinellid sponges. External morphology was examined based on preserved specimens and photographs, and included lengths and diameters of individual polyps, tentacle numbers, coloration of polyps and coenenchyme and relative development of coenenchyme. Diameters of oral disks and polyp dimensions were measured when polyps were expanded. The internal morphology was observed based on histological sections of 8–10 µm thickness, stained with haematoxylin and eosin after decalcification with Morse solution for 24–48 h (1 : 1 vol; 20% citric acid : 50% formic acid). We additionally observed hand-cut polyps for marginal musculature position and type, and mesenterial arrangement and number of mesenteries. Classification of marginal musculature shapes followed Swain *et al.* (2015). Cnidae were observed in the tentacles, column, actinopharynx and mesenterial filaments under a Nikon Eclipse80i stereomicroscope (Nikon, Tokyo). Cnidae sizes were measured using ImageJ v.1.45s (Rasband, 2012). Cnidae classification followed England (1991) and Ryland & Lancaster (2004), except for the treatment of basitrichs and microbasic b-mastigophores, as mentioned in Kise *et al.* (2019). Associated hexactinellid sponges were identified based on morphology (Reiswig & Wheeler, 2002a, b).

**Abbreviations:** NSMT: National Science Museum, Tsukuba, Ibaraki, Japan. QM: Queensland Museum, Queensland, Australia. RMNH: Rijksmuseum van Natuurlijke Historie (now at Naturalis Biodiversity Center), Leiden, the Netherlands. RUMF: Ryukyuu University Museum (Fujukan), University of the Ryukyus, Okinawa, Japan. USNM: Natural Museum of Natural History, Washington, DC, USA. ZMA: Zoological Museum Amsterdam (now at Naturalis Biodiversity Center), Leiden, the Netherlands.

## RESULTS

### MOLECULAR PHYLOGENY

All phylogenetic analyses (ML, BI) using the concatenated dataset show that the new genera described below (*Churabana* and *Vitrumanthus*) are part of the family Parazoanthidae (Fig. 2). The basic topologies between ML and BI phylogenetic trees are congruent, although there are a few differences. In the ML phylogenetic tree, *Vitrumanthus* is sister to *Churabana* and a clade containing the Demospongiae sponge-associated genera *Bergia* Duchassaing & Michelotti, 1860, *Parazoanthus* Haddon & Shackleton, 1891a and *Umimayanthus* Montenegro *et al.*, 2015 with weak nodal support (ML = 40%), while in the BI phylogenetic tree, *Churabana* is basal to *Vitrumanthus* and the clade of Demosponge-associated zoantharian genera with strong support (BI = 0.99). *Churabana* and *Vitrumanthus* are monotypic and monophyletic clades, respectively, with strong nodal support (ML = 100%, BI = 1; ML = 98%, BI = 1). *Kauluzoanthus* sp. associated with *Hyalonema* sp. is sister to *Ka. kerbyi* with moderate support (ML = 73%, BI = 1).

In Epizoanthidae, all hexactinellid sponge-associated species (*Epizoanthus fatuus*, *E. stellaris*, *E. aff. fatuus* and *E. aff. armatus*) formed a monophyletic clade including *E. aff. illoricatus sensu* Swain (2010) with moderate nodal support (ML = 59%, BI = 0.99), and this clade was sister to a clade consisting of sequences of eunicid polychaete-associated species (*E. illoricatus* and *E. beriber*).

### SYSTEMATICS

PHYLUM CNIDARIA HATSCHEK, 1888

CLASS ANTHOZOA EHRENBERG, 1834

ORDER ZOANTHARIA RAFINESQUE, 1815

FAMILY EPIZOANTHIDAE DELAGE & HÉROUARD, 1901

GENUS *EPIZOANTHUS* GRAY, 1867

*Type species:* *Dysidea papillosa* Johnston, 1842, by monotypy (see also Opinion 1689 in ICZN, 1992).

*Diagnosis:* Macrocnemic zoantharians with simple mesogleal musculature, readily distinguishable from *Palaeozoanthus* by the presence of non-fertile micromesenteries (Sinniger & Häussermann 2009).

*EPIZOANTHUS FATUUS* (SCHULTZE, 1860)

(FIG. 3A, B)

*Synonymy:* *Palythoa fatua* Schultze, 1860: 36, taf. 2, figs 1–2; *Palythoa fatua*—Andres, 1884: 311; *Sidisia*

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

Vouvier number	Specimen ID	Specimen ID	Family	species	Collection locality	Coordinates	
						Latitude	Longitude
RMNH. COEL.42620	9234	2K	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Cargill Pier, Bonaire, Netherlands	12°04'47.9"N	68°17'37.7"W
RMNH. COEL.42621	9251	24I	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Cargill Pier, Bonaire, Netherlands	12°04'47.9"N	68°17'37.7"W
RMNH. COEL.42622	9219	10I	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	Kralendijk Pier, Bonaire, Netherlands	12°08'48.9"N	68°16'55.6"W
RMNH. COEL.42624	7293	16A	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Cape Verde Islands, São Tiago, Ilheus Rombos, E of Cima	14°56'59"N	24°37'59"W
RMNH. COEL.42623	10224	4L	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Cape Verde Islands, SãoTiago, Ilheus Rombos, E of Cima	14°56'59"N	24°37'59"W
RMNH. COEL.42625	15756	3L	Parazoanthidae	<i>Vitrumanthus vanderlandi</i> sp. nov.	Gulf of Guinea, Guinea	4°25'N	8°29'W
CMNH ZG 4785	4785	JDR307	Parazoanthidae	<i>Vitrumanthus oligomyarius</i> comb. nov.	off Katsuura, Chiba, Japan	34°50'N-35°00'N	140°20'W-140°30'W
NSMT-Co 1756	50J	50J	Parazoanthidae	Parazoanthidae sp.	Nanpo Trough, Kikaijima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E
NSMT-Co 1754	51J	51J	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Nanpo Trough, Kikaijima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E
NSMT-Co 1755	52J	52J	Parazoanthidae	<i>Vitrumanthus</i> sp.	Nanpo Trough, Kikaijima, Kagoshima, Japan	28°20'21.64"N	129°57'14.56"E
RMNH. COEL.42430	22K	6BH	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	SubStation, Curaçao	12°05'04"N	68°53'54"W
RMNH. COEL.42429	23K	5BH	Parazoanthidae	<i>Vitrumanthus schrieri</i> sp. nov.	SubStation, Curaçao	12°14'01"N	68°53'32"W
RUMF-ZG-04447	MZ1	MZ1	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Near Iejima, Okinawa, Japan	26°54'53.6"N	127°37'50.9"E
RUMF-ZG-04448	MZ3	MZ3	Parazoanthidae	<i>Churabana kuroshioae</i> sp. nov.	Near Iejima, Okinawa, Japan	26°54'53.6"N	127°37'50.9"E
NSMT-Co 1759	HPD1323	HPD1323	Epizoanthidae	<i>Epizoanthus</i> aff. <i>armatus</i>	Kuroshima Island, Kagoshima, Japan	24°13'36.1"N	124°06'18.0"E
NSMT-Co 1758	HK132	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

Date	Depth (m)	Collector	COI	12S	16S-rDNA	18S-rDNA	ITS-rDNA	28S-rDNA
June 1, 2013	223	L. Becking & E. Meesters	---	---	---	---	MZ329711	---
June 1, 2013	248	L. Becking & E. Meesters	---	---	---	---	MZ329714	---
May 30, 2013	140	L. Becking & E. Meesters	---	---	---	---	MZ329710	---
August 24, 1986	580	R/V HNIMS Tydeman	---	---	---	---	MZ329717	---
August 24, 1986	700–800	R/V HNIMS Tydeman	MZ313327	---	---	MZ329700	MZ329716	MZ329734
October 31, 1963	380–510	ICITA	---	---	---	---	MZ329718	---
January 19, 2006	390	A. Tamura	MZ313324	---	MZ329732	MZ329699	MZ329715	MZ329733
October 14, 2011	450	J. Montenegro	MZ313320	MZ329751	---	MZ329696	MZ329709	MZ329737
October 14, 2011	520	J. Montenegro	MZ313325	---	MZ329727	MZ329695	MZ329721	---
October 14, 2011	520	J. Montenegro	MZ313326	---	MZ329728	MZ329704	---	---
April 21, 2014	200	B.W. Hoeksema	---	---	---	---	MZ329713	---
March 31, 2014	161–243	B.W. Hoeksema	---	MZ329750	MZ329726	MZ329701	MZ329712	MZ329735
March 2, 2018	600–650	T. Higashiji	MK385649	MZ329753	---	MZ329698	MK377416	MZ329743
March 2, 2018	600–650	T. Higashiji	MZ313323	MZ329752	MZ329731	MZ329697	MK377415	MZ329744
September 19, 2011	484	J.D. Reimer	MZ313321	MZ329745	MZ329730	MZ329703	MZ329706	MZ329741
February 12, 2015	133–274	H. Kotsuka	MZ313316	MZ329749	---	MZ329691	MZ329707	MZ329742



Table 1. Continued

Voucher number	Specimen ID	Specimen ID	Family	species	Collection locality	Coordinates	
						Latitude	Longitude
NSMT-Co 1757	HK33-2	HK33-2	Epizoanthidae	<i>Epizoanthus fatuus</i>	Amakusa, Kumamoto, Japan	32°24'44.8"N	129°28'01.3"E
QM G337590	337590	G337590	Epizoanthidae	<i>Epizoanthus</i> aff. <i>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
QM G337585	337585	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
USNM 1424050	OK1	OK1	Parazoanthidae	<i>Kauluzoanthus</i> sp.	French Frigate Shoals, Hawaii	23°56.649' N	166°02.187' W

*fatus*—Lwowsky, 1913: 589–596, taf. 19, figs 5–8; *Sidisia fatua* var. *alba*—Lwowsky, 1913: 597.

**Material examined:** MISE-HK33-2 (NSMT-Co 1757), off Amakusa, Kumamoto, Japan, 32°24'44.8"N 129°28'01.3"E (position approximate, exact location unknown), 1000 m depth, beat trawl, coll. D. Uyeno on the training vessel *Nagasaki-maru*, 2011, fixed in 99.5% ethanol. MISE-HK132 (NSMT-Co 1758), Sagami Bay, Kanagawa, Japan, 35°08'27.5"N–35°08'33.5"N, 139°32'12.2"E–139°32'44.3"E, 133–274 m depth, dredging, coll. H. Kotsuka on vessel *Rinkai-maru*, 12 Feb 2015, fixed in 99.5% ethanol.

**Description: External morphology.** Ten to 50 cylindrical polyps connected by strongly developed dark brown and light beige coenenchyme on stalks of hexactinellid sponges (*Hyalonema* sp.) in preserved specimens. Column of preserved polyps dark brown and light beige in coloration. Colonies covered upper part of stalks, but not around the spiculous anchor. Contracted preserved polyps 0.8–2.9 mm in height, 1.9–3.6 mm in diameter. Capitulum swollen, and diameter of capitulum larger than scapus when contracted. Capitular ridges present and strongly pronounced when contracted, 14 in number. The number of tentacles of each polyp in this study 28, and tentacles arranged in two rows. Ectoderm and mesoglea of polyps and coenenchyme heavily encrusted with numerous sand and silica particles.

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

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

Associated host. *Hyalonema* spp.

**Remarks:** This species has been reported in several studies, which indicate the presence of intraspecific variation (Andres, 1884; Lwowsky, 1913, Carlgren, 1923). In fact, we observed several morphological differences, such as coloration and polyp dimensions, between the two examined specimens (NSMT-Co 1757 and NSMT-Co 1758). Also, some genetic variation of *E. fatuus* was observed in ITS-rDNA sequences (8 bp), and thus the possibility remains that *E. fatuus* contains cryptic species, as ITS-rDNA has been demonstrated to be among the most variable genetic regions currently utilized to delineate species within Zoantharia (e.g. Reimer et al., 2007; Montenegro et al., 2015). However, the sequences of the two examined specimens formed a strongly supported monophyletic clade and we therefore identify the examined specimens as a single species in this study. Additional specimens and fine-scale genetic analyses are required to better examine if there is any cryptic diversity within this group.

