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PRELIMINARY LIST OF MACROCNEMIC ZOANTHID DIVERSITY (ANTHOZOA: HEXACORALLIA: ZOANTHARIA) FROM SOUTHERN SHIKOKU, JAPAN

By

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Abstract

The oceans of southwestern Shikoku are known for their high levels of marine biodiversity due to the presence of temperate, subtropical, and tropical species. In a previous report, we examined the species diversity of brachycnemic zoanthids (Brachycnemina: Zoantharia: Anthozoa: Cnidaria) in southwestern Shikoku in order to provide basic data for future zoanthid research in the region. However, little information on the other suborder of zoanthids, Macrocnemina, exists for this region. This study, based on surveys conducted between 2006 and 2012, aims to fill this gap by providing a species list of macrocnemic zoanthid species from this region, along with informal descriptions, habitat information, and in situ images. In total, these surveys have recorded eight species from five genera in the southern Shikoku region; *Epizoanthus ramosus*, *Epizoanthus* sp. C, *Epizoanthus* sp. S, *Parazoanthus* sp., *Parazoanthus* aff. *puertoricense*, *Savalia* sp., *Antipathozoanthus* sp., and *Hydrozoanthus gracilis*. However, as most specimens were acquired by snorkeling and SCUBA, only the shallowest and most accessible areas have been examined, and it is very likely that the total species diversity of macrocnemic zoanthids in southern Shikoku is much higher than indicated here.

Introduction

Zoanthids are an order (Zoantharia; =Zoanthidea, Zoanthiniaria) of hexacorals similar in many regards to anemones (order Actiniaria), with soft-tissued bodies. Most zoanthid

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species are colonial, and all have tentacles forming two cycles around the oral disks of polyps. Unlike other benthic cnidarians, most zoanthid species take up detritus from the surrounding environment and incorporate this into their structure for strength and protection. However, due to this, sectioning and histology of zoanthids can be problematic (Reimer et al. 2010b), and traditional morphological examinations and identifications are often very difficult. However, in the past three decades, examinations using molecular methods have re-invigorated zoanthid taxonomic research (e.g. Burnett et al. 1995; 1997; Reimer et al. 2004; Sinniger et al. 2005; Swain & Wulff 2007). Currently, the order Zoantharia is one of the few cnidarian groups for which the molecular phylogeny largely reflects taxonomic understanding.

The suborder Macrocnemina, despite being paraphyletic (Sinniger et al. 2005), contains zoanthid species with a "macrocnemic" mesentery arrangement; in which the fifth mesentery from the dorsal directive in these zoanthids is complete (=large, ="macro"). Macrocnemic zoanthids are often epizoic upon other benthos such as hermit crabs, gastropods, sponges or hydrozoans, and the group has undergone recent revision (Sinniger et al. 2010) with the host taxa playing an important role in supraspecific (e.g. generic) determination.

In Japan, many surveys of zoanthids have been carried out in recent years, from the Yaeyama Islands of southern Japan to Miyake-jima Island, south of Tokyo. The majority of the surveys have focused on the suborder Brachycnemina, which includes the conspicuous and common shallow water zooxanthellate genera *Zoanthus* and *Palythoa*. On the other hand, knowledge on the zoanthids in the suborder Macrocnemina, which includes species often found at deeper depths and at higher latitudes (Ryland et al. 2001; Swain 2010), is incomplete for Japan, despite some historical records and descriptions (e.g. Lwowsky 1913; Carlgren 1934).

The waters of southern Shikoku are known for their high biodiversity due to the confluence of the colder temperate waters of the Bungo Suido Channel and Seto Inland Sea with the warmer, northwardly flowing Kuroshio Current that travels north along the Pacific coast of Japan (Fig. 1). Southern Shikoku is noted for its extensive hermatypic coral communities and mixture of tropical, subtropical and temperate fauna.

In 2007, a preliminary report of the zoanthids of the suborder Brachycnemina was published to provide baseline data for future zoanthid research in the southern Shikoku region (Reimer 2007). Here, as a follow-up to Reimer (2007), a preliminary list of the macrocnemic zoanthids of the same region is given.

