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First Detailed Record of Symbiosis Between a Sea Anemone and Homoscleromorph Sponge, With a Description of *Tempuractis rinkai* gen. et sp. nov. (Cnidaria: Anthozoa: Actiniaria: Edwardsiidae)

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A new species in a new genus of sea anemone, *Tempuractis rinkai* gen. et sp. nov., was discovered at several localities along the temperate rocky shores of Japan. The new species is approximately 4 mm in length and has been assigned to family Edwardsiidae, because it has eight macrocnemes, lacks sphincter and basal muscles, and possesses rounded aboral end. The sea anemone, however, also has a peculiar body shape unlike that of any other known taxa. This new species resembles some genera, especially *Drillactis* and *Nematostella*, in smooth column surface without nemathybomes or tenaculi, but is distinguishable from them by several morphological features: the presence of holotrichs and absence of nematosomes. Furthermore, this edwardsiid species exhibits a peculiar symbiotic ecology with sponges. Therefore, a new genus, *Tempuractis*, is proposed for this species. In the field, *T. rinkai* sp. nov. was always found living inside homosclerophorid sponge of the genus *Oscarella*, which suggests a possible obligate symbiosis between Porifera and Actiniaria. The benefit of this symbiosis is discussed on the basis of observations of live specimens, both in the aquarium and field. This is the first report of symbiosis between a sea anemone and a homoscleromorph sponge.

Key words: taxonomy, edwardsiid, marine invertebrates, species description, symbiotic relationship, transmission electron microscopy (TEM), Japan, intertidal, overhang

INTRODUCTION

Edwardsiid sea anemones, which are characterized by their wormlike bodies, are a major taxon in the order Actiniaria and comprise ~90 species (Williams, 1981; Fautin, 2013; WoRMS, 2017). The group is characterized by eight perfect mesenteries in the first cycle, even in adults, whereas almost all other sea anemones have 12 or more (Carlgren, 1949). The mesenterial arrangement of edwardsiids is traditionally regarded as an ancestral character among the Actiniaria since the arrangement is similar to that of "*Edwardsia*-stage" larvae (Duerden, 1899) from several sea anemone species (e.g., reviewed in Daly, 2002a; Uchida and Soyama, 2001), and as a result, the Edwardsiidae had been presumed to be the most ancestral extant form of sea anemones. However, this view has been challenged by several researchers (e.g., Manuel, 1981a; Daly, 2002b), who assert that the simplified mesenterial arrangement in this family may be a secondary condition and correlated with being vermiform for adaptation to infaunal life. This hypothesis is reinforced by the result of a recent phylogenetic study (Rodríguez et al., 2014).

In Japan, 11 species in four genera of edwardsiids have been identified (Yanagi, 2006; Sanamyan and Sanamyan, 2013) (Table 1), although it has recently advocated that *Metedwardsia akkeshi* (Uchida, 1932) and the genus *Metedwardsia* Carlgren, 1947 do not belong to Edwardsiidae (Gusmão et al., 2016). Of these 11 species, only six have been formally described (Carlgren, 1931, 1940; Sanamyan and Sanamyan, 2013; Stimpson, 1856; Uchida, 1932). The remaining five are included in field guides, but without precise morphological information (Uchida, 1965; Uchida and Soyama, 2001). Considering the previously reported richness of living organisms around Japan (e.g., Fujikura et al., 2010; Motokawa and Kajihara, 2016), it is quite possible that Japanese waters are also home to a variety of undescribed or unidentified edwardsiids. Even among the known species, taxonomic revision is greatly needed, owing to insufficient documentation of these species' mor-

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Table 1. All Edwardsiidae sea anemone recorded in Japan. Some researchers recently had advocated that *Metedwardsia akkeshi* (and the genus *Metedwardsia*) do not belong to Edwardsiidae (Gusmão et al., 2016).

	Species	Localities in Japan	Source
1	*Edwardsia japonica* Carlgren, 1931	(Type locality) Misaki, Sagami Bay	Carlgren, 1931
2	*Edwardsia octoradiata* Carlgren, 1931	(Type locality) Japan	Carlgren, 1931
3	*Edwardsia arctica* Carlgren, 1921	2300 m depth in Sea of Japan	Carlgren, 1940
4	*Edwardsia sojabio* Sanamyan and Sanamyan, 2013	(Type locality) 500–3500 m depth at Sea of Japan	Sanamyan and Sanamyan, 2013
5	*Paraedwardsia cretata* (Stimpson, 1856)	(Type locality) Kagoshima Bay, Japan	Stimpson, 1856
6	*Metedwardsia akkeshi* (Uchida, 1932)	(Type locality) Akkeshi, Hokkaido	Uchida, 1932
(Species as below were only included in field guidebook)			
7	*Edwardsia sipunculoides* (Stimpson, 1853)	Tohoku region	Uchida, 1965
8	*Edwardsianthus* cf. *pudica* (Klunzinger, 1987)	Sagami Bay, Onagawa Bay (as *E. japonica* in Uchida, 1941 and 1965)	Uchida and Soyama, 2001
9	*Edwardsianthus gilbertensis* (Carlgren, 1931)	Kabira Bay, Okinawa	Uchida and Soyama, 2001
10	*Edwardsia hantuensis* England, 1987	Boso Pninsula, Chiba	Uchida and Soyama, 2001
11	*Edwardsia* aff. *norvegica* (Carlgren, 1942)	Kii Peninsula, Wakayama	Uchida and Soyama, 2001

phological characters. Accordingly, our research group recently conducted an extensive local faunistic survey around Japan.

During the course of our faunal survey, we identified tiny peculiar sea anemones living inside a possibly undescribed species of homoscleromorph sponge of the genus *Oscarella*. The newly identified sea anemone possessed the characteristic features of edwardsiid anemones, but its morphological characters did not correspond to the diagnosis of any other genus in the family. We propose a new genus and species, *Tempuractis rinkai*, and describe the taxon herein. The discovery of *T. rinkai* provides a new insight into the symbiosis of sea anemones and sponges.

MATERIALS AND METHODS

Sample collection and preservation

Specimens of *Tempuractis rinkai* sp. nov. were collected from intertidal to subtidal rocky shores at four localities in Japan (Fig. 1) by wading, snorkeling, and SCUBA diving. The specimens were collected together with the host sponge *Oscarella* sp. and were kept undisturbed in an aquarium for several hours to several days, until they relaxed and completely spread and elongated their tentacles. The relaxed specimens were then anesthetized with magnesium chloride solution prior to preservation.

Because each host sponge contained many *T. rinkai* polyps, the sponges were cut into pieces such that each contained one or a few polyps. Some of the pieces were preserved in 99% (v/v) ethanol for DNA analyses, and some other pieces were preserved in 10% (v/v) formalin solution with seawater for whole body specimens, others were preserved in a prefixative solution (0.45 M sucrose, 2.5% glutaraldehyde, 0.1 M sodium cacodylate; pH 7.4) for observation by transmission electron microscopy (TEM), and the remaining pieces were preserved in Bouin's fluid (picric acid : formalin : acetic acid = 15:5:1) for both cnida specimens and histological sections. The specimens used in the present study (Table 2) were living in a single *Oscarella* sp. collected from each locality. Type specimens were deposited at the National Museum of Nature and Science, Tokyo (NSMT) and the Coastal Branch of the Natural History Museum and Institute, Chiba (CMNH).

