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Redescription of *Synactinernus flavus* for the First Time After a Century and Description of *Synactinernus churaumi* sp. nov. (Cnidaria: Anthozoa: Actiniaria)

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Two species of *Synactinernus* sea anemones were found in Japanese waters. *Synactinernus flavus* Carlgren, 1918, the only described species of this genus, is rediscovered from off the Goto Islands a century after the original description. *Synactinernus flavus* was once synonymized with *Isactinernus quadrilobatus* Carlgren, 1918; however, we show that, based on morphological (including examination of type specimens) and molecular (using nuclear 18S rDNA) evidence, these species are completely different. The other species, *Synactinernus churaumi* sp. nov., was found off Ishigaki Island and Okinawa Island by a remotely operated vehicle (ROV), and had been kept for 15 years in a tank at the Okinawa Churaumi Aquarium. There are clear differences between these two species; therefore, we describe the second species and revise the diagnosis of *Synactinernus*.

**Key words:** Endocoelanthae, Anenthemonae, Actinernidae, mesogleal thickening, mesenterial arrangement, fertile mesentery, Goto Islands, Okinawa Churaumi Aquarium

INTRODUCTION

The family Actinernidae Stephenson, 1922 comprises four genera and seven species (Fautin, 2013; Daly and Fautin, 2018). This family is characterized by the following three characteristics: more than 20 mesenteries; mesenteries of the second and younger cycles born in the endocoels of the first cycle; and actinopharynx with siphonoglyphs both on dorsal and ventral sides (Stephenson, 1922; Carlgren, 1949; Uchida, 2007). The peculiar mesenterial arrangement shared by Actinernidae and Halcuriidae Carlgren, 1918 in the suborder Endocoelantheae Carlgren, 1925 is traditionally regarded as the most important character for the classification of Actiniaria; for this reason, Carlgren (1925) established the suborder to accommodate these two families, and distinguished them from all other actinarians. Although this classification by Carlgren (1925) was followed until quite recently, Rodríguez et al. (2014) revealed that Endocoelanthae should be a part of the larger suborder Anenthemonae, but maintained the taxon combining Actinernidae and Halcuriidae by reviving the superfamily Actinernoidea Stephenson, 1922.

The genus *Synactinernus* was described in Carlgren (1918) monotypically with a single species, *Synactinernus flavus* Carlgren, 1918, collected from Japan. However, there have been no newly collected specimens of this genus for approximately a century (Fautin, 2013); hence, the study of *Synactinernus* has not advanced. Meanwhile, Fautin and den Hartog (2003) stated that *S. flavus* is a junior synonym of *Isactinernus quadrilobatus* Carlgren, 1918. Rodriguez et al. (2014) concluded that Endocoelanthae should be a part of the larger suborder Anenthemonae, and considered the genus *Synactinernus* remains valid to this day (Fautin et al., 2007; Fautin, 2016). This contradiction results from the difficulty to grasp the real nature of *S. flavus*; thus, new specimens are needed to resolve this problem. Yanagi (2006) and Uchida (2007) recognized *S. flavus* as a junior synonym of *I. quadrilobatus* following Fautin and den Hartog (2003), and considered *Actinernus robustus* (Hertwig, 1882),
**Isactinernus quadrilobatus**, and *Synhalcurias elegans* (Wassilieff, 1908) as the only three currently valid species of Actinernidae from Japanese waters.

During the 21st Century, several specimens identified as *S. flavus* were collected from Goto-nada Sea, near the type locality. By analyzing these newly collected specimens, we discovered several morphological differences from *I. quadrilobatus* and realized that *S. flavus* cannot be accommodated in *Isactinernus* or any other genera of Actinernidae. Molecular phylogenetic analyses suggest that *Synactinernus* is less closely related to *Isactinernus* than the other genera in Actinernidae. Moreover, we also found other specimens of undescribed species of *Synactinernus* from off Okinawa Island. Some new characteristics of this new species suggest that the diagnosis of the genus *Synactinernus* is in need of revision. In addition, we observed some ecological features of *Synactinernus* anemones: transverse fission of *S. flavus* for the first time in Actinernoidea sea anemones and aggregation of *S. churaumi* sp. nov., the first observation of the ecology of Actinernidae in nature.