*EPIZOANTHUS* AFF. *FATUUS* (SCHULTZE, 1860)

(FIG. 3C)

**Material examined:** QM G337590. Hunter CMR, Australia, New South Wales, 32°34'30.0"S–32°37'53.8"S, 153°08'31.2"E–153°09'42.1"E, depth 1006–1036 m, beam trawl, coll. M. Ekins on *RV Investigator*, Cruise IN2017\_V03, 3 June 2017, fixed in 99.5% EtOH.

**Description: External morphology.** Circa 40 cylindrical polyps connected by strongly developed light beige

Date	Depth (m)	Collector	COI	12S	16S-rDNA	18S-rDNA	ITS-rDNA	28S-rDNA
2011	1000	D. Uyeno	MZ313317	MZ329747	MZ329729	MZ329693	MZ329705	MZ329740
June 3, 2017	2595–2474	M. Ekins	MZ313319	MZ329748	MZ329725	MZ329692	MZ329720	MZ329739
June 3, 2017	1006–1036	M. Ekins	MZ313318	MZ329746	MZ329724	MZ329694	MZ329719	MZ329738
February 28, 2016	1227	Okeanos Explorer expedition, NOAA	MZ313315	---	---	MZ329702	MZ329708	MZ329736

coenenchyme on stalks of hexactinellid sponges (*Hyalonema* sp.) in preserved specimen. Colony covered upper part of the stalks, but not around the spiculous anchor. Contracted preserved polyps 1.0–3.0 mm in height, 1.5–3.5 mm in diameter.

**Remarks:** The capitulum of this specimen (QM G337590) were not swollen, while capitulum of the examined specimens of *Epizoanthus fatuus* (NSMT-Co 1757, NSMT-Co 1758) were swollen when contracted. On the other hand, numbers of tentacles and mesenteries were the same between QM G337590 and the examined specimens of *E. fatuus*. Therefore, we here preliminarily identify the examined specimen as *Epizoanthus* aff. *fatuus*.

*EPIZOANTHUS STELLARIS* HERTWIG, 1888  
(FIG. 3D)

**Material examined:** QM G337585. Hunter CMR, New South Wales, Australia, 32°28'44.4"S–32°30'25.2"S, 152°59'27.6"E–52°59'38.4"E, depth 1006–1036 m, beam trawl, coll. M. Ekins on *RV Investigator*, Cruise IN2017\_V03, 3 June 2017, fixed in 99.5% EtOH.

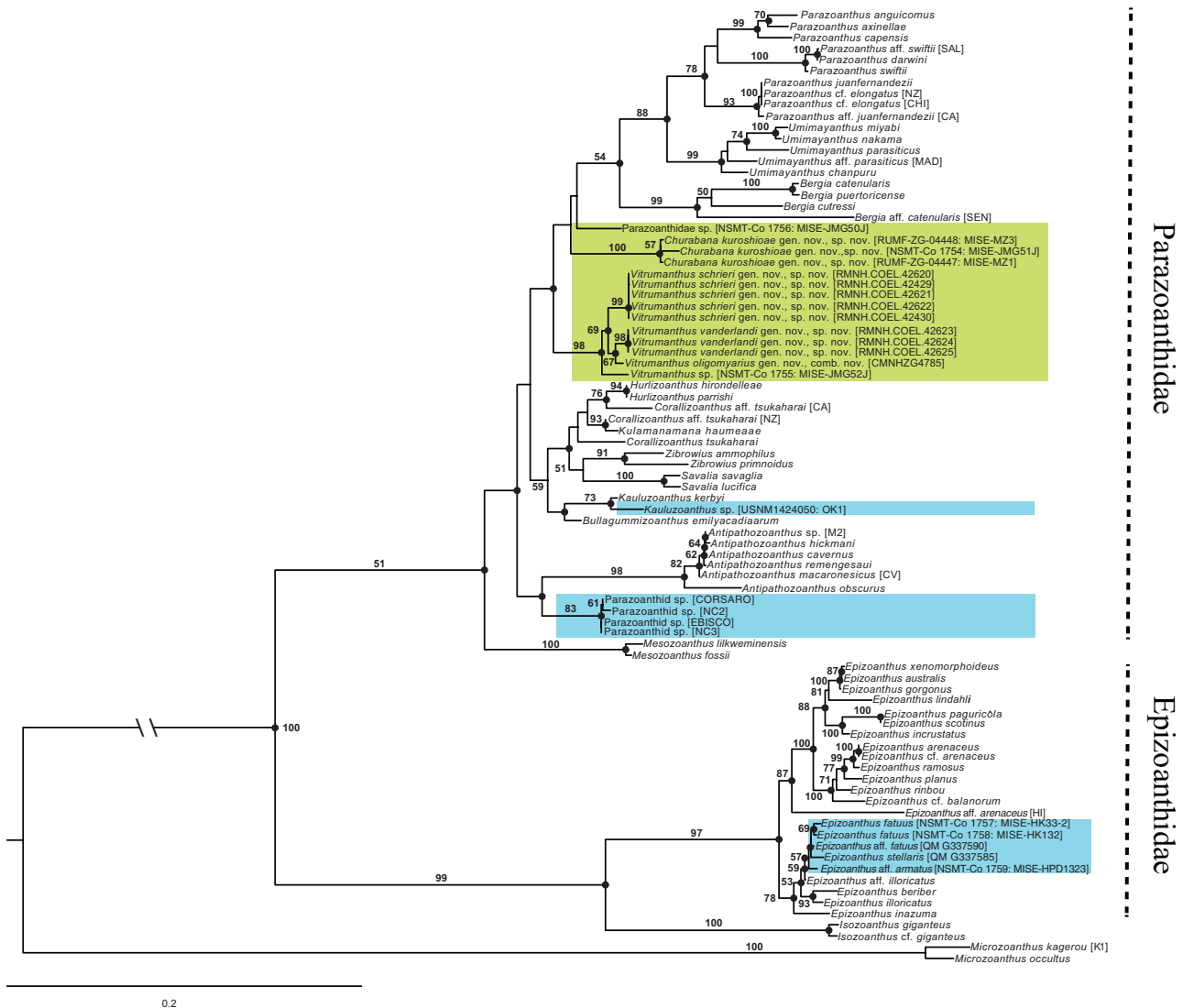
**Description: External morphology.** Circa 40 nearly saucer-shaped polyps connected by strongly developed dark-brownish coenenchyme on stalks of hexactinellid sponges (*Hyalonema* sp.) in preserved specimen. Colony covered the upper part of the stalks, but not around the spiculous anchor. Contracted preserved polyps only rise a little from the coenenchyme and flat, 0.4–1.1 mm in height, 3.0–6.9 mm in diameter. Capitular ridges present and well pronounced when contracted, approximately 14–18 in number. Ectoderm and mesoglea of polyps and coenenchyme heavily encrusted with numerous sand and silica particles.

**Internal morphology.** Zooxanthellae absent. Number of mesenteries 28–36, in macrocnemic arrangement. Mesogleal thickness *c.* 0.1–0.3 mm. Numerous and various size of pigment cells in the ectoderm and mesoglea. Mesoglea thicker than ectoderm and endoderm in column, actinopharynx and mesenteries. Reticulate mesogleal musculature short and poorly developed. Siphonoglyph distinct and V-shaped. Mesenterial filaments present.

**Habitat and distribution:** Tasman Sea at depths of 1006–1036 m in this study. The type locality of this species is off Samboangan [Zamboanga City], Philippines at a depth of 150 m (82 fathoms). This species has been recorded from the Indian Ocean (Reimer & Sinniger, 2021), New Zealand (NIWA, 2018), the East Pacific Ocean (Beaulieu, 2001; Reimer & Sinniger, 2021) and the Caribbean Sea (Dueñas & Puentes, 2018).

**Associated host:** *Hyalonema* sp.

**Remarks:** *Epizoanthus stellaris* can be distinguished from other *Epizoanthus* species found on stalks of hexactinellid sponges: the strongly lamellated polyps of *E. stellaris* are not observed in *E. fatuus*, *E. armatus* or *E. longiceps* (Lwowsky, 1913) (2.0–10.0 mm in height; Lwowsky, 1913; Carlgren, 1923). Lwowsky (1913) synonymized *E. stellaris* as *E. fatuus* on account of extreme growth forms of *E. fatuus* due to the high amounts of morphological variability that are commonly found within zoantharian species. However, the results of our molecular phylogenetic analyses support that *E. stellaris* and *E. fatuus* are distinct species. Beaulieu (2001) observed *E. stellaris* frequently in the East Pacific Ocean, although it should be noted that several species may be contained in *E. stellaris*, as observed by Beaulieu (2001), based on the results of the present study.



**Figure 2.** Maximum likelihood tree based on combined dataset of *COI*, 12S-rDNA, 16S-rDNA, 18S-rDNA, 28S-rDNA and ITS-rDNA. Green-coloured box indicates Hexasterophora sponge-associated zoantharians and blue coloured boxes indicate Amphidiscophora sponge-associated zoantharians. Number at nodes represent ML bootstrap values (> 50% are shown). Black circles on nodes indicate high support of Bayesian posterior probabilities (> 0.95).

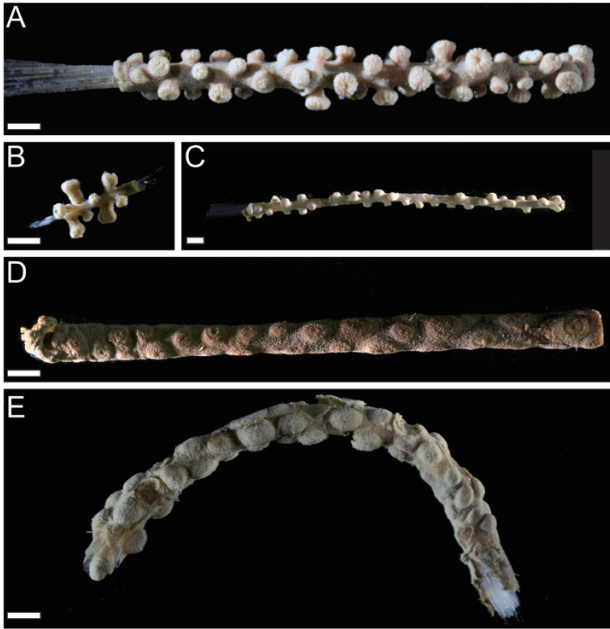
*EPIZOANTHUS* AFF. *ARMATUS* CARLGRÉN, 1923  
(FIG. 3E)

**Material examined:** MISE-HPD1323 (NSMT-Co 1759), Kuroshima Island, Kagoshima, Japan, 24°13'36.1"N, 124°06'18.0"E, depth 468 m, ROV, coll. J. D. Reimer on RV *Natsushima*, 19 Sep 2011, fixed in 90% ethanol.

**Description: External morphology.** Circa 80 cylindrical polyps connected by strongly developed light-brownish coenenchyme on stalks of hexactinellid sponges (*Hyalonema* sp.) in preserved specimen. Colony covered the upper part of the stalks, but not around the spiculous

anchor. Contracted preserved polyps well developed and 0.5–4.9 mm in height, 2.5–7.8 mm in diameter. Capitulary ridges present but weakly pronounced when contracted, c. 14–16 in number. The numbers of tentacles of each polyp c. 28–32 and tentacles arranged in two rows. **Internal morphology.** Zooxanthellae absent. Number of mesenteries 28–32, in macrocnemic arrangement. Reticulate mesogleal musculature.

**Habitat and distribution:** Off Kuroshima, Okinawa, in the Ryukyu Archipelago, Japan at a depth of 468 m. *Epizoanthus armatus* has previously been



**Figure 3.** 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.

reported from off Somalia in the Indian Ocean (Carlgren, 1923).

*Associated host:* *Hyalonema* sp.

*Remarks:* Polyp dimensions of the examined specimen are larger than those of *E. fatuus* and *E. stellaris*, and this specimen resembles *E. armatus* as described by Carlgren (1923). The morphological characters and dimensions observed in the examined specimen agree well with the original description by Carlgren (1923). *Epizoanthus armatus* was originally described from East Africa (Carlgren, 1923). Kise *et al.* (2018) reported the existence of *E. planus* Carlgren, 1923 in Japanese waters, and this species was also originally described from East Africa. Therefore, it is possible that *E. armatus* may also be distributed in Japanese waters. However, the collected depths of the examined specimen in this study and the specimens Carlgren (1923) examined are different (468 m vs. 741–1362 m deep). We also could not observe internal morphology of the examined specimen due to its poor preserved condition. Therefore, we here preliminarily identified the examined specimen as *Epizoanthus* aff. *armatus*. Examination of additional specimens combined with molecular analyses should help confirm the identity of this specimen.

