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Morphological and molecular characterisation 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

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Abstract. The order Zoantharia (= Zoanthiniaria, Zoanthidea) remains one of the most taxonomically neglected anthozoan groups, primarily owing to the difficulty of examining the internal morphology of sand-encrusted zoanthids, upon which classification has been largely based until very recently. Additionally, relatively few zoanthid species (all belonging to the genus *Epizoanthus*) have been properly described from the deep-sea. Numerous individuals of an unidentified sediment-encrusting zoanthid-like species were observed and sampled during a *Shinkai 6500* deep-sea submersible dive at a methane cold seep (depth 3259 m) off Muroto at the Nankai Trough, Japan. Unlike previously described deep-sea zoanthids, *Abyssoanthus nankaiensis*, gen. *et* sp. nov. (Abyssoanthidae fam. nov.) was found to be non-colonial, free-living (non-commensal), and, uniquely, on mudstone in the vicinity of a methane cold seep. Morphologically, *A. nankaiensis*, gen. *et* sp. nov. is characterised by its relatively uniform polyp diameter from oral to aboral end with 19–22 mesenteries. Additionally, DNA (mitochondrial 16S rDNA and mitochondrial cytochrome oxidase c subunit I DNA) sequences obtained from these samples also unambiguously place this specimen in a previously undescribed and new family within the order Zoantharia. This is the first reported zoanthid species from a methane cold seep or other so-called 'extreme' environment, and the first molecular characterisation of any such deep-sea zoanthid.

Introduction

The order Zoantharia (= Zoanthiniaria, Zoanthidea) is found worldwide in most marine environments. Zoanthids are characterised by the presence of two rows of tentacles and one siphonoglyph, with the majority of species described thus far being colonial and encrusted with sand and/or other detritus. Despite such conspicuous morphological characteristics, Zoantharia remains a poorly described, understood and inventoried group. Until recently, Zoantharia was divided into two suborders, Macrocnemina and Brachycnemina, based on the organisation of septa (Haddon and Shackleton 1891). Septa data are only obtainable by cross-sections, which are unusually difficult to obtain from small encrusted zoanthids. However, Sinniger *et al*. (2005) showed that based on molecular data these two suborders are invalid taxonomic groupings, with Macrocnemina paraphyletic. Currently, no single morphological characteristic can be reliably used to classify zoanthid specimens. However, recent work combining both molecular and morphological techniques has begun to bring taxonomic order to some groups of zoanthids (Reimer *et al*. 2004; Sinniger *et al*. 2005; Reimer *et al*. 2006*a*, 2006*b*).

Deep-sea zoanthids have been reported worldwide at depths of up to 5000 m (reviewed by Ryland *et al*. 2000), and all deep-sea zoanthids identified until now have been characterised as belonging to the genus *Epizoanthus* (family Epizoanthidae). Both shallow water and deep-sea *Epizoanthus* species have been characterised generally to be: *1*, azooxanthellate (although zooxanthellate species exist), *2*, epizoic on a wide variety of substrate organisms (including mollusks, pagurid crabs

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(Muirhead *et al.* 1986), and hyalonematid glass sponges (Beaulieu 2001) (except non-commensal species such as *E. couchii* and *E. paxi*), and *3*, colonial, with individual polyps connected by a stolon or coenenchyme.

During a recent *Shinkai 6500* deep-sea submersible dive (Dive #884, June 18, 2005) at the Nankai Trough off Japan (32°34.945′N, 134°41.545′E), numerous polyps of a sediment-encrusted zoanthid-like species were discovered on blocks of mudstone at 3259 m. Unlike most previously reported deep-sea Zoantharia species of the family Epizoanthidae, observed specimens were non-colonial and free-living, and also uniquely inhabited a methane cold-seep chemosynthetic environment. Specimens were collected and compared morphologically and genetically (using mitochondrial 16S rDNA and cytochrome oxidase c subunit I (COI) DNA markers) with samples from the other described families in the order Zoantharia: Epizoanthidae, Parazoanthidae, Sphenopidae, and Zoanthidae. As ecological, morphological, and molecular characteristics were all significantly different from known families in the order Zoantharia, our specimens were attributed to a new family, new genus, and new species. Observed novel characteristics of *Abyssoanthus nankaiensis*, gen. *et* sp. nov. (Abyssoanthidae, fam. nov.) are discussed in relation to other families in the order Zoantharia.

