



A record of *Neozoanthus* cf. *uchina* Reimer, Irei & Fujii, 2012 from the Yaeyama Islands, southern Ryukyu Islands, Japan

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Abstract. A single zoanthid specimen closely resembling *Neozoanthus uchina* Reimer, Irei & Fujii, 2012 (Cnidaria: Anthozoa: Zoantharia: Neozoanthidae) was recently collected from shallow mesophotic waters (38 m) in Nagura Bay, Ishigaki Island, southern Ryukyu Islands, Japan. Phylogenetic analyses indicate a small but significant difference in mitochondrial 16S ribosomal DNA sequences between this specimen and the other two Pacific species of *Neozoanthus*, *N. uchina* from the central Ryukyu Islands and *N. caleyi* Reimer, Irei & Fujii, 2012 from the Great Barrier Reef in Australia. This single specimen is the first distributional record of this genus in the northern hemisphere south of Okinawa Island, Japan. We briefly discuss the possibility identity of this specimen, and preliminarily designate it as *Neozoanthus* cf. *uchina* based on morphological similarities (partial sand encrustation, tentacle count) with *N. uchina*. Future research into *Neozoanthus* in the Yaeyama Islands is needed to clearly ascertain its true status. Furthermore, this research demonstrates deeper mesophotic regions may harbor unknown zoanthid diversity.

Introduction

The zoanthid family Neozoanthidae Herberts, 1972 was first described as a monotypic group based on a single species from Madagascar, *Neozoanthus tulearensis* Herberts, 1972. Uniquely for zoanthids, Neozoanthidae is only partially encrusted with sand and detritus (Reimer et al. 2012), unlike other zoanthids, which are either completely encrusted or have no encrustation at all. Internally, *Neozoanthus* has an endodermal sphincter but a brachynermic mesentery arrangement (Herberts 1972). Thus, *Neozoanthus* has a unique morphology among zoanthids, and due to its unique encrustation pattern, is relatively easy to identify in the field. Recently, putative *Neozoanthus* specimens were collected

from both the Great Barrier Reef (GBR) and Okinawa, Japan (Reimer et al. 2011a), and these were formally described as *N. caleyi* Reimer, Irei & Fujii, 2012 in the GBR and *N. uchina* Reimer, Irei & Fujii, 2012 from Okinawa and Kagoshima Prefectures, Japan.

Neozoanthus uchina is known to be distributed from Okinawa Island, Japan northwards to Amami-Oshima Island in Kagoshima, but has not been found further south in the Miyako and Yaeyama Islands (=Sakishima Islands), Japan, nor in Taiwan, despite zoanthid-focused surveys in these areas (e.g. Reimer et al. 2011b), nor has it been seen from more northerly Yakushima, Kyushu, or Shikoku in zoanthid surveys in these regions (Reimer 2007; Ono et al. 2008).

In this study, we report on a newly found *Neozoanthus* specimen from Ishigaki Island, southern Ryukyu Islands, Japan and its possible identity.

Materials and methods

Specimen collection. The single specimen in this study was collected by hand during a diving with the aid of a closed circuit rebreather (Evolution, AP Diving Ltd.), and photographed in situ from a depth of 38 m at Nagura Bay, Ishigaki Island, southern Ryukyu Islands, Japan (24° 24.402' N, 124° 6.827' E) on 23 January 2013. After collection, it was preserved in 99.5% ethanol until further analyses.

DNA extraction and PCR. DNA extraction was performed following Sinniger et al. (2010). For the specimen in this study, two DNA markers were sequenced; mitochondrial 16S ribosomal DNA (mt 16S rDNA) and the Folmer region of cytochrome oxidase subunit I (COI). Primers and PCR reaction conditions followed as in Sinniger et al. (2005) for mt 16S rDNA and Reimer et al. (2007a) for COI, respectively. The amplified products were visualized by 1.5 % agarose gel electrophoresis.

PCR products were treated with Exonuclease I and Alkaline Phosphatase (Shrimp) (Takara) prior to sequencing. Sequences were outsourced and products analyzed by Fasmac Japan (Kanagawa, Japan).