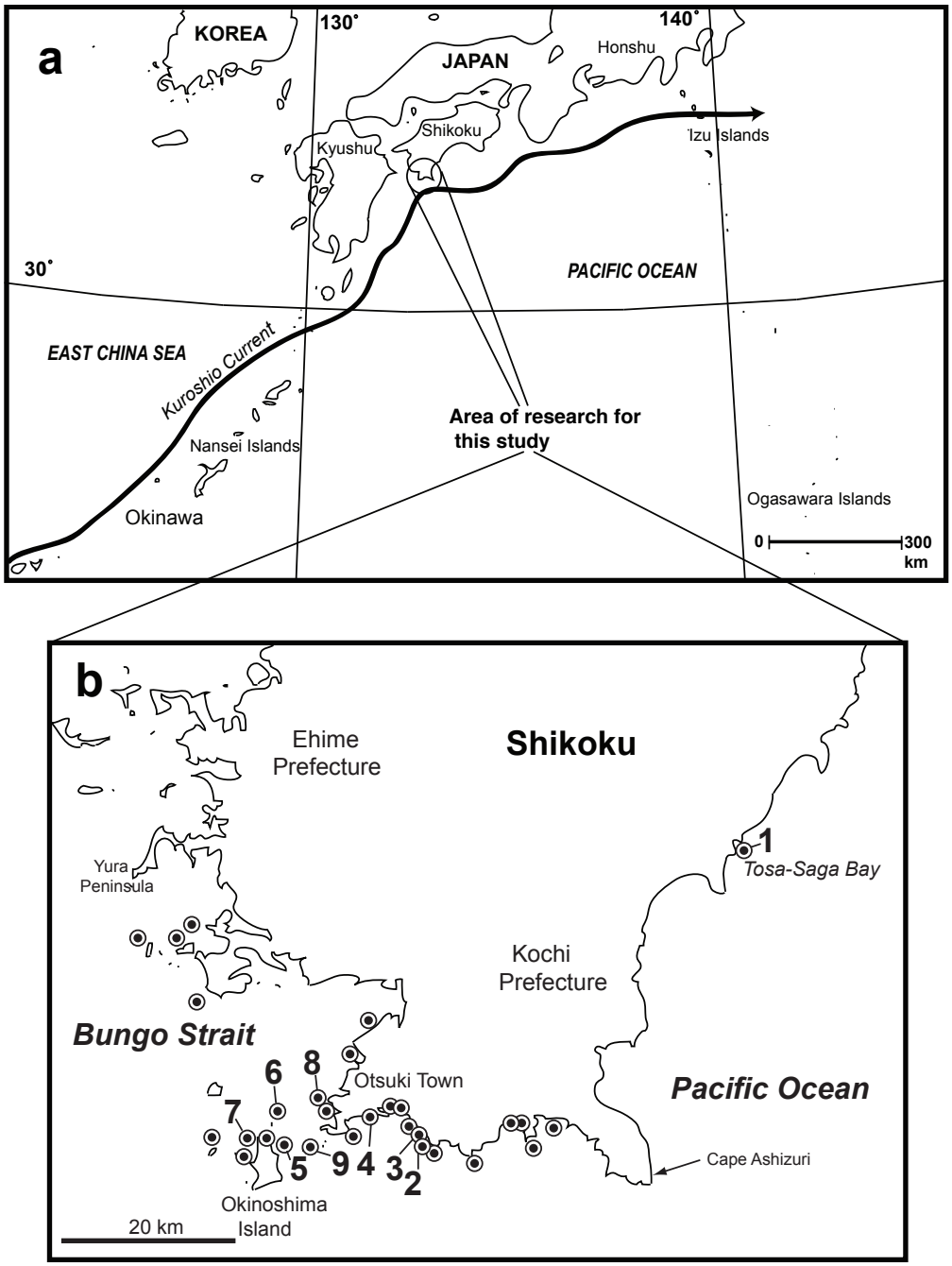

Fig. 1 Map of area investigated in this study. A. Overall region of Shikoku and Japan, showing the pathway of the Kuroshio Current. B. Sampling area of southern Shikoku. Circles indicate locations that were investigated. Numbered locations mentioned in text as follows:

1 = Tosa-Saga Bay; 2 = Nishidomari; 3= Arch at Nishidomari; 4 = Odo Kaigan;

5 = Sotogashira; 6 = Futanarabijima; 7 = Akasaki-niban; 8 = Komo-ichi,

9 = Asabae-zaki, Birojima.

Materials and methods

Specimen observation, collection, and examicnation

Specimens were searched for during snorkeling or SCUBA diving between September 2006 and September 2012 (n=>40 dives). The region directly investigated by the authors spanned from Ainan in the Bungo Suido Channel of Ehime across to the Pacific coast and Cape Ashizuri in Tatsukushi, Kochi, including Okinoshima and surrounding small islands (Figure 1). Zoanthids were photographed *in situ* using a digital camera with underwater housing. At the same time, depth and surrounding environment were noted. Collected specimens Table 1) were placed in a numbered Ziploc plastic bag or sampling jar. Specimens were kept in seawater (SW) until returning the Biological Institute on Kuroshio. Some specimens were kept for a short period (<1 d) in a running SW tank for further observation, and all specimens were ultimately preserved in 70-99.5% ethanol.