Materials and methods

Sample collection

The zoanthid samples were all obtained at a depth of 3259 m from the Nankai Trough off Muroto, Japan (32°34.945′N, 134°41.545′E), during a dive of the deep-sea submersible *Shinkai 6500* (Dive #884) on June 18, 2005. The zoanthids were unintentionally obtained during collection of other samples using a manipulator. The zoanthids (total 12 polyps) were photographed and immediately preserved (in 99.5% ethanol, Bouin's fixative, or glutaraldehyde) by T. Sato and Y. Fujiwara aboard the R/V Yokosuka. Since the specimens were initially unintentionally acquired during sampling of other organisms, no high-resolution *in situ* images were obtained.

DNA extraction, PCR amplification, and sequencing

Deoxyribonucleic acid was extracted from three of the collected zoanthid samples (5–20 mg) following procedures outlined in Reimer *et al.* (2004) by using a DNAEasy Tissue Kit for animals (Qiagen, Tokyo, Japan).

The mitochondrial cytochrome oxidase c subunit I (COI) gene was amplified using the newly designed zoanthid-specific primers COIZoanF (3′ TGATAAGGTTAGAACTTTCTGCCCCGGAAC 5′) and COIZoanR (3′ TGATAAATATAGCCATGTCCACG 5′), because previously reported general COI primers (HCO2198 and LCO1490; see Folmer *et al.* 1994) did not successfully amplify the target molecule. The following thermal cycle conditions were used: 35 cycles of: 1 min at 94°C, 1 min at 40°C, 1min 30 s at 72°C, and followed by a 7 min extension at 72°C.

Mitochondrial 16S rDNA (mt 16S rDNA) was amplified using primers described by Sinniger *et al.* (2005), with the following thermal cycle conditions: 40 cycles of: 30 s at 94°C, 1 min at 52°C, 2 min at 72°C, and followed by a 5 min extension at 72°

The amplified PCR products were checked by 1.5% agarose gel electrophoresis. The PCR-amplified DNA fragments were sequenced with an ABI PRISM 3700 DNA Analyser (PE Biosystems, Foster City, CA, USA) using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems, Foster City, CA, USA). The sequences were analysed using GENETYX-MAC version 8.0 (Software Development, Tokyo, Japan) and DNASIS Mac v3.6 (Hitachi Software Engineering Co. Ltd, Tokyo, Japan).

Light microscope analyses

Initial observation of samples and polyp surfaces were made using a dissecting microscope. Owing to the presence of sand grains in the tissue of zoanthids (except in the family Zoanthidae), obtaining sections is unusually difficult unless potentially dangerous fluorohydric acid is used. We attempted both paraffin embedding (see Ono *et al.* 2005) and embedding in low-viscous hydrophilic epoxy resin Quetol 651 (Nissin EM Co. Ltd, Tokyo, Japan) as improved by Kushida *et al.* (1988) and specified for geological samples by Takizawa *et al.* (1995), but unfortunately no clear slides, usable as figures, were obtained. Further morphological examination has been limited owing to the low number of specimens obtained (12 polyps). We were able, however, to observe mesentery number and mesogleal thickness. Additionally, polyp dimension data (height, oral end maximum width, aboral end maximum width, and mid-polyp minimum width) were obtained.

Sources of material

Abbreviations for collections used in the text are as follows:

JAMSTEC	Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan
NHM	Natural History Museum, London, UK
NSMT	National Science Museum of Tokyo, Japan
USNM	Smithsonian National Museum of History, Washington DC, USA

Phylogenetic analyses

New sequences obtained in the present study were deposited in the DNA Data Bank of Japan (DDBJ) (accession numbers AB247343–AB247364). By using CLUSTAL X version 1.8 (Thompson *et al.* 1997), the nucleotide sequences of the COI gene and mt 16S rDNA from our samples were separately aligned with Zoantharia obtained from known families (Epizoanthidae, Parazoanthidae, Sphenopidae, and Zoanthidae) and coral/anemone outgroups (Table 1). The alignments were inspected by eye and manually edited. All ambiguous sites of the alignments were removed from the dataset for phylogenetic analyses. Consequently, we generated two alignment datasets: (*1*) 310 sites of 30 taxa (the COI gene); and (*2*) 582 sites of 23 taxa (mt 16S rDNA). All alignment data are available on request from the corresponding author or as accessory material from the *Invertebrate Systematics* website (http://www.publish.csiro.au/nid/120.htm). For the phylogenetic analyses of the COI gene and the mt 16S rDNA sequences the same methods were independently applied. Maximum likelihood (ML) analyses were performed using PhyML (Guindon and Gascuel 2003). The datasets were tested for optimal fit of various models of

