The LINNEAN SOCIETY of London ZOOLOGICAL Journal of the Linnean Society

*Zoological Journal of the Linnean Society*, 2013, **169**, 509–522. With 5 figures

# A new family of diminutive zooxanthellate zoanthids (Hexacorallia: Zoantharia)

TAKUMA FUJII[1]* and JAMES DAVIS REIMER[2,3]*

[1]*Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Sciences, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*
[2]*Molecular Invertebrate Systematics and Ecology Laboratory, Rising Star Program, Trans-disciplinary Organization for Subtropical Island Studies, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*
[3]*Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

*Received 9 April 2013; revised 25 July 2013; accepted for publication 30 July 2013*

A new family, genus, and species of zooxanthellate macrocnemic zoanthid is described from Okinawa, Japan. The diminutive zoanthid ***Nanozoanthus harenaceus* sp. nov.** occurs in sandy 'pools' upon hard substrates in coral reefs. The results of molecular phylogenetic analyses of mitochondrial *16S* ribosomal DNA and cytochrome *c* oxidase subunit I suggests that **Nanozoanthidae fam. nov.** is genetically close to family Microzoanthidae and *Isozoanthus sulcatus* at the intrafamily–suborder level. The **Nanozoanthidae fam. nov.**–Microzoanthidae clade is clearly highly divergent from all other known zoanthid families and from the order Actiniaria at the suborder level or higher. These results demonstrate that much high-level (e.g. above genus) diversity remains to be described within the order Zoantharia, and until such work is complete it will be difficult to completely understand their biodiversity.


doi: 10.1111/zoj.12075

ADDITIONAL KEYWORDS: *16S* – *COI* genetics – mtDNA – new classification – phylogenetics – phylogeny – taxonomy.

## INTRODUCTION

Zoanthids (Phylum Cnidaria; Class Anthozoa; Subclass Hexacorallia; Order Zoantharia = Zoanthidea) are marine benthic animals distributed widely throughout the world's oceans. The species diversity of this order has historically been poorly known because of the difficult species-level identification based on morphology. Zoanthid polyps do not have many useful diagnostic morphological characters, as they show a remarkable similarity within the order (Carlgren, 1913; Swain, 2010; Reimer *et al*., 2011). Furthermore, most zoanthids do not produce skeletons or sclerites as in other hexacorallian orders such as Scleractinia or Antipatharia. Further compounding the accurate identification is that it is often difficult to observe the internal morphology of zoanthids as they have the unique character of encrusting sand particles into their body wall (Haywick & Muller, 1997). These hard particles often make histological observations impossible (Reimer *et al*., 2010b). Moreover, high levels of morphological plasticity within some species have been demonstrated in recent years, and this may also contribute to taxonomic confusion (Burnett *et al*., 1994, 1997; Reimer *et al*., 2004; Ong, Reimer & Todd, 2013).

In recent years molecular techniques have helped advance the taxonomy and phylogeny of marine

*Corresponding author. E-mail: takumagnum0213@hotmail.co.jp; jreimer@sci.u-ryukyu.ac.jp

invertebrates. In Zoantharia, at least three new families and ten new genera have been described during last 10 years, in large part from molecular phylogenetic analyses (e.g. Reimer *et al*., 2007; Sinniger, Reimer & Pawlowski, 2008; Fujii & Reimer, 2011; Sinniger, Ocaña & Baco, 2013). Current biodiversity studies using molecular analyses suggest zoanthid species diversity may be higher in tropical and subtropical areas (Swain, 2009); however, much more research is required to fully understand zoanthid diversity, particularly in under-examined regions such as the coral reefs of the Indo-Pacific.

Zooxanthellate animals (e.g. hard corals, soft corals, anemones, zoanthids, foraminifers, and giant clams) that are symbiotic with photosynthetic dinoflagellates of the genus *Symbiodinium* are major components of coral reef ecosystems, and many ecological studies have focused on these symbiotic algae. Zooxanthellate zoanthids are very common in shallow subtropical and tropical waters (Sinniger, 2006; Reimer, 2010). Zooxanthellate zoanthids have primarily been reported from the suborder Brachycnemina, which consists of three families (Sphenopidae Hertwig, 1882; Zoanthidae Rafinesque, 1815; and Neozoanthidae Herberts, 1972). Additionally, some species of suborder Macrocnemina are also zooxanthellate, and all zooxanthellate macrocnemic species are currently known only from the Atlantic (Manuel, 1979; Swain, 2009). The suborder Brachycnemina is currently considered to be a monophyletic group within Zoantharia based on both morphological and molecular studies, in contrast to polyphyletic suborder Macrocnemina (Sinniger *et al*., 2005; Swain, 2010).

Herein we formally describe a new family, genus, and species of zooxanthellate macrocnemic zoanthid from Japan. The species is considered to be one of the smallest species in order Zoantharia ever described. This diminutive new species is unique in that the majority of each polyp (excepting the oral disc) is buried in small sandy patches in coral reef environments. It is likely that this new group was not discovered until now because of their cryptic habitat and tiny size (average polyp diameter = 1.1 mm). Aside from a formal description, we also provide information on the phylogenetic position of this new group within the order Zoantharia. These findings combined with the recent description of the family Microzoanthidae Fujii & Reimer 2011 have greatly widened our understanding of the total diversity of Zoantharia. These results further demonstrate that much marine biodiversity remains to be discovered, and we suggest that higher (suborder) level taxonomic reorganization within the order Zoantharia will have to be considered in the near future.

## MATERIAL AND METHODS

### Sample collection

Specimens from Okinawa Island, Japan (see Taxonomy), and Ningaloo Reef, Australia, were collected by scuba. As specimens were collected, *in situ* digital images were taken to assist in morphological analyses (oral disc and polyp diameter, colour, polyp form, etc.). The specimens collected were fixed and preserved in 99.5% ethanol. Some large colonies (colonies of more than six polyps) were subdivided into subsamples. Polyps of subsamples were relaxed with magnesium chloride ($MgCl_2$) and subsequently fixed in 5–10% seawater (SW) formalin to be used in observing cnidae and making anatomical sections for internal morphological analyses.

### Morphological analyses

The length of individual polyps and maximum column diameter of preserved specimens were measured using a caliper. The overall shape of colonies, colour of the polyps, and number and length of the tentacles were recorded from *in situ* images. Horizontal and vertical sections of polyps were made with paraffin embedding following the method described by Reimer *et al*. (2010b). After decalcification with Bouin's fluid for 24 h, sand and other detritus remaining on the surface of the column were removed as much as possible by tweezers under a dissecting microscope. The specimens were dehydrated through an ethanol–xylene series and then embedded in paraffin. Sections (8-μm thick) made by embedding were stained with haematoxylin and eosin.

### Cnidae

Undischarged cnidae were measured from tentacles, column, and mesenterial filaments of a polyp of the holotype. As only a few colonies could be obtained and because of the very small size of the polyps, only one polyp in total was examined. Moreover, the actinopharynx was too small to successfully isolate without contamination of cnidae from surrounding tissues. Images of the cnidae were obtained by differential interference contrast microscopy, and measured using the software ImageJ (National Institutes of Health, Bethesda, MD, USA). Cnidae nomenclature generally followed that of England (1991) and Ryland & Lancaster (2003); however, Schmidt (1974), Hidaka, Miyazaki & Yamazato (1987), and Hidaka (1992) have suggested basitrichs and microbasic b-mastigophores are the same type of nematocyst, and in this study the two types were treated as the same.

### DNA extraction and polymerase chain reaction amplification

DNA was extracted from ethanol-preserved specimens by following a guanidine extraction protocol (Sinniger, Reimer & Pawlowski, 2010). Polymerase chain reaction (PCR) amplifications were performed for mitochondrial cytochrome *c* oxidase subunit I (*COI*), mitochondrial *16S* ribosomal DNA (mt *16S* rDNA), and the internal transcribed spacer region of ribosomal DNA (*ITS* rDNA) using the primer pairs HCO and LCO (Folmer *et al*., 1994), 16SarmL (modified primer for mt 16S rDNA used in Sinniger *et al*., 2008, see Fujii & Reimer, 2011), and 16SBmoH (Sinniger *et al*., 2005), respectively. Amplified PCR products were sequenced by Fasmac (Atsugi, Kanagawa, Japan).

