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 nematobomes 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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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 prefixed 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).

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 prefixed 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...
0.1 M sodium cacodylate (pH 7.4), the specimens were postfixed with 1% OsO₄ 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).

Cnidae 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). 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 nemathymbomes 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: spirocrysts, 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 suffix -acts 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, Nematomella Stephenson, 1935, and Metedwardsia Carlgren, 1947 in possessing a smooth scapus with no nemathymbomes or tenaculi. The following genera are distinguishable from this new genus: Edwardsia de Quatrefages, 1842, Scolanthus Gosse, 1853, and Edwardsianthus England, 1897 have nemathymbomes (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 Halcampogiton 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 hexameroerously 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

<table>
<thead>
<tr>
<th>Type</th>
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<th>Detail of specimens</th>
<th>Locality</th>
<th>Depth</th>
<th>Histological section</th>
<th>Cnidae specimens</th>
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<td>Holo</td>
<td>NSMT-Co 1873</td>
<td>specimens extracted from Oscarella sp.,</td>
<td>Araihama, Aburatsubo (Misaki)</td>
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<td>×</td>
<td>○</td>
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<td>A whole specimen extracted from Oscarella sp.</td>
<td>Araihama, Aburatsubo (Misaki)</td>
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<td>×</td>
<td>×</td>
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<td>CMNH-ZG 08970</td>
<td>A whole specimen in the portion of Oscarella sp.</td>
<td>Araihama, Aburatsubo (Misaki)</td>
<td>intertidal</td>
<td>×</td>
<td>×</td>
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<td>CMNH-ZG 08971</td>
<td>Histological sections (longitudinal).</td>
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<td>〇</td>
<td>×</td>
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<td>Histological sections (transverse).</td>
<td>Shukunegi, Sado</td>
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<td>×</td>
</tr>
<tr>
<td>Para</td>
<td>CMNH-ZG 08973</td>
<td>Histological sections (transverse).</td>
<td>Tohama, Toba</td>
<td>intertidal</td>
<td>×</td>
<td>×</td>
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<tr>
<td>Para</td>
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<td>A whole specimen in the portion of Oscarella sp.</td>
<td>Sugashima, Toba</td>
<td>2 m</td>
<td>×</td>
<td>×</td>
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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.*
inkai. 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 Edwardsiidae. 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.](https://bioone.org/journals/Zoological-Science on 07 Sep 2019 Terms of Use: https://bioone.org/terms-of-use)
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Fig. 5. The images of sections between Tempuractis rinkai gen. et sp. nov. and Oscarella sp.; CMNH-ZG 08969. Whole specimen left in a portion of 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.

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 nemathymbomes or tenacula. Epidermis 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 of mesenterial arrangement in two octamerous cycles, a quite tiny body surface without nemathybmomes or cuticles, stenticular characterized by possessing holotrichs, a smooth body 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.

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 Abarutsubo, 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 Abarutsubo, 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 “jikkenjo” 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).

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)
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 endoecolic. Retractor muscles comparatively weak, restricted or reniform in upper part, but diffuse 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 siphoglyphs (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 sphenicter 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, invading 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 Tempuractis 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, undersides 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.
Tempuractis rinkai gen. et sp. nov.

*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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**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 mastigophore. (F) column spirocyst. (G) column basitrich. (H) column holotrich. (I) column microbasic mastigophore. (J) filament basitrich. (K) filament microbasic 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.

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<th>Cnidae type</th>
<th>Size</th>
<th>Mean</th>
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<td>(A) Spirocyct</td>
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<td>(B) Basitrichs</td>
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<td>16.1 × 1.6</td>
<td>2.82 × 0.30</td>
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<td>Common</td>
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<td>(C) Holotrichs</td>
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<tr>
<td>(D) Basitrichs</td>
<td>6.7–19.6 × 1.0–4.1</td>
<td>14.6 × 4.0</td>
<td>2.84 × 0.88</td>
<td>71</td>
<td>Common</td>
</tr>
<tr>
<td>(E) Microbasic</td>
<td>21.9–27.7 × 3.7–5.6</td>
<td>24.6 × 4.6</td>
<td>4.66 × 0.40</td>
<td>52</td>
<td>Common</td>
</tr>
<tr>
<td><em>p</em>-mastigophores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Column</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(F) Spirocyct</td>
<td>17.2–20.7 × 2.6–3.8</td>
<td>18.8 × 3.5</td>
<td>1.39 × 0.40</td>
<td>6</td>
<td>Rare</td>
</tr>
<tr>
<td>(G) Basitrichs</td>
<td>8.2–13.6 × 1.7–2.9</td>
<td>11.7 × 2.4</td>
<td>0.94 × 0.29</td>
<td>57</td>
<td>Common</td>
</tr>
<tr>
<td>(H) Holotrichs</td>
<td>13.0–30.2 × 3.1–7.0</td>
<td>25.0 × 5.8</td>
<td>3.20 × 0.79</td>
<td>126</td>
<td>Common</td>
</tr>
<tr>
<td>(I) Microbasic</td>
<td>21.0–27.0 × 4.3–5.7</td>
<td>23.2 × 4.8</td>
<td>1.77 × 0.41</td>
<td>8</td>
<td>Rare</td>
</tr>
<tr>
<td><em>p</em>-mastigophores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filament</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(J) Basitrichs</td>
<td>10.1–29.8 × 2.1–7.0</td>
<td>13.1 × 2.7</td>
<td>4.57 × 1.00</td>
<td>69</td>
<td>Common</td>
</tr>
<tr>
<td>(K) Microbasic</td>
<td>21.0–27.0 × 4.3–5.7</td>
<td>22.3 × 4.2</td>
<td>1.92 × 0.52</td>
<td>47</td>
<td>Common</td>
</tr>
</tbody>
</table>

In conclusion, the present study revealed the second known symbiosis between members of Actiniaria and Porifera; provided the first detailed record between 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 *Spongias sp. nov.* Although these structures resemble the bump-like parts of *Oscarella sp.* where *Tempuractis 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 Oscarella, 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 epiderm (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 cnidian 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 Oscarella, 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*).
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 (nemathysts) in the mesogela. ........................................... C
C1. Physa present, without nematohymes. ............... D
D1. Microcnemes of the first cycle present. ............. E

................................................................. Edwardsia
D2. Microcnemes of the first cycle absent. .......... F
................................................................. Scolanthus

B2. Scapus without nematohymes. ...................... E
E1. Scapus with 8 rows of solid papillae forming nematocyst batteries. .................... Halicampogelon
E2. Scapus has tentaculi, and often attaching grains of sand or mud. ....................... Paraedwardsia
E3. Scapus covered by a strong cuticle (periderm), scalus distinct. .................... Edwardsiella
E4. Scapus smooth ........................................... F
F1. Nematosomes in the coelenteron. .......... G
F2. Nematosomes absent. ............................... H
G2. Scapus holotrich absent. Inhabits in sand. .... I

................................................................. Drilactis
H1. Column divisible into scapus, 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 Edwardsiathus England, 1987 from Edwardsia. In addition, Edwardsiodies Danielssen, 1890, 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 Lubjenkov, 2008), and Fagesia Delphyl, 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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