Table 1. Outgroup and Order Zoantharia sequence data used in phylogenetic analyses

n/a, not available

Order (sub-order)	Family	Genus and species	Locality	Collected by	Accession numbers (mt 16S rDNA/COI)	Reference
Scleractinia	Acroporidae	*Montipora cactus*	n/a	n/a	AY903296/—	Tseng *et al*. (unpublished)
		Acropora tenuis	n/a	n/a	—/AF338425	van Oppen *et al*. 2002
Actinaria	Aipstasiidae	*Aipstasia pulchella*	n/a	n/a	AY345875/—-	Daly *et al*. 2003
	Metridiidae	*Metridium senile*	n/a	n/a	AF000023/—	Beagley *et al*. 1998
			n/a	n/a	—/U36783	Beagley *et al*. 1998
Zoantharia (Macrocnemina)	Epizoanthidae	*Epizoanthus paguricola*	Tyrrhenian Sea, Italy	H. Zibrowius	AY995928/—	Sinniger *et al*. 2005
					—/AB247347	this study
		Epizoanthus couchii	Rathlin I., Ireland	B. Picton	AB247343/—	this study
		Epizoanthus paxi	Marseille, France	F. Sinniger	AY995926/—	Sinniger *et al*. 2005
					—/AB247348	this study
		Epizoanthus illoricatus	N. Sulawesi, Indonesia	M. Boyer	—/AB247349	this study
	Parazoanthidae	*Parazoanthus swiftii*	Utila, Honduras	F. Sinniger	AY995936/—	Sinniger *et al*. 2005
					—/AB247350	this study
		Parazoanthus puertoricense	Utila, Honduras	F. Sinniger	AY995933/—	Sinniger *et al*. 2005
					—/AB247351	this study
		Parazoanthus aff. *puertoricense* [1]	Izu, Japan	J. Reimer	—/AB247352	this study
		Parazoanthus parasiticus	Utila, Honduras	F. Sinniger	AY995938/—	Sinniger *et al*. 2005
		Parazoanthus tunicans	Utila, Honduras	F. Sinniger	AY995940/—	Sinniger *et al*. 2005
					—/AB247353	this study
		Parazoanthus gracilis	N. Sulawesi, Indonesia	M. Boyer	AY995942/—	Sinniger *et al*. 2005
		Parazoanthus axinellae	Marseille, France	F. Sinniger	—/AB247355	this study
		Savalia savaglia	Gran Canaria, Canary Islands	P. Wirtz	AY995930/—	Sinniger *et al*. 2005
		Savalia savaglia	Marseille, France	F. Sinniger	—/AB247356	this study
					AY995925/—	this study
		Cape Verde zoanthid	Sal Island, Cape Verde	P. Wirtz	—/AB247357	this study
		Yellow polyps	Aquarium trade[2]	n/a	—/AB247358	this study
Zoantharia (Brachycnemina)	Sphenopidae	*Palythoa* sp.	Aquarium trade[2]	n/a	AY995943/—	Sinniger *et al*. 2005
		Palythoa heliodiscus	Erabu, Japan	J. Reimer	AB219224/—	Reimer *et al*. 2006*b*
			Lau Lau, Saipan	J. Reimer	—/ AB219201	Reimer *et al*. 2006*b*
		Palythoa mutuki 2	Amami, Japan	J. Reimer	AB219221/AB219212	Reimer *et al*. 2006*b*
		Palythoa sp. 289	Sakatia, Madagascar	F. Sinniger	—/AB247359	this study
		Palythoa sp. 296 (Protopalythoa)	Sakatia, Madagascar	F. Sinniger	—/AB247360	this study
		Isaurus sp.	Aquarium trade[2]	n/a	AY995945/—	Sinniger *et al*. 2005
		Isaurus sp. IKO1	Otsuki, Japan	F. Iwase	—/AB247361	this study
	Zoanthidae	*Acrozoanthus* sp.	Aquarium trade[2]	n/a	AY995946/—	Sinniger *et al*. 2005
		Zoanthus gigantus (AmamiZ4)	Amami, Japan	J. Reimer	—/AB128893	Reimer *et al*. 2004
		Zoanthus gigantus (ZgYS1)	Yakushima, Japan	J. Reimer	AB219192/—	Reimer *et al*. 2006*a*
		Zoanthus gigantus (ZgAT2)	Amami, Japan	J. Reimer	— AB219184	Reimer *et al*. 2006*a*
		Zoanthus sansibaricus (ZSH2)	Sakurajima, Japan	J. Reimer	—/AB194021	Reimer *et al*. 2004
		Zoanthus sansibaricus (ZSH23)	Sakurajima, Japan	J. Reimer	AB219187/—	Reimer *et al*. 2006*a*
		Zoanthus sansibaricus (AmamiZ1)	Amami, Japan	J. Reimer	—/AB128897	Reimer *et al*. 2004
		Zoanthus kuroshio (ZYS1b)	Yakushima, Japan	J. Reimer	—/AB214177	Reimer *et al*. unpub.
		Zoanthus kuroshio (ZYS1)	Yakushima, Japan	J. Reimer	—/AB214176	Reimer *et al*. unpub.
Zoantharia (suborder not assigned)	Abyssoanthidae	*Abyssoanthus nankaiensis* (1)	Nankai Trough, Japan	Y. Fujiwara and T. Sato	AB247344/AB247362	this study
		Abyssoanthus nankaiensis (2)	Nankai Trough, Japan	Y. Fujiwara and T. Sato	AB247345/AB247363	this study
		Abyssoanthus nankaiensis (3)	Nankai Trough, Japan	Y. Fujiwara and T. Sato	AB247346/AB247364	this study