Anemone extraction from a sponge

One holotype and two paratypes were carefully extracted from sponge tissues using tweezers. The holotype specimen was cut transversely, and tiny tissues were excised for cnida observation (see below).

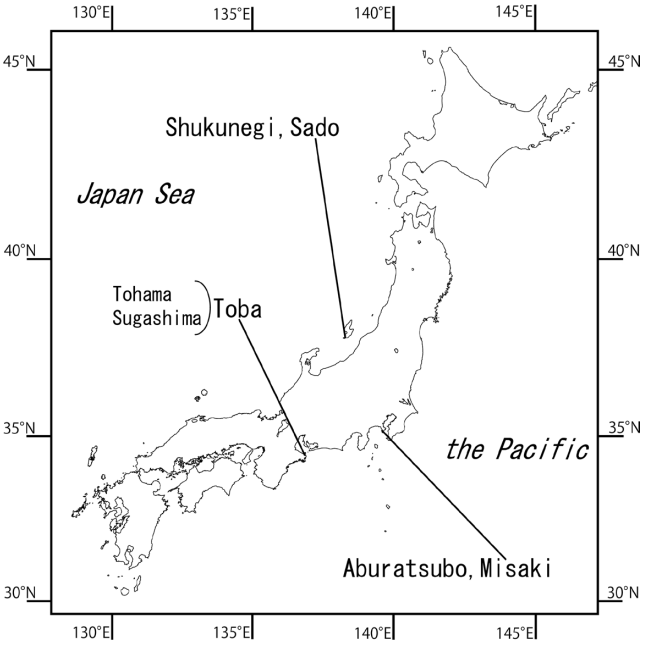

Fig. 1. Sampling localities of *Tempuractis rinkai* gen. et sp. nov.

Preparation of histological sections

Histological sections were made following a standard protocol. Three paratypes (two from Misaki and one from Sado) that were preserved in Bouin's fluid were dehydrated using ethanol and xylene, embedded in paraffin, sliced into serial sections (8 μm thick) using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (Presnell and Schreibman, 1997).

Transmission electron microscope (TEM) observation

The specimens were fixed in prefixative solution (0.45 M sucrose, 2.5% glutaraldehyde, 0.1 M sodium cacodylate; pH 7.4) at 4°C for 2 h. After three washes with 0.45 M sucrose buffered with

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Table 2. *Tempuractis rinkai* sp. nov. specimens used in the present study.

Type	Deposit number	Detail of specimens	Locality	Depth	Histological section	Cnidae specimens
Holo	NSMT-Co 1573	specimens extracted from *Oscarella* sp., being cut into three parts, and cnidae specimens (on slide glasses).	Araihama, Aburatsubo (Misaki)	intertidal	×	○
Para	CMNH-ZG 08969	A whole specimen extracted from *Oscarella* sp.	Araihama, Aburatsubo (Misaki)	intertidal	×	×
Para	CMNH-ZG 08970	A whole specimen in the portion of *Oscarella* sp.	Araihama, Aburatsubo (Misaki)	intertidal	×	×
Para	CMNH-ZG 08971	Histological sections (longitudal).	Araihama, Aburatsubo (Misaki)	intertidal	○	×
Para	CMNH-ZG 08972	Histological sections (transverse).	Araihama, Aburatsubo (Misaki)	intertidal	○	×
Para	CMNH-ZG 08973	Histological sections (transverse).	Shukunegi, Sado	8 m	○	×
Para	CMNH-ZG 08974	A whole specimen in the portion of *Oscarella* sp.	Tohama, Toba	intertidal	×	×
Para	CMNH-ZG 08975	A whole specimen extracted from *Oscarella* sp.	Sugashima, Toba	2 m	×	×

0.1 M sodium cacodylate (pH 7.4), the specimens were postfixed with 1% OsO_4 buffered with 0.1 M sodium cacodylate (pH 7.4) on ice for 1 h. Then they were washed with 0.1 M sodium cacodylate (pH 7.4) on ice for 10 min, dehydrated through an ethanol and propylene oxide series, and embedded in Quetol 812 (Nisshin EM Co., Tokyo, Japan). The resin was solidified sequentially at 37°C overnight, at 45°C for 12 h, at and 60°C for 48 h and thin-sectioned with an average thickness of 70 nm. Sections were stained with uranyl acetate and lead citrate and were observed under a transmission electron microscope (JEM 1200EX; JEOL, Tokyo, Japan).

Cnida observation

Cnidae of the tentacles, column, actinopharynx, and filaments were imaged using differential interference contrast microscopy (Yanagi et al., 2015). The length and width were then measured using ImageJ v. 1.49 (Rasband, 1997–2012), and the cnidae were classified following Mariscal (1974).

RESULTS

Order **ACTINIARIA** Hertwig, 1882
Family **Edwardsiidae** Andres, 1881
Tempuractis gen. nov. Izumi, Ise and Yanagi
(Japanese name: tempura-isoginchaku-zoku)

Diagnosis. Edwardsiid with very tiny column, not differentiated into the capitulum, scapus, and physa. Surface of long column smooth, lacking nemathybomes or tenaculi. Tentacle sixteen, in two cycles, arranged octamerously, with eight axes of symmetry on the tentacular circle; inner cycle tentacles comparatively longer than outer ones. There is no siphonoglyph. Sphincter muscle not present. Aboral end tapered or rounded but not differentiated into a physa. Inhabits only in homoscleomorph sponge symbiotically and never lives independently. Cnidae: spirocysts, basitrichs, holotrichs, and microbasic *p*-mastigophores.

Etymology. *Tempura* is a deep-fried, batter-coated nugget of seafood and/or vegetables in Japanese cuisine. This word comprises the first half of the Japanese name of the type species of this genus, as the shape of the actiniarian when embedded in a sponge tissue resembles shrimp *tempura*. The siffix *-actis* is commonly used in actiniarian genus names, meaning radiation of sunshine in Greek. The new genus name is feminine in gender.

Remarks. Within the Edwardsiidae, *Tempuractis* gen. nov. morphologically resembles the valid genera *Edwardsiella* Andres, 1881, *Drillactis* Verrill, 1922, *Nematostella* Stephenson, 1935, and *Metedwardsia* Carlgren, 1947 in possessing a smooth scapus with no nemathybomes or tenaculi. The following genera are distinguishable from this new genus: *Edwardsia* de Quatrefages, 1842, *Scolanthus* Gosse, 1853, and *Edwardsianthus* England, 1987 have nemathybomes (Gosse, 1853; Carlgren, 1949; England, 1987); *Paraedwardsia* Nordgaard, 1905 and *Synhalcampella* Carlgren, 1921 have tenaculi on the scapus, and the former generally adhere grains of sand on the column; and *Halcampogeton* Carlgren, 1937 has 12 longitudinal rows of solid papillae (Carlgren, 1937). The most prominent difference between *Tempuractis* and *Edwardsiella* is periderm; species in *Edwardsiella* bear periderm on the scapus, but the column in *Tempuractis rinkai* is naked and has no periderm. In addition, tentacular arrangement is useful for reference; in contrast with *Tempuractis rinkai* which has *Edwardsia*-like tentacular arrangement, *Edwardsiella* species possess three or more cycles of tentacles that are hexamerously arranged, with the innermost cycle being the longest (Daly et al., 2013). *Drillactis* species are most similar to *Tempuractis rinkai*, but there are several differences between them: holotrichs are abundant in *T. rinkai*, but are absent in *Drillactis* (Carlgren, 1949, 1954). As a reference, all *Drillactis* species are distinguishable from *Tempuractis* by the difference of characters as below; tentacles in *Drillactis* species has vertical rows of white spots (Carlgren, 1954; Verrill, 1880) while there are no patterns on tentacles of *T. rinkai*; the two *Drillactis* species have far larger bodies than *T. rinkai* (e.g., the body lengths of *T. rinkai* are even much smaller than the tentacle lengths of *Drillactis pallida*) (Verrill, 1922; Carlgren, 1954; Fautin, 2013); *T. rinkai* inhabits