**MATERIALS AND METHODS**

**Sample collection and preservation**

Six specimens of *Synactinernus* were collected in this study: three each of *Synactinernus flavus* and *Synactinernus churaumi* sp. nov. Of the three specimens of *S. flavus*, two were collected from south off the Goto Islands, Nagasaki Prefecture, using a biological dredge during a cruise of R/V Nagasaki-Maru, and the other was from Uragami, Wakayama Prefecture, by a net for coral fishing (Fig. 1). Of the three specimens of *Synactinernus churaumi* sp. nov., two were collected from the sea northwest of Ishigaki Island and the other was from the sea east of Okinawa Island, Okinawa Prefecture (Fig. 1); all were collected by a remotely operated vehicle LEO (Kowa Corporation), operated from Dai-2 Kuroshio-Maru. All but the one from Uragami were kept alive in aquarium tanks at Okinawa Churaumi Aquarium (Okinawa, Japan) for several years before fixation. Prior to fixation, *in vitro* images of living polyps were taken to record external form of the oral disc, as well as the color and size of the polyps. From three of the five specimens—one of *S. flavus* from the Goto-nada Sea (NSMT-Co 1660) and two *S. churaumi*, one from off Ishigaki Island (NSMT-Co 1661) and one from off Okinawa Island (CMNH-ZG 09733)—tentacle tissues were dissected and preserved in 99% ethanol for DNA extraction; the remaining whole polyps were fixed in 10–20% (v/v) formalin sea water solution after approximately half a day of anesthetic...
tization using magnesium chloride solution. For another two speci-
mens—one each of *S. flavus* (the other one from the Goto Islands) and *S. churaumi* (the other one from Ishigaki Island)—only tentacle tissues were dissected for molecular analyses (Table 1), with the rest of the bodies still kept alive in a tank, along with additional other specimens, at the Okinawa Churaumi Aquarium for exhibition. The other specimen of *S. flavus* from Uragami had been kept alive at the Kushimoto Marine Park (Wakayama, Japan), and processed using the same method as described above.

For morphological comparison and molecular analyses with *Synactinernus* species, we also examined specimens of *Isactinernus quadrilobatus* and *Actinernus robustus*: *I. quadrilobatus* (NSMT-Co 1662), collected on 8 March 2016, from the Kumano-nada Sea off Hamajima Island, Mie Prefecture, around a depth of 350 m, by the fishing boat *Kiei-Maru*; *I. quadrilobatus* (CMNH-ZG 09734), collected on 18 April 2018, from the East China Sea off Koshikijima Island, Kagoshima Prefecture, around a depth of 380 m, by the fishing boat *Koei-Maru*; and *A. robustus* (CMNH-ZG 09735), collected on 28 April 2002, from Okinawa trough off Kumejima Island, Okinawa Prefecture (27°02.89′N, 126°59.09′E), around a depth of 1550 m, by an ORE beam trawl during a cruise of R/V *Tanser-Maru*. The specimens were preserved by the same method described earlier in this section. The specimens examined have been deposited at either the National Museum of Nature and Science, Tokyo (NSMT) or the Coastal Branch of Natural History Museum and Institute, Chiba (CMNH).

**Examination of type specimens**

Observation of type specimens were performed at the Museum of Zoology, Lund University (MZLU) and the Museum of Evolution Zoology, Uppsala University (UUZM) in September–October 2014, at the Zoological Museum, University of Copenhagen (ZMUC) in October 2013, and the Natural History Museum, London (BM) in March 2016. We observed the specimens of *S. churaumi* (the other one from Ishigaki Island) only tentacle tissues were dissected for molecular analyses (Table 1), with the rest of the bodies still kept alive in a tank, along with additional other specimens, at the Okinawa Churaumi Aquarium for exhibition. The other specimen of *S. flavus* from Uragami had been kept alive at the Kushimoto Marine Park (Wakayama, Japan), and processed using the same method as described above.

**Preparation of histological sections**

Histological sections were prepared following standard proto-
cols. The specimens of *Synactinernus flavus* (NSMT-Co 1660) and *S. churaumi* sp. nov. (NSMT-Co 1661) were dissected to obtain some tissues. The tissues were dehydrated by ethanol and cleared in xylene, embedded in paraffin, sliced into serial sections (7–10 μm thick) using a microtome, mounted on glass slides, and stained with hematoxylin and eosin (Presnell and Schreibman, 1997). Because *S. churaumi* was too large to be mounted on slide glasses, the specimen was cut into blocks including some mesenteries before embedding. For identification and comparison, the specimens of *A. robustus* and *I. quadrilobatus* were processed in the same way as *Synactinernus* specimens.