[1] Corresponds to *Parazoanthus* aff. *puertoricense* as described in Uchida (2001), but our sequence data clearly shows this specimen is not conspecific with *P. puertoricense* from the Utila, Honduras, in the Caribbean.

[2] As noted in Sinniger *et al*. (2005), samples were acquired in the aquarium trade but are assumed to be from Indonesia.

nucleotide evolution using ModelTest version 3.06 (Posada and Crandall 1998). The PhyML analysis was performed using an input tree generated by BIONJ with the Hasegawa–Kishino–Yano (HKY) model (Hasegawa *et al.* 1985) of nucleotide substitution incorporating invariable sites and a discrete gamma distribution (four categories) (HKY + I + Γ) selected by ModelTest. The proportion of invariable sites and base frequencies of the model were estimated from the dataset. PhyML bootstrap trees (500 replicates) were constructed using the same parameters as the individual ML trees.

Bayesian trees were also reconstructed by using the program MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) under HKY + I + Γ. One cold and three heated Markov chains Monte Carlo (MCMC) with default-chain temperatures were run for 1 000000 generations, sampling log-likelihoods (InLs), and trees at 100-generations intervals (10000 InLs and trees were saved during MCMC). The likelihood plot for COI and mt 16S rDNA datasets suggested that MCMC reached the stationary phase after the first 30000 generations for both analyses (potential scale reduction factor [PSRF] = 1.000 for both datasets; standard deviation of split frequencies = 0.010895 and 0.00566 respectively). Thus, the remaining 970000 trees of COI and mt 16S rDNA were used to obtain clade probabilities and branch-length estimates.

The neighbour-joining (NJ) method (Saitou and Nei 1987) was performed using PAUP* Version 4.0 (Swofford 1998), with ML distances (HKY + I + Γ). Neighbour joining bootstrap trees (1000 replicates) were constructed using the same model.

New taxa

Order **ZOANTHARIA** Gray, 1870

Family **ABYSSOANTHIDAE**, fam. nov. Reimer & Fujiwara

Diagnosis

Sand/detritus/sediment-encrusted Zoantharia with unitary (non-colonial) free-living polyps, attached to hard substrates at abyssal (non-continental shelf deep-sea) depths surrounding methane cold seeps. Polyps dully coloured, colouration mostly from encrusted sand/detritus/sediment.

Remarks

As only one species for this family is described herein, the above diagnosis may change if additional species and/or genera within Abyssoanthidae are described.

Genus *Abyssoanthus*, gen. nov. Reimer & Fujiwara

Type species: *Abyssoanthus nankaiensis*, sp. nov. Reimer & Fujiwara

Diagnosis

As for family above.

Etymology

Named for the abyssal depth at which it was found and its relation to other Zoantharia genera.

Abyssoanthus nankaiensis, sp. nov. Reimer & Fujiwara

(Figs 1, 2; Table 1, 2)

Material examined

Holotype. 1, Japan, Nankai Trough off Muroto, 32°34.945′N, 134°41.545′E, depth 3259 m, 16.vi.2005, coll. Takako Sato and Yoshihiro Fujiwara (JAMSTEC) (NSMT-Co 1500).