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only in a homoscleomorph sponge, an extraordinary place for the sea anemone, with symbiotic ecology, while both *Drillactis* species live in sand, very ordinary habitat for edwardsiids, and without symbiosis (Verrill, 1922). *Nematostella* is characterized by having nematosomes, the spherical structures, 15–45 μm in diameter, and flagellated bodies containing nematocysts (Hand and Uhlinger, 1992), which are structures that are present only in this genus in Edwardsiidae (Hand, 1994) and are the origin of the name of this genus. However, there is no structure like a nematosome observed both from the outside when they were living and in their coelenteric cavity on transversal sections in *T.*

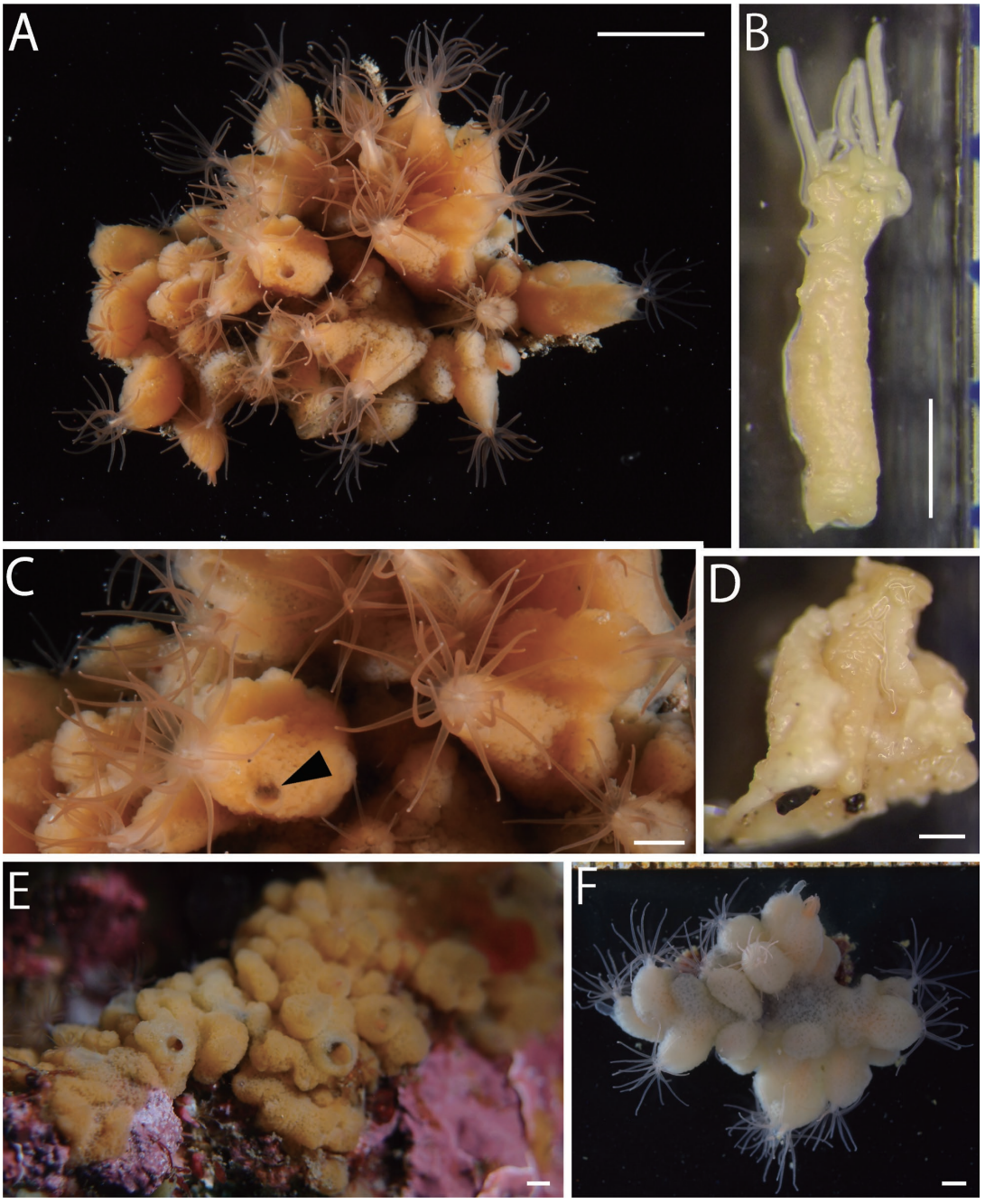

Fig. 2. External view of *Tempuractis rinkai* gen. et sp. nov. and its host sponge *Oscarella* sp. **(A)**, **(C)**, **(E)**, and **(F)**: living colony of *T. rinkai* sp. nov. **(A–D)**: specimens collected from Misaki, Kanagawa. **(A)** a living colony of *T. rinkai* gen. et sp. nov. with *Oscarella* sp., including a holotype (NSMT-Co 1573) and four paratypes (CMNH-ZG 08969 to 08972). **(B)** excised and preserved *T. rinkai* gen. et sp. nov. specimen (NSMT-Co 1573) with elongated tentacles. **(C)** *T. rinkai* gen. et sp. nov. living in a bunch-like part of *Oscarella* sp. Arrowhead indicates the oscular opening of the host sponge. **(D)** dissected host sponge, showing whole body of a *T. rinkai* gen. et sp. nov. specimen (NSMT-Co 1573) with shrunken tentacles that was totally buried in the host sponge. **(E)** field image of *Oscarella* sp. collected from Shukunegi, Sado, Niigata. Numerous *T. rinkai* are buried in the sponge, including paratype CMNH-ZG 08973. **(F)** living colony of *T. rinkai* gen. et sp. nov., including paratype CMNH-ZG 08974, collected from Tohama, Toba, Mie. All scale bars represent 1 mm.

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rinkai. Therefore, this new species does not belong to *Nematostella*. *Metedwardsia* is monotypic for *Metedwardsia akkeshi* (Uchida, 1932). This species, the only edwardsiid without nemathybomes in Japan, is obviously distinguished from all other edwardsiids by the distribution of microcnemes; microcnemes of *M. akkeshi* are elongated from distal to proximal end (Carlgren, 1947; Uchida, 1932), while all other edwardsiids' microcnemes are limited to the distal end. This is the most unique character of *Metedwardsia*, so *T. rinkai*, in which the elongation of microcnemes is the same that in other edwardsiids, also cannot be included this genus.