### Phylogenetic analyses

*Zoanthids*

New sequences obtained in this study were deposited in GenBank (accession numbers KF499599-KF499611; Table 1). The DNA sequences obtained were aligned using BIOEDIT 7.1.3.0 (Hall, 1999) and the attached application ClustalW, using default parameters (Thompson, Higgins & Gibson, 1994). The nucleotide sequences of mt *16S* rDNA and *COI* from the specimens were separately aligned with previously obtained zoanthid sequences from each zoanthid family (the GenBank accession numbers are given in the resulting phylogenetic trees). For out-groups, sequences of Actiniaria were used for both mt *16S* rDNA and *COI* trees. Indels were kept unedited in the alignments of mt *16S* rDNA. All phylogenetic alignments are available from the corresponding author. For phylogenetic analyses of mt *16S* rDNA and *COI*, the same methods were independently applied. The neighbour-joining (NJ) and maximum-likelihood (ML) methods were performed using MEGA 5 (Tamura *et al*., 2007), with 1000 replicates of bootstrapping for NJ, and 500 replicates for ML, performed using an input tree generated by BIONJ with the general time-reversible model (Rodriguez *et al*., 1990) of nucleotide substitution, incorporating invariable sites and a discrete gamma distribution (eight categories) (GTR + I + C). The proportion of invariable sites, a discrete gamma distribution, and base frequencies of the model were estimated from the data set. Bayesian trees were made by Mr Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003) under GTR + I + C. One cold and three heated Markov chain Monte Carlo (MCMC) analyses with default-chain temperatures were run for 10 million generations, sampling log-likelihoods (lnLs) and trees at 100-generation intervals (10 000 lnLs and trees were saved during the MCMC). The likelihood plots for *COI* and mt *16S* rDNA data sets suggest that

**Table 1. Nanozoanthidae fam. nov.** specimens examined in this study with GenBank accession numbers of cytochrome *c* oxidase subunit I and mitochondrial *16S* ribosomal DNA sequences

| Specimen number | Collection date | Location | Depth (m) | *COI* accession number | mt *16S* rDNA accession number | Species identification |
|---|---|---|---|---|---|---|
| RMNH Coel. 41502 (paratype3) | 24 Oct 2010 | Zatsun, Okinawa, Japan | 23 | KF499611 | KF499603 | ***Nanozoanthus hareneceus* sp. nov.** |
| TF101 | 17 Jun 2011 | Sunabe, Okinawa, Japan | 15 | KF499599 | KF499596 | ***Nanozoanthus hareneceus* sp. nov.** |
| USNM-1221444 (paratype1) | 21 Dec, 2011 | Cape Maeda, Okinawa, Japan | 22 | KF499600 | KF499597 | ***Nanozoanthus hareneceus* sp. nov.** |
| TF134 | 21 Dec, 2011 | Cape Maeda, Okinawa, Japan | 10 | NA | NA | ***Nanozoanthus hareneceus* sp. nov.** |
| NSMT-Co1555 (Holotype) | 15 Feb, 2012 | Cape Maeda, Okinawa, Japan | 9 | KF499609 | KF499601 | ***Nanozoanthus hareneceus* sp. nov.** |
| RUMF-2G-04372 (paratype2) | 13 Mar, 2012 | Oura Bay, Okinawa, Japan | 20 | KF499610 | KF499602 | ***Nanozoanthus hareneceus* sp. nov.** |
| TF143 | 20 Apr, 2012 | Oura Bay, Okinawa, Japan | 10 | NA | NA | ***Nanozoanthus hareneceus* sp. nov.** |
| YI-NR15 | 20 May 2010 | Ningaloo Reef, Australia | 5.5 | KF499606 | KF499604 | *Nanozoanthus* sp. |
| YI-NR16 | 20 May 2010 | Ningaloo Reef, Australia | 5 | KF499607 | KF499605 | *Nanozoanthus* sp. |

MCMC reached the stationary phase after the first 100 000 generations for *COI* and mt *16S* rDNA (standard deviation of split frequencies = 0.008085 and 0.0065, respectively). Thus, the remaining 9000 trees of *COI* and mt *16S* rDNA were used to obtain clade probabilities and branch-length estimates.

*Zooxanthellae*

For *Symbiodinium ITS* rDNA sequences, an alignment of 'clade C' (*sensu* LaJeunesse 2001) sequences was generated from alignments of previous *Symbiodinium* analyses (Reimer, Takishita & Maruyama, 2006a; Reimer *et al*., 2006b; Reimer, Hirose & Wirtz, 2010a) using zoanthid-associated zooxanthellae sequences plus sequences of other clade-C subclades from recent studies (the GenBank accession numbers are given in the resulting phylogenetic tree). The alignment consisted of primarily the second internal ribosomal space of ribosomal DNA (*ITS2*, which has been widely used in the identification of *Symbiodinium* types, e.g. LaJeunesse, 2002). An alignment of 315 sites of 25 taxa was generated, and is available upon request from the corresponding author.

Maximum-likelihood (ML) analysis with PhyML (Guindon *et al*., 2010) was performed using an input tree generated by BIONJ, with the general time-reversible model (Rodriguez *et al*., 1990) of nucleotide substitution incorporating invariable sites and eight categories. The proportion of invariable sites, discrete gamma distribution, and base frequencies of the model were estimated. A PhyML bootstrap tree (1000 replicates) was constructed using the same parameters. Distances were calculated using Kimura's two-parameter model (Kimura, 1980). Support for NJ branches was tested by bootstrap analysis (Felsenstein, 1985) of 1000 replicates in CLC Free Workbench 3.2.2 (Aarhus, Denmark).

## RESULTS

### SYSTEMATICS

#### NANOZOANTHIDAE FAM. NOV.

*Type genus*

*Nanozoanthus* gen. nov.

*Etymology*

As for the type genus, with ending as in other zoanthid families.

*Diagnosis*

Well-developed polyps connected by narrow stolon. Mineral particles encrusted in column from aboral end to the edge of the oral disc. Irregularly sized sand particles encrusted into ectoderm and slightly into mesoglea. Zigzagged, white-coloured pattern following outside edge of oral disc. Macrocnemic mesenterial arrangement. Sphincter muscle mesogleal. No lacunae or ring sinus. Zooxanthellate. Mitochondrial *COI* and *16S* ribosomal DNA sequences significantly differ from all other known zoanthid genera (Figs 1, 2).

*Remarks*

Only a few other macrocnemic zoanthids symbiotic with zooxanthellae are known, primarily from the genera *Parazoanthus* and *Isozoanthus*. It is easy to distinguish this family from these two genera by the position of sphincter muscle and by the phylogenetically highly divergent *COI* and mt *16S* rDNA sequences.

#### *NANOZOANTHUS* GEN. NOV.

*Type species*

*Nanozoanthus harenaceus* sp. nov.

*Etymology*

Named from the latin 'nano', meaning 'dwarf', as polyp size in specimens of this group are generally too small to clearly observe *in situ* with the naked eye, with ending as in other zoanthid genera. Gender is masculine.

*Diagnosis*

Only one genus of family Nanozoanthidae, as for family above.

#### *NANOZOANTHUS HARENACEUS* SP. NOV.

(FIGS 3A, B AND 4)

*Holotype*

Specimen number NSMT-Co-1555. Cape Maeda, Onna, Okinawa, Japan (26°26′36″ N, 127°46′22″ E), 9 m depth, collected by Takuma Fujii (T.F.), 15 February 2012, half of colony fixed in 5–10% formalin, other half of colony fixed in 99% EtOH, deposited in National Museum of Nature and Science, Tokyo, Japan (NSMT). GenBank accession numbers: mt *COI*, KF499609; *16S* rDNA, KF499601.