Additional *Savalia* and *Epizoanthus* spp. specimens were acquired by fishery nets and trawling. *Savalia* specimens and one specimen of *Epizoanthus ramosus* were collected by precious coral nets from depths of 80-129 m (*Savalia*) or 100-200 m (*E. ramosus*) in October 2007 in Otsuki, Kochi. *Epizoanthus* spp. were collected in 2009 as described in Reimer et al. (2010) from Tosa-Saga Bay, Kochi. All additional specimens were also preserved in 70-99.5% ethanol, except for some specimens (n=3) preserved in 10% SW formalin for morphological analyses. All specimens examined in this study and their collection information are listed in Table 1.

Results and Discussion

Systematics and Species List

The list below is given as a general guide and introduction to macrocnemic species of southern Shikoku. It is not intended as a formal guide or taxonomic revision, but to serve as a basis for future zoanthid research in the region.

Suborder Macronemina Haddon and Shackleton, 1891

Zoanthids with the fifth mesenteries from the dorsal directive being complete. This suborder of Zoantharia is not monophyletic (Sinniger et al. 2005).

Family Epizoanthidae Delage and Hérouard, 1901

Type genus: *Epizoanthus* Gray, 1867

Macrocnemic zoanthids with a simple mesogleal sphincter muscle.

Genus *Epizoanthus* Gray, 1867

Type species: *Epizoanthus papillosus* (Johnston, 1842), by monotypy.

General diagnosis as for family but distinguishable from the other genus on the family, *Palaeozoanthus,* by the presence of non-fertile micromesenteries (Carlgren 1928, see also Sinniger and Haüssermann 2009). Species found on rocky substrata, or on gastropod shells inhabited by gastropods or pagurid crabs.

1. *Epizoanthus ramosus* Carlgren, 1934

Plate 1A (reproduced from Reimer et al. 2010a)

Colony morphology: Like the other two *Epizoanthus* spp. below, has a well-developed coenenchyme.

Size and polyp morphology: *Epizoanthus ramosus* has polyps that are comparatively smaller (fixed polyps <4 mm in diameter, <3 mm in height) than the other two *Epizoanthus* spp. listed below. This species has been reported to have between 26 (Reimer et al. 2010a) to 36 (Carlgren, 1934) white tentacles.

Associations: This species was originally described as associated with hermit crab species living within snail shells, although colonies have been reported to spread to hard substrata in aquaria (J.D. Reimer, personal observation), and there are specimens associated with the gastropod *Guildfordia triumphans* that have been also identified as *Epizoanthus ramosus* (mentioned in Reimer et al. 2010a), as was the specimen in this study.

Locations and depth in this study: Odo Kaigan, Otsuki, Kochi, 100 to 200 m.

Notes: *Epizoanthus ramosus* was first described from specimens from Misaki Marine Station (locality = "Tutamachiga") at depths of 30 to 45 m. Given the paucity of data on *Epizoanthus* in Japan and the Pacific, and based on the results of recent works (e.g. Reimer et al. 2010a) it is possible the original *E. ramosus* and the specimens on *G. triumphans* could be different species.

2. *Epizoanthus* sp. C *sensu* Reimer et al. (2010a)

Plate 1B (reproduced from Reimer et al. 2010a)

Colony morphology: Polyps may be up to 10 mm above the surrounding, well-developed

coenenchyme, which encusts the majority of the upper surface of *Gemmula* spp. shells.

Size and polyp morphology: This species has approximately 24 translucent red tentacles, with a fixed polyp diameter of 4-6mm.

Associations: Found on the outer surfaces of shells of *Gemmula* spp. gastropods inhabited by *Paguristes palythophilus* hermit crabs.

Locations and depth in this study: This undescribed species has only been reported from Tosa Saga Bay region of Kochi (Fig. 1), where it is found at 40 to 60 m in sandy bays.

Notes: As this putative species was first discovered in 2010, very little is known about its range, ecology, biology, or whether it associates with other crab species. This situation is indicative of the state of *Epizoanthus* spp. research not only in Japan but for much of the Indo-Pacific.

3. *Epizoanthus* sp. S *sensu* Reimer et al. (2010a)

Plate 1C (reproduced from Reimer et al. 2010a)

Colony morphology: Like *Epizoanthus* sp. C, this species has a well developed coenenchyme. Polyps are generally spaced more tightly together than in *Epizoanthus* sp. C.

Size and polyp morphology: Tentacles are translucent red, and number approximately 24, although in situ images are scarce. Fixed polyps are up to 6 mm in height, and 2-5 mm in diameter.

Associations: This species is found on the outer surface of live *Gemmula unedo* gastropods.

Locations and depth in this study: Like *Epizoanthus* sp. C above, this species has only been reported from Tosa Saga Bay (Fig. 1), where it is found at similar depths of 40 to 60 m.