FAMILY PARAZOANTHIDAE DELAGE & HÉROUARD, 1901

GENUS *CHURABANA* GEN. NOV.

(FIG. 4A–H)

*Zoobank registration:* urn:lsid:zoobank.org:act:EACFCC05-EA56-4F04-94A2-FB716F22004C.

*Type species:* *Churabana kuroshioae* sp. nov. by original designation.

*Diagnosis:* Parazoanthidae with obligate symbiotic relationship with massive hexasterophoran sponges. Preserved polyps 3.0–4.0 mm in height, 2.8–4.0 mm in diameter. Azooxanthellate. Cteniform endodermal marginal musculature.

*Remarks:* *Churabana* and other already described sponge-associated zoantharian genera can be easily distinguished from each other by their host sponges (Hexactinellida sponges vs. Demospongiae sponge) and depths; the former can be found at > 140 m, while the latter are found in shallow coral reefs. Although *Churabana* and several species within *Isozoanthus* and *Epizoanthus* are associated with Hexactinellida sponges, these three genera can be distinguished from each other by their hosts: the latter two genera are associated with species within the subclass Amphidiscophora, while species of *Churabana* are associated with sponge species within the subclass Hexasterophora. *Churabana* has a unique deletion of 15 bp (from position 168 to 182 in our alignment) in its 16S-rDNA region.

*Etymology:* The generic name is derived from the Ryukyuan language words *chura*, beautiful, and *bana*, flower, referring to the appearance of this species. Gender feminine. The Japanese name is 'Chura-tama-sunaginchaku'.

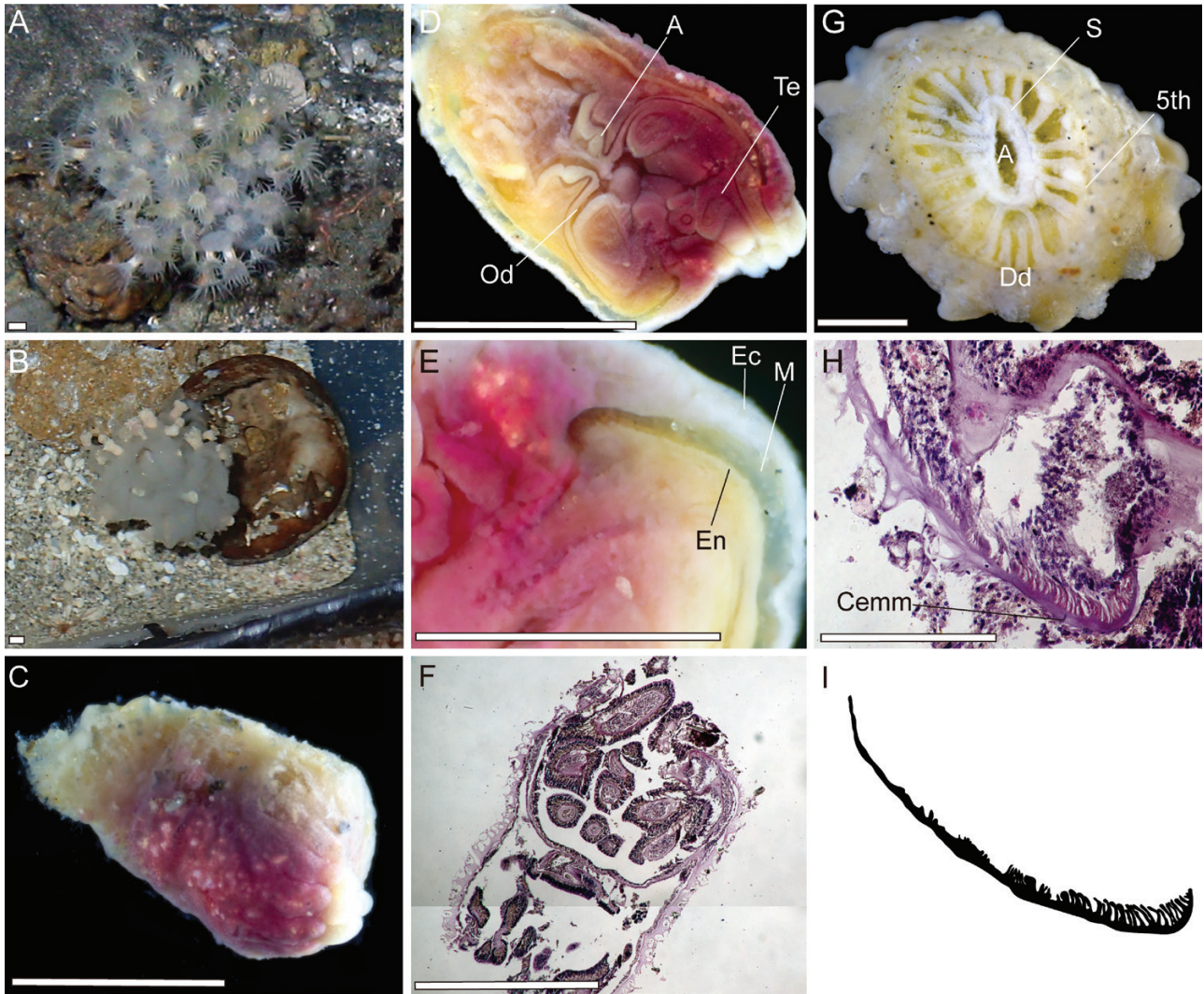
*CHURABANA KUROSHIOAE* SP. NOV.

(FIG. 4A–I)

*Synonymy:* Parazoanthidae sp. 1—Reimer *et al.*, 2019: 7, fig. 2A.

*Zoobank registration:* urn:lsid:zoobank.org:act:FC503255-BDC2-45C5-9788-41F52BC40FA5.

*Material examined:* Holotype: RUMF-ZG-04447, near Iejima Island, Motobu, Okinawa, Japan, 26°54'53.6"N, 127°37'50.9"E, 600–650 m, baskets, coll. T. Higashiji on vessel *Daini-kuroshio-maru*, 2 March 2018, divided into two pieces, one portion fixed in 5–10% saltwater formalin and other in 99.5% ethanol.



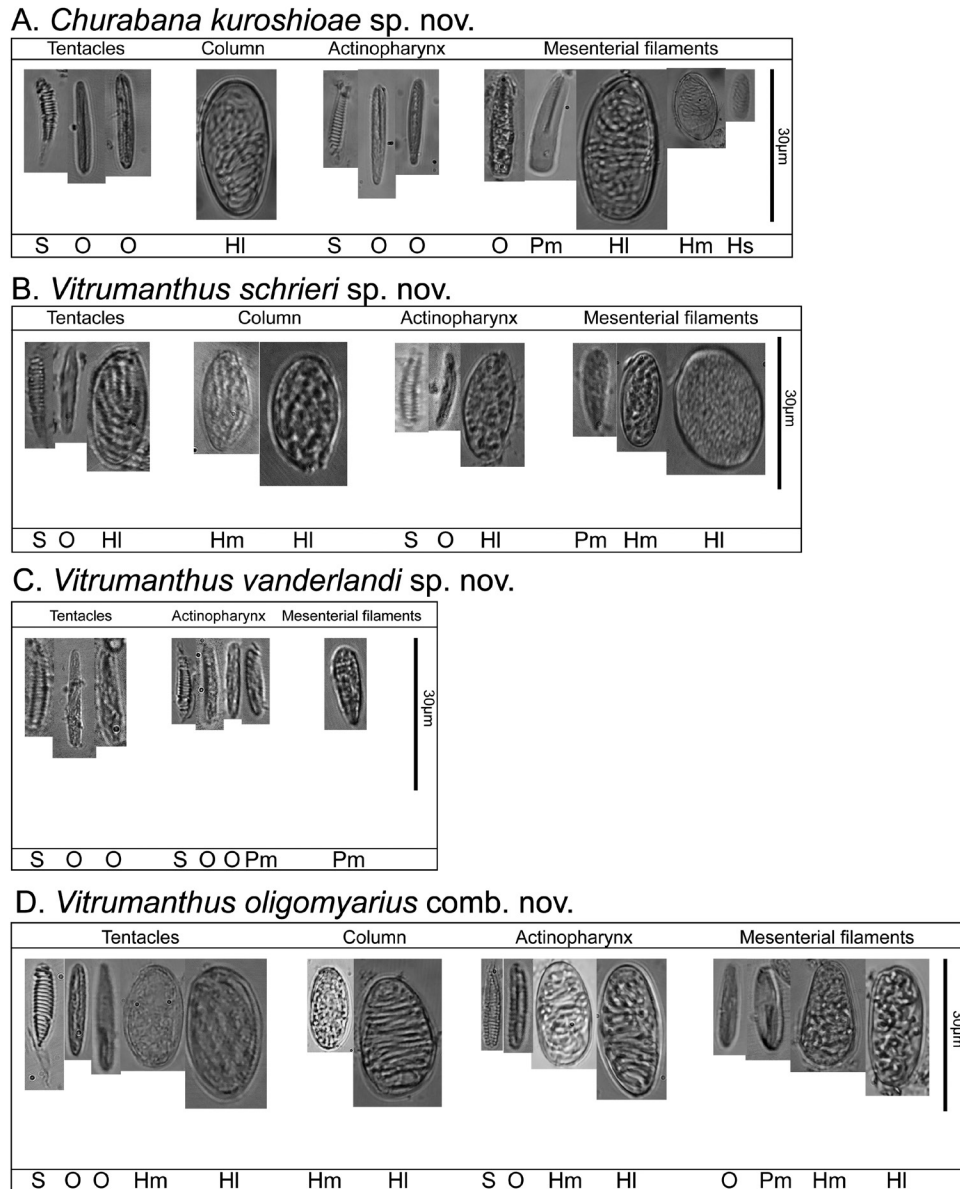
**Figure 4.** Images of external and internal morphology of *Churabana kuroshioae* (A, paratype: NSMT Co-1754; B–I, holotype: RUMF-ZG-04447). A, living polyps on *Pararete* sp.1 *in situ* at Nanpo Trough, Kikaijima Island; B, living polyps on *Pararete* sp.2 in an aquarium at Okinawa Churaumi Aquarium, 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; 5<sup>th</sup>, fifth mesentery from dorsal directives. Scales: 5 mm (A, B), 2 mm (C–H).

*Paratype:* RUMF-ZG-04448, near Iejima Island, Motobu, Okinawa, Japan, 26°54'53.6"N, 127°37'50.9"E, 600–650 m, baskets, coll. T. Higashiji on vessel *Daini-kuroshio-maru*, 2 March 2018, fixed in 99.5% ethanol. MISE-JMG51J (NSMT-Co 1754), Nanpo Trough, Kikaijima Island, Kagoshima, Japan, 28°20'21.64"N, 129°57'14.56"E, depth 520 m, ROV, coll. Javier Montenegro on RV *Natsushima*, 14 Oct 2011, fixed in 99.5% ethanol.

*Etymology:* The species is named after the *Daini-kuroshio-maru*, as the type specimens were

collected by this vessel. The Japanese name is 'Beni-chura-tama-sunaginchaku'.

*Description: External morphology.* Four truncated cone-shaped or cylindrical polyps in preserved specimen. The polyp bases embedded within the sponge *Pararete* Ijima, 1927. Solitary polyps arise irregularly from *Pararete* specimens. The living polyps cream-pink or beige and tentacles cream or whitish transparent in coloration. Preserved polyps beige and partially red. Surface of column rough and ectoderm continuous. Ectoderm and mesoglea of polyps



**Figure 5.** Cnidae in the tentacles, column, actinopharynx and mesenterial filaments of holotypes of new species in this study. A, cnidae of *Churabana kuroshioae*; B, cnidae of *Vitrumanthus schrieri*; C, cnidae of *Vitrumanthus vanderlandi*; D, cnidae of *Vitrumanthus oligomyarius*. Abbreviations: HI, holotrichs large; Hm, holotrichs medium; Hs, holotrich small; O, basitrichs and microbasic b-mastigophores; Pm, microbasic p-mastigophores; S, spirocysts.

encrusted with numerous and various sizes of sand and silica particles. The living expanded oral disks *c.* 1.5–2.0 mm in diameter, expanded polyps *c.* up to 10 mm in height, 4.0–5.0 mm in diameter. Preserved contracted polyps 3.0–4.0 mm in height, 2.8–4.0 mm in diameter. Capitulary ridges discernible, 15–16 in number when contracted. 30–32 tentacles in number.