*Description of holotype*

Substrate of colony fragment of dead coral: dimensions 30 × 30 × 20 mm for formalin-fixed specimen; dimensions 30 × 20 × 15 mm for EtOH-fixed specimen. Polyps cylindrical, connected by narrow stolon. Height of polyps ($n$ = 37) 1.2–4.5 mm, diameter 0.4–1.8 mm. Mineral particles encrusted in column from aboral end to edge of oral disc.

*Paratypes (all from Japan)*

*Paratype 1:* Specimen number USNM-1221444. Cape Maeda, Okinawa, Japan (26°26′36″ N, 127°46′22″ E),

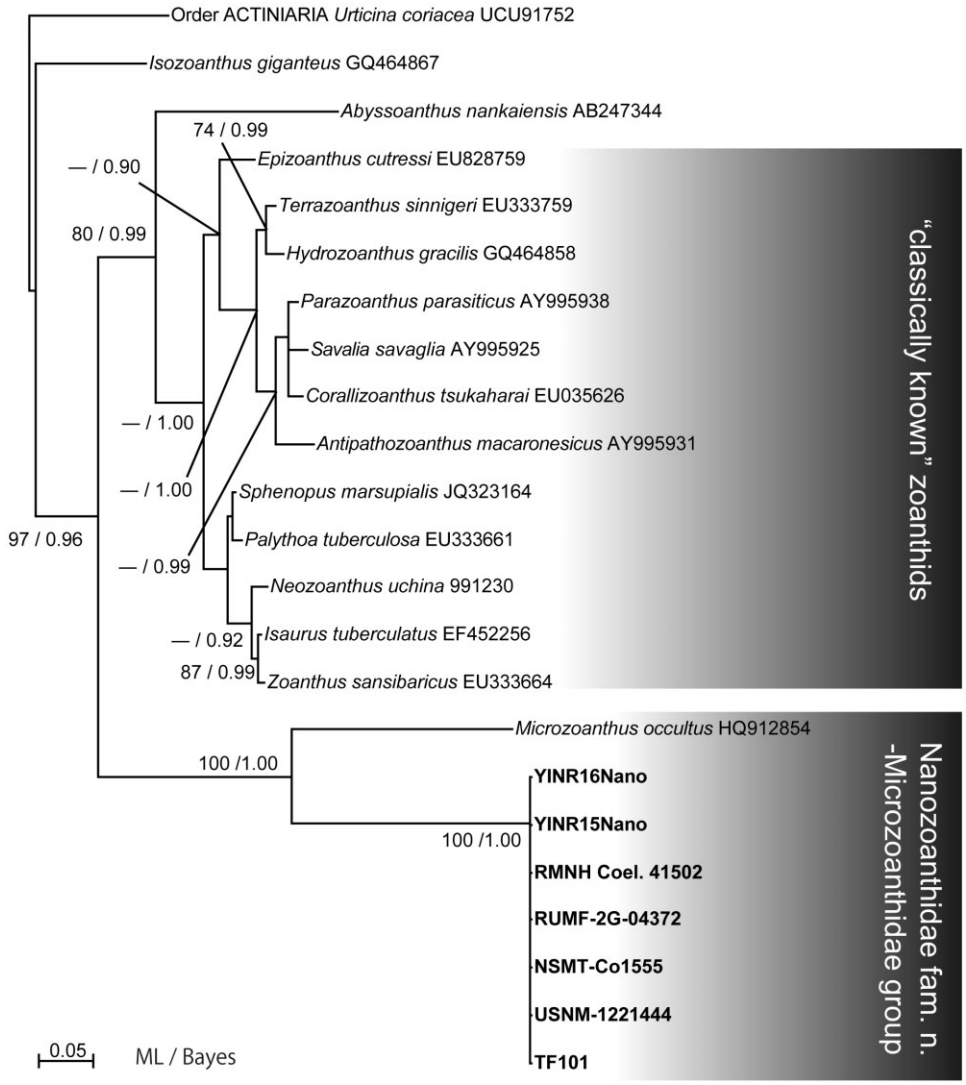



**Figure 1** Bayesian tree of mitochondrial *16S* ribosomal DNA for newly obtained sequences from zoanthid specimens in this study along with previously published GenBank sequences. Values of Bayesian posterior priorities > 0.95 and bootstrap values > 60% are shown at respective nodes. Sequences of **Nanozoanthidae fam. nov.** are set in bold. Species names of sequences from previous studies are set in normal (non-bold) font.

22 m depth, collected by T.F., 21 December 2011, half of colony fixed in 5–10% formalin, other half of colony fixed in 99% EtOH, deposited in National Museum of Natural History, Smithsonian Institution, Washington, D.C., USA (USNM).

*Paratype 2:* Specimen number RUMF-2G-04372. Oura Bay, Okinawa, Japan (26°32′16″ N, 128°4′48″ E), 20 m depth, collected by T.F., 13 March 2012, fixed by 99% EtOH, deposited in Ryukyu University Museum Fujukan, Okinawa, Japan (RUMF).

*Paratype 3:* Specimen number RMNH Coel. 41502. Zatsun, Okinawa, Japan (26°49′42″ N, 128°14′33″ E), 23 m depth. Collected by T.F., 24 October 2010, fixed by 99% EtOH, deposited in Naturalis Biodiversity Center, Leiden, the Netherlands (RMNH).

*Other materials*

Specimen number MISE-TF-101. Sunabe Beach, Okinawa, Japan, 15 m depth. Collected by T.F., 16 June 2011, half of colony fixed in 5–10% formalin, other half of colony fixed in 99% EtOH. Specimen number MISE-TF-143. Oura Bay, Okinawa, Japan. 10 m depth. Collected by T.F., 20 April 2012, fixed by 99% EtOH.

*Common name*

Kakure-sunaginchaku (new Japanese name), Okinawan nanozoanthid.