Paratypes. **Japan. Nankai Trough off Muroto**: 1, data as for holotype (NSMT-Co 1501); 2, data as for holotype (NHM 2007.80); 3, data as for holotype (USNM-1084304).

Additional material. **Japan**. **Nankai Trough off Muroto**: data as for holotype, polyp #10 (JAMSTEC # 058332); Polyp #11 (JAMSTEC # 058333); Polyp #12 (JAMSTEC # 058334).

Diagnosis

Unitary (non-colonial), free-living polyps found in large groups of tens to thousands of individuals on mudstone surrounding methane cold seep (Fig. 1). Body wall encrusted, containing white and dark coloured sand particles up to ~200 μm in diameter. Outside of the polyps light coffee brown/grey; upon close inspection mottled black, brown and white owing to encrusted particles and not pigment (Fig. 2). No capitular ridges clearly visible, outer polyp surface rough with encrusted particles. Outer polyp surface region around oral disk (polyp closed) slightly lighter than the rest of the external surface of polyp encrusting fewer particles (Fig. 2). Oral disk and tentacles light cream in color, but existing images of low resolution so exact oral disk and tentacle colour uncertain (Fig. 1). Mesogleal thickness 250–850 μm, with polyp diameter (closed polyps) of 1.60–3.30 mm (Table 2). Closed polyp height 3.50–5.65 mm (Table 2). Expanded oral disks estimated (from *in situ* images) at ~6–10 mm in diameter and expanded polyps up to 3 cm in height. Polyps relatively uniform in diameter towards both oral opening and base, slightly narrower in middle of the polyp (Fig. 2). Tentacle count unknown (no high resolution *in situ* images exist), but based on other zoanthid species, likely close to the mesentery count (~19–22, although the number of complete mesenteries unknown owing to damage incurred during sectioning).

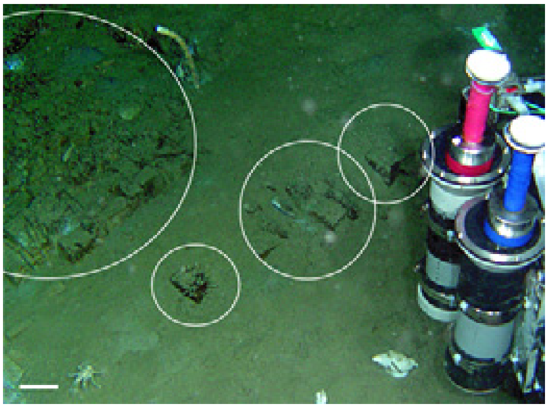

Fig. 1. *In situ* image of numerous *Abyssoanthus nankaiensis*, gen. *et* sp. nov. polyps (circled areas) on mudstone at the Nankai Trough, Japan (32°34.945′N, 134°41.545′E) during dive #884 of the deep-sea submersible *Shinkai 6500* (depth = 3259 m). Scale bar = 15 cm. Unfortunately no higher resolution *in situ* images exist.

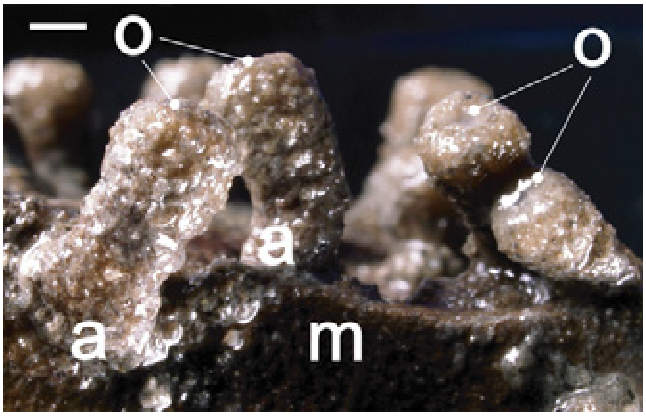

Fig. 2. *Abyssoanthus nankaiensis*, gen. *et* sp. nov. polyps on mudstone (m), showing several individual closed polyps. Encrusted sediment is evident on the polyp surface. Scale bar = 1 mm. o, oral opening/oral end; a, aboral end.

Description of the holotype

Single whole polyp, oral maximum diameter 2.45 mm, mid-polyp minimum diameter 1.85 mm, aboral maximum diameter 2.25 mm; 3.50 mm in height. Oral disk dark grey in color, polyp cylindrical. Sediment visibly encrusted on and in mesoglea surrounding ectoderm (Fig. 2). Holotype fixed in Bouin's fluid and then transferred to 70% ethanol.