Phylogenetic analyses. The two new sequences obtained in the present study were deposited in GenBank (accession number KF914141). For phylogenetic analyses of mt 16S rDNA, acquired sequences were aligned with recently published sequences of *N. uchina* and *N. caleyi* (Reimer et al. 2011a) (GenBank Accession Numbers HM991227-229, HM991230, HM991235-236), with sequences from *Zoanthus sansibaricus* (AB219188) and *Isaurus tuberculatus* (EF452253) (both species within Family Zoanthidae) as outgroups. The alignment was inspected by eye and manually edited. No ambiguous sites in the alignment were observed. An alignment dataset of 740 sites of 9 sequences was generated. The alignment data are available on request from the corresponding author.

The mt 16S rDNA alignment was analyzed with

maximum-likelihood method (ML) using PhyML (Guindon et al. 2010). PhyML was performed using an input tree generated by BIONJ with the general time-reversible (GTR) model of nucleotide substitution incorporating fixed invariable sites and eight categories (GTR + I). The discrete gamma distribution, and base frequencies of the model were estimated from the dataset. PhyML bootstrap trees (1000 replicates) were constructed using the same parameters as the individual ML tree. CLC Free Workbench 3.2.2 (Aarhus, Denmark) was used for NJ phylogenetic analyses (1000 replicates).

Systematics

Family Neozoanthidae Herberts, 1972

Genus Neozoanthus Herberts, 1972

***Neozoanthus cf. uchina* Reimer, Irei & Fujii, 2012**

(Figs. 1–3)

Specimen examined. Specimen RUMF-ZG-04382. Four individual polyps from a small colony