Notes: As this species was reported for the first time in Reimer et al. (2010a), very little is known of its range, ecology, and biology.

Family Parazoanthidae Delage and Hérouard, 1901

Type genus: *Parazoanthus* Haddon and Shackleton, 1891

This family includes macrocnemic zoanthids possessing an endodermal sphincter (according to the original description). Species are often associated with other benthic organisms, which are utilized as substrata.

Genus *Parazoanthus* Haddon and Shackleton, 1891

Type species: *Parazoanthus axinellae* (Schmidt, 1862)

Parazoanthids with a well-developed canal system in the mesoglea of the column, forming a

ring sinus. These zoanthids usually associated with sponges, and this genus seems to be not monophyletic, potentially consisting of three major clades (A to C) (Sinniger et al. 2010).

4. *Parazoanthus* sp.

Plate 2A

Colony morphology: Colonies consist of polyps connected by stolons embedded within sponge tissue. Colonies generally cover the entire host sponge.

Size and polyp morphology: Diameter of polyps were <2 mm, and details were difficult to observe by the naked eye in situ. The colors of polyps and tentacles, regardless of sponge color, were light brown or tan, with no obvious markings or patterns. Tentacles numbered approximately 28, and were transparent, with a length of approximately 40-60% of the expanded oral disc.

Associations: This species was associated with several species of demosponges, with polyps protruding from the sponge tissue, and connected by stolons inside the sponge.

Locations and depth in this study: This species was the most common macrocnemic zoanthid observed during our research, and was noticed at many of the locations surveyed. Specimens were found even from very shallow depths (2 m), associated with sponges in overhangs and crevices and not directly exposed to sunlight. Specimens observed and collected from numerous locations around Nishidomari, Okinoshima, and the Bungo Channel coast of Ehime.

Notes: Based on recent phylogenetic research (J. Montenegro, pers. comm.) in Okinawa, Japan, this group may include more than one species.

5. *Parazoanthus* aff. *puertoricense sensu* Uchida, 2001

Plate 2B

Colony morphology: Similar to *Parazoanthus* sp. above, with polyps generally connected by stolons within the sponge tissue and not visible from the outside. Occasionally, two or three polyps were located close together and joined by coenenchyme on the sponge surface. Colonies had high amounts of encrustation, with sediment often visible in polyps.

Size and polyp morphology: Polyps of this species were slightly bigger than those of *Parazoanthus* sp. above, up to 3 mm in diameter.

Associations: This species was associated with several species of demosponges, with polyps protruding from the sponge tissue, and connected by stolons inside the sponge.

Locations and depth in this study: In this study, this species was observed at several sites but

appears to be not as abundant as *Parazoanthus* sp. above, and was generally found at deeper depths (approximately 20 m). Specimens from Sotogashira at Okinoshima, and observed in Nishidomari.

Notes: Although similar in morphology to *P. puertoricense* from the Caribbean, from distributional and phylogenetic data it is clear that this species is not *P. puertoricense* and does not match any other sponge-associated species, and is likely an undescribed species. This species has also been observed from Izu Penninsula (Reimer et al. 2007) and Wakayama (Uchida 2001) in Japan.

Genus *Savalia* Nardo, 1844

Type species: *Savalia savaglia* (Bertolini, 1819)

Parazoanthids with no mesogleal canal system in the column. Secretes a black or dark brown horny skeleton, and is azooxanthellate.

6. *Savalia* sp.

Plate 2C

Colony morphology: The polyps are all connected by a continuous ceonenchyme covering the skeleton.

Size and polyp morphology: The polyp observed were about 1-2 mm high and 3-4 mm diameter. However, the appearance of the colony suggested a strong reduction of polyp size resulting from the long storage (several months) in running seawater without feeding (F. Sinniger. personal observation). Based on the knowledge on other specimens belonging to this genus, the expected size of the polyp would be around 4-5 mm height and diameter for contracted polyps.

Associations: Like for other species in the genus, this species seems to colonize gorgonian octocorals. However, the paucity of the sampling and the absence of in situ observation did not allow an precise identification of the host organism.

Locations and depth in this study: This putative species is known only from several specimens at Birojima/Asabae-zaki, Otsuki, Kochi, from depths of 80 to 129 m.

Notes: This zoanthid is one of the rare zoanthid able to secrete its own scleroproteic skeleton. *Savalia* species are notable for their abundant mucus secretion after collection, with colonies literally dripping mucus once taken out of water.

Genus *Antipathozoanthus* Sinniger, Reimer and Pawlowski, 2010

Type species. *Antipathozoanthus macaronesicus* (Ocaña and Brito, 2003)

Sinniger et al. (2010) originally described this genus as being found exclusively on antipatharians. Subsequently, species of this genus have been found on gorgonian octocorals (Bo et al. 2012).

7. *Antipathozoanthus* sp.