**Cnidae observation**

Cnidae were observed in the tentacle, actinopharynx, column, and filament. Tissue from each organ was placed on slide glasses and mounted using 50% (v/v) glycerin seawater solution. Images of the cnidae were observed by differential interference contrast microscopy (Yanagi et al., 2015). The length and width were measured using the software ImageJ ver. 1.49 (Rasband, 1997–2012), Cnidae nomenclature followed Mariscal (1974).

**Phylogenetic analyses**

DNA was extracted from the tissues of three specimens of *S. flavus* (NSMT-Co 1660, CMNH-ZG 09732, and the specimen pre-
served only tentacle tissue) and *S. churaumi* sp. nov. (NSMT-Co 1661, and the specimen only tissue) preserved in 99% EIOH follow-
ing a guanidine extraction protocol (Sinniger et al., 2010) or by ChargeSwitch gDNA Micro Tissue Kit (Invitrogen). PCR amplifi-
cations were performed for mitochondrial 16S rDNA primers, 16Santi0a and 16Sbm0H (Sinniger et al., 2005), and nuclear 18S rDNA primers, 18SA and 18SB (Medlin et al., 1988). The PCR reac-
tion was performed in a 10-μL reaction volume, consisting of 0.4 μL of forward and reverse primers (25 μM), 2.0 μL of EmeraldAmp PCR Master Mix (TaKaRa), and 3.4 μL of distilled water. The ampli-
fication conditions followed Sinniger et al. (2005) for 16S rDNA and Medlin et al. (1988) for 18S rDNA. The PCR products were pro-
cessed with Exonuclease I and shrimp alkaline phosphate (Exo-SAP) prior to sequencing. Sequencing reaction was per-
formed using PCR primers (16S) or PCR primers and internal primers (18S). We used four primers (Apakupakul et al., 1999): two forwards, 18SC and 18SO and two reverses, 18SL and 18SY, and BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems). Sequencing was performed by ABI 3130xL or 3500xL Genetic Analyzer (Applied Biosystems). The two sequences of 16S and six sequences of 18S were individually assembled by GeneStudio ver. 2.2.0.0 (http://genestudio.com). The new sequences obtained in this study have been deposited in GenBank (Table 1).

For phylogenetic analyses, sequence data of four species of Actineroidea (Actinidae and Halicuridae) and *Nematostella vectensis* Stephenson, 1935 (belonging to Edwardsiidae in Edwardsioidea) as an outgroup, were obtained from GenBank (Table 1). The data set was aligned by MAFFT ver. 7.402 (Katoh and Standley, 2013) under the default settings. Ambiguously aligned regions were eliminated by Gblocks ver. 0.91b (Castresana, 2002): type of sequences was DNA: the parameter was default except allowing small final blocks and gap positions within the final blocks. Next, the file was processed by Kukusan 4 (Tanabe, 2011) to test its substitution models for analyses of both RAxML and MrBayes (the alignment is available from the corresponding author upon request). Maximum-likelihood (ML) analyses were performed by RAxML-VI-HPC (Stamatakis, 2006), with the GTR+Γ evolutionary model recommended by Kukusan 4 and evaluated by 100 bootstrap replicates. Bayesian inference (BI) was conducted by using MrBayes ver. 3.2.6 (Ronquist and Huelsenbeck, 2003) with HKY85_Gamma as the substitution parameter. Two independent runs of the Markov Chain Monte Carlo were carried out simultaneously for 3,000,000 generations, sampling trees every 100 generations and calculating average standard deviation of split frequencies (ASDSFs) every 100,000 generations. As ASDSF was calculated based on the last 75% of the samples, the initial 25% of the sampled trees were discarded as burn-in.

Finally, two resultant trees were combined by FigTree ver. 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/) and low bootstrap (<50) and posterior probability values (<0.90) were manually deleted on each node.