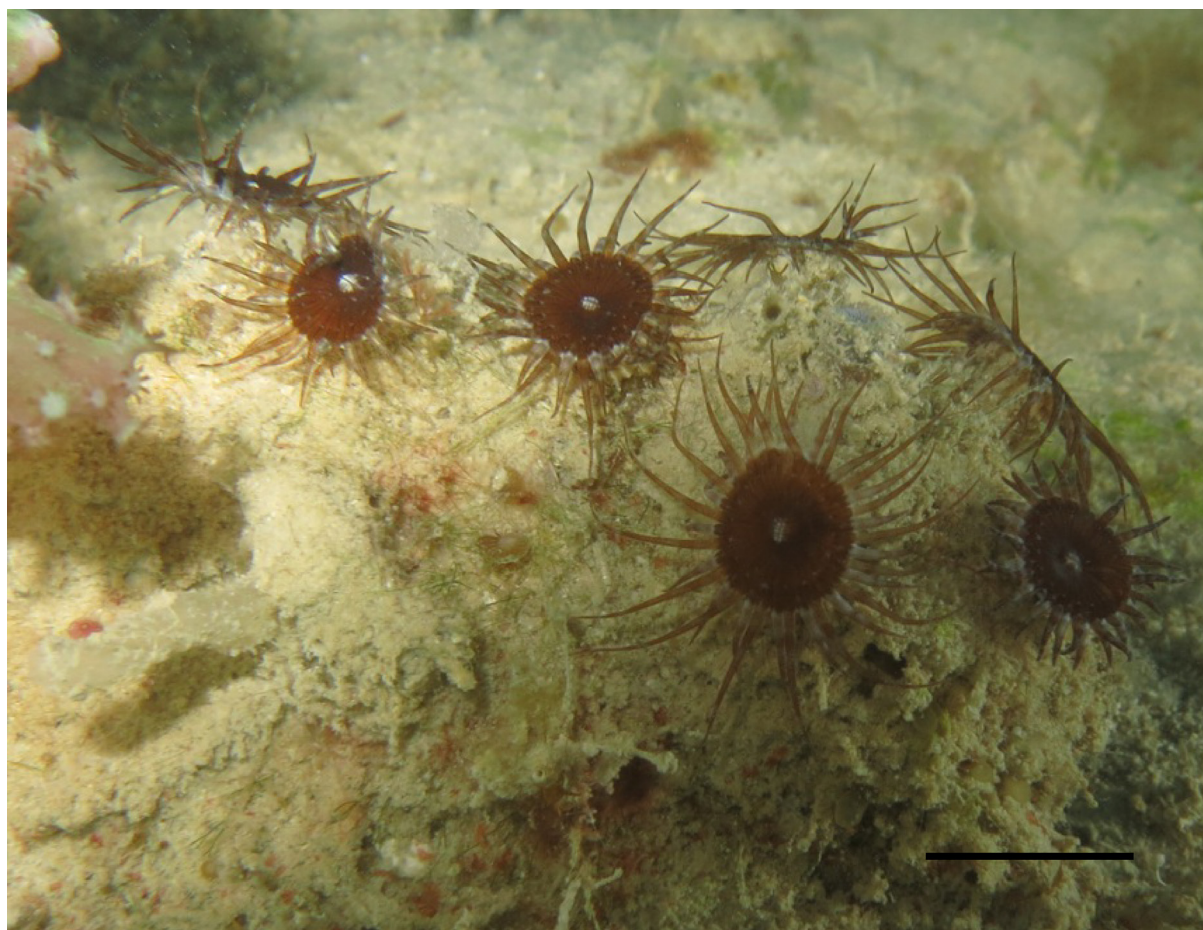


Fig. 1. *Neozoanthus cf. uchina* in situ at Nagura Bay, Ishigaki Island, southern Ryukyu Islands. Depth = 38m, 23 January 2013. Scale bar = approximately 1 cm.

図 1 *Neozoanthus cf. uchina*. 石垣島名蔵湾産、水深 38m にて 2013 年 1 月 23 日に撮影。スケールバーは約 1cm.

of seven polyps originally connected by stolon, on piece of coral rubble. RUMF= Ryukyu University Museum, Fujukan, University of the Ryukyus, Japan.

Morphological aspects. Preserved (99% ethanol), closed polyps 2.5–3.0 mm in diameter, 2.5–4.0 mm in height, coated in sand and sediment except for oral end (not visible as polyps are closed). From in situ images, polyps with 36–38 tentacles, tentacles approximately as long as oral disc diameter (estimated at approximately 7 mm). Oral disc color wine red with small blue spots towards

margins, tentacles translucent with faint, alternating grayish-blue and brown stripes (Fig. 1). Oral opening (=mouth) white.

Phylogenetic aspects. Acquired mitochondrial cytochrome oxidase subunit I sequences match 100% with previously reported sequences from *Neozoanthus uchina*. However, there was a single base pair difference (1/740 base pairs; =0.013%) between previous *N. uchina* mitochondrial 16S ribosomal DNA sequences and this specimen's sequence (Figs. 2, 3), and therefore phylogenetic analyses were carried out only using this marker.