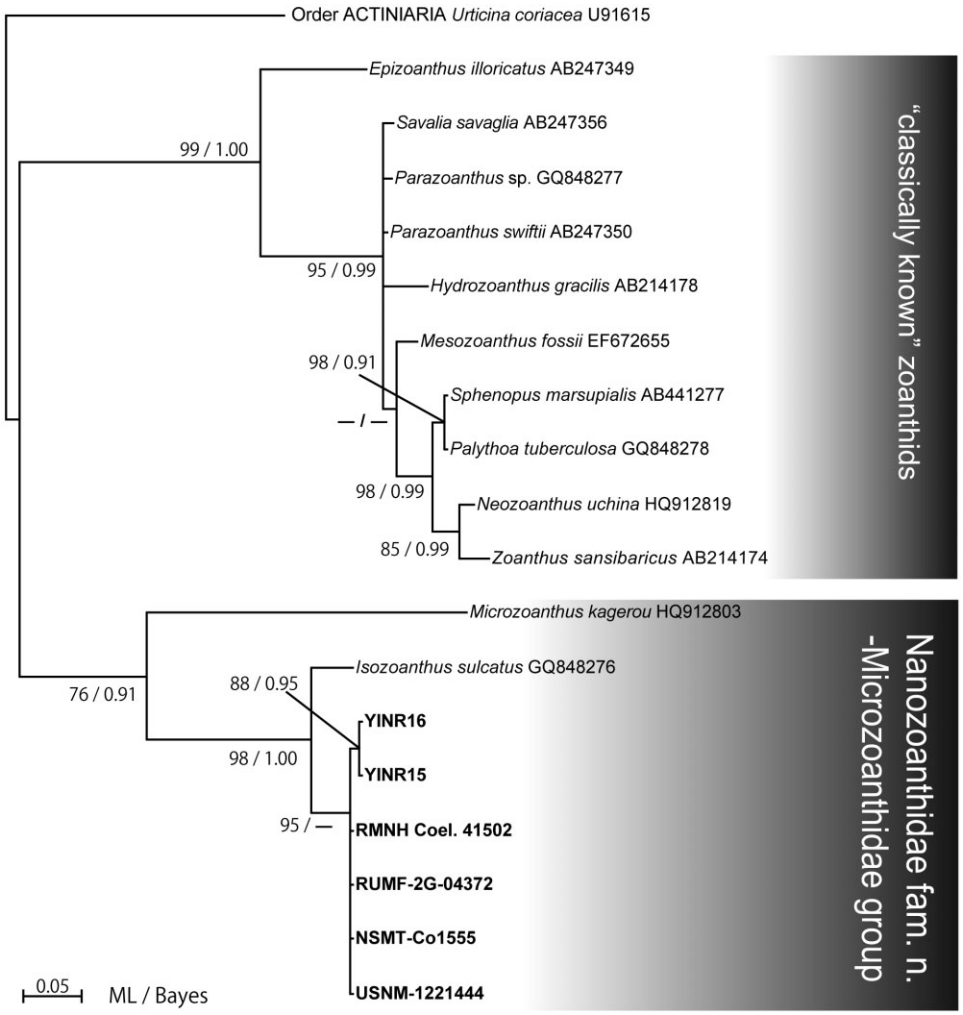



**Figure 2.** Bayesian tree of mitochondrial cytochrome *c* oxidase subunit I for newly obtained sequences from zoanthid specimens in this study along with previously published GenBank sequences. Values of Bayesian posterior priorities > 0.95 and bootstrap values > 60% are shown at respective nodes. Sequences of **Nanozoanthidae fam. nov.** are set in bold. Species names of sequences from previous studies are set in normal (non-bold) font.

*Diagnosis*

*Morphology:* Colonies with up to 20 well-developed polyps connected by narrow stolons. Polyps cylindrical. Polyp diameter up to 1.8 mm, average 1.1 mm ($n$ = 30 polyps from five colonies, standard variation = 0.38 mm), height up to 4.5 mm, average 2.35 mm ($n$ = 30 polyps from five colonies, standard variation = 0.96 mm). Between 16 and 20 tentacles ($n$ = 9 polyps), 1.0–2.5 times as long as diameter of oral disc. Oral disc forming concave depression. Distal tip of contracted polyps rounded. Mineral particles encrusted in column from aboral end to edge of oral disc. Irregularly sized particles encrusted into ectoderm and few particles in mesoglea. Tentacles transparent, with tiny brown dots visible upon magnification (= individual zooxanthellae). Zigzagged, white colour pattern following outside edge of oral disc. Oral disc brown, occasionally white.

*Internal morphology:* Macrocnemic. Mesentery number same as tentacle number. Ten perfect mesenteries, eight macrocnemes. Marginal mesogleal sphincter muscle. No lacunae or ring sinus.

*Symbiosis:* Zooxanthellate. *Symbiodinium* subclade C3 in three polyps from three different sites around Okinawa Island, Japan (Fig. 5).

*Cnidae:* Holotrichs in column; basitrichs and microbasic p-mastigophores in filaments; basitrichs, microbasic p-mastigophores, and holotrichs in tentacles (Table 2).

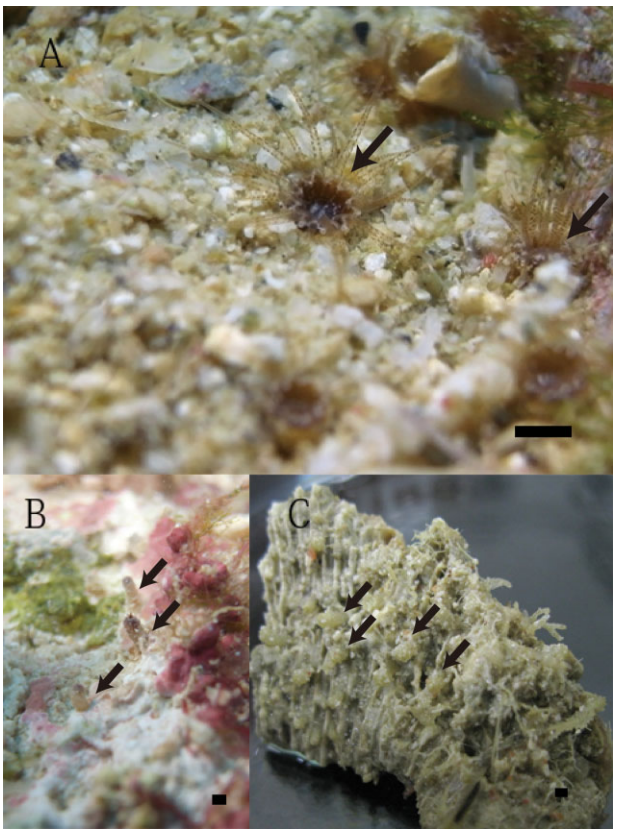



**Figure 3.** ***Nanozoanthus harenaceus*** **sp. nov.** from Okinawa Island. A, colony with open polyps *in situ*, specimen RUMF-2G-04372 (paratype2), Oura Bay, Okinawa, Japan, 20 m depth, 13 May 2012. B, contracted polyps *in situ*, specimen RUMF-2G-04372 (paratype2), Cape Maeda, Okinawa, Japan, 22 m depth, 21 December 2011. C, part of a colony of preserved specimen fixed by 5–10% formalin seawater, specimen NSMT-Co1555 (holotype), Cape Maeda, Okinawa, Japan, 9 m depth, 15 February 2012. Scale bars: approx. 1 mm.

*Habitat: Nanozoanthus harenaceus* sp. nov. occurs on rocky substrates on the slopes of coral reefs. Almost all observed colonies almost completely buried in sand 'pools' that formed in small pockets or depressions in the hard coral substrate of reef slopes, with only tentacles and oral disc protuding above the surface of sand when polyps open (Fig. 3A). Found from the east and west coasts of Okinawa Island, Japan. Depth 9–27 m.

*DNA sequences:* GenBank accession numbers: *COI*, KF499609; *16S* rDNA, KF499601, with *Symbiodinium ITS* rDNA sequences KF499598.

*Remarks*

Only one species has been described in Nanazoanthidae fam. nov., and the diagnostic characters are as for family above. Internal morphology is unavailable for the holotype because the encrusted sand inhibited making useable sections of the polyps.

The distribution of family Nanozoanthidae fam. nov. is currently known from only a few sites in Okinawa and from one site in western Australia. The lack of reports or other information undoubtedly results from the difficulty in finding colonies because of their tiny size and the cryptic appearance of the polyps. This species is one of the smallest zoanthids described, along with Caribbean *Parazoanthus parasiticus* (Duchassaing and Michelotii, 1860) and some unidentified sponge-associated Parazoanthidae spp. from the Pacific, with all of these species having polyp diameters of < 2 mm. Additionally, the transparent and sandy body colour make *N. harenaceus* sp. nov. very cryptic, in contrast to the bright body colours of many sponge-associated zoanthids. Therefore, this small and cryptic zoanthid is likely to have a wider distribution in the Indo-Pacific than is described here.

The two specimens from Ningaloo Reef, Western Australia, are considered to be an undescribed species of genus *Nanozoanthus* gen. nov. by the results of molecular analyses. Additional specimens from regions other than Okinawa of colonies with enough polyps to properly observe both molecular and morphological characters are needed to more fully explore the species diversity of *Nanozoanthus* gen. nov.

*Etymology*

Named from the latin 'harenaceus' meaning 'sandy', as living polyps are buried in sand and resemble particles or clumps of sand because of their small size, and for the coloration of the oral disc and tentacles.

ZooBank: urn:lsid:zoobank.org:act:8AF484C9-B2EC-41D5-BA3F-A6764CE34DB8.