Plate 2D

Colony morphology: Forming a well-developed, flat coenenchyme that completely envelopes the antipatharian axes upon which this species grows. Polyps generally spaced regularly over axes. Polyps, coenenchyme, oral disk, and tentacles generally all bright yellow; color not due to encrusted particles but most likely pigments in zoanthid tissue.

Size and polyp morphology: Outer polyp surface rough with encrusted particles. Aboral maximum polyp diameter (closed preserved polyps) 1.7-3.9 mm. Expanded oral disks estimated (from in situ images) to average 6.5 mm in diameter, expanded polyps up to approximately 1.2 cm in height. Polyps relatively uniform in diameter towards both oral opening and base, slightly wider at oral end of polyp when both closed and expanded. 18 - 24 capitular ridges clearly visible in closed polyps. Tentacle count 18-22, mesentery number likely close to tentacle and capitular ridge numbers based on observations of number of complete mesenteries (9 – 11)

Associations: Found growing on the axis of black corals (anthipatharians).

Locations and depth in this study: Observed at only two locations, at Komo-ichi, Tachibana and Sotogashira, Okinoshima, at depths of 24-26 m. More specimens may be present at deeper depths based on observations from other locations in Japan (J. Reimer, F. Sinniger, pers. obs.).

Notes: Similar zoanthids growing on antipatharians have been observed from Kagoshima Bay, Kagoshima, Japan (J.D. Reimer, T. Fujii, unpubl. data.).

Family Hydrozoanthidae Sinniger, Reimer and Pawlowski, 2010

Type genus: *Hydrozoanthus* Sinniger, Reimer and Pawlowski, 2010

This family was created for former family Parazoanthidae species sharing specific insertions and deletions in mt-16S rDNA, especially in the V5 region (as defined in Sinniger et al. 2005). Species in this family are phylogenetically more closely related to brachycnemic zoanthids (especially in the genus *Palythoa*) than to other parazoanthids. (Sinniger et al. 2010). Hydrozoanthidae groups several tropical and sub-tropical macrocnemic zoanthid species

including species associated with hydrozoans and also several other non-hydrozoan associated species. (Sinniger et al. 2010).

Genus *Hydrozoanthus* Sinniger, Reimer and Pawlowski, 2010
Type species. *Parazoanthus tunicans* Duerden, 1900
Hydrozoanthids associated with hydrozoans.

8. *Hydrozoanthus gracilis* (Lwowsky, 1913)
Plate 2E
Colony morphology: Thin but well-developed coenenchyme covering hydroid main axes and branches surface.
Size and polyp morphology: In southern Shikoku, polyps were always bright yellow in color, unlike at other localities in southern Japan where a darker brown morphotype, potentially an undescribed species, was also observed. Polyps were 2-5 mm in diameter, and of similar height above the coenenchyme.
Associations: Found exclusively on plumulariid hydrozoans.
Locations and depth in this study: Locally common at sites with strong levels of current. Observed and collected from Nishidomari, Okinoshima, at depths from 12 to 26 m.
Notes: This easily identifiable zoanthid has been observed widely in the west Pacific Ocean, from Japan in the north to the Great Barrier Reef.

Discussion

Over the course of this study, eight species representing five macrocnemic zoanthid genera were found in the waters of southern Shikoku. However, most surveys were limited to depths easily accessible by SCUBA (approx. 30 m), and much of the region remains to be examined, particularly at deeper depths. It is indicative that from the only two deeper surveys, both utilizing fishery nets, two species not seen in shallower locations were found (*Savalia* sp. and *Epizoanthus ramosus*). Combined with previous observations that Macrocnemina species may be more common and diverse at deeper depths and in colder waters (Ryland et al. 2001), it is reasonable to assume that this report only partially represents the macrocnemic zoanthid diversity actually present in the southern Shikoku region.

Another demonstration of how little is known of macrocnemic zoanthids from this

region is the fact that of the eight species collected, only two have been formally described. Thus, while we recommend continuing surveys into the deeper (>30m) waters of Shikoku, we also hope that taxonomic efforts to clear this "backlog" of undescribed species are conducted. As previously suggested by many recent studies, a combination of molecular and morphological analyses should be able to properly place these undescribed species into the correct supraspecific grouping, and eliminate future confusion over the identity of species by providing reproducible DNA sequences tied to voucher specimens, to go along with the more 'traditional' morphological descriptions.