Given the above, it is inappropriate to include this new species in existing genera. To begin with, this species has several peculiar morphological features for Edwardsiidae; *T. rinkai* has large holotrichs, which is one of the most recognizable characteristics of this genus; there is no description of holotrich in any recent genus diagnosis of Edwardsiidae (e.g., Carlgren, 1949; Daly and Ljubenkov, 2008; Daly et al., 2013; Gusmão et al., 2016). *Tempuractis rinkai*, especially in the column, is rich in prominently large holotrichs, so the cnidom can be said to be a unique character of this genus. In addition, the new species also possesses a few microbasic *p*-mastigophores and spirocysts in its column, which is peculiar to Edwarsiidae. Moreover, the small size of this species, less than 5 mm in whole body length even for adults, is prominent in this family. Ultimately, the habitat of this spe-

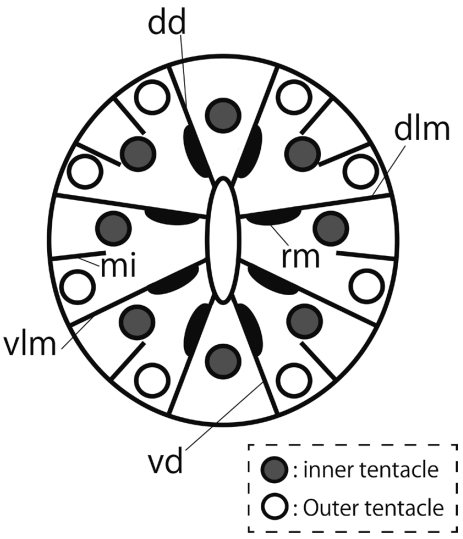

Fig. 3. Arrangement of *Tempuractis rinkai* gen. et sp. nov. tentacles and mesenteries. Two pairs of macrocnemes are directives, and the others are lateral mesenteries. Retractor muscles on lateral mesenteries are all facing ventral side. There are eight micronemes and two circles of tentacles. Two tentacles on the inner circle are located at the endocoel between both directives. Circles indicate tentacle positions; white circles indicate outer tentacles and grey circles indicate inner ones. Abbreviations: dd, dorsal directive; dlm, dorso-lateral mesentery; mi, microcneme; rm, retractor muscle; vd, ventral directive; vlm, ventro-lateral mesentery.

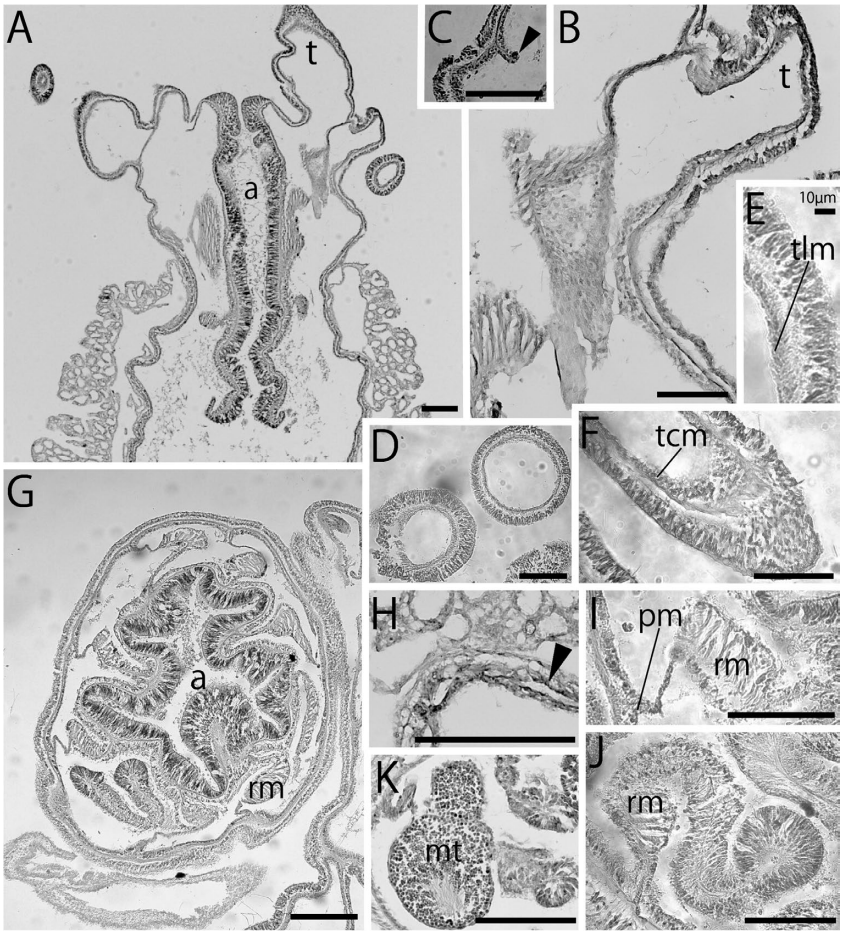

Fig. 4. Internal anatomy of *Tempuractis rinkai* gen. et sp. nov. **(A, B)** paratype CMNH-ZG 08971. **(C–J)** paratype CMNH-ZG 08972. K: specimen CMNH-ZG 08973. **(A)** longitudinal section. **(B)** enlarged view of the longitudinal section, showing absence of sphincter muscle. **(C)** transverse section of a microcneme (arrowhead). **(D)** transverse section of the tentacles. **(E)** enlarged view of the tentacle transverse section. **(F)** longitudinal section of a tentacle tip. **(G)** cross section of column, showing eight macrocnemes and no micronemes at the actinopharlynx. **(H)** longitudinal section of *T. rinkai* adhered to the inner surface of the sponge (arrowhead). **(I)** macrocnemes and retractor muscle at the actinopharlynx. **(J)** macrocnemes in the gastral cavity. **(K)** cross section of a macrocneme, showing retractor muscle and gonad (mature testis) with mature sperm cells. Abbreviations: a, actinopharynx; mt, matured testis cyst; pbm, parietal basilar muscle; rm, retractor muscle; t, tentacle; tcm, tentacular circular muscle; tlm, tentacular longitudinal muscle. All scale bars represent 100 μm unless otherwise noted.