**RESULTS**

**Description**

Order **ACTINIARIA** Hertwig, 1882

Suborder **ANENTHEMONAE** Rodríguez and Daly, 2014

Superfamily **Actineroidea** Stephenson, 1922

Family **Actinidae** Stephenson, 1921

Genus **Synactinernus** Carlgren, 1918

(New Japanese name: Kuroba-kawari-ginchaku-zoku)

**Diagnosis** (revised from Carlgren, 1918; revised parts are shown in *italics*). Actinidae with cylindrical body, which is distally drawn out into eight distinct lobes, *all eight of same size or four larger and four smaller alternating. Column without papillae. No sphincter. Tentacles in at least two
cycles without distinct mesogloeleal thickenings, all same length or largest at apexes of the lobes, numerous. Longitudinal muscles of tentacles ectodermal, radial muscles of oral disc chiefly ectodermal, strong. Two broad siphonoglyphs on actinopharynx. 36 macrocnemes cyclic in arrangement, beyond them weak mesenteries of unequal size in upper part of the body. Retractors weak, parietal muscles weak or rather well developed.

**Type species.** Synactinernus flavus Carlgren, 1918.

**Remarks.** The genus Synactinernus was established in the family Actinernidae by Carlgren (1918) monotypically for Synactinernus flavus Carlgren, 1918. Recently, Fautin and den Hartog (2003) argued that Synactinernus flavus, the only species of Synactinernus, cannot be distinguished from Isactinernus quadrilobatus and synonymized Synactinernus with Isactinernus. However, Synactinernus was listed as a valid genus in later studies (Fautin, 2013, 2016). Therefore, the actual status of *S. flavus* and the validity of Synactinernus remained unclear. Additional specimens are needed to resolve this taxonomical problem, but no new specimen of Synactinernus had been found for a century after the original description of Carlgren (1918). The present study is the first revision of the genus Synactinernus, as we obtained a second specimen of *S. flavus* and the second species of this genus.

Our morphological analyses of the newly collected specimens of two Synactinernus species and *I. quadrilobatus* and of the type specimens of *I. quadrilobatus* showed that tentacles of *S. flavus* and *S. churaumi* sp. nov. are simple in shape, apparently lacking thickening of the aboral side (Figs. 2C, G, 3C, 6B, 7C), while those of *I. quadrilobatus*, both in our collection and type specimens, are apparently thickened at the base of the aboral side (Fig. 6C). This clear difference suggests that Synactinernus species can be distinguished from *I. quadrilobatus* by their tentacles. In addition, Synactinernus species had a fixed number (36) of macrocnemes (Figs. 3A, 7A), and this prominent feature in the mesentery structure should be a diagnostic characteristic of the genus, while *I. quadrilobatus* has many indistinguishable mesenteries. In contrast, the alternate arrangement of larger and smaller oral lobes of *S. flavus*, considered as an important morphological feature of this monotypic genus (Carlgren, 1918, 1949), turned not to be a diagnostic characteristic, because the new species *S. churaumi* has eight lobes of the same size.

The result of the current molecular phylogenetic analyses that Synactinernus formed a well-supported independ-
Figure 3. Internal morphology of *Synactinernus flavus* (NSMT-Co 1660). (A) Transverse section of actinopharynx of the gross specimen. (B) Transverse section of macrocnemes and microcnemes. (C) Transverse section of the tentacle. Arrowheads indicate tentacular longitudinal muscle. (D) Longitudinal section of the most basal part of the tentacle. (E) Transverse section of filaments and gonads. (F) Enlarged view of gonad. Abbreviations: a, actinopharynx; ma, macrocneme; me, mesoglea; mi, microcneme; oo, oocyte; ov, ovary; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph. Scale bars indicate 5 mm in (A), 1 mm in (B, E), 500 μm in (C, D), and 200 μm in (F).