## Molecular phylogeny

*16S rDNA (Fig.1; Table 3)*

The results of the phylogenetic analyses of *16S* rDNA showed *N. harenaceus* sp. nov. and the specimens from Australia (*Nanozoanthus* sp., specimen numbers YINR16 and YINR15) forming a strongly supported large clade with family Microzoanthidae Fujii and Reimer, 2011 (ML = 100%, Bayes = 1.00). The genetic distance between the two separate subclades of Nanozoanthidae fam. nov. and Microzoanthidae was higher than the intrafamilial distance levels observed between other known zoanthid genera (p-distance = 0.411; see Table 4). This large Nanozoanthidae fam. nov.–Microzoanthidae clade was sister to order Zoantharia, excepting one basal sequence of *Isozoanthus giganteus* Chun, 1903, and is highly divergent from other zoanthid genera. The 'classically

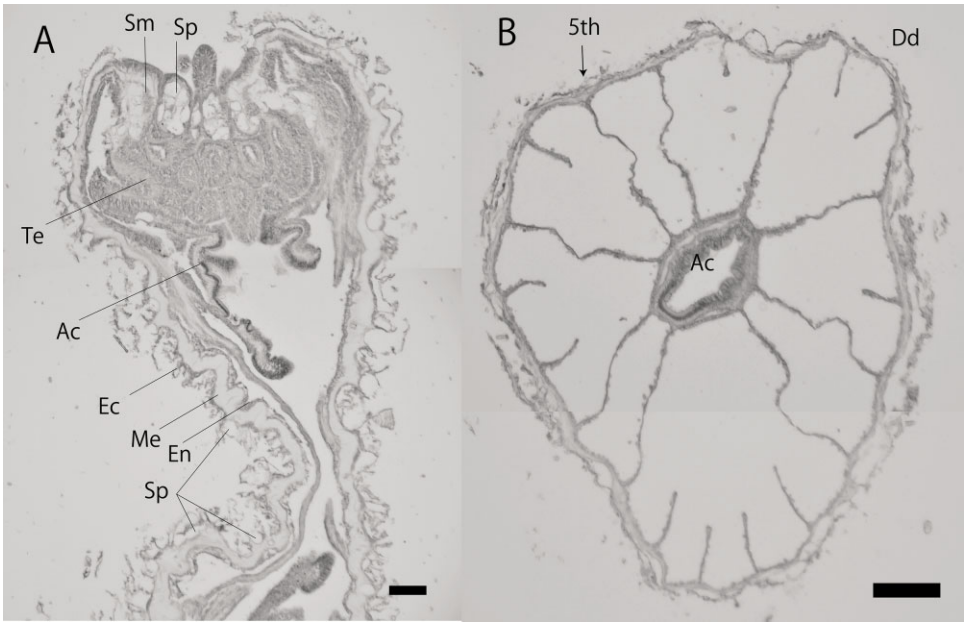



**Figure 4.** Histological section of specimen USNM-1221444 (paratype1). A, longitudinal section with mesogleal sphincter muscles at capitulum. B, cross section with macrocnemic mesenterial arrangement. Asymmetric arrangement caused by bias of cutting angle (perfect mesenteries appear similar to imperfect mesenteries). Abbreviations: 5th, 5th mesentery; Ac, actinopharynx; Dd, dorsal directive; Ec, ectoderm; En, endoderm; Me, mesoglea; Sm, sphincter muscles; Sp, existence of incrusted sand particles.

known zoanthids' (e.g. families Epizoanthidae, Parazoanthidae, Hydrozoanthidae, Zoanthidae, Sphenopidae, Neozoanthidae) and the deep-sea genus *Abyssoanthus* Reimer and Fujiwara, 2007 formed a well-supported clade (ML = 80%, Bayes = 0.99), and were highly divergent from both the Nanozoanthidae fam. nov.–Microzoanthidae clade and *I. giganteus* (Table 2). Most clades within the 'classically known zoanthid' grouping had low statistical support. Genetic distances between Actiniaria (out-group), *I. giganteus*, and the 'known zoanthid' clade were less than or equal to the distance between them and the Nanozoanthidae fam. nov.–Microzoanthidae clade (p-distance < 0.328; see Table 4).

*COI (Fig. 2; Table 3)*

The phylogenetic tree of mitochondrial *COI* showed order Zoantharia divided into two strongly supported clades. The distance between these two clades (p-distance = 0.213–0.271) was as much as that to the out-group Actiniaria (p-distance = 0.2139–0.250). One of these clades was the 'known zoanthid' clade, consisting of families Epizoanthidae, Parazoanthidae, Hydrozoanthidae, Sphenopidae, Neozoanthidae, and Zoanthidae (ML = 99%, Bayes = 1.00). Genus *Epizoanthus* Gray, 1897 was highly divergent from other genera. The remaining 'known zoanthids' formed a well-supported subclade (ML = 95%, Bayes = 0.99), but most of the bootstrap values within this subclade were low. The other clade in the *COI* tree consisted of family Microzoanthidae, *Isozoanthus sulcatus* (Gosse, 1859), and Nanozoanthidae fam. nov. (ML = 76%, Bayes = 0.91). Family Micorozoanthidae was highly divergent within this clade (p-distance = 0.182–0.191), and *I. sulcatus* and Nanozoanthidae fam. nov. formed a strongly supported subclade with genetic distances at interfamily or genus levels (ML = 96%, Bayes = 1.00, p-distance = 0.044–0.047; see Table 4). Specimens of family Microzoanthidae fam. nov. formed a well-supported clade (ML = 95%, Bayes = 0.93). The sequences of the *Nanozoanthus* gen. et sp. nov. specimen from Ningaloo Reef were only different by one base pair from the sequences of *N. harenaceus* sp. nov., and they formed a well-supported subclade within the Nanozoanthidae clade (ML = 88%, Bayes = 0.95).

## DISCUSSION

### Phylogeny of order Zoantharia

Order Zoantharia is currently separated into two suborders, Macrocnemina and Brachycnemina, with discrimination by the complete or incomplete condition of the fifth mesentery from the dorsal directive; however, as mentioned in Sinniger *et al*. (2005) and Swain (2010), the classification of these two suborders

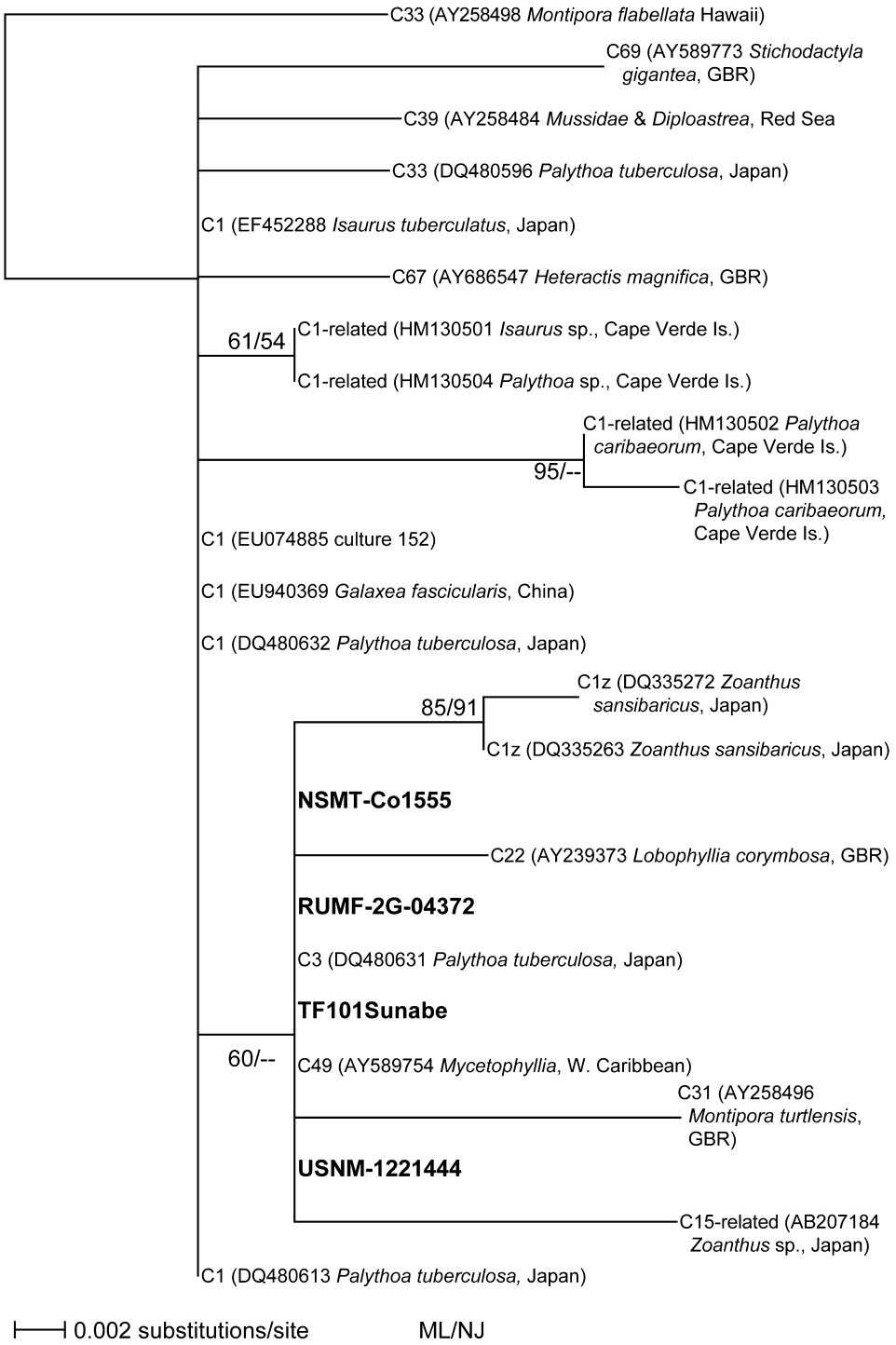
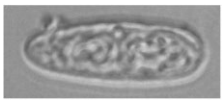
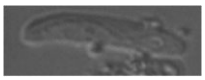
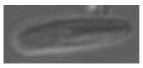
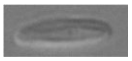