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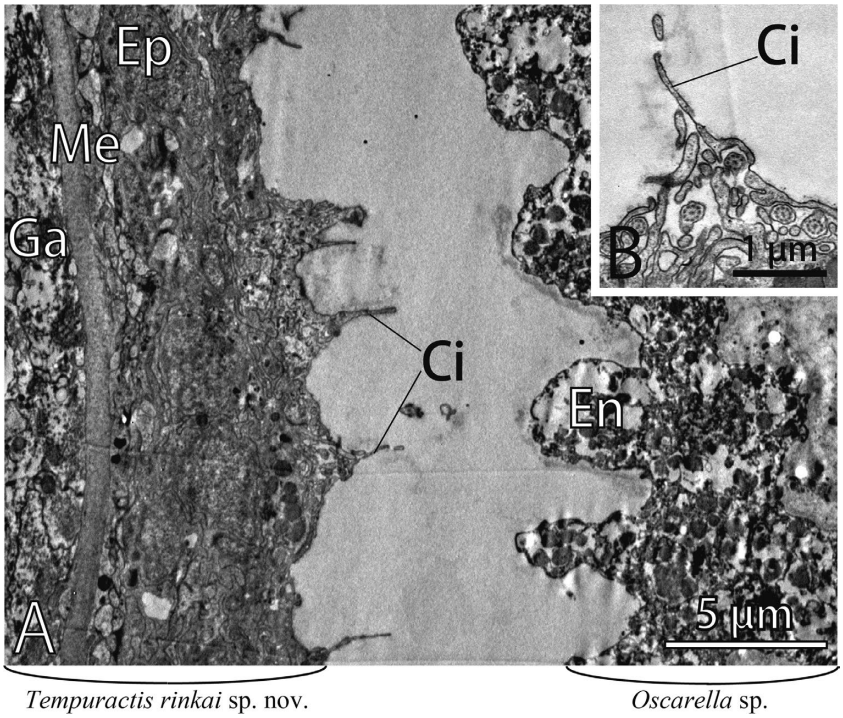

Fig. 5. The images of sections between *Tempuractis rinkai* gen. et sp. nov. and *Oscarella* sp. obtained by transmission electron microscope (TEM). **(A)** structure between the sea anemone and the sponge. The obtruding cilia of the sea anemone are corresponding to the sites of depression of endopinacocytes of the sponge. **(B)** Enlarged view of the obtruding cilia of sea anemone. Several cilia are twisting around each other. Abbreviations: Ci, cilia; En, endopinacocyte of *Oscarella* sp.; Ep, epiderm of *T. rinkai*; Ga, gastroderm of *T. rinkai*; Me, mesoglea of *T. rinkai*.

cies is very unique for not only edwardsiids but also for sea anemones in general; only *Spongiactis japonica* Sanamyan, Sanamyan and Tabachnick, 2012 is recorded to be symbiotic with sponges (Sanamyan et al., 2012). *Tempuractis rinkai* is quite different from *S. japonica* in morphological features and, of course, belongs to a quite different taxon at the family level.

Therefore, we establish the new genus *Tempuractis* to accommodate the newly identified sea anemone, which is characterized by possessing holotrichs, a smooth body surface without nemathybomes or cuticles, s tenticular arrangement in two octamerous cycles, a quite tiny body and inhabiting symbiotically in a sponge.

Type species. *Tempuractis rinkai* sp. nov. Izumi, Ise and Yanagi 2017 fixed by original designation.

Tempuractis rinkai sp. nov. Izumi, Ise and Yanagi, 2017
(Figs. 2–8, Tables 2, 3)
(New Japanese name: tempura-isoginchaku)

Material examined. *Holotype*. NSMT-Co 1573. One specimen cut into three parts and prepared for nematocyst observation, collected by wading on 7 June 2013 from the intertidal zone of Aburatsubo, Misaki, Kanagawa, Japan by Yuji Ise. *Paratypes.* CMNH-ZG 08969. Whole specimen extracted from a sponge; CMNH-ZG 08970. Whole specimen left inside a sponge; CMNH-ZG 08971. Series of histological longitudinal sections; CMNH-ZG 08972. Series of histological cross sections, all specimens collected by wading on June 7, 2013 from the intertidal zone of Aburatsubo, Misaki, Kanagawa, Japan by Yuji Ise; CMNH-ZG 08973. Histological sections. Collected by SCUBA diving on 3 October 2013 at a depth of 8 m in Shukunegi, Sado Island, Niigata, Japan by Yuji Ise; CMNH-ZG 08974. Whole specimen left in a portion of the sponge, collected by wading on August 22, 2013 from the intertidal zone of Tohama, Toba City, Mie, Japan by Takeya Moritaki.; CMNH-ZG 08975. Whole specimen, collected by snorkeling on 13 October 2014 at a depth of 2 m in Sugashima, Toba City, Mie, Japan by Yuji Ise.

Note. Series of histological sections were prepared from a specimen from the same host sponge from which the holotype and paratypes were collected.

Etymology. The species epithet is dedicated to marine biological stations around Japan. The first specimens of this species were collected from a rocky shore in front of the Misaki Marine Biological Station (the University of Tokyo). This station is called “Misaki rinkai jikkenjo” in Japanese (“rinkai” means seaside and “jikkennjo” means research facility). Other specimens were collected during a subsequent faunistic survey in collaboration with other marine biological stations: Sugashima Marine Biological Laboratory (Nagoya University) and Sado Marine Biological Station (Niigata University).

Description. *External anatomy.* Column naked, smooth, very small, ca. 3.0–5.0 mm in length (3.0 mm in the holotype) and 0.7–1.2 mm in width (0.8 mm in the holotype), and pipe-like in form (Fig. 2B). The surface of column simple, no nemathybomes or tenaculi. Epiderm adhesion with endopinacocytes of *Oscarella* sp. very tight. Aboral end tapered, not differentiated from scapus, but more or less adherent (see Ecological remarks below). Tentacles slender, without acrospheres, but bearing white patches on each tip, ca. 2.5–4.0 mm in length in living and 1.0–2.0 mm (1.2–1.8 mm in the holotype) in fixed specimens, longer than diameter of oral disk, but well contractible. Tentacles 16 in number, arranged in two concentric cycles of eight inner and eight outer ones positioned alternately (Fig. 3), inner tentacles a little longer than outer ones. Oral disk 0.7–1.2 mm in diameter (0.8 mm in the holotype). Column and tentacles pale orange or pale pink, semitransparent so that mesenterial insertion visible in upper part when alive, no pattern or patches. Area around mouth and actinopharynx white. Body completely surrounded by the tissue of host sponge *Oscarella* sp. except tentacles and capitulum (Fig. 2A, C–F), so the color of column unidentifiable in alive.

Internal anatomy. Mesenterial arrangement as typical as that of *Edwardsia*. Eight macrocnemes, four dorsal and ventral directives, and the other four lateral mesenteries (Figs. 3, 4G). All macrocnemes perfect, present along whole length

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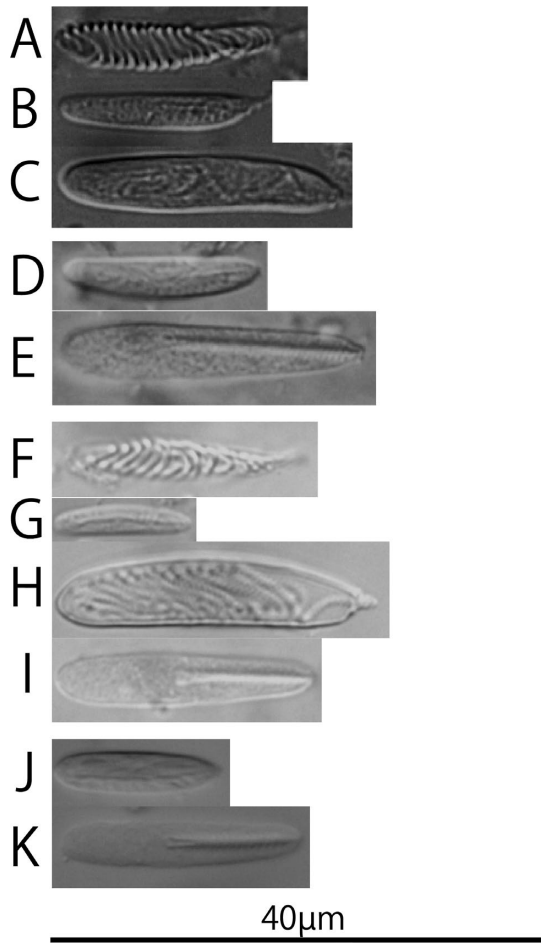