Distribution

Has been found only at one location in the Nankai Trough, off Muroto, Japan.

Remarks

Paratypes 1–3 and other examined polyps do not show any significant differences in polyp appearance from the holotype aside from differing dimensions (Table 2).

Abyssoanthus nankaiensis was found on hard mudstone substrate in areas surrounding methane cold seeps. Additional populations of *A. nankaiensis* or other species of *Abyssoanthus* may await discovery and description in so-called 'extreme environments' in other locations in both the Pacific Ocean and other areas. Based on images from similar depths taken from the Atlantic (C. Kato, unpub. data), very similar (non-colonial, tentacle counts of ~22, mesentery counts of 19–22, polyp form and dimensions) yet unidentified zoanthids appear to exist at hydrothermal vents, although there is no published information on such organisms.

Etymology

The species has been named for the type locality, the Nankai Trough. *Nankai* means 'south sea' in Japanese.

Genetic sequences

Cytochrome oxidase c subunit I: AB247362–AB247364
mt 16S rDNA: AB247344–AB247346

Sequence results

The ML trees resulting from the analyses of COI (Fig. 3) and mt 16S rDNA (Fig. 4) sequences both show monophyly of *Abyssoanthus nankaiensis* unambiguously within Zoantharia, and sister to the clade Zoanthidae–Sphenopidae–Parazoanthidae. Bootstrap support for the monophyly of *A. nankaiensis* samples was very high for both COI and mt 16S rDNA (both markers ML = 100%, NJ = 100%, Bayesian posterior probability = 100%).

Discussion and classification

Classification of deep-sea zoanthids up until now has been relatively limited, with all observed samples placed within the genus *Epizoanthus* (family Epizoanthidae). Most described *Epizoanthus* spp. are colonial and epizoitic, differing from *Abyssoanthus*. Similar to *A. nankaiensis*, *E. vagus*, described from the Mediterranean Sea (Herberts 1972), is a non-colonial free-living zoanthid, but is found at depths of only 30–120 m.

No published reports of zoanthids from so-called 'extreme' environments (for example cold seeps and hydrothermal vents etc.) exist, although this may be the result of a lack of proper investigation and/or sampling effort. According to anecdotal evidence, organisms similar to *A. nankaiensis* have been observed at numerous cold seeps and hot water vents in the deep-sea around Japan (K. Fujikura pers. comm.; H. Miyake pers. comm.) and in the Atlantic (C. Kato unpub. data). Further sampling followed by morphological and molecular examination of deep-sea zoanthids from a variety of environments is needed to understand this long-neglected and potentially diverse group of organisms.

Table 2. Dimensions of *Abyssoanthus nankaiensis*, gen. *et* sp. nov. polyps

Polyp number*	Height (mm)	Oral maximum width (mm)	Mid-polyp minimum width (mm)	Aboral maximum width (mm)
Holotype NSMT-Co 1500	3.50	2.45	1.85	2.25
Paratype 1 NSMT-Co 1501	4.70	2.30	1.60	2.75
Paratype 2 NHM 2007.80	4.50	2.90	2.70	3.20
Paratype 3 USNM 1084304	4.35	2.10	1.70	2.25
10 JAMSTEC 058332	5.65	2.60	2.15	3.30
11 JAMSTEC 058333	3.70	2.15	1.60	2.15
12 JAMSTEC 058334	3.70	2.80	2.15	2.60
Mean ± s.d.	4.3 ± 0.75	2.5 ± 0.31	2.0 ± 0.40	2.6 ± 0.47

*All polyps were preserved in 70% ethanol at time of measurement and closed. Expanded *in situ* polyps are considerably larger (see text).

How *A. nankaiensis* acquires nutrition remains in question. A wide range of cold seep organisms from the Nankai Trough are in symbioses with autothiotrophic bacteria (for example *Lamellibrachia* sp. vestimentiferans in Kojima *et al*. (2001) and *Calyptogena* sp. bivalves (M. Kawato pers. comm.)), suggesting that this may also be the case with *A. nankaiensis*. Initial results investigating potential symbiotic bacteria in our *A. nankaiensis* samples were inconclusive. The presence of potentially endosymbiotic epsilon protobacteria was molecularly confirmed from two of three polyps (in one of twelve investigated cloned sequences [polyp 2] and five of twelve cloned sequences [polyp 3]), but their intracellular presence in *A. nankaiensis* could not be confirmed owing to a limited number of available samples, and the potential of bacterial contamination from neighbouring *Lamellibrachia* sp. (visible in Fig. 1) and *Calyptogena* sp. Thus, whether *A. nankaiensis* is in symbiosis with bacteria or free-living remains to be clearly determined.