| Table 2. Size and distribution of cnidae in *Synactinernus flavus* (NSMT-Co 1660), and *Synactinernus churaumi* sp. nov. (holotype, NSMT-Co 1661). Size range, mean, and SD are dictated as length x width. A–T indicate the figures of each cnidae in Fig. 5; n indicates number of nematocysts measured. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Synactinernus flavus |                 |                 | Synactinernus churaumi sp. nov. |                 |                 |
|                 | NSMT-Co 1660 |                 |                 | NSMT-Co 1661 |                 |                 |
| **Cnidales**    | Abundance | Size range (μm) | Mean (μm) | SD (μm) | n | Abundance | Size range (μm) | Mean (μm) | SD (μm) | n |
| Tentacles       |            |                 |            |        |    |            |                 |            |        |    |
| Sporocytes      | A numerous | 18.5–45.7 x 3.0–9.8 | 32.3 x 5.9 | 6.74 x 1.53 | 88 | K numerous | 17.8–56.8 x 2.6–8.3 | 33.5 x 4.8 | 10.41 x 1.74 | 105 |
| Basitrichs      | B numerous | 15.3–33.4 x 2.3–4.4 | 28.7 x 3.3 | 2.59 x 0.42 | 55 | L numerous | 30.0–42.2 x 2.1–3.3 | 35.1 x 2.9 | 2.58 x 0.33 | 56 |
| Actinopharynx   |            |                 |            |        |    |            |                 |            |        |    |
| Sporocytes      | C numerous | 25.8–40.2 x 5.4–8.0 | 34.4 x 6.4 | 3.02 x 0.57 | 55 | M numerous | 34.4–43.1 x 5.8–8.9 | 39.6 x 7.1 | 2.17 x 0.70 | 21 |
| Basitrichs      | D numerous | 23.8–39.6 x 2.6–4.2 | 31.1 x 3.5 | 3.32 x 0.35 | 65 | N numerous | 26.5–42.5 x 3.2–4.6 | 35.7 x 3.9 | 2.27 x 0.34 | 80 |
| Microbasitrichs | E rare     | 23.0–23.9 x 4.0–5.0 | 23.4 x 4.5 | 0.42 x 0.47 | 2  | O numerous | 34.3–39.5 x 5.6–6.8 | 36.4 x 6.3 | 1.56 x 0.38 | 12 |
| Column          |            |                 |            |        |    |            |                 |            |        |    |
| Sporocytes      | F numerous | 20.9–47.1 x 4.1–7.6 | 35.2 x 6.6 | 6.14 x 1.11 | 17 | P numerous | 28.6–39.2 x 4.3–6.0 | 33.2 x 5.2 | 2.82 x 0.46 | 15 |
| Basitrichs      | G numerous | 27.9–42.7 x 2.9–5.0 | 34.9 x 3.9 | 2.50 x 0.40 | 100 | Q numerous | 36.1–46.4 x 2.9–4.6 | 41.8 x 3.6 | 2.02 x 0.33 | 98 |
| Filament        |            |                 |            |        |    |            |                 |            |        |    |
| Sporocytes      | H numerous | 19.9–31.0 x 3.4–6.5 | 26.7 x 5.2 | 2.58 x 0.72 | 24 | R numerous | 24.5–36.1 x 4.6–7.3 | 31.8 x 5.9 | 2.31 x 0.60 | 79 |
| Basitrichs      | I numerous | 28.5–38.5 x 2.5–4.3 | 32.3 x 3.7 | 1.90 x 0.39 | 59 | S numerous | 21.6–36.3 x 2.4–3.7 | 27.8 x 3.1 | 3.82 x 0.38 | 29 |
| Microbasitrichs | J numerous | 20.5–27.5 x 4.5–6.7 | 23.9 x 5.7 | 1.64 x 0.57 | 37 | T numerous | 28.1–39.4 x 4.8–7.1 | 33.5 x 6.0 | 2.22 x 0.52 | 56 |

**Description.** *External anatomy.* Body cylindrical (Fig. 2A), up to ca. 5–8 cm in length and ca. 2–4 cm in width when alive, and 2–4 cm in length and 1–2 cm in width on specimen. Column surface comparatively smooth, without tentacles, with numerous discontinuous, wavy wrinkles running in a transverse direction, pale surface with pale orange ectoderm layer. Ectoderm of column without nematocyst batteries, nematocysts very sparsely distributed containing numerous spirocysts. Upper part of column widely expanded and thrown into eight lobes, four larger and four smaller ones (Fig. 2B, C). Upper margin of the lobes fluorescent yellow in color (Fig. 2B). Tentacles simple, all marginal, ca. 2–5 mm in length, no thickenings including their aboral base, pale white in color, and ca. 120–200 in number on oral disk; inner and outer ones alternatingly bared. The tip of tentacles pointed. Pedal disk in aboral end, semitransparent, and mesenterial insertion visible. Oral disk diameter ca. 3–6 cm, same color as tentacles, mouth at center of oral disk, appa-
**Synactinernus churaumi** sp. nov.

**Internal anatomy.** 36 (18 pairs of) macrocnemes (Figs. 3A, 4) on actinopharynx; twelve, including four directives, in the first cycle; eight in the second cycle; 16 in the third cycle. Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, an arrangement obeying the rule of mesenterial arrangement of Actinernidae (Fig. 4). Pairs of microcnemes, in the fourth cycle, observed in transverse section (Fig. 3A, arrowhead). Judged by the numbers of tentacles, mesenteries of *Synactinernus flavus* in the first–sixth cycles.