**Internal morphology.** Zooxanthellae absent. Cteniform endodermal marginal musculature. Encircling sinus present and basal canals of mesenteries absent. Mesenteries thin, 30–32 in macrocnemic arrangement.

Mesoglea thickness 0.5–1.6 mm. Mesoglea thicker than ectoderm and endoderm. Siphonoglyph distinct and U-shaped. Mesenterial filaments present.

**Cnidae.** Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs and spirocysts (Fig. 5A; Table 2).

**Distribution and habitats:** Ryukyu Archipelago, Japan: Near Iejima Island, Okinawa and Nanpo Trough, Kikaijima Island, Kagoshima, Japan at depths of 520–650 m.

**Table 2.** 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 kuroshioae</i> sp. nov.				<i>Vitrumanthus schrieri</i> sp. nov.
		Length (min-max, mean)	Width (min-max, mean)	Frequency	<i>N</i>	Length (min-max, mean)
Tentacle	Spirocysts	13.0-26.0, 20.2	2.0-4.0, 3.0	Numerous	58	12.0-21.0, 16.0
	Basitrichs and microbasics b-mastigophores	20.0-30.0, 25.3	2.0-5.0, 4.0	Numerous	105	13.0-18.0, 15.1
	Holotrichs (M)					
	Holotrichs (L)					24.0-26.0, 25.3
	Microbasics p-mastigophores					
Column	Holotrichs (L)	33.0-34.0, 33.3	17.0-19.0, 17.7	Rare	3	22.0-26.0, 23.7
	Holotrich (M)					20.0
Actinopharynx	Spirocysts	13.0-17.0, 15.0	3.0	Rare	2	14.0-20.0, 16.5
	Basitrichs and microbasics b-mastigophores	20.0-28.0, 22.7	2.0-4.0, 3.4	Common	18	15.0-17.0, 16.0
	Holotrichs (M)					
	Holotrichs (L)					23.0
	Microbasics p-mastigophores					
Mesenterial filaments	Basitrichs and microbasics b-mastigophores	21.0-27.0, 23.3	4.0-5.0, 4.3	Rare	4	
	Microbasics p-mastigophores	18.0-27.0, 22.7	4.0-7.0, 5.4	Numerous	79	10.0-17.0, 15.0
	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
	Holotrichs (L)	28.0-35.0, 32.6	14.0-21.0, 17.8	Common	20	21.0-25.0, 23.4

*Associated host:* *Pararete* sp. 1 and *Pararete* sp. 2.

*Remarks:* *Churabana kuroshioae* resembles *Vitrumanthus* species, but they can be easily separated by differences in their marginal musculatures (see also Remarks under *Vitrumanthus*).

#### GENUS *VITRUMANTHUS* GEN. NOV.

(FIGS 6–8)

*Type species:* *Vitrumanthus schrieri* sp. nov. by original designation.

*Zoobank registration:* urn:lsid:zoobank.org:act:00F773E4-4535-49F4-8CDA-30F569190BEF.

*Diagnosis:* Parazoanthidae with obligate symbiotic relationship with massive hexasterophoran and Demospongiae sponges. Preserved polyps 0.3–3.1 mm in length, 0.8–3.4 mm in diameter.

Azooxanthellate. Cyclically transitional marginal musculature.

*Remarks:* *Vitrumanthus* is distinguished from genus *Churabana* by its marginal musculature. In *Vitrumanthus*, cyclically transitional marginal musculature with several mesogleal lacunae was observed, while in *Churabana* cteniform endodermal marginal musculature with comb-like mesogleal pleats was seen. Molecular phylogenetic analyses clearly support the distinctiveness between *Churabana* and *Vitrumanthus*. In the 16S-rDNA region, *Vitrumanthus* has a unique deletion of 15 bp (positions 136–150 in our alignment).

*Etymology:* The generic name is derived from the Latin word *vitrum*, glass, and Greek word *anthos*, flower, referring to the appearance of this genus. Gender masculine. The Japanese name is 'Ruri-sunaginchaku'.

<i>Vitrumanthus vanderlandi</i> sp. nov.						<i>Vitrumanthus oligomyarius</i> comb. nov.				
Width (min-max, mean)	Frequency	<i>N</i>	Length (min-max, mean)	Width (min-max, mean)	Frequency	<i>N</i>	Length (min-max, mean)	Width (min-max, mean)	Frequency	<i>N</i>
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
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
							18.0-20.0, 19.0	9.0-12.0, 10.5	Occasional	6
9.0-11.0, 10.3	Rare	3					29.0	14.0	Rare	4
11.0-12.0, 11.3	Occasional	7					22.0-28.0, 25.5	12.0-14.0, 13.0	Rare	4
11.0	Rare	1					11.0-14.0, 12.5	2.0-4.0, 3.0	Rare	4
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
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
							17.0-18.0, 17.5	9.0-11.0, 10.0	Rare	2
11.0	Rare	1					23.0-28.0, 25.6	11.0-15.0, 12.9	Occasional	11
			13.0-14.0, 13.5	3.0-5.0, 4.0	Rare	2				
							10.0-20.0, 15.3	3.0-4.0, 3.7	Rare	3
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
9.0	Rare	1					20.0	11.0	Rare	2
9.0-19.0, 11.3	Rare	11					23.0-28.0, 25.5	11.0-16.0, 13.1	Common	22

**VITRUMANTHUS SCHRIERI SP. NOV.**

(FIG. 6A–F)

*Synonymy:* Parazoanthidae sp. – Montenegro *et al.*, 2020: 7–8, fig. 2; ‘zoanthid symbionts’, ‘zoanthids’ – Reiswig & Dohrmann, 2014: 241–242, fig. 4A, B; ‘white zoanthids’ – Van Soest *et al.*, 2014: 438.

*Zoobank registration:* urn:lsid:zoobank.org:act:D326729E-6617-4C5F-8F43-31C5C7133EF4.

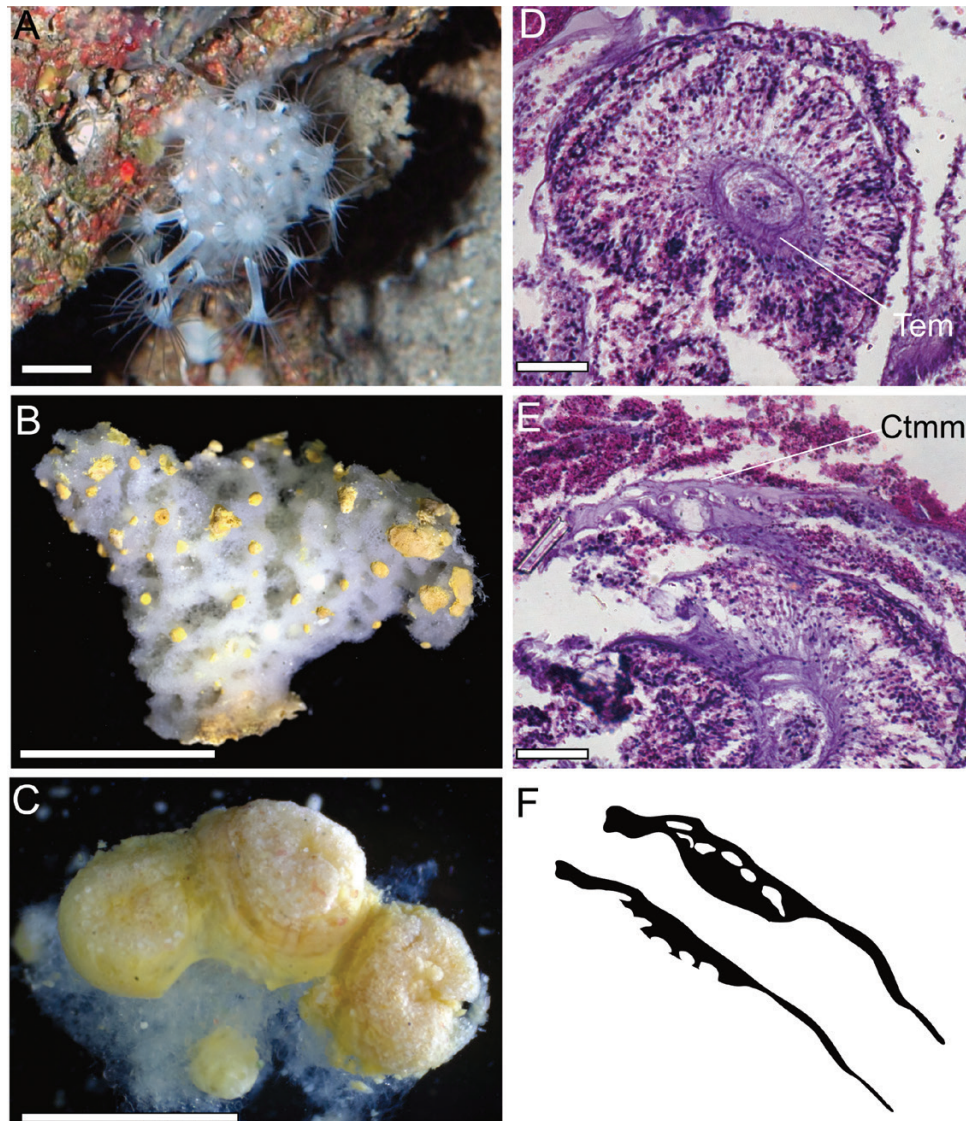
*Material examined:* Holotype: RMNH.COEL.42429, SubStation, Curaçao, 12°14′01″N, 68°53′32″W, depth range 161–243 m, manned submarine *Curasub*, coll. B. W. Hoeksema, 31 Mar 2014, fixed in 99.5% ethanol.

*Paratype:* RMNH.COEL.42430, SubStation, Curaçao, 12°05′04″N, 68°53′54″W, c. 200 m deep, manned submarine *Curasub*, coll. B. W. Hoeksema, 21 Apr 2014, fixed in 99.5% ethanol.

*Other material examined:* RMNH.COEL.42620, Cargill Pier, Bonaire, Caribbean Netherlands, 12°04′47.9″N, 68°17′37.7″W, depth 223 m, manned submarine *Curasub* based on RV *Chapman*, coll. L. Becking and E. Meesters, 1 Jun 2013, fixed in 99.5% ethanol. RMNH.COEL.42621, Cargill Pier, Bonaire, Caribbean Netherlands, 12°04′47.9″N, 68°17′37.7″W, depth 248 m, manned submarine *Curasub* based on RV *Chapman*, coll. L. Becking and E. Meesters, 1 Jun 2013, fixed in 99.5% ethanol. RMNH.COEL.42622, Kralendijk Pier, Bonaire, Caribbean Netherlands, 12°08′48.9″N, 68°16′55.6″W, depth 140 m, manned submarine *Curasub* based on RV *Chapman*, coll. L. Becking and E. Meesters, 30 May 2013, fixed in 99.5% ethanol.

*Etymology:* The species is named for Adriaan ‘Dutch’ Schrier, owner of the manned submersible *Curasub* and RV *Chapman*, operated from Substation Curaçao, for his generosity in allowing BWH to sample specimens. The Japanese name is ‘Amime-ruri-sunaginchaku’.