Based on morphological characteristics and obtained genetic sequences, the family Abyssoanthidae is the first zoanthid group described from a chemosynthetic ecosystem. Our study highlights the glaring need for further investigation of deep-sea zoanthids. Return trips to the sampling location at Nankai Trough and

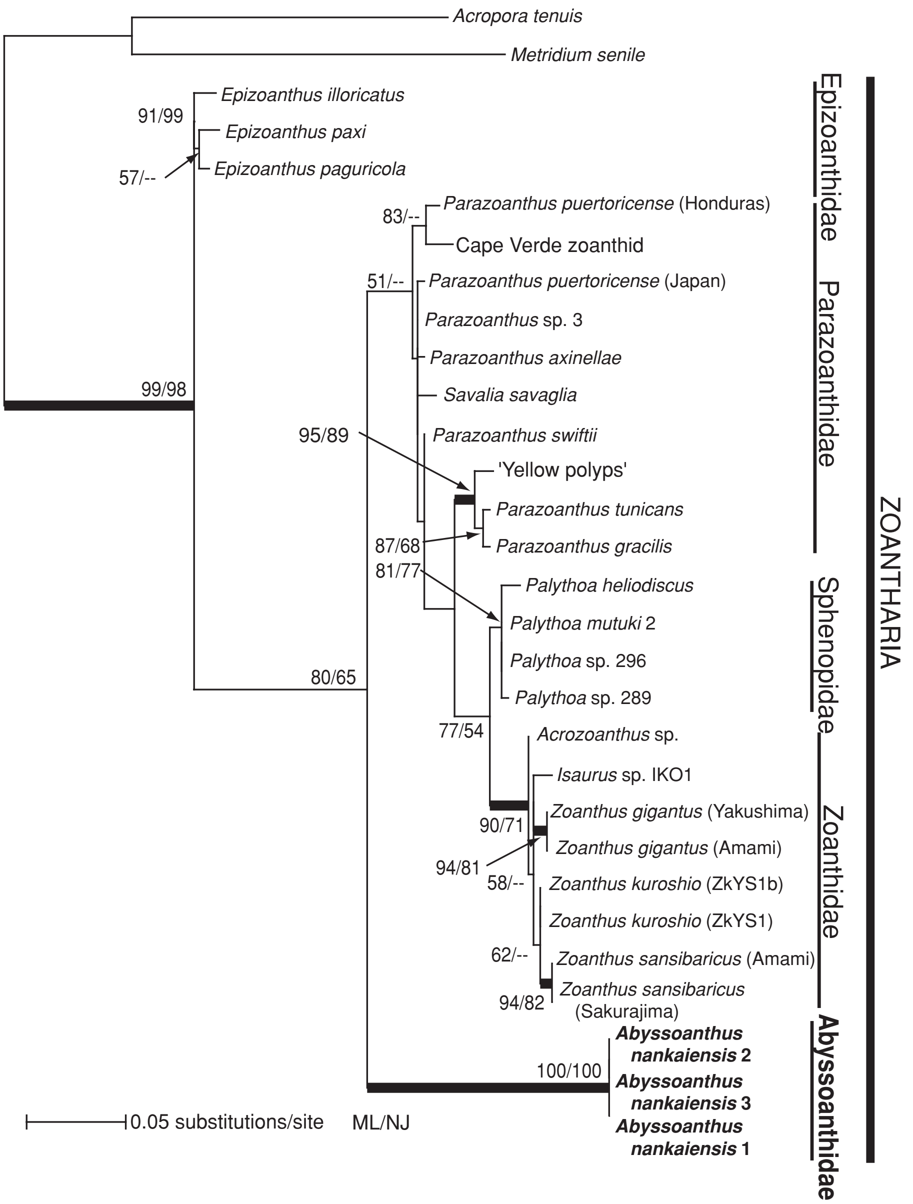

Fig. 3. Maximum likelihood tree of mitochondrial cytochrome oxidase c subunit I sequences from the Order Zoantharia with Hexacorallia outgroups highlighting the novel phylogenetic position of Abyssoanthidae (sample names in bold). Values at branches represent ML and NJ bootstrap probability, respectively (>50%). Monophylies with more than 95% Bayesian posterior probability are shown by thick branches. (For sample name information refer to Table 1.)

in-depth *in situ* observation of *A. nankaiensis* are required to further characterise this intriguing new family. The continued use of a combination of morphological and molecular analyses may help uncover more new zoanthid groups in the future.