Fig. 6. Cnidae of *Tempuractis rinkai* gen. et sp. nov., holotype, NSMT-Co 1573. **(A)** tentacle spirocyst. **(B)** tentacle basitrich. **(C)** tentacle holotrich. **(D)** actinopharynx basitrich. **(E)** actinopharynx microbasic *p*-mastigophore. **(F)** column spirocyst. **(G)** column basitrich. **(H)** column holotrich. **(I)** column microbasic *p*-mastigophore. **(J)** filament basitrich. **(K)** filament microbasic *p*-mastigophore.

of body (extending from oral to aboral end). Retractor muscle of lateral mesenteries all facing ventrally (Fig. 4G). Eight tiny microcnemes, without muscles, only in distal-most part of column, extending about 30 μm under the base of tentacles (Fig. 4C). Four micronemes between dorsal directives and dorso-lateral mesenteries, two between the dorso- and ventro-lateral mesenteries and two between the ventro-lateral mesenteries and ventral directives (Fig. 3). Each tentacle between either exo- or endocoelic. Retractor muscles comparatively weak, restricted or reniform in upper part, but diffused in lower part (Fig. 4G, I, J). Muscle processes mostly simple, around 10 in each muscle pennon. Parietal muscles of macrocnemes not distinct. Actinopharynx short, without distinct siphonoglyphs (Fig. 4A). Tentacular circular muscle endodermal and longitudinal muscle ectodermal (Fig. 4B, D–F). Mesoglea in body wall, mesenteries, and actinopharynx thin, < 10 μm in thickness (Fig. 4G). Marginal sphincter muscle and basilar muscle absent. All parts of body wall, except capitulum, tightly adhered to endopinacocytes of *Oscarella* sp. (Fig. 4A, H). Many cilia from epiderm of sea anemone, invaginating into endopinacocytes of the sponge, this structure may strengthen the adhering between epiderm of *T. rinkai* and endopinacocytes of *Oscarella* sp. (Fig. 5). No mature gametes in holotype. Mature testes observed in paratype (CMNH-ZG 08973; Fig. 4K).

Cnidom. Spirocysts (in tentacles and column), basitrichs (in every tissue), holotrichs (in tentacle and column) and microbasic *p*-mastigophores (in actinopharynx, column and filament; Table 3, Figs. 6, 7).

Ecological remarks. Colonies of *Tempractis rinkai* sp. nov. were always found in the host sponge *Oscarella* sp., and no living individual was found independently outside of a sponge. The position of *T. rinkai* sp. nov. inside the sponge was unique, as the oscula of the sponge opens beside the oral disc of sea anemone (Fig. 2C), which means that the sea anemones do not utilize the spongocoel or central cavity of the host sponge as do other temporary visitors. Each polyp of *T. rinkai* sp. nov. was isolated from other polyps and was completely buried in the sponge body, exhibiting a bunch-like shape (Fig. 2A). The epidermis of *T. rinkai* sp. nov. was strongly adherent to the endopinacocytes of *Oscarella* sp. Although the majority of individuals were completely buried in the sponge, some sea anemones were piercing their body through *Oscarella* sp. and adhered to the substrate by their aboral end. Thus, the aboral end of this sea anemone is more or less adhesive.

Tempuractis rinkai sp. nov. and the host sponges were found in cryptic habitats, such as the underside of overhangs, underside of rocks, or interstices of beach rocks. This phenomenon is probably the result of the habitat preference of the host sponge, rather than that of *T. rinkai* sp. nov., and allows these sea anemones to live in habitats that differ from those of other edwardsiids, which are usually buried in sandy or muddy sea bottom.

Tempuractis rinkai sp. nov. elongated its tentacles outside its host sponge when relaxed, and when exposed to various external stimuli, such as being touched by something, being exposed to a strong current, or when a shadow of something fell on them, they retracted their tentacles and hid themselves inside the sponge (Fig. 8). Tentacles of *T. rinkai* sp. nov. were sometimes observed to be in contact with those of other polyps because colonies of *T. rinkai* sp. nov. were densely distributed (Fig. 2A, C, F); however, they did not retract their tentacles or attack each other.

DISCUSSION

Symbiosis between *Tempuractis rinkai* sp. nov. and *Oscarella* sp.

Tempuractis rinkai sp. nov. and its host sponge *Oscarella* sp. are likely to be involved in a symbiotic relationship. Histological sections suggested that the epiderm of *T. rinkai* sp. nov. and the endopinacocytes of *Oscarella* sp. were strongly adhered (Fig. 4H), in that they were difficult to separate in both live and ethanol-preserved samples. Based on TEM observation (Fig. 5), it appeared that the surface structures of both the sea anemone and the sponge are closely related. The cilia of the sea anemone are projecting to the depressions of endopinacocytes of *Oscarella* sp, and twisting each other (Fig. 5). This structure suggests that there is specific mechanism of adhesion between the sponge endopinacocytes and sea anemone epiderm. Transmission electron microscopy images suggest that the epiderm of *T.*