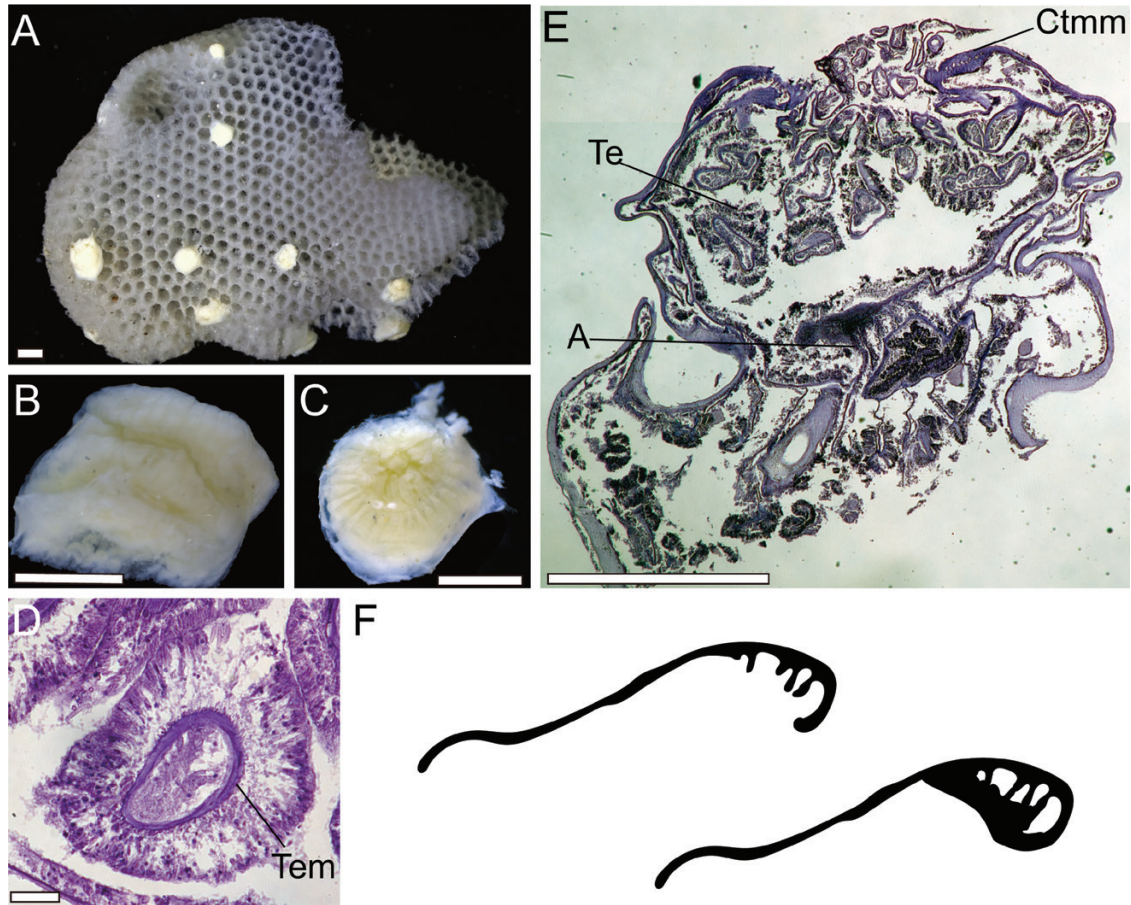


**Figure 6.** Images of external and internal morphology of *Vitrumanthus schrieri* (A, photographic record: image was taken at the entrance of Piscadera Bay, 12°07'06"N, 68°58'34"W; B–F, holotype: RMNH.COEL.42429). A, living polyps on *Verrucocoeloidea liberatorii* in situ; B, preserved polyps; C, close-up 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).

**Description: External morphology.** Circa 50 cylindrical polyps in preserved specimen. Solitary or colonial polyps rise irregularly from the hexactinellid sponge *Verrucocoeloidea liberatorii* Reswig & Dohrmann, 2014. Living polyps white and tentacle transparent in coloration. Preserved polyps dark beige. Surface of column rough and ectoderm continuous. Ectoderm and mesoglea of polyps encrusted with numerous and various sizes of sand and silica particles. The living expanded oral disks c. up to 8.0 mm in diameter, expanded polyps

c. up to 10 mm in height, 1.0–5.0 mm in diameter. Preserved contracted preserved polyps 0.3–2.1 mm in height, 0.8–2.8 mm in diameter. Capitulatory ridges indiscernible. Tentacles 20–24 in number.

**Internal morphology.** Zooxanthellae absent. Cyclically transitional marginal musculature. Encircling sinus present and basal canals of mesenteries absent. Mesenteries thin. Mesoglea thickness c. 0.6 mm. Mesoglea thicker than ectoderm and endoderm. Siphonoglyph distinct and V-shaped. Mesenterial filaments present.



**Figure 7.** Images of external and internal morphology of *Vitrumanthus vanderlandi* (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).

*Cnidae*. Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs and spirocysts (Fig. 5B; Table 2).

*Distribution and habitats*: Caribbean Netherlands: known from Curaçao and Bonaire at depths of 140–248 m.

*Associated hosts*: *Verrucocoeloidea liberatorii* Reswig & Dohrmann, 2014, *Parahigginsia strongylifera* Van Soest, Meesters & Becking, 2014 and *Cyrtaulon sigsbeeii* (Schmidt, 1880).

*Remarks*: In terms of host hexactinellid sponges, *Vitrumanthus schrieri* can be distinguished from *V. vanderlandi* and *V. oligomyarius* (Wassilieff, 1908) because only *V. schrieri* is associated with *Verrucocoeloidea* sponges. *Vitrumanthus schrieri* is not only associated with hexactinellid sponges, but also with Demospongiae species in the genus *Parahigginsia* (Van Soest *et al.*, 2014). On the other hand, *V. vanderlandi*

and *V. oligomyarius* are associated with *Tretochone duplicata* (Topsent, 1928). In addition, the column of *V. schrieri* is rough, due to heavy encrustation, while columns of *V. vanderlandi* and *V. oligomyarius* are smooth with almost no encrustations. *Vitrumanthus* sp. (NSMT-Co 1755) and *V. vanderlandi* are associated with *Cyrtaulon* species.

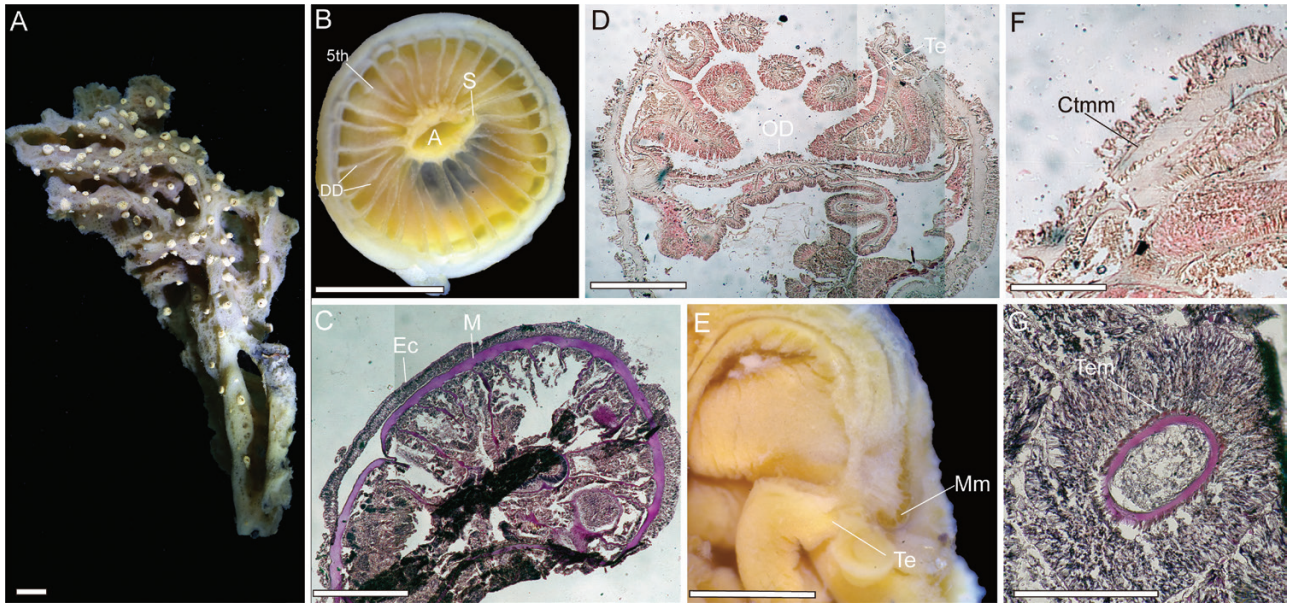
Reiswig & Dohrmann (2014) reported zoantharians were found on *Verrucocoeloidea liberatorii*, and we judge that these are likely *V. schrieri* based on external morphology and the host hexactinellid sponge species.

#### VITRUMANTHUS VANDERLANDI SP. NOV.

(FIG. 7A–F)

*Zoobank registration*: urn:lsid:zoobank.org:act:CE659473-CB9F-462B-97F7-4B974FAE96B9.

*Material examined*: Holotype: RMNH.COEL.42623, CANCAP-VII Expedition Station CV 7.041, Cape



**Figure 8.** Images of external and internal morphology of *Vitrumanthus oligomyarius* (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, fifth 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).

Verde Islands, SãoTiago, Ilheus Rombos, east of Cima, 14°56'59"N, 24°37'59"W, Agassiz trawl, at a depth of 700–800 m, coll. RV *HNIMS Tydeman*, 24 Aug 1986, fixed in 99.5% ethanol. Paratype: RMNH.COEL.42624, CANCAP-VII Expedition Station CV 7.041, Cape Verde Islands, SãoTiago, Ilheus Rombos, east of Cima, 14°56'59"N, 24°37'59"W, Agassiz trawl, at a depth of 580 m depth, coll. RV *HNIMS Tydeman*, 24 Aug 1986. *Other material examined*: RMNH.COEL.42625, Equalant II Expedition to the Gulf of Guinea, as part of the ICITA Programme (International Cooperative Investigations of the Tropical Atlantic), Sta. 18/8, 38 km off Liberia, 4°25'N, 8°29'W, trawl at a depth range of 380–510 m, on RV *Geronimo* Cruise 2, 31 Oct 1963, fixed in 99.5% ethanol.

*Etymology*: This species is named in honour of Dr Jacob van der Land, expedition leader of the CANCAP-VII expedition off the coast of West Africa (Van der Land, 1987), during which the type specimens of this species were collected. The Japanese name is 'Hime-ruri-sunaginchaku'.

*Description*: *External morphology*. Thirty-two cylindrical polyps in preserved specimen. Base of polyps embedded in *Aphrocallistes* sponge. Solitary polyps arise irregularly from host hexactinellid sponge. Preserved polyps cream white in coloration. Surface of column smooth, and

ectoderm continuous. Ectoderm of polyps partially encrusted with small size of sand and silica particles. Transparent capitulum contracted and rounded. Contracted preserved polyps 0.3–2.5 mm in height, 0.8–3.0 mm in diameter. Capitular ridges indiscernible when contracted. Approximately 20 tentacles in number.

*Internal morphology*. Zooxanthellae absent. Cyclically transitional marginal musculature. Mesoglea thickness 0.2–1.1 mm. Mesoglea thicker than ectoderm and endoderm. Siphonoglyph distinct and V-shaped. Mesenterial filaments present.

*Cnidae*. Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores and spirocysts (Fig. 5C; Table 2).

*Distribution and habitats*: West Africa: Guinea and Cape Verde at depths of 580–800 m.

*Associated host*: *Aphrocallistes beatrix* Gray, 1858, *Aphrocallistes* sp.

*Remarks*: *Vitrumanthus vanderlandi* is phylogenetically close to *V. oligomyarius*, but these two species can be distinguished by dimensions of polyps and the numbers of tentacles. *Vitrumanthus vanderlandi* and *V. oligomyaria* are also associated with different host hexactinellid sponges (*Aphrocallistes* species vs.

*Tretochone duplicata*). Moreover, *V. vanderlandi* does not have any holotrich nematocysts in all tissues we examined, while other species of *Vitrumanthus* have holotrich nematocysts present in some tissues.

Although the *in situ* polyp colour of *V. vanderlandi* is unknown, [Buhl-Mortensen et al. \(2017\)](#) have reported yellowed zoantharians that were observed on *A. beatrix* collected from the Gulf of Guinea. Therefore, the coloration of polyps of this species may be yellow. Further investigations with newly collected specimens are needed to confirm the *in situ* morphology of *V. vanderlandi*.

**VITRUMANTHUS OLIGOMYARIUS (WASSILIEFF, 1908) COMB. NOV.**

(FIG. 8A–G)

*Basionym:* *Gemmaria oligomyaria* [Wassilieff, 1908](#): 47, fig. 29; taf. I, fig. 29.