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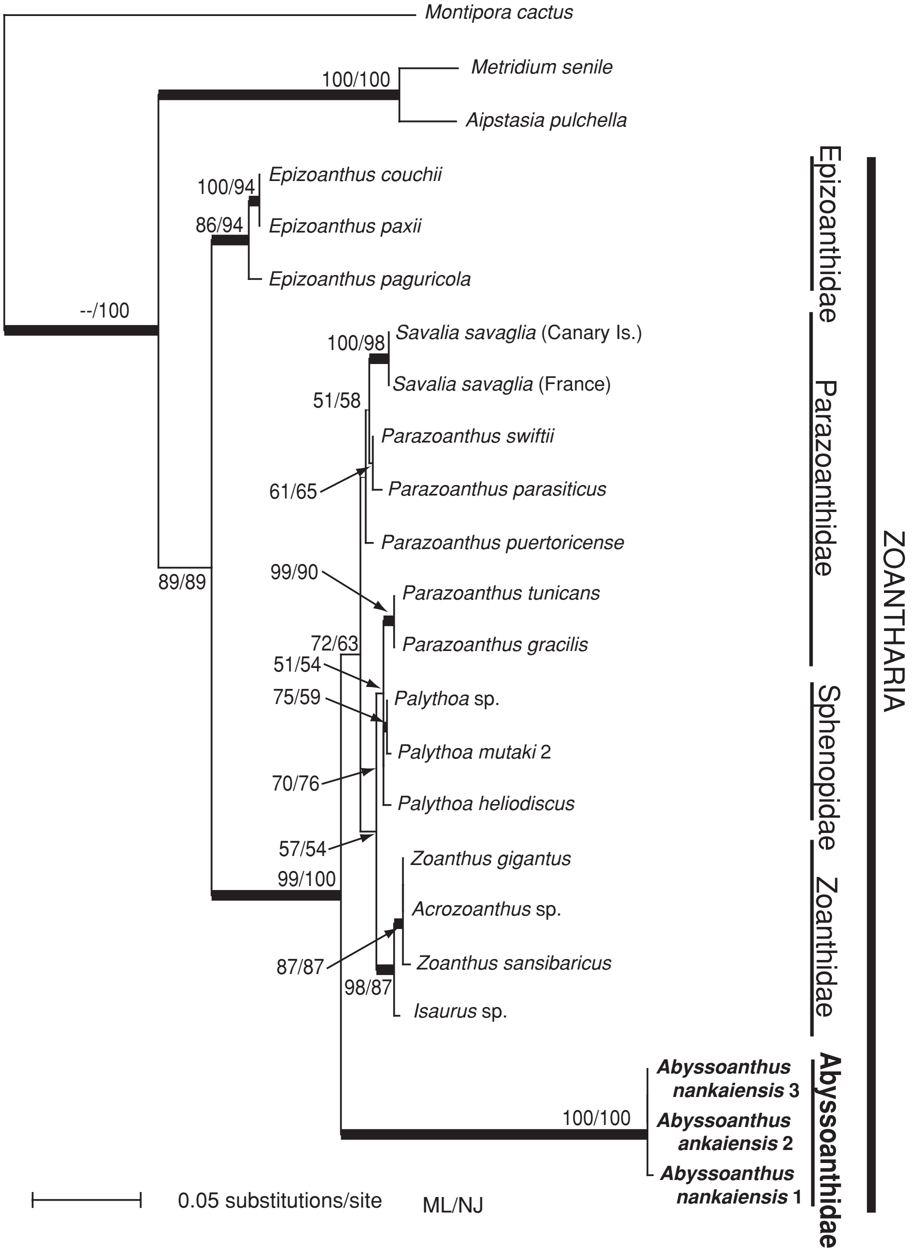

Fig. 4. Maximum likelihood tree of mitochondrial 16S rDNA sequences from the Order Zoantharia with Hexacorallia outgroups highlighting the novel phylogenetic position of Abyssoanthidae (sample names in bold). Values at branches represent ML and NJ bootstrap probability, respectively (>50%). Monophylies with more than 95% Bayesian posterior probability are shown by thick branches. (For sample name information refer to Table 1.)

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