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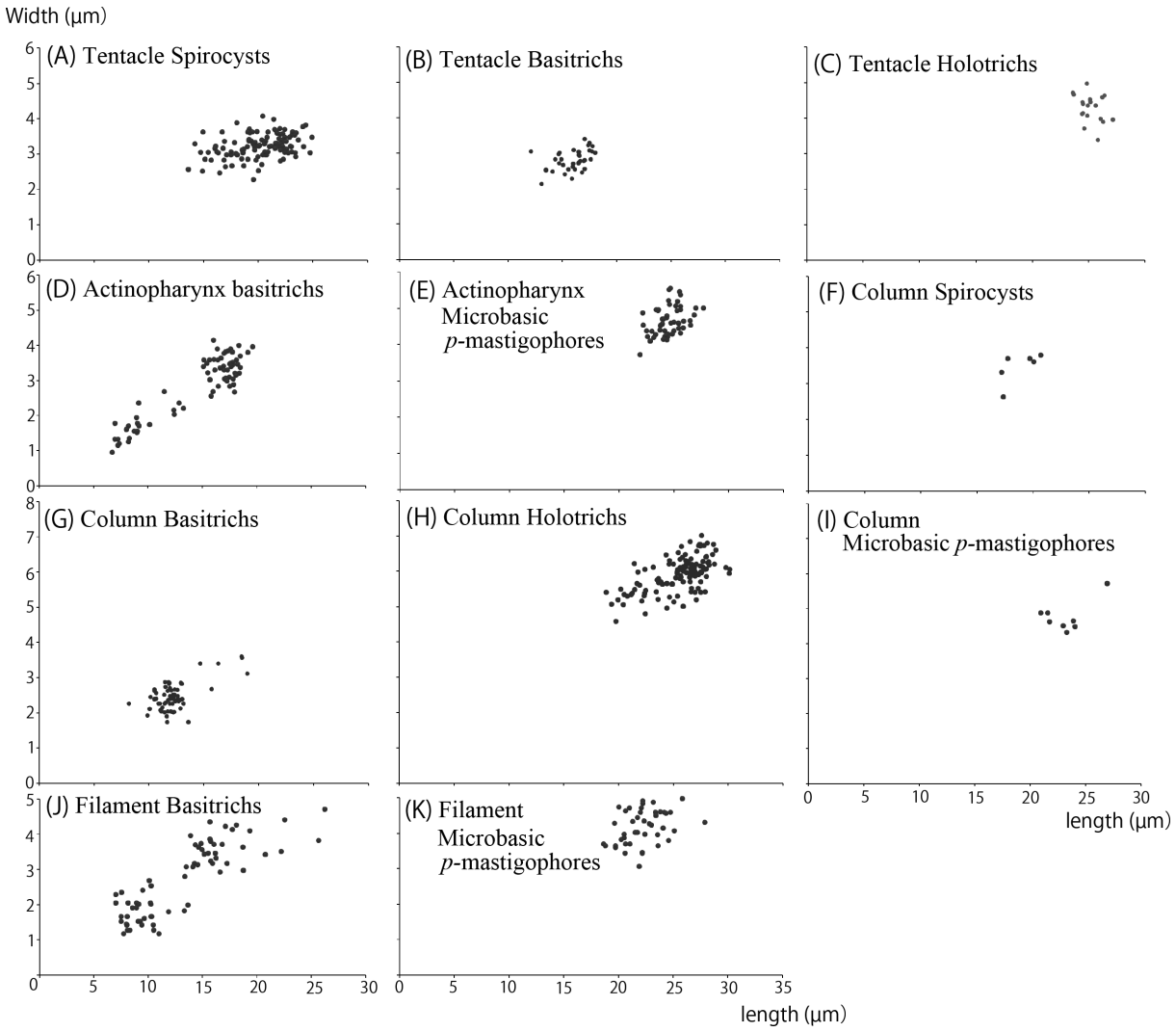

Fig. 7. Size distribution of cnidae of *Tempuractis rinkai* gen. et sp. nov., holotype, NSMT-Co 1573. **(A)** tentacle spirocyst. **(B)** tentacle basitrich. **(C)** tentacle holotrich. **(D)** actinopharynx basitrich. **(E)** actinopharynx microbasic *p*-mastigophore. **(F)** column spirocyst. **(G)** column basitrich. **(H)** column holotrich. **(I)** column microbasic *p*-mastigophore. **(J)** filament basitrich. **(K)** filament microbasic *p*-mastigophore. Abbreviation: N, number of measured cnida capsules.

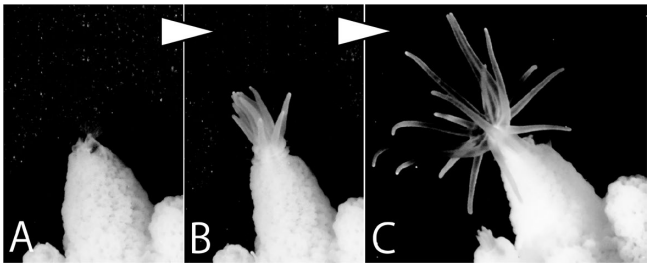

Fig. 8. A series of images of behavior of *Tempuractis rinkai* gen. et sp. nov. **(A)** specimen completely buried in the host sponge *Oscarella* sp. **(B)** tentacles gradually elongating out of the sponge. **(C)** tentacles and oral disc emerging from the sponge body. Reverse action (**(C)** to **(A)**) occurs rapidly upon stimulation.

rinkai anchors to endopinacocytes of *Oscarella* sp. by bundles of cilia. This may stabilize their position. When *T. rinkai* sp. nov. shrinks, its column is totally encased by sponge tissue (Fig. 8A). During this process, it seems that the *T. rinkai* sp. nov. epiderm pulls the endopinacocytes of *Oscarella* sp., thereby completely closing the holes that the sea anemones live in. So far, no *Tempuractis rinkai* sp. nov. has been found outside the host sponge *Oscarella* sp., and all of the *Oscarella* sp. sponges collected during the present study contained several *T. rinkai* sp. nov., suggesting that these animals are involved in an obligate symbiotic relationship. The benefit of this symbiosis has not been precisely determined yet; however, it is expected that *T. rinkai* sp. nov. hide their body in the host sponge when they are attacked by unknown predators. The advantage of this symbiosis for the host sponge *Oscarella* sp. is unclear; however, the possible role of *T. rinkai* sp. nov. in this symbiosis can be assumed from the following observations in the field: sea slugs, *Berthella stellata* (Risso, 1826) (Pleurobranchidae, Notaspidea, Gastropoda, Mollusca), were sometimes observed to feed on *Oscarella* sp. inhabited by few polyps of *T. rinkai* sp. nov. in the present sampling localities, and there have been several studies showing that *Berthella* spp. feed on homosclero-

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Table 3. Cnidae of *Tempuractis rinkai* sp. nov., holotype, NSMT-Co 1573. A: tentacle spirocyst. B: tentacle basitrich. C: tentacle holotrich. D: actinopharynx basitrich. E: actinopharynx microbasic *p*-mastigophore. F: column spirocyst. G: column basitrich. H: column holotrich. I: column microbasic *p*-mastigophore. J: filament basitrich. K: filament microbasic *p*-mastigophore. For letters **(A)–(K)**, refer to Fig. 6. Abbreviations: N, number of measured cnidae capsules; SD, standard deviation.

Cnidae type		Size	Mean	SD	N	Frequency
Tentacle						
(A)	Spirocyst	13.6–24.9 × 2.3–4.1	20.1 × 3.2	2.55 × 0.33	117	Common
(B)	Basitrichs	12.1–18.0 × 2.1–3.4	16.1 × 1.6	2.82 × 0.30	37	Common
(C)	Holotrichs	23.6–27.2 × 3.4–5.0	25.1 × 4.3	0.93 × 0.41	19	Rare
Actinopharynx						
(D)	Basitrichs	6.7–19.6 × 1.0–4.1	14.6 × 4.0	2.84 × 0.88	71	Common
(E)	Microbasic *p*-mastigophores	21.9–27.7 × 3.7–5.6	24.6 × 4.6	4.66 × 0.40	52	Common
Column						
(F)	Spirocyst	17.2–20.7 × 2.6–3.8	18.8 × 3.5	1.39 × 0.40	6	Rare
(G)	Basitrichs	8.2–13.6 × 1.7–2.9	11.7 × 2.4	0.94 × 0.29	57	Common
(H)	Holotrichs	13.0–30.2 × 3.1–7.0	25.0 × 5.8	3.20 × 0.79	126	Common
(I)	Microbasic *p*-mastigophores	21.0–27.0 × 4.3–5.7	23.2 × 4.8	1.77 × 0.41	8	Rare
Filament						
(J)	Basitrichs	10.1–29.8 × 2.1–7.0	13.1 × 2.7	4.57 × 1.00	69	Common
(K)	Microbasic *p*-mastigophores	21.0–27.0 × 4.3–5.7	22.3 × 4.2	1.92 × 0.52	47	Common

morph sponges (Delaloi and Tardy, 1977; Willan, 1984; Picton, 2002; Rudman, 2005, 2007, 2010; Goddard, 2007), and thus *Berthella* species are thought to be specific predators on homoscleromorph sponge (Goddard, 2007). Therefore, *T. rinkai* sp. nov. might protect its host sponge from *Berthella* species using the cnidae on its tentacles, because the nudibranch may not approach the area where the tentacles of *T. rinkai* extend, protecting the sponge from being totally eaten.