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

*Description: External morphology.* Circa > 300 cylindrical polyps. Solitary polyps arise from *Tretochone duplicata* ([Topsent, 1928](#)) in preserved specimen. Preserved polyps dark-yellow in coloration and trapezoid when opened. Polyps located all over the three-dimensional structured hexactinellid sponge body. Surface of column smooth, and ectoderm continuous. Ectoderm of polyps partially encrusted with small sized sand and silica particles (*c.* < 0.1 mm). The transparent capitulum contracted and rounded. Contracted preserved polyps 0.5–3.1 mm in height, 1.2–3.4 mm in diameter. Capitulary ridges indiscernible when contracted. Tentacles 32–36 in number.

*Internal morphology:* Zooxanthellae absent. Cyclically transitional marginal musculature from endoderm to mesogleal, and lacunae confined toward endoderm proximally. Encircling sinus or mesogleal canal usually imperceptible and basal canals of mesenteries absent. Mesenteries thin, 32–36 in number, in brachycnemic arrangement. Mesoglea thickness 0.7–1.0 mm. Mesoglea thicker than endoderm but thinner than ectoderm in the body wall. Siphonoglyph distinct and U-shaped. Mesenterial filaments present.

*Cnidae:* Basitrichs and microbasic b-mastigophores, microbasic p-mastigophores, holotrichs and spirocysts ([Fig. 5D](#); [Table 2](#)).

*Distribution and habitats:* Pacific coast of Japan: Suruga Bay, Shizuoka and off Katsuura, Chiba at depths of < 390 m.

*Associated host:* *Tretochone duplicata* ([Topsent, 1928](#)).

*Remarks:* The morphological characteristics of *Vitrumanthus oligomyarius* collected in this study are identical to those of the original description by [Wassilieff \(1908\)](#). The musculature of the sphincter of the examined specimen is in a cyclically transitional arrangement containing several mesogleal lacunae; identical musculature was also mentioned by [Wassilieff \(1908\)](#). Moreover, the examined specimen by [Wassilieff \(1908\)](#) and this study both have brachycnemic mesenterial arrangements, with incomplete mesenteries in the fifth mesenterial pair from the dorsal directive, a diagnostic characteristic of the suborder Brachycnemina. This finding is unusual as *V. oligomyarius* is clearly phylogenetically placed in the suborder Macrocnemina, which have complete fifth mesentery pairs from the dorsal directive. On the other hand, the other morphological characteristics of *V. oligomyarius* are similar to those of other macrocnemic species. It should be noted a similar case has been reported in the past. *Parazoanthus douglasi* [Haddon & Shackleton, 1891b](#), which likely belongs to the genus *Hydrozoanthus* [Sinniger et al., 2010](#) ([Reimer & Sinniger, 2021](#)), also has a brachycnemic mesenterial arrangement, while its other characteristics, such as being epizoic on hydroids and its marginal musculature, are similar to other macrocnemic species. *Vitrumanthus oligomyarius* can be easily distinguished from other *Vitrumanthus* species by the numbers of tentacles and its unique mesenterial arrangement. This study reports on the existence of this species for the first time since its original description. The Japanese name is 'Subesube-ruri-sunaginchaku'.

GENUS *KAULUZOANTHUS* [SINNIGER ET AL., 2013](#)

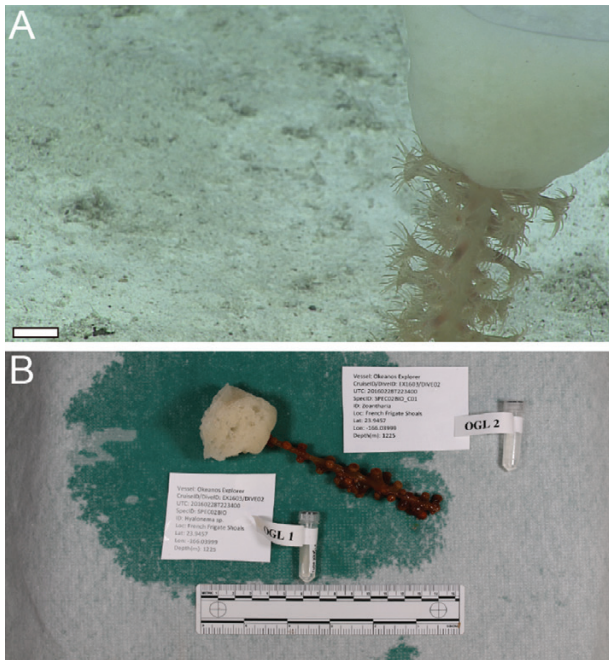
*Type species:* *Kauluzoanthus kerbyi* [Sinniger et al., 2013](#)

*Diagnosis:* Polyps do not contract when fixed. Characteristic insertion/deletion pattern in the 16S V5 region *sensu* [Sinniger et al. \(2005\)](#) ([Sinniger et al., 2013](#)).

*KAULUZOANTHUS* SP.

(FIG. 9A–B)

*Material examined:* USNM 1424050, French Frigate Shoals, Hawaii (23°56.649'N, 166°02.187'W), 1225 m, ROV, coll. RV *Okeanos Explorer*, NOAA on 28 February 2016, fixed in 99.5% EtOH.



**Figure 9.** 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.

**Description:** Non-encrusted azooxanthellate zoantharians. The examined specimen associated with *Hyalonema* sp. Preserved polyps cylindrical and c. 1.8–9.6 mm in height, 3.9–6.7 mm in diameter, with brown column in coloration. Thin coenenchyme completely covering stalk of *Hyalonema* sp. Capitulary ridges discernible, 14–16 in number when contracted. Tentacles relatively short, as long as expanded oral disk diameter. Numbers of tentacles 28–32.

**Associated host:** *Hyalonema* sp.

**Distribution and habitats:** North Pacific Ocean: French Frigate Shoals, Hawaii, at a depth of 1225 m.

**Remarks:** The genus *Kauluzoanthus* is known to associate with the gold coral *Kulamanamana haumea* and with several octocoral species (Sinniger *et al.*, 2013). However, our examined specimen was associated with *Hyalonema* sponges, and was genetically close to *Ka. kerbyi* based on the results in this study. This finding suggests that *Kauluzoanthus* species are not host-specific to *Ku. haumea* or other octocorals. However, we could not obtain any 16S-rDNA sequences, which contain the V5 region *sensu* Sinniger *et al.* (2005). Therefore, further integrated studies

including morphological and ecological studies are needed to describe this putative new species.

## DISCUSSION

### PHYLOGENY AND TAXONOMY OF HEXACTINELLID SPONGE-ASSOCIATED ZOANTHARIANS

Our phylogenetic analyses focused on hexactinellid sponge-associated zoantharians, conducted for the first time, confirmed that the diversity of hexactinellid sponge-associated zoantharians is higher than previously thought. Two new genera and three new species are formally described above. Our study confirms that hexactinellid sponge-associated zoantharians are located in both families Epizoanthidae and Parazoanthidae: zoantharians are found on the stalks of Amphidiscophora sponges are found in both families, while zoantharians found on the bodies of Hexasterophora are unique to the Parazoanthidae (Supporting Information, Table S3).

In Epizoanthidae, Amphidiscophora sponge-associated zoantharians were identified as *Epizoanthus* species, and the monophyly of this association is confirmed. The phylogenetic positions of *E. fatuus* and *E. stellaris* were established for the first time in Epizoanthidae, with additional information on their morphology provided by the present study.

In our phylogenetic analyses, within Parazoanthidae, previously reported sequences of Amphidiscophora sponge-associated zoantharians Parazoanthidae sp. CORSARO, Parazoanthidae sp. EBISCO, Parazoanthidae sp. NC2 and Parazoanthidae sp. NC3 (Sinniger *et al.*, 2010) formed a monophyletic clade, supporting previous studies (Sinniger *et al.*, 2010; Swain, 2010, 2018). These parazoanthid specimens may possibly correspond to one or more of the *Isozoanthus* species described on the stalks of Amphidiscophora sponges: *I. africanus* Carlgren, 1923, *I. arenosus* Carlgren, 1923 and *I. valdiviae* Carlgren, 1923, described from East Africa and the Andaman Sea. Currently, no DNA sequences exist for these *Isozoanthus* species. However, the taxonomic status of the genus *Isozoanthus* is uncertain, and has been debated in several studies (e.g. Williams, 2000; Sinniger *et al.*, 2010; Low *et al.*, 2016). In fact, the species *Hydrozoanthus antumbrosus* (Swain, 2009b) and *Zibrowius primnoidus* (Carreiro-Silva *et al.*, 2010) were both originally described in the genus *Isozoanthus* based on available ecological and morphological data. The type species of the genus is *Isozoanthus giganteus* Carlgren in Chun, 1903, and this species is not associated with stalked glass-sponges, but it is a large solitary species (Carlgren, 1938). Moreover, the phylogenetic position of *I. giganteus* is closer to Epizoanthidae than to that of Parazoanthidae,

although comparatively phylogenetically distant from all other zoantharians (Swain, 2010, 2018). Thus, we consider that the phylogenetic clade of Parazoanthidae specimens found on stalks of hexactinellid sponges are probably distinct from *Isozoanthus* and represent an as-of-yet undescribed group. Further molecular and morphological analyses with specimens from this clade are needed to clarify the taxonomy of this group.

The new genera, *Churabana* and *Vitrumanthus*, are genera consisting of Hexasterophora sponge-associated zoantharian species. In comparison to *Churabana*, *Vitrumanthus* is a geographically widespread genus and its host range is extensive. Such geographic and ecological diversity patterns may drive diversification within *Vitrumanthus*, although the diversity of Hexasterophora sponge-associated zoantharian remains to be explored further.

The phylogenetic results correspond with their marginal musculatures: the position of *Churabana* is relatively closer to demosponge-associated zoantharian genera (*Bergia*, *Parazoanthus* and *Umimayanthus*) and they share analogous marginal musculatures (cteniform endodermal arrangement), while *Vitrumanthus* has cyclically transitional marginal musculature, as has been reported in specimens of *Corallizoanthus* and *Savalia* (Swain *et al.*, 2015). Thus, these findings clearly support the importance of marginal musculature characters for use in the higher taxonomy of Zoantharia, as has been recently emphasized by Swain & Swain (2014) and Swain *et al.* (2015).

Although the taxonomic position of *Thoracactis topsenti* is still not clear, *Thoracactis* and the two new genera in this study can be easily distinguished from each other by their external morphology. *Thoracactis topsenti* has an obligate association with the Hexasterophora sponge *Sarostegia oculata*, and polyps of *T. topsenti* are completely embedded in *S. oculata* and surrounded by sponge ectosomal formations (Topsent, 1904; Gravier, 1918). On the other hand, only the bases of polyps of *Churabana* and *Vitrumanthus* are embedded in host hexactinellid sponges, but in this study, no *Thoracactis* specimens were available for examination, and thus further studies are necessary to determine the exact taxonomic position of *T. topsenti*.

#### THE ORIGIN AND EVOLUTION OF HEXACTINELLID SPONGE-ASSOCIATED ZOANTHARIANS

Our results do not support a single origin for the symbioses between hexactinellid sponges and zoantharians. Phylogenetic analyses presented here suggest at least two independent origins. Although Amphidiscophora sponge-associated *Epizoanthus* and Parazoanthidae can be easily distinguished from each other by their marginal musculatures, the appearance of the columns and mesoglea of both

groups are similar (H. Kise, M. Ekins and J. D. Reimer unpubl. data). This similarity may reflect convergent adaptations associated with similar environmental constraints in the sponge-zoantharian associations of *Epizoanthus* and Parazoanthidae species. According to our analyses, Hexasterophora sponge symbioses appear to have been independently gained in Parazoanthidae (Fig. 2).

The new taxa described in this work confirm that the deep-sea harbours high levels of undescribed zoantharian diversity, as has been recently speculated (Sinniger *et al.*, 2013; Carreiro-Silva *et al.*, 2017; Reimer *et al.*, 2019). More than 60 zoantharian species have been described from the deep-sea (e.g. Hertwig, 1888; Lwowsky, 1913; Carlgren, 1923; Sinniger *et al.*, 2013; Carreiro-Silva *et al.*, 2017). However, other recent work has clearly highlighted the lack of information on deep-sea zoantharian data (Reimer *et al.*, 2020) and even small numbers of new specimens may yet have a great influence on our understanding of the diversity of this early diverging hexacorallian order. The present research also demonstrates that museum collections may contain important specimens of associated zoantharian species that await discovery, and that a continued study of this material would be a welcome addition to field sampling in order to obtain a more complete image of the zoantharian faunal composition in various regions around the world, in both shallow and in deep waters (Reimer *et al.*, 2014, 2015; Montenegro *et al.*, 2020). With their wide range of associations, zoantharians can serve as an important model marine invertebrate group to understand how host associations have evolved and changed over time. The current research clarifies multiple independent origins of zoantharian-sponge associations in the deep sea. The repeated evolution of similar associations across evolutionary time suggests common molecular symbiotic mechanisms or pathways within zoantharians that should be examined in the future.