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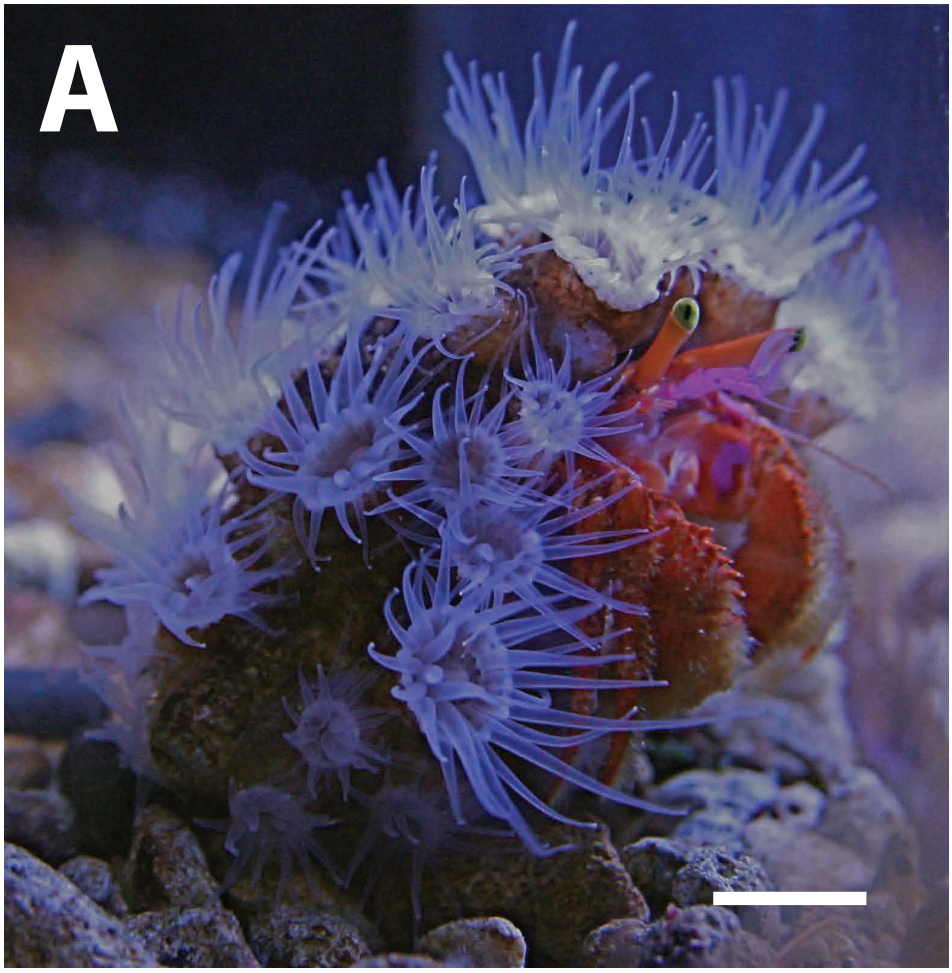
Explanation of plate 1