This is the first report of symbiosis among members of the Actiniaria (and also Cnidaria) and Homoscleromorpha both ecologically and morphologically. As for *Oscarella* sponge, or the family Oscarellide, this is undoubtedly first record of symbiosis with any other metazoans. Concerning cnidarians, symbiotic relationships between order Zoantharia and Porifera have been documented far more frequently than those between the Actiniaria and Porifera. For example, several species of *Epizoanthus* Grey, 1867, *Parazoanthus* Haddon and Shackleton, 1891, and *Umimayanthus*, Montenegro, Sinniger and Reimer, 2015 inhabit in/on sponges (Swain and Wulff, 2007; Montenegro and Acosta, 2010; Montenegro et al., 2015). Among these, species of *Epizoanthus* bury their bodies much deeper in the sponge than the other genera (Swain and Wulff, 2007; Montenegro and Acosta, 2010; Montenegro et al., 2015), like *Tempuractis rinkai* sp. nov. However, there has been no report of zoanthids inhabiting in/on homoscleomorph sponges.

In contrast to the diversity of sponge-symbiotic zoanthids, only a single species of Actiniaria is known to live in sponges. The first detailed report of a symbiotic relationship between members of Actiniaria and Porifera was about an actiniarian and a hexactinellid sponge (Sanamyan et al., 2012). According to Sanamyan et al. (2012), the host sponge *Hyalonema sieboldi* Gray, 1832 forms "specific minute volcano-like rises" above the sea anemone *Spongiactis japonica*. Although these structures resemble the bunch-like parts of *Oscarella* sp. where *Tempractis rinkai* sp. nov. lives, the adhesion mechanisms and lineages involved in the two symbioses are completely different. In the *S. japonica*/*H. sieboldi* symbiosis, the sea anemones adhere to the host sponge *via* the perforation of their columns by long spicules of the sponge (Sanamyan et al., 2012). However, the family Oscarellidae, including *Oscarella* spp., totally lack spicules (Muricy and Diaz, 2002; Gazave et al., 2010, 2012; Ruiz et al., 2017), thereby precluding a similar adhesion mechanism. The different adhesion mechanisms might be a consequence of the different surface structures of the host sponges because the pinacocytes of homoscleromorph sponges form an epithelium (e.g., Ereskovsky et al., 2009), whereas those of hexactinellid sponges do not (e.g., Leys et al., 2007).

In homoscleromorph sponges, the zoanthid "*Epizoanthus* sp. nov." sensu Crocker and Reiswig (1981) was reported to live exclusively inside three species of the sponge genus *Plakortis* Schulze, 1880 (Crocker and Reiswig, 1981; Swain and Wulff, 2007), but this cnidarian species was revealed to be an edwardsiid species, Edwardsiidae sp, by molecular phylogenetic study (Swain, 2009). However, although the embedded form of Edwardsiidae sp., of which only the tentacles protrude from the sponge (Swain and Wulff, 2007; Montenegro and Acosta, 2010), resembles that of *T. rinkai* sp. nov., this sea anemones were just ascertained to belong to the family Edwardsiidae only by molecular phylogeny. And there was no subsequent study showing the details of its morphological characters, and so the detailed taxonomy of this sea anemone is still unknown. We presume that "Edwardsiidae sp." in Swain (2009) is a different species from *T. rinkai* sp. nov. because the host sponges belong to different families; *Oscarella* sp. belongs to Oscarellide, while the host sponges of "Edwardsiidae sp." in Swain (2009) belong to Plakinidae. Furthermore, the localities of sampling sites are very different and distant; *T. rinkai* sp. nov. and its host sponge *Oscarella* sp. were found from temperate rocky shores of Japan, but "Edwardsiidae sp." in Swain (2009) and its host sponges were found from coral reefs of the Caribbean (Crocker and Reiswig, 1981).

In conclusion, the present study revealed the second known symbiosis between members of Actiniaria and Porifera and provided the first detailed record between an actiniarian and a homoscleromorph sponge. Moreover, this study suggests that a brand new and more diverse symbiotic rela-

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tionships among members of the Cnidaria and Porifera.

Updated taxonomic key to genera of Edwardsiidae

A1. Microcnemes are limited nearby the distal end.
  B1. Scapus with batteries of nematocysts (nemathybomes) sunk in the mesoglea. C
    C1. Physa present, without nemathybomes. ..... D
      D1. Microcnemes of the first cycle present. ..... ***Edwardsia***
      D2. Microcnemes of the first cycle absent. ..... ***Edwardsianthus***
    C2. Physa absent, aboral end with nemathybomes. ..... ***Scolanthus***
  B2. Scapus without nemathybomes. ..... E
    E1. Scapus with 8 rows of solid papillae forming nematocyst batteries. ..... ***Halcampogeton***
    E2. Scapus has tenaculi, and often attaching grains of sand or mud. ..... ***Paraedwardsia***
    E3. Scapus covered by a strong cuticle (periderm), scapulus distinct. ..... ***Edwardsiella***
    E4. Scapus smooth ..... F
      F1. Nematosomes in the coelentron ..... ***Nematostella***
      F2. Nematosomes absent. ..... G
        G1. Scapus rich in holotrichs. Inhabits in sponge symbiotically. ..... ***Tempuractis* gen. nov.**
        G2. Scapus holotrich absent. Inhabits in sand. ..... ***Drillactis***
A2. Microcnemes are along the whole body. ..... H
  H1. Column divisible into scapulus, scapus and physa. Scapus with tenaculi. ..... ***Synhalcampella***
  H2. Column smooth, indivisible parts ..... ***Metedwardsia***

After Carlgren (1949), this is the most recent genus-level taxonomic key of Edwardsiidae. Since Carlgren (1949), Manuel (1981a) restored *Scolanthus* Gosse, 1853, and England (1987) separated *Edwardsianthus* England, 1987 from *Edwardsia*. In addition, *Edwardsioides* Danielssen, 1890, which was once regarded as invalid (Carlgren, 1921), was resurrected by England (1987) and then invalidated again by Fautin et al. (2007). *Isoedwardsia* Carlgren, 1921 was stated as a junior synonym of *Scolanthus* (Manuel, 1981a; Daly and Ljubenkov, 2008), and *Fagesia* Delphy, 1938 was synonymized with *Edwardsiella* by Manuel (1981b) and Daly (2002b). All genera mentioned in this key were regarded as valid in Fautin (2016), except *Tempuractis* gen. nov. which is established in this report.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

TI mainly worked at this study and wrote article. YI discovered this symbiont and wrote discussion part with TI. KY tutored the methods of analyzing sea anemones to TI. DS operated TEM observation. RU supervised this study.

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