#### ACKNOWLEDGEMENTS

We are grateful to Drs Kensuke Yanagi (Coastal Branch of Natural History Museum and Institute, Chiba) and Daisuke Uyeno (Kagoshima University), as well as Messrs Mamoru Sekifuji and Hisanori Kohtsuka (Misaki Marine Biological Station, The University of Tokyo) for providing valuable specimens. We thank Dr Tim O'Hara (CSIRO Marine National Facility) and the crew and scientific staff from the IN2017\_V03 cruise on the RV *Investigator*.

Project funding for collection on the RV *Investigator* was provided by the Marine Biodiversity Hub, supported through the Australian Government's National Environmental Science Program (NESP). The material was lawfully collected under the following permits: (1)

Access to Biological Resources in a Commonwealth Area for Non-Commercial Purposes, AU-COM2017-352; (2) Approval for Activity in a Commonwealth Marine Reserve, CMR-17-000455; (3) Australian Fisheries Management Authority Scientific Permit, 100339+3; (4) Queensland Government General Fisheries Permit, 191670. Permission for sampling during the CANCAP-VII Expedition at the Cape Verde Islands in 1986 was granted by the Portuguese government (Van der Land, 1987). BWH is indebted to Adriaan 'Dutch' Schrier, Laureen Schenk and the crews of the *Curasub* and *R/V Chapman*, based at Substation Curaçao, for their generosity and help during the collecting of specimens from deep water, and the use of photographs taken during earlier submersible surveys. This publication is Ocean Heritage Foundation/Curaçao Sea Aquarium/Substation Curaçao contribution #OHF/CSA/SC43. We thank JAMSTEC, the crew of the *RV Natsushima* and *ROV Hyper-Dolphin* operators who made expedition NT11-21 possible. We are also grateful to Annie Evankow (Northeastern University) for assistance in searching images of the Okeanos Explorer expedition. Mr Honmei Kishimoto of *Daini-kuroshio-maru* and the Motobu Fisheries Cooperative are thanked for their assistance in specimen collection from Okinawa, Japan. The first author thanks Dr Akihiro Takemura (UR) for allowing us to use a microtome. The first author was supported by the Sasakawa Scientific Research Grant from the Japan Science Society grant number 2018–5021, Japan Society for the Promotion of Science grant number 19J12174 and the JAMBIO Coastal Organism Joint Survey. The second author was supported by a Martin Fellowship from Naturalis Biodiversity Center to visit and analyse relevant specimens in the Porifera collection. The senior author was supported by Temminck Fellowships from Naturalis Biodiversity Center to visit and analyse relevant specimens in the Zoantharia collection and to conduct field work in the Caribbean.

#### DATA AVAILABILITY

All alignment datasets underlying this article are available at the Figshare repository at <https://dx.doi.org/10.6084/m9.figshare.14616657>.

#### REFERENCES

- Andres A. 1884.** Le Attinie. *Fauna und Flora des Golfes von Neapel* **9**: 1–459.
- Apakupakul K, Siddall ME, Burreson EM. 1999.** Higher level relationships of leeches (Annelida: Clitellata: Euhirudinea) based on morphology and gene sequences. *Molecular Phylogenetics and Evolution* **12**: 350–359.
- Beaulieu SE. 2001.** Life on glass houses: sponge stalk communities in the deep sea. *Marine Biology* **138**: 803–817.
- Bigatti G. 2015.** Marine Invertebrate from Argentina, Uruguay and Chile. v1.4. ArOBIS Centro Nacional Patagónico. Puerto Madryn, Chubut, Argentina. Dataset/Occurrence. <http://arobis.cenpat-conicet.gob.ar:8081/resource?r=arobis-marineinvertebrate&v=1.4> <https://doi.org/10.15468/xntwha> accessed via GBIF.org (accessed on 2020-10-01).
- Buhl-Mortensen L, Serigstad B, Buhl-Mortensen P, Olsen MN, Ostrowski M, Błażewicz-Paszkowycz M, Appoh E. 2017.** First observations of the structure and megafaunal community of a large *Lophelia* reef on the Ivorian-Ghanaian margin (the Gulf of Guinea). *Deep Sea Research Part II: Topical Studies in Oceanography* **137**: 148–156.
- Burnett WJ. 2002.** Longitudinal variation in algal symbionts (zooxanthellae) from the Indian Ocean zoanthid *Palythoa caesia*. *Marine Ecology Progress Series* **234**: 105–109.
- Carlgrén O. 1923.** Ceriantharia und zoantharia der deutschen tiefsee-expedition. Zoantharia. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer 'Valdivia' 1898–1899* **19**: 252–337.
- Carlgrén O. 1938.** South African Actiniaria and Zoantharia. *Kungliga Svenska Vetenskapsakademiens Handlingar* **17**(3): 1–148.
- Carreiro-Silva M, Braga-Henriques A, Sampaio I, de Matos V, Porteiro FM, Ocaña O. 2010.** *Isozoanthus primnooides*, a new species of zoanthid (Cnidaria: Zoantharia) associated with the gorgonian *Callogorgia verticillata* (Cnidaria: Alcyonacea). *ICES Journal of Marine Science* **68**: 408–415.
- Carreiro-Silva M, Ocaña O, Stanković D, Sampaio Í, Porteiro FM, Fabri MC, Stefanni S. 2017.** Zoantharians (Hexacorallia: Zoantharia) associated with cold-water corals in the Azores region: new species and associations in the deep sea. *Frontiers in Marine Science* **4**: 1–21.
- Chen CA, Wallace CC, Wolstenholme J. 2002.** Analysis of the mitochondrial 12S rRNA gene supports a two-clade hypothesis of the evolutionary history of scleractinian corals. *Molecular Phylogenetics and Evolution* **23**: 137–149.
- Chun C. 1903.** *Aus den Tiefen des Weltmeeres Schilderungen von der Deutschen Tiefsee-Expedition*. Jena: Gustav Fisher.
- Delage Y, Hérouard E. 1901.** Zoanthidés – Zoanthidea. In: *Traité de Zoologie concrète. Tome II – 2me Partie. Les Coelentérés*. Paris: C. Reinwald, **654–667**. Available at: <https://gallica.bnf.fr/ark:/12148/bpt6k5597996v.texteImage>
- Dendy A. 1916.** Report on the Hexactinellid sponges (Triaxonida) collected by H.M.S. 'Sealark' in the Indian Ocean. *Transactions of the Linnean Society of London* **17**: 211–224.
- Dohrmann M, Janussen D, Reitner J, Collins AG, Worheide G. 2008.** Phylogeny and evolution of glass sponges (porifera, hexactinellida). *Systematic Biology* **57**: 388–405.
- Dohrmann M, Göcke C, Janussen D, Reitner J, Lüter C, Wörheide G. 2011.** Systematic and spicule evolution in dictyonal sponges (Hexactinellida: Sceptrulophora) with description of two new species. *Zoological Journal of the Linnean Society* **163**: 1003–1025.

- Duchassaing P, Michelotti J. 1860.** Mémoire sur les coralliaires des Antilles. *Memorie della Reale Accademia delle Scienze di Torino Serie Seconda* **23**: 270–365.
- Dueñas LF, Puentes V. 2018.** *Aportes al conocimiento de la biodiversidad de aguas profundas del mar Caribe colombiano.* Anadarko Colombia Company, Bogotá, Colombia. Occurrence dataset <https://doi.org/10.15472/kr6lqa> accessed via GBIF.org (accessed on 2020-10-01).
- Duerden JE. 1903.** West Indian sponge incrusting actinians. *Bulletin of the American Museum of Natural History* **19**: 495–503.
- Ehrenberg CG. 1834.** Beiträge zur physiologischen Kenntniss der Corallenthiere im allgemeinen, und besonders des rothen Meeres, nebst einem Versuche zur physiologischen Systematik derselben. *Abhandlungen der Koniglichen Akademie der Wissenschaften in Berlin* **1832**: 225–380.
- England KW. 1991.** Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* **216**: 691–697.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994.** DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* **3**: 294–299.
- Fujii T, Reimer JD. 2011.** Phylogeny of the highly divergent zoanthid family Microzoanthidae (Anthozoa, Hexacorallia) from the Pacific. *Zoologica Scripta* **40**: 418–431.
- Gravier C. 1918.** Note sur une actinie (\**Thoracactis*\* n. g., \**topsenti*\* n. sp.) et un annélide polychète (\**Hermadion Fauveli*\* n. sp.), commensaux d'une Éponge siliceuse (\**Sarostegia oculata*\* Topsent). *Bulletin de l'Institut océanographique de Monaco* **344**: 1–20.
- Gray JE. 1832.** *Synopsis of the contents of the British Museum, 27th edn, Vol 4.* London: British Museum, 1–212.
- Gray JE. 1858.** On *Aphrocallistes*, a new genus of Spongiadae from Malacca. *Proceedings of the Zoological Society of London* **26**: 114–115.
- Gray JE. 1867.** Notes on Zoanthinae, with descriptions of some new genera. *Proceedings of the Zoological Society of London* **15**: 233–240.
- Haddon AC, Shackleton AM. 1891a.** A revision of the British Actiniae. Part II. The Zoantheae. *Scientific Transactions of the Royal Dublin Society* **4**: 609–672.
- Haddon AC, Shackleton AM. 1891b.** Actiniae: I. Zoantheae. In: Reports on the zoological collections made in the Torres Straits by Professor A.C. Haddon, 1888–1889. *Scientific Transactions of the Royal Dublin Society* **4**: 673–701, pls 61–64.
- Haeckel E. 1896.** Systematische Phylogenie der wirbellosen Thiere (*Invertebrata*). zweiter theil. *Des Entwurfs einer systematischen Stammesgeschichte.* Berlin: G. Reimer.
- Hajdu E, Castello-Branco C, Lopes DA, Sumida PYG, Perez JAA. 2017.** Deep-sea dives reveal an unexpected hexactinellid sponge garden on the Rio Grande Rise (SW Atlantic). A mimicking habitat? *Deep Sea Research Part II Topical Studies in Oceanography* **146**: 93–100.
- Hatschek B. 1888.** *Lehrbuch der Zoologie: eine morphologische Übersicht des Thierreiches zur Einführung in das Studium dieser Wissenschaft.* Jena: Gustav Fischer.
- Hertwig R. 1882.** Report on the Actiniaria dredged by H.M.S. 'Challenger' during the years 1873–1876. In: Thomson CW, Murray J, eds. *Report on the scientific results of the voyage of H.M.S. Challenger during the years 1873–76.* *Zoology* **6**: 1–136.
- Hertwig R. 1888.** Report on the Actiniaria dredged by H.M.S. 'Challenger' during years 1873–1876. Supplement. In: Thomson CW, Murray J, eds. *Report on the scientific results of the voyage of H.M.S. Challenger during the years 1873–76.* *Zoology* **26**: 39–41.
- ICZN 1992.** Opinion 1689. *Epizoanthus* Gray, 1867 (Cnidaria, Anthozoa): conserved. *Bulletin of Zoological Nomenclature* **49**: 236–237.
- Ijima I. 1927.** Hexactinellida of the Siboga expedition. In: Weber M, ed. *Uitkomsten op zoologisch, botanisch, oceanographisch en geologisch gebied verzameld in Nederlandsch Oost-Indië 1899–1900, 6.* Leiden: Brill, 1–138.
- Johnston G. 1842.** *A history of British sponges and lithophytes.* Edinburgh, London & Dublin: Publisher
- Kahn AS, Pennelly CW, McGill PR, Leys SP. 2020.** Behaviors of sessile benthic animals in the abyssal northeast Pacific Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* **173**: 104729.
- Katoh K, Standley DM. 2013.** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**: 772–780.
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012.** Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics (Oxford, England)* **28**: 1647–1649.
- Kise H, Reimer JD. 2016.** Unexpected diversity and a new species of *Epizoanthus* (Anthozoa, Hexacorallia) attached to eunicid worm tubes from the Pacific Ocean. *ZooKeys* **562**: 49–71.
- Kise H, Reimer JD. 2019.** A new epizoanthus species (Cnidaria: Anthozoa: Epizoanthidae) associated with the gastropod mollusk *Guildfordia triumphans* from southern Japan. *Zoological Science* **36**: 259–265.
- Kise H, Dewa N, Reimer JD. 2018.** First record of sea urchin-associated *Epizoanthus planus* from Japanese waters and its morphology and molecular phylogeny. *Plankton and Benthos Research* **13**: 136–141.
- Kise H, Maeda T, Reimer JD. 2019.** A phylogeny and the evolution of epizoism within the family Hydrozoanthidae with description of a new genus and two new species. *Molecular Phylogenetics and Evolution* **130**: 304–314.
- Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019.** RAXML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics (Oxford, England)* **35**: 4453–4455.
- Lamouroux JVF. 1816.** *Histoire des Polypiers coralligenes flexibles, vulgairement nommés zoophytes.* Caen: F. Poisson.
- Leys SP, Mackie GO, Reisinger HM. 2007.** The biology of glass sponges. *Advances in Marine Biology* **52**: 1–145.