PLATE 1

Epizoanthus spp. found in southern Shikoku, including A) *Epizoanthus ramosus* associated with the hermit crab *Paguristes balanophilus*, B) *Epizoanthus* sp. C sensu Reimer et al. (2010) associated with the hermit crab *Paguristes palythophilus*, and C) *Epizoanthus* sp. S sensu Reimer et al. (2010) associated with the gastropod *Gemmula unedo*. Images reproduced from Reimer et al. (2010) with permission. All scale bars = approximately 1 cm.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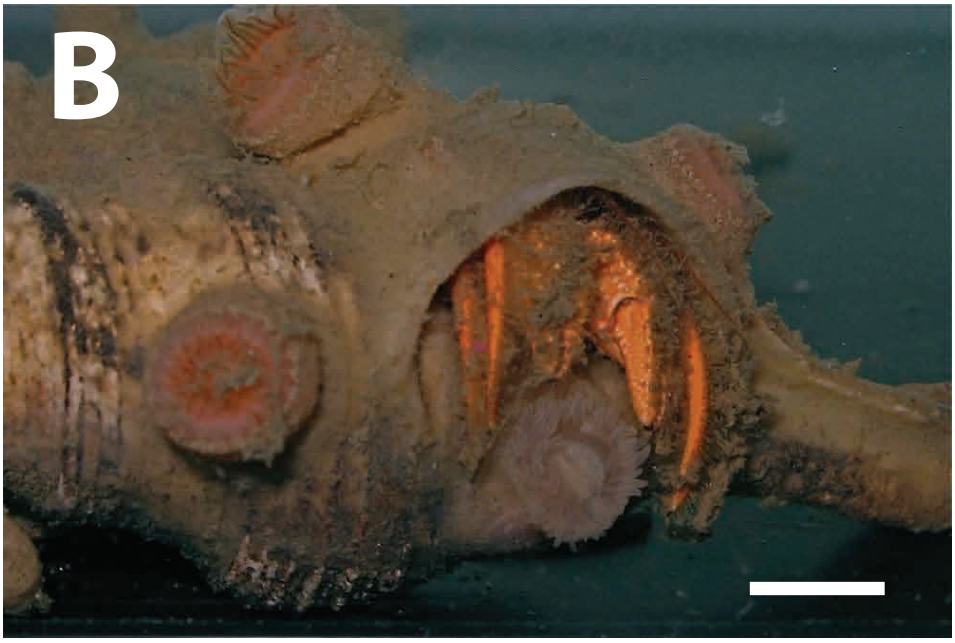
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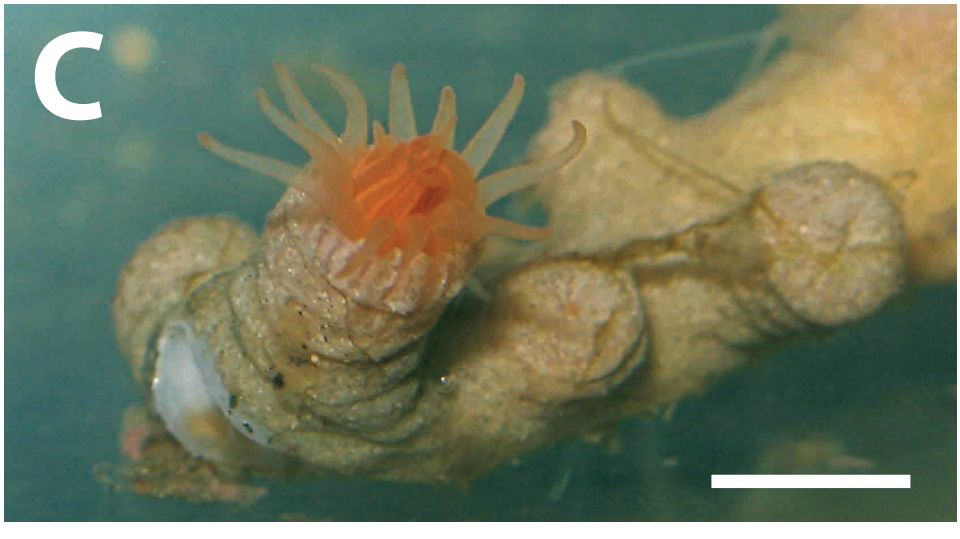
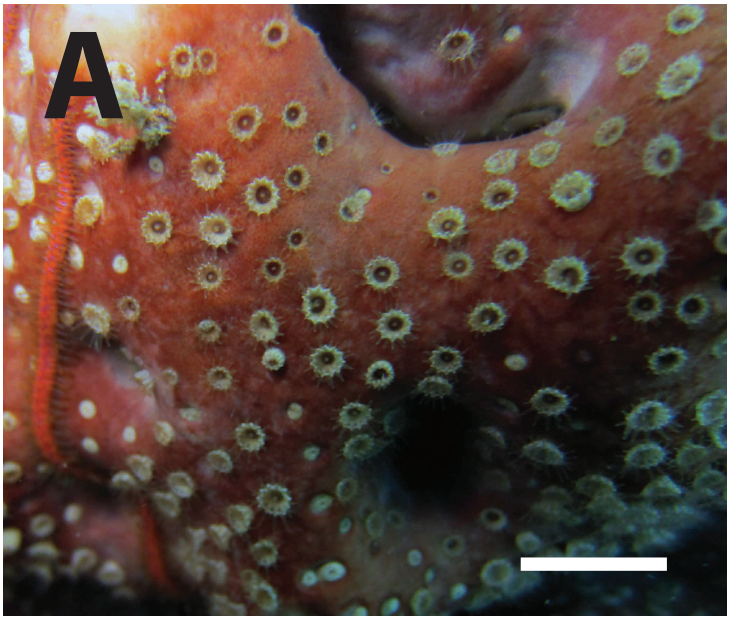