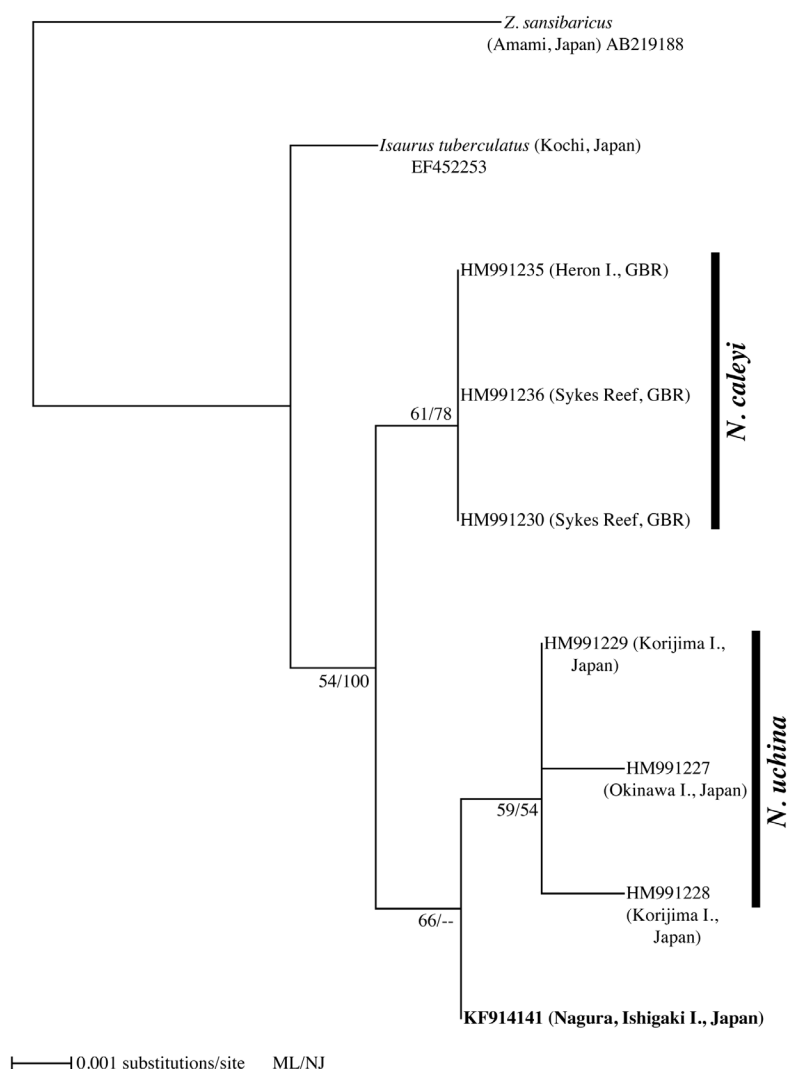


Fig. 2. Maximum likelihood (ML) trees of mitochondrial 16S ribosomal DNA sequences of *Neozoanthus* species examined. The new sequence from this study in **bold**. Sequences from previous studies with GenBank Accession Numbers and sampling locations. Values at nodes represent ML and neighbor-joining (NJ) values, respectively. Abbreviation: GBR=Great Barrier Reef (Australia).

図2. *Neozoanthus* 類のミトコンドリア 16S rDNA を用いた最尤法による系統解析. 本研究により新たに得られた配列を太字で示す. 先行研究により得られた配列は, GenBank の登録番号と標本の産地を示す. 分岐点の値は, 最尤法と近隣結合法のブートストラップ値を示す. GBR: グレートバリアリーフ (オーストラリア).



Fig. 3. Alignment of a portion of mitochondrial 16S ribosomal DNA (mt 16S rDNA) showing sequences from the specimen in this study, *Neozoanthus uchina*, and *N. caleyi*. Areas and red fonts in boxes highlight regions of difference between sequences. Alignment position numbers are identical to numbers in the mt 16S rDNA alignment used in analyses.

図3 石垣産 *Neozoanthus cf. uchina*, リュウキュウニセマメスナギンチャク *Neozoanthus uchina* 及びオーストラリア産 *N. caleyi* におけるミトコンドリア 16S rDNA のアライメント結果。

四角で囲まれた部分は配列間に違いがあった箇所を示し、*N. cf. uchina* において異なる部位を赤字で示す。アラインメントの位置番号は、解析に使用した mt16S rDNA のアラインメントと同じである。

Remarks. This specimen was initially identified as belonging to the genus *Neozoanthus* based on being zooxanthellate and having partial encrustation of outer body wall with no encrustation around the oral end of polyps. Oral disc coloration, tentacle number, polyp sizes, and encrustation pattern all agree well with both *Neozoanthus uchina* and *N. caleyi* as originally described (Table 1). Phylogenetically, the specimen was closer to *N. uchina* than to *N. caleyi* (Fig. 2).

Discussion. The specimen from Ishigaki Island was found in a somewhat different environment than has been reported for *N. uchina* previously (Reimer et al. 2012). Nagura Bay, Ishigaki Island, is relatively muddy with heavy sedimentation, and is a sheltered bay with a generally low level of water energy (waves, tidal currents, etc.). Finally, the specimen was found at 38 m, deeper than has been previously reported for *N. uchina*. Around Okinawa-jima Island and other locations, *N. uchina* has been reported from locations with sandy sedimentation, high levels of water flow, and has been found from the intertidal zone to 25 m. Thus, the discovery of *Neozoanthus* in Nagura Bay is somewhat surprising.

The identity of this specimen remains somewhat ambiguous. Although morphologically this specimen agrees perfectly with *N. uchina*, the mt 16S rDNA data presents an unexpected 1 base pair difference with *N. uchina* (Figs. 2, 3). This low level of variation has previously indicated

species-level differences in Anthozoa due to the slow evolution of mitochondrial DNA (Shearer et al. 2002; Huang et al. 2008). For example, the zoanthid species *Palythoa tuberculosa* (Esper, 1805) and *P. mutuki* (Haddon & Shackleton, 1891) can be distinguished by 1 base pair of difference over the combined COI+mt 16S rDNA data set (Reimer et al. 2006c; 2007b), as can *Zoanthus kuroshio* Reimer & Ono in Reimer, Ono, Iwama, Takishita, Tsukahara & Maruyama, 2006 and *Z. vietnamensis* Pax & Müller, 1957 (Reimer et al. 2006b; 2013). Thus, based on COI and mt 16S rDNA data alone, it is possible conclude the specimen is either an undescribed *Neozoanthus* sp., or a subspecies of *N. uchina*. Therefore, given the sum of the evidence, and with examination of only a single specimen, for now we designate this specimen as *Neozoanthus cf. uchina*.