**Figure 5.** Maximum-likelihood (ML) tree of internal transcribed spacer of ribosomal DNA (*ITS* rDNA) regions from *Symbiodinium*. Values at branches represent ML and neighbour-joining (NJ) bootstrap probabilities, respectively (> 50%). Specimens collected in this study are set in bold. Sample names with GenBank accession numbers are from a previous study.

is not reflected by their phylogeny, as Brachycnemina is apparently polyphyletic. Because of this problem, and in addition the difficulty in observing internal morphology caused by encrusted mineral particles in their bodies (Reimer *et al*., 2010b), some recently described families and genera of order Zoantharia have not been assigned to either suborder (Reimer *et al*., 2007; Sinniger *et al*., 2010).

**Table 2.** Cnidae types and size of different area of the polyps of ***Nanozoanthus harenaceus* sp. nov.**

| | | 0 10 20 30 40 (μm) | Length* | Width* | Frequency† |
|---|---|---|---|---|---|
| Column | Holotrich |  | 19.3 (11–24) | 7.0 (5–8) | $n$ = 11, occasional |
| Filaments | Basitrich |  | 16.8 (13–20) | 3.4 (3–4) | $n$ = 11, common |
| | p-mastigophore |  | 12.9 (11–15) | 4.1 (3–5) | $n$ = 21, numerous |
| Tentacles | Holotrich |  | 8.3 (7–10) | 3.7 (3–4) | $n$ = 10, rare |
| | Basitrich |  | 11.0 (10–12) | 3.2 (3–4) | $n$ = 11, common |
| | Spirocyst |  | 12.3 (10–16) | 3.8 (3–5) | $n$ = 21, numerous |

* Length and width: average, minimum–maximum (μm).
† Frequency: $n$ = number of examined cnidae in this analysis. Frequency in decreasing order: numerous, common, occasional, rare.

From the results of this research, it is clear that family Nanozoanthidae fam. nov. is divergent from all known zoanthid families at the family level or higher. Molecular phylogenetic trees of the two DNA markers used in this study showed that this new family is closest to Microzoanthidae, and this is supported by high bootstrap values (*16S* rDNA, ML = 100%, Bayes = 1.00; *COI*, ML = 76%, Bayes = 0.91). The Nanozoanthidae fam. nov.–Microzoanthidae clade is highly divergent from all other known zoanthids (excepting *I. sulcatus* in the *COI* tree), potentially at the level of suborder or even order, suggesting that these groups are possibly not zoanthids (see Table 4); however, because of their morphological affinity with other zoanthids, we place this group within the order Zoantharia.

In the *COI* analyses, Nanozoanthidae fam. nov. and *I. sulcatus* formed a statistically well-supported clade. These two taxa have some similarities, with small polyp sizes, transparent long tentacles, similar numbers of tentacles, and both are zooxanthellate (Manuel, 1979; Davy, Lucas & Turner, 1997; Williams, 2000). The most significant morphological difference is the sphincter muscle in *Nanozoanthus* gen. nov. is mesogleal and in *I. sulcatus* is endodermal. The position of the sphincter muscle has previously been considered to be diagnostic at the family level in Zoantharia. Our results support the idea suggested in previous studies that morphological features should often not be used for phylogenetically classifying zoanthid families (Sinniger *et al*., 2005; Swain, 2010).

Another result of this study is that the species *I. sulcatus* is likely to belong to Nanozoanthidae fam. nov. and is not within genus *Isozoanthus*. The type species of genus *Isozoanthus* is *I. giganteus*, for which mt *16S* rDNA sequences (from Swain, 2010) were used in this study. From the phylogenetic tree of *16S* rDNA, *I. giganteus* is genetically distant from Nanozoanthidae fam. nov. However, no taxonomic study has focused on re-examining the genus *Isozoanthus* (= type species *I. giganteus*) in detail using both morphological and molecular phylogenetic methods. Such research could further ascertain the position of *I. giganteus* (and therefore the genus *Isozoanthus*), and confirm the placement of *I. sulcatus* within Nanozoanthidae fam. nov. Given the genetic distance between *Nanozoanthus* and *I. sulcatus* (4.4%, *COI*), it is likely that *I. sulcatus* should belong to another undescribed genus within Nanozoanthidae fam. nov.

Comparing the branch lengths in the *COI* tree between the two current zoanthid suborders, Brachycnemina and Macrocnemia, it is clear that the genetic distances between the Nanozoanthidae fam. nov.–Microzoanthidae clade and all other zoanthids are at least as high as the suborder level (Fig. 2; Table 5). Morphological similarities between Nanozoanthidae fam. nov. and Microzoanthidae are few, and creating a new suborder based on classical morphological taxonomy is difficult. Nanozoanthidae fam. nov. and Microzoanthidae share white zigzag outside edges of oral discs, long transparent tentacles, mesogleal sphincter muscles, and rough, irregular

**Table 3.** Genetic distances (p-distance) for mitochondrial *16S* ribosomal DNA (*16S* rDNA) within specimens of Zoantharia and out-group Actiniaria