All mesenteries perfect near the mouth, and each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle ectodermal (Fig. 3C); tentacular circular muscle too weak to observe in histological sections (Fig. 3D). Retractor muscles comparatively weak and restricted actinopharynx or filament side (Fig. 3B, E). Muscle processes short, mostly simple or slightly branched, around 30–50 in each muscle pennon. Parietal muscles of macrocnemes weak, distinct with approximately 10 muscle fibers in upper part near actinopharynx (Fig. 3B), but indistinct in lower part near filaments (Fig. 3E). Mesoglea thickest in body wall and actinopharynx (Fig. 3A), reaching 2–3 mm in thickness, and far thicker than the ectoderm and endoderm. However, mesoglea thinner in mesenteries (Fig. 3E), and thinnest in tentacles (Fig. 3C, D). Actinopharynx, with siphonoglyphs on dorsal and ventral sides (Figs. 3A, 4), always connected to actinopharynx, and with 10 longitudinal grooves as deep as siphonoglyphs. Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, matured eggs in gonads in NSMT-1660 (Fig. 3F).

**Cnidom.** Basitrichs, spirocysts, and microbasic p-mastigophores. See Table 2 and Fig. 5 for size and distribution.

**Derivation of new Japanese name.** “Kuroba” means the plant clover. The oral disk with four large and foursmall lobes resembles the four-leaf clover, which is considered a symbol of good luck.

**Taxonomic remarks.** We provide a detailed description of *S. flavus* based on the holotype and our new specimens in this study. We presented cnidom data of every part of the body (Table 2) and the figures of external features (Fig. 2), which were lacking in Carlgren (1918). Almost all morphological features of the present specimen (NSMT-Co 1660) corresponded to Carlgren (1918) and the holotype (U0ZM 232); cylindrical body (Figs. 2A, 6A); ca. 120–200 tentacles with no thickening even on base (Figs. 2B, C, 3D, 6B); about 10 longitudinal grooves on actinopharynx (Fig. 3A); mesoglea on the actinopharynx are thick (Fig. 3B); ectodermal but weak tentacular muscle (Fig. 3D); numerous spirocysts in column (Fig. 5F, Table 2). The size of spirocysts, 20.9–47.1 μm in length and 4.1–7.6 μm in width in our specimens (Table 2), is similar to that of the syntype, 26–38 μm in length and 3–5 μm in width (Carlgren, 1918).
closely related on the phylogenetic tree and the difference mentioned above; species based on the several morphological differences discuss that they are independent groups, and our molecular morphological differences of both species are sufficient to two times that of \( I. \) quadrilobatus (Fig. 6A, C). These several addition, the size of \( I. \) quadrilobatus \( I. \) quadrilobatus has many fertile mesenteries (Fig. 6D). In \( S. \) flavus fertilizes only the first and second cycle mesenterial pairs among 81 specimens when the two species were synonymized. However, we confirmed that there are several differences between the two species other than the above-mentioned differences between the two genera; whether as mentioned in remarks of genus Synactinernus, thickening on the base of tentacle is only observed on \( I. \) quadrilobatus. At the oral view. All scale bars indicate 1 cm.

The number of macrocnemes is fixed to 36 in \( S. \) flavus (Fig. 3A), while over 100 in \( I. \) quadrilobatus (Fig. 6D; there are the same number of mesenterial pairs next to the actinopharynx), but it is not a strong evidence to divide the species and genus because Fautin and den Hartog (2003) reported a range (18–40) in the number of mesenterial pairs among 81 specimens when the two species were synonymized. However, we confirmed that there are several differences between the two species other than the above-mentioned differences between the two genera; whether as mentioned in remarks of genus Synactinernus, thickening on the base of tentacle is only observed on \( I. \) quadrilobatus. At the oral view. All scale bars indicate 1 cm.

Ecological remarks. It was observed that Synactinernus flavus reproduced several times by transverse fission in the tank at Okinawa Churaumi Aquarium. When the bodies were mechanically or thermally stimulated, for example when their basal disc was detached from the substrate, it cracked on the surface of the column, and transverse fission of the body occurred soon thereafter (Fig. 2D). Both halves became independent full-grown individuals. This is the first observation of transverse fission in Actinernoidea, while fission from the basal disc has been observed in Halcuriidae (Uchida, 2004).