- Low MEY, Sinniger F, Reimer JD. 2016.** The order Zoantharia Rafinesque, 1815 (Cnidaria, Anthozoa: Hexacorallia): supraspecific classification and nomenclature. *ZooKeys* **641**: 1–80.
- Lwowsky FF. 1913.** Revision der gattung *Sidisia* (*Epizoanthus* auct.), ein beitrag zur kenntnis der zoanthiden. *Zoologische Jahrbücher (Systematik)* **34**: 557–613.
- Medlin L, Elwood HJ, Stickel S, Sogin ML. 1988.** The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**: 491–499.
- Milne I, Lindner D, Bayer M, Husmeier D, McGuire G, Marshall DF, Wright F. 2009.** TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics (Oxford, England)* **25**: 126–127.
- Montenegro J, Sinniger F, Reimer JD. 2015.** Unexpected diversity and new species in the sponge-Parazoanthidae association in southern Japan. *Molecular Phylogenetics and Evolution* **89**: 73–90.
- Montenegro J, Hoeksema BW, Santos MEA, Kise H, Reimer JD. 2020.** Zoantharia (Cnidaria: Hexacorallia) of the Dutch Caribbean with historical distribution records from the Atlantic and one new species of *Parazoanthus*. *Diversity* **12**(5): 190.
- NIWA. 2018.** NIWA Invertebrate collection, v.1.1. The National Institute of Water and Atmospheric Research (NIWA). Dataset/Occurrence. <https://nzobisipt.niwa.co.nz/resource?r=obisspecify&v=1.1> (accessed on 2020-09-15).
- Pei Z. 1998.** *Fauna Sinica. Coelenterata: Actiniaria, Ceriantharia, Zoanthidea*. Beijing: Science Press, 200–201.
- Rafinesque CS. 1815.** *Analyse de la nature ou tableau de l'univers et des corps organisés*. Palermo: Publisher, 224.
- Rasband WS. 2012.** ImageJ: image processing and analysis in Java. *Astrophysics Source Code Library* **1**: 06013.
- Reimer J, Sinniger F. 2021.** World List of Zoantharia. Zoantharia. Available at: World Register of Marine Species: <http://marinespecies.org/aphia.php?p=taxdetails&id=607338> (accessed on 2020-10-01)..
- Reimer JD, Takishita K, Ono S, Maruyama T. 2007.** Diversity and evolution in the zoanthid genus *Palythoa* (Cnidaria: Hexacorallia) based on nuclear ITS-rDNA. *Coral Reefs* **26**: 399–410.
- Reimer JD, Nonaka M, Sinniger F, Iwase F. 2008.** Morphological and molecular characterization of a new genus and new species of parazoanthid (Anthozoa: Hexacorallia: Zoantharia) associated with Japanese Red Coral. *Coral Reefs* **27**: 935–949.
- Reimer JD, Hirose M, Wirtz P. 2010.** Zoanthids of the Cape Verde Islands and their symbionts: previously unexamined diversity in the northeastern Atlantic. *Contributions to Zoology* **79**: 147–163.
- Reimer JD, Obuchi M, Irei Y, Fujii T, Nozawa Y. 2011.** Shallow water brachycnemic zoanthids (Cnidaria: Hexacorallia) from Taiwan: a preliminary survey. *Zoological Studies* **50**: 363–371.
- Reimer JD, Poliseno A, Hoeksema BW. 2014.** Shallow-water zoantharians (Cnidaria, Hexacorallia) from the Central Indo-Pacific. *ZooKeys* **444**: 1–57.
- Reimer JD, Wee HB, Ang P, Hoeksema BW. 2015.** Zoantharia (Cnidaria: Anthozoa: Hexacorallia) of the South China Sea and Gulf of Thailand: a species list based on past reports and new photographic records. *Raffles Bulletin of Zoology* **63**: 334–356.
- Reimer JD, Kise H, Santos MEA, Lindsay DJ, Pyle RL, Copus JM, Bowen BW, Nonaka M, Higashiji T, Benayahu Y. 2019.** Exploring the biodiversity of understudied benthic taxa at mesophotic and deeper depths: examples from the order Zoantharia (Anthozoa: Hexacorallia). *Frontiers in Marine Science* **6**: 305.
- Reimer JD, Yanagi K, Kise H, Poliseno A, Kushida Y, Saeedi H, Lindsay D. 2020.** Review of deep-sea cnidaria and ctenophora in the NW Pacific Ocean. In: Saeedi H, Brandt A, eds. *Biogeographic atlas of the deep NW Pacific fauna*. Sofia: Pensoft Publishers, 67–88.
- Reiswig HM, Dohrmann M. 2014.** Three new species of glass sponges (Porifera: Hexactinellida) from the West Indies, and molecular phylogenetics of Euretidae and Auloplacidae (Sceptrulophora). *Zoological Journal of the Linnean Society* **171**: 233–253.
- Reiswig HM, Wheeler B. 2002a.** Family Euretidae Zittel, 1877. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Plenum, 1301–1331.
- Reiswig HM, Wheeler B. 2002b.** Family Tretodictyidae Schulze, 1886. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera. A guide to the classification of sponges*. New York: Plenum, 1341–1354.
- Ronquist F, Huelsenbeck JP. 2003.** MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* **19**: 1572–1574.
- Ryland JS, Lancaster JE. 2004.** A review of zoanthid nematocyst types and their population structure. *Hydrobiologia* **530**: 179–187.
- Sanamyan NP, Sanamyan KE, Tabachnick KR. 2012.** The first species of Actiniaria, *Spongeactis japonica* gen. n. (Cnidaria: Anthozoa), an obligate symbiont of a glass sponge. *Invertebrate Zoology* **9**: 127–141.
- Schmidt O. 1870.** *Grundzüge einer Spongien-Fauna des atlantischen Gebietes*. Leipzig: W. Engelmann, iii–iv, 1–88.
- Schmidt O. 1880.** Die Spongien des meerbusen von Mexico (Und des caraibischen Meeres). Heft II. Abtheilung II. Hexactinelliden. Abtheilung III. Tetractinelliden. Monactinelliden und Anhang. Nachträge zu Abtheilung I (Lithistiden). In: *Reports on the dredging under the supervision of Alexander Agassiz, in the Gulf of Mexico, by the USSCS 'Blake'*. Jena: Gustav Fischer, 33–90.
- Schultze MJS. 1860.** *Die Hyalonemen. Ein Beitrag zur Naturgeschichte der Spongien*. Bonn: A. Marcus, i–iii, 1–46.
- Sinniger F, Häussermann V. 2009.** Zoanthids (Cnidaria: Hexacorallia: Zoantharia) from shallow waters of the southern Chilean fjord region, with descriptions of a new genus and two new species. *Organisms Diversity & Evolution* **9**: 23–36.
- Sinniger F, Montoya-Burgos JI, Chevaldonne P, Pawlowski J. 2005.** Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. *Marine Biology* **147**: 1121–1128.

- Sinniger F, Reimer JD, Pawlowski J. 2010.** The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: description of two new genera. *Marine Biodiversity* **40**: 57–70.
- Sinniger F, Ocaña OV, Baco AR. 2013.** Diversity of zoanthids (Anthozoa: Hexacorallia) on Hawaiian seamounts: description of the Hawaiian gold coral and additional zoanthids. *PLoS One* **8**: e52607.
- Swain TD. 2009a.** *Isozoanthus antumbrosus*, a new species of zoanthid (Cnidaria: Anthozoa: Zoanthidea) symbiotic with Hydrozoa from the Caribbean, with a key to hydroid and sponge-symbiotic zoanthid species. *Zootaxa* **2051**: 41–48.
- Swain TD. 2009b.** Phylogeny-based species delimitations and the evolution of host associations in symbiotic zoanthids (Anthozoa, Zoanthidea) of the wider Caribbean region. *Zoological Journal of Linnean Society* **156**: 223–238.
- Swain TD. 2010.** Evolutionary transitions in symbioses: dramatic reductions in bathymetric and geographic ranges of Zoanthidea coincide with loss of symbioses with invertebrates. *Molecular Ecology* **19**: 2587–2598.
- Swain TD. 2018.** Revisiting the phylogeny of Zoanthidea (Cnidaria: Anthozoa): Staggered alignment of hypervariable sequences improves species tree inference. *Molecular Phylogenetics and Evolution* **118**: 1–12.
- Swain TD, Swain LM. 2014.** Molecular parataxonomy as taxon description: examples from recently named Zoanthidea (Cnidaria: Anthozoa) with revision based on serial histology of microanatomy. *Zootaxa* **3796**: 81–107.
- Swain TD, Schellinger JL, Strimaitis AM, Reuter KE. 2015.** Evolution of anthozoan polyp retraction mechanisms: convergent functional morphology and evolutionary allometry of the marginal musculature in order Zoanthidea (Cnidaria: Anthozoa: Hexacorallia). *BMC Evolutionary Biology* **15**: 123.
- Topsent E. 1904.** *Sarostegia oculata*, Hexactinellide nouvelle des îles du Cap-Vert. *Bulletin du Musée Océanographique de Monaco* **10**: 1–8.
- Topsent E. 1928.** Sur deux Eurétides du Japon. (Note préliminaire). *Bulletin de l'Institut océanographique de Monaco* **515**: 1–4.
- Van der Land J. 1987.** Report on the CANCAP-project for marine biological research in the Canarian – Cape Verdean region of the North Atlantic Ocean. Part i. list of stations. *Zoologische Verhandlungen* **243**: 3–94.
- Van Soest RW, Meesters EH, Becking LE. 2014.** Deep-water sponges (Porifera) from Bonaire and Klein Curaçao, Southern Caribbean. *Zootaxa* **3878**: 401–443.
- Wassilieff A. 1908.** Japanische actinien, in Doflein. *Abhandlungen der Mathematisch-Physikalischen Klasse der Königlich Bayerischen Akademie der Wissenschaften* **1**: 1–49.
- West DA. 1979.** Symbiotic zoanthids (Anthozoa: Cnidaria) of puerto rico. *Bulletin of Marine Science* **29**: 253–271.
- Williams RB. 2000.** A redescription of the zoanthid *Isozoanthus sulcatus* (Gosse, 1859), with notes on its nomenclature, systematics, behavior, habitat and geographical distribution. *Ophelia* **3**: 193–206.
- Zapalski MK, Kise H, Dohnalik M, Yoshida R, Izumi T, Reimer JD. 2021.** Hexacoral-crinoid associations from the modern mesophotic zone: ecological analogues for Palaeozoic associations. *Palaeogeography, Palaeoclimatology, Palaeoecology* **572**: 110419.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

**Table S1.** Description of PCR amplification and corresponding primers used in this study.

**Table S2.** GenBank accession numbers used for phylogenetic analyses in this study.

**Table S3.** Diversity of symbiotic interactions between hexactinellid sponges and zoantharians. Morphological characteristics and distribution of hexactinellid sponges-associated zoantharians are listed in the table.