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *Urticina crassicornis* | | | | | | | | | | | | | | | | | |
| 2. *Isozoanthus giganteus* | 0.212 | | | | | | | | | | | | | | | | |
| 3. *Abyssoanthus nankaiensis* | 0.323 | 0.350 | | | | | | | | | | | | | | | |
| 4. *Epizoanthus cutressi* | 0.235 | 0.232 | 0.218 | | | | | | | | | | | | | | |
| 5. *Terrazoanthus sinnigeri* | 0.248 | 0.222 | 0.189 | 0.046 | | | | | | | | | | | | | |
| 6. *Hydrogracilis gracilis* | 0.241 | 0.213 | 0.203 | 0.052 | 0.020 | | | | | | | | | | | | |
| 7. *Parazoanthus parasiticus* | 0.252 | 0.241 | 0.228 | 0.065 | 0.033 | 0.039 | | | | | | | | | | | |
| 8. *Savalia savaglia* | 0.240 | 0.181 | 0.157 | 0.034 | 0.027 | 0.031 | 0.025 | | | | | | | | | | |
| 9. *Corallizoanthus tsukaharai* | 0.260 | 0.248 | 0.218 | 0.072 | 0.037 | 0.046 | 0.023 | 0.018 | | | | | | | | | |
| 10. *Antipathozoanthus macaronesicus* | 0.257 | 0.247 | 0.230 | 0.056 | 0.052 | 0.058 | 0.042 | 0.041 | 0.055 | | | | | | | | |
| 11. *Sphenopus marsupialis* | 0.234 | 0.224 | 0.204 | 0.038 | 0.030 | 0.032 | 0.056 | 0.025 | 0.060 | 0.067 | | | | | | | |
| 12. *Palythoa tuberculosa* | 0.237 | 0.222 | 0.210 | 0.043 | 0.037 | 0.039 | 0.063 | 0.027 | 0.065 | 0.074 | 0.006 | | | | | | |
| 13. *Neozoanthus uchina* | 0.231 | 0.217 | 0.215 | 0.059 | 0.078 | 0.080 | 0.094 | 0.053 | 0.096 | 0.112 | 0.029 | 0.034 | | | | | |
| 14. *Isaurus tuberculatus* | 0.221 | 0.212 | 0.206 | 0.047 | 0.048 | 0.048 | 0.067 | 0.039 | 0.071 | 0.083 | 0.025 | 0.029 | 0.012 | | | | |
| 15. *Zoanthus sansibaricus* | 0.222 | 0.209 | 0.213 | 0.052 | 0.052 | 0.052 | 0.070 | 0.042 | 0.076 | 0.087 | 0.029 | 0.034 | 0.016 | 0.004 | | | |
| 16. *Microzoanthus occultus* | 0.389 | 0.402 | 0.473 | 0.379 | 0.394 | 0.388 | 0.444 | 0.304 | 0.452 | 0.382 | 0.345 | 0.349 | 0.403 | 0.346 | 0.355 | | |
| 17. ***Nanozoanthus* gen. et sp. nov.** | 0.398 | 0.430 | 0.458 | 0.359 | 0.383 | 0.385 | 0.420 | 0.328 | 0.430 | 0.396 | 0.376 | 0.385 | 0.407 | 0.364 | 0.358 | 0.411 | |
| 19. ***Nanozoanthus harenaceus* sp. nov.** | 0.398 | 0.430 | 0.458 | 0.359 | 0.383 | 0.385 | 0.420 | 0.328 | 0.430 | 0.396 | 0.376 | 0.385 | 0.407 | 0.364 | 0.358 | 0.411 | 0.000 |

**Table 4.** Genetic distances (p-distance) for mitochondrial cytochrome oxidase *c* subunit I (*COI*) within specimens of Zoantharia and out-group Actiniaria

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. *Urticina coriacea* | | | | | | | | | | | | | | |
| 2. *Epizoanthus illoricatus* | 0.219 | | | | | | | | | | | | | |
| 3. *Savalia savaglia* | 0.242 | 0.125 | | | | | | | | | | | | |
| 4. *Parazoanthus swiftii* | 0.238 | 0.122 | 0.012 | | | | | | | | | | | |
| 5. *Parazoanthus* sp. | 0.231 | 0.125 | 0.014 | 0.007 | | | | | | | | | | |
| 6. *Hydroazoanthus gracilis* | 0.249 | 0.133 | 0.042 | 0.032 | 0.037 | | | | | | | | | |
| 7. *Mesozoanthus fosii* | 0.250 | 0.127 | 0.022 | 0.024 | 0.027 | 0.055 | | | | | | | | |
| 8. *Sphenopus marsupialis* | 0.249 | 0.130 | 0.044 | 0.034 | 0.042 | 0.042 | 0.042 | | | | | | | |
| 9. *Palythoa tuberculosa* | 0.249 | 0.130 | 0.044 | 0.034 | 0.042 | 0.042 | 0.042 | 0.000 | | | | | | |
| 10. *Neozoanthus uchina* | 0.245 | 0.130 | 0.060 | 0.050 | 0.055 | 0.062 | 0.053 | 0.029 | 0.029 | | | | | |
| 11. *Zoanthus sansibaricus* | 0.234 | 0.142 | 0.074 | 0.063 | 0.068 | 0.070 | 0.071 | 0.037 | 0.037 | 0.027 | | | | |
| 12. *Micro*zoanthus *kagerou* | 0.229 | 0.244 | 0.260 | 0.260 | 0.259 | 0.261 | 0.271 | 0.257 | 0.257 | 0.260 | 0.268 | | | |
| 13. *Isozoanthus_sulcatus* | 0.226 | 0.223 | 0.222 | 0.212 | 0.215 | 0.229 | 0.222 | 0.222 | 0.222 | 0.226 | 0.226 | 0.182 | | |
| 14. ***Nanozoanthus* gen. et sp. nov.** | 0.212 | 0.221 | 0.227 | 0.216 | 0.220 | 0.224 | 0.230 | 0.220 | 0.220 | 0.224 | 0.231 | 0.191 | 0.047 | |
| 16. ***Nanozoanthus harenaceus* sp. nov.** | 0.219 | 0.224 | 0.223 | 0.213 | 0.216 | 0.220 | 0.227 | 0.216 | 0.216 | 0.220 | 0.227 | 0.188 | 0.044 | 0.005 |

**Table 5.** Genetic distances for *16S* rDNA and cytochrome *c* oxidase subunit I (*COI*) between ***Nanozoanthus harenaceus* gen. et sp. nov.** and other zoanthids

| *16S* | Genetic distance | Taxonomic difference |
|---|---|---|
| ***Nanozoanthus harenaceus* sp. nov.**–*Nanozoanthus* sp. | 0 | Conspecific, or intra-generic |
| **Nanozoanthidae fam. nov.**–*Microzoanthus occultus* | 0.411 | Same suborder |
| **Nanozoanthidae fam. nov.**–*Abyssoanthus nankaiensis* | 0.458 | Same order |
| **Nanozoanthidae fam. nov.**–classically known zoanthids | 0.328–0.430 | Same order |
| **Nanozoanthidae fam. nov.**–*Urticina coriacea* | 0.398 | Same subclass |
| **Examples from described group comparisons** | | |
| *Terrazoanthus sinnigeri–Hydrozoanthus gracilis* | 0.02 | Same family |
| *Terrazoanthus sinnigeri–Epizoanthus cutressi* | 0.046 | Same suborder |
| *Terrazoanthus sinnigeri–Zoanthus sansibaricus* | 0.052 | Same order |
| Classically known zoanthids–*Urticina coriacea* | 0.221–0.260 | Same subclass |
| **COI** | | |
| ***Nanozoanthus harenaceus* sp. nov.**–*Nanozoanthus* sp. | 0.005 | Same genus |
| Nanozoanthidae fam. nov.–*Isozoanthus sulcatus* | 0.044 | Same family? |
| ***Nanozoanthus harenaceus* sp. nov.**–*Microzoanthus kagerou* | 0.188 | Same suborder? |
| **Nanozoanthidae fam. nov.**–classically known zoanthids | 0.213–0.227 | Same order |
| **Nanozoanthidae fam. nov.**–*Urticina coriacea* | 0.219 | Same subclass |
| **Examples from described group comparisons** | | |
| *Savalia savaglia–Parazoanthus swiftii* | 0.012 | Same family |
| *Savalia savaglia–Epizoanthus illoricatus* | 0.125 | Same suborder |
| *Savalia savaglia–Zoanthus sansibaricus* | 0.074 | Same order |
| *C*lassically known zoanthids–*Urticina coriacea* | 0.219–0.250 | Same subclass |

encrustations of mineral particles into their ectoderm and mesoglea (Fujii & Reimer, 2011). However, considering that zoanthids have few morphological characters remarkable for taxonomy, and given the large divergence between these two families in the molecular phylogenies and the small divergence of morphology, we consider it likely that the two groups form an isolated suborder. Because of the low bootstrap values at the genus level within the 'known zoanthid' clade and the uncertain phylogenetic placement of *Isozoanthus giganteus*, we refrain from formally describing the Nanozoanthidae fam. nov.–Microzoanthidae group as a suborder. However, this group will have to be described and characterized in the near future. This study demonstrates that in order to reconstruct the classification within the order Zoantharia it is urgent to more completely understand their biodiversity.