C

Explanation of plate 2

PLATE 2

Zoanthids of the families Parazoanthidae and Hydrozoanthidae found in southern Shikoku, including A) *Parazoanthus* sp. in situ associated with demosponge, at Nishidomari, depth 2.3 m, B) *Parazoanthus* aff. *puertoricense* in situ associated with demosponge, at Sotogashira, depth 20 m, C) *Savalia* sp. specimen in the laboratory, growing over antipatharian, collected from Saeki Bay, Oita, depth 80 m, D) *Antipathozoanthus* sp. in situ associated with antipatharians at Komo-ichi, depth 26 m, E) *Hydrozoanthus gracilis* in situ associated with plumulariid hydrozoan at Nishidomari, depth = 12 m. All scale bars = approximately 1 cm, except in C), which is 5 cm.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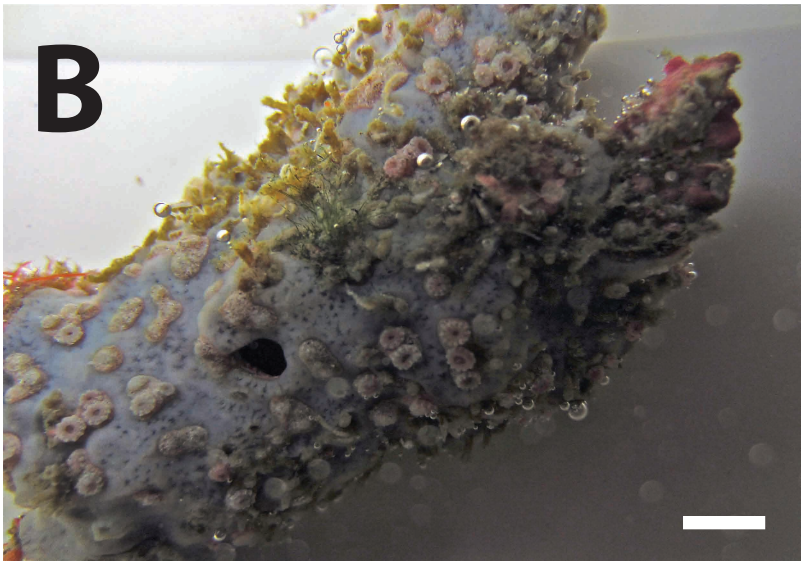
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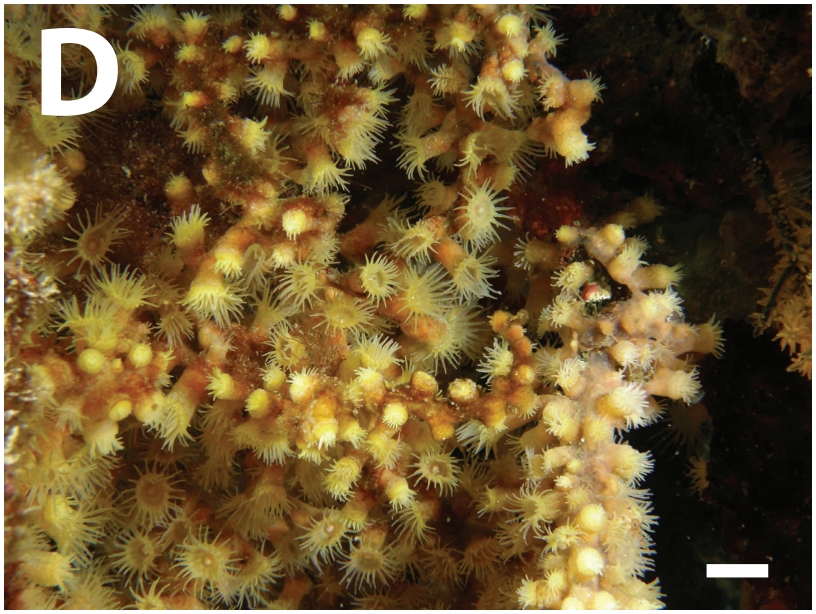
C
5 cm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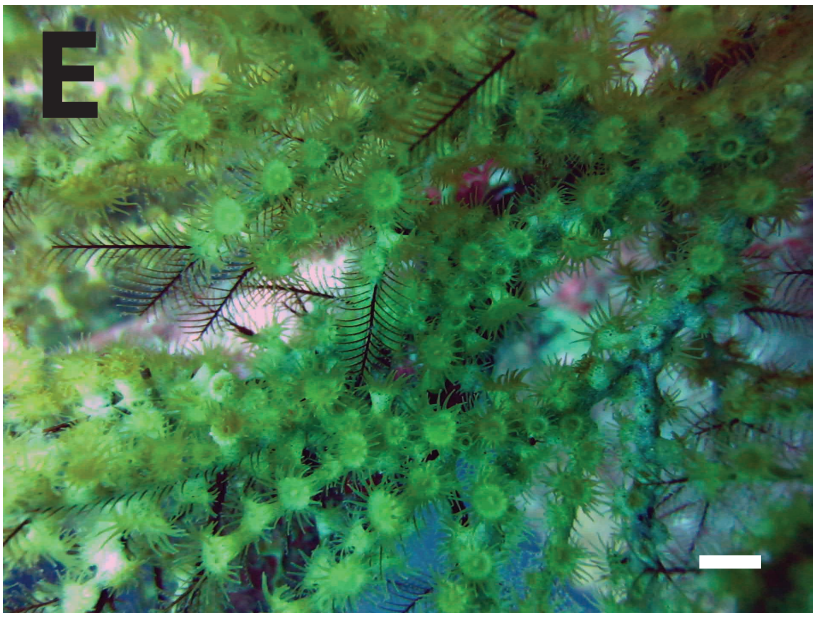
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