Although there are some small discrepancies between the conclusions that can be reached based on either the morphological and molecular data of this specimen, such a situation is not uncommon in zoanthids, as overlap in morphological characters between different species is possible (Burnett et al. 1997; Reimer & Sinniger 2010). For the time being, more specimens of *Neozoanthus* from similar muddy, shallow mesophotic environments are needed to confirm or refute the findings here.

If this specimen is truly identifiable as *N. uchina*, it represents the southernmost record of *Neozoanthus uchina*, and would extend the southern

Table 1. Comparison of various features of species of *Neozoanthus* and specimen in this study.

表 1. ニセマメスナギンチャク属各種の諸特徴の比較.

	<i>N. tulearensis</i> ¹	<i>N. uchina</i> ²	<i>N. caleyi</i> ²	This specimen (RUMF-ZG-04382)
Distribution	NE Madagascar	Central Ryukyu Islands, Japan	Heron Island, Great Barrier Reef, Australia	Nagura Bay, Ishigaki I., Japan
Depth (m)	No data	0-25	4-29	38
Oral disk color	Greenish-beige to yellow	Light gray-blue, white, rust or deep wine red	Light gray-blue, white, or deep wine red	Wine-red with small blue dots towards margin, oral opening white
Polyp diameter (mm)	1.5-5.0	2.2-5.1	2.3-5.0	2.5-3.0 (n=4)
Polyp height (mm)	2.0-12.0	2.0-8.5	2.5-3.0	2.5-4.0 (n=4)
Number of tentacles (avg. ± SE)	38-44	32-42 (38±3.0)	28-40 (33±3.9)	36-38 (n=2)

¹From Herberts (1972).²From Reimer et al. (2012).

limit of distribution of this species from Okinawa Island to Ishigaki Island, an extension of approximately 400 km. Although this report describes only one specimen, this finding indicates *Neozoanthus* inhabits a wider range of environments than previously reported (Reimer et al. 2011a; 2012). Future research into *Neozoanthus* in Japan and Taiwan would benefit from examining the shallow mesophotic zone (30-50 m) in more detail, and examining the endosymbiotic *Symbiodinium* of *Neozoanthus* from such depths, as some other zoanthids are known to be somewhat flexible in their symbiont association (Burnett 2002; Kamezaki et al. 2013).

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リュウキュウニセマメスナギンチャク *Neozoanthus uchina* Reimer Irei & Fujii, 2012 の八重山諸島からの報告

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要旨. 石垣島名蔵湾の中深度上部 (水深 38m) より, リュウキュウニセマメスナギンチャク *Neozoanthus uchina* Reimer, Irei & Fujii, 2012 (刺胞動物門: 花虫綱: スナギンチャク目: Neozoanthidae 科) に似た標本が得られた. 系統解析の結果, この標本と 中琉球産リュウキュウニセマメスナギンチャク, 及びオーストラリ

ア・グレートバリアリーフ産 *N. caleyi* の間には、ミトコンドリア 16S リボゾーマル DNA に若干の違いがみられた。今回のニセマメスナギンチャク属の石垣島からの報告は、北半球の沖縄島以南では最初の分布記録である。本報告では、石垣産標本の分類学的位置について、リュウキュウニセマメスナギンチャク、あるいはその亜種、もしくは未記載種である可能性についても検討したが、形態的な類似性と標本数が限られていることから、暫定的に *Neozoanthus cf. uchina*

として扱うことにした。八重山諸島に分布するニセマメスナギンチャク類の分類学的な位置を正確に決定するためにはさらなる調査が必要であるが、本研究により、中深度深部における未知なスナギンチャク類が存在する可能性が示唆された。

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