## ACKNOWLEDGEMENTS

The first author thanks Yuka Irei (University of the Ryukyus), the Census of Coral Reef Ecosystems' Australian node (CReefs: a field project of the Census of Marine Life), Dr Julian Caley, and Shawn Smith (both Australian Institute of Marine Science) for help in providing specimens from Ningaloo Reef, Australia, and Shin Nishihara for helping to collect specimens in Okinawa, Japan. Specimens from Ningaloo Reef were collected under Western Australia's Department of Environment and Conservation Permit #SF007428.

The first author was supported by a research fellowship from the Japan Society for the Promotion of Science for young scientists, and JSPS KAKENHI (grant number 24-3048). This work was also supported by Rising Star Program and International Research Hub Project for Climate Change (both at the University of the Ryukyus) grants to the second author.

## REFERENCES

**Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS. 1994.** High genetic variability and patchiness in a common Great barrier reef zoanthid (*Palythoa caesia*). *Marine Biology* **121:** 153–160.

**Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS. 1997.** Zoanthids (Anthozoa, Hexacorallia) from the Great Barrier Reef and Torres Strait, Australia: systematics, evolution and a key to species. *Coral Reefs* **16:** 55–68.

**Carlgren O. 1913.** Zoanntharia. *The Danish Ingolof-Expedition* **4:** 1–62.

**Davy SK, Lucas IAN, Turner JR. 1997.** Uptake and persistence of homologous and heterologous zooxanthellae in the temperate sea anemone *Cereus pedunculatus* (Pennant). *The Biological Bulletin* **2:** 208–216.

**England KW. 1991.** Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* **216/217:** 691–697.

**Felsenstein J. 1985.** Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **4:** 783–791.

**Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994.** DNA primers for amplification of mitochondrial cytochrome 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* **4:** 418–431.

**Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010.** New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59:** 307–321.

**Hall TA. 1999.** BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95 / 98 / NT. *Nucleic Acids Symposium Series* **41:** 95–98.

**Haywick DW, Muller EM. 1997.** Sediment retention in encrusting *Palythoa* spp.-a biological twist to a geological process. *Coral Reefs* **16:** 39–46.

**Herberts C. 1972.** Etude systématique de quelques zoanthaires tempérés et tropicaux. Paris. *Tethys Supplement* **3:** 69–156.

**Hertwig R. 1882.** Report on the Actiniaria dredged by H.M.S. Challenger during the years 1873–1876. *Zoology* **6:** 108–123.

**Hidaka M. 1992.** Use of nematocyst morphology for taxonomy of some related species of scleractinian corals. *Galaxea* **11:** 21–28.

**Hidaka M, Miyazaki I, Yamazato K. 1987.** Nematocysts characteristic of the sweeper tentacles of the coral *Galaxea fascicularis* (Linnaeus). *Galaxea* **6:** 195–207.

**Kimura M. 1980.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16:** 111–120.

**LaJeunesse TC. 2001.** Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a "species" level marker. *Journal of Phycology* **37:** 866–880.

**LaJeunesse TC. 2002.** Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. *Marine Biology* **141:** 387–400.

**Manuel RL. 1979.** Some new records of Anthozoa from British waters. *Journal of the Marine Biological Association of the United Kingdom.* **59:** 393–397.

**Ong CW, Reimer JD, Todd PA. 2013.** Morphologically plastic responses to shading in the zoanthids *Zoanthus sansibaricus* and *Palythoa tuberculosa*. *Marine Biology* **160:** 1053–1064.

**Rafinesque CS. 1815.** *Analyse de la nature, ou tableau de l'univers et des corps organisés*. Palermo: L'Imprimerie de Jean Barravecchia, 1–224.

**Reimer JD. 2010.** Key to field identification of shallow water brachycnemic zoanthids (Order Zoantharia: Suborder Brachycnemina) present in Okinawa. *Galaxea* **12:** 23–39.

**Reimer JD, Hirose M, Irei Y, Obuchi M, Sinniger F. 2011.** The sands of time: rediscovery of the genus Neozoanthus (Cnidaria: Hexacorallia) and evolutionary aspects of sand incrustation in brachycnemic zoanthids. *Marine Biology* **5:** 983–993.

**Reimer JD, Hirose M, Wirtz P. 2010a.** Zoanthids of the Cape Verde Islands and their symbionts: previously unexamined diversity in the Northeastern Atlantic. *Contributions to Zoology* **4:** 147–163.

**Reimer JD, Nakachi S, Hirose M, Hirose E, Hashiguchi S. 2010b.** Investigations of zoanthids (Cnidaria: Anthozoa): a critical assessment of methodology and necessity. *Marine Biotechnology* **12:** 605–617.

**Reimer JD, Ono S, Fujiwara Y, Takishita K, Tsukahara J. 2004.** Reconsidering *Zoanthus* spp. diversity: molecular evidence of conspecifity within four previously presumed species. *Zoological Science* **21:** 517–525.

**Reimer JD, Sinniger F, Fujiwara Y, Hirano S, Maruyama T. 2007.** Morphological and molecular characterization of *Abyssoanthus nankaiensis*, a new family, new genus and new species of deep-sea zoanthid (Anthozoa: Hexacorallia: Zoantharia) from a north-west Pacific methane cold seep. *Invertebrate Systematics* **21:** 255–262.

**Reimer JD, Takishita K, Ono S, Maruyama T, Tsukahara J. 2006b.** Latitudinal and intracolony ITS-rDNA sequence variation in the symbiotic dinoflagellate genus *Symbiodinium* (Dinophyceae) in *Zoanthus sansibaricus* (Anthozoa: Hexacorallia). *Phycological Research* **2:** 122–132.

**Reimer JD, Takishita T, Maruyama T. 2006a.** Molecular identification of symbiotic dinoflagellates (*Symbiodinium* spp.) from *Palythoa* spp. (Anthozoa: Hexacorallia) in Japan. *Coral Reefs* **4:** 521–527.

**Rodriguez F, Oliver JL, Marin A, Medina JR. 1990.** The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142:** 485–501.

**Ronquist F, Huelsenbeck JP. 2003.** Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford)* **19:** 1572–1574.

**Ryland JS, Lancaster JE. 2003.** Revision of methods for separating species of *Protopalythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. *Invertebrate Systematics* **17:** 407–428.

**Schmidt H. 1974.** On evolution in the Anthozoa. *Proceedings of the 2nd International Coral Reef Symposium* **1:** 533–560.

**Sinniger F. 2006.** Zoantharia of New Caledonia. In: Payri C, Richier De Forges B, eds. *Compendium of marine species from New Caledonia. Documents scientifiques et techniques II (7)*. Noumea: IRD Editions, 127–128.

**Sinniger F, Montoya-Burgos JI, Chevaldonné P, Pawlowski J. 2005.** Phylogeny of the order Zoantharia (Anthozoa, Hexacorallia) based on the mitochondrial ribosomal genes. *Marine Biology* **147:** 1121–1128.

**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* **1:** e52607.

**Sinniger F, Reimer JD, Pawlowski J. 2008.** Potential of DNA sequences to identify zoanthids (Cnidaria: Zoantharia). *Zoological Science* **25:** 1253–1260.

**Sinniger F, Reimer JD, Pawlowski J. 2010.** The Parazoanthidae (Hexacorallia: Zoantharia) DNA taxonomy: description of two new genera. *Marine Biodiversity* **1:** 57–70.

**Swain TD. 2009.** Phylogeny-based species delimitations and the evolution of host associations in symbiotic zoanthids (Anthozoa, Zoanthidea) of the wider Caribbean region. *Zoological Journal of the Linnaean 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.

**Tamura K, Dudley J, Nei M, Kumar S. 2007.** MEGA4: molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24:** 1596–1599.

**Thompson JD, Higgins DG, Gibson TJ. 1994.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* **22:** 4673–4680.

**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.