Synactinernus churaumi sp. nov. Izumi and Fujii, 2019
(New Japanese name: Churaumi-kawari-ginchaku)
(Figs. 2, 5, 7; Table 2)

Material examined. Holotype: NSMT-Co 1661: specimen dissected, tissues embedded in paraffin, histological sections (20 slides) prepared, nematocysts prepared (four slides); originally collected on February 22, 2004, off north-western Ishigaki Island, Okinawa Prefecture, at a depth of 281–312 m, using LEO, ROV of Okinawa Churaumi Aquarium, by Takuo Higashiji, and fixed by Takuma Fujii and Hiroko Takaoka from the aquarium tank on 25 January 2013. Paratype: CMNH-ZG 09733: originally collected on 5 September 2018, off Onna Village, Okinawa Island, Okinawa Prefecture (26°34.22′N, 127°47.96′E), at a depth of 320 m, using ROV of the Okinawa Churaumi Aquarium by Takuo Higashiji, and fixed by Takato Izumi from the aquarium tank on 10 September 2018 (Fig. 1).
**Synactinernus churaumi** sp. nov.

**Description.** *External anatomy.* Body cylindrical (Fig. 2E), up to 20–25 cm in length and 15 cm in width when alive. Regarding specimens, 12 cm in length and 10 cm in width on holotype, and 13 cm in length and 9 cm in width on paratype. Column surface smooth, without tentaculi, pale surface with orange or yellow ectoderm layer. Ectoderm of column without nematocyst batteries and nematocysts very sparsely distributed but contain numerous spirocysts. Upper part of column widely expanded and thrown into eight lobes of the same size (Fig. 2F). Upper margin of the lobes same as column in color (Fig. 2E). Tentacles simple, all marginal, 5–20 mm in length, no thickenings including their aboral base, pale white in color, and ca. 350–500 in number on oral disk; inner and outer ones alternatingly bared (Fig. 2F). The tip of tentacles pointed. Pedal disk in aboral end, opaque and mesenterial insertion invisible. Oral disk diameter ca. 12–20 cm in living specimen, same color as tentacles or pale orange, Mouth at center of oral disk, exceedingly swollen, lip-like, pale white to yellow (Fig. 2F).

**Internal anatomy.** 36 (18 pairs of) macrocnemes (Figs. 4, 7A) on actinopharynx; 12, including four directives, in the first cycle; eight in the second cycle; and 16 in the third cycle. Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries; their arrangement obeying the rule of mesenterial arrangement of Actinernidae (Fig. 4). Mesenteries in younger than the fourth cycle microcnemes, and judged by the numbers of tentacles, mesenteries of *Synactinernus churaumi* sp. nov. in the first–seventh cycles. All mesenteries perfect near the mouth, and each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle ectodermal (Fig. 7C, D); tentacular circular muscle too weak to observe in histological section; retractor muscles comparatively weak and restricted on the middle of mesenteries (Fig. 7B); or diffused and integrated to parietal muscle (Fig. 7E). Muscle processes short, mostly simple or slightly branched, around 60–90 in each muscle pennon. Parietal muscles of macrocnemes weak, distinct with approximately 10 muscle fibers in upper part near actinopharynx (Fig. 7B) but integrated to retractor muscle near filaments (Fig. 7E). Mesoglea thickest in body wall and actinopharynx (Fig. 7A, B), reaching to 8–10 mm in thickness, and far thicker than ectoderm and endoderm. However, mesoglea thinner in mesenteries (Fig. 7B, E) and thinnest in tentacle (Fig. 7C, D). Actinopharynx, with siphonoglyphs on dorsal and ventral sides (Figs. 7A, 4), always connected to actinopharynx, and with 10 longitudinal grooves as deep as siphonoglyphs. Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, matured testes in NSMT-1661; a variety of stages of maturation in the same testis (Fig. 7F). Mesenteries in the first and second cycles only fertile. The release of eggs and sperm by *S. churaumi* was observed in the tank at the Okinawa Churaumi Aquarium.

**Cnidom.** Basitrichs, spirocysts, and microbasic p-mastigophores. See Table 2 and Fig. 5 for size and distribution

**Etymology.** The species epithet “churaumi” is named after Okinawa Churaumi Aquarium, an aquarium in Okinawa, Japan, which provided us the specimens of this species. “Chura” means beautiful in Okinawan language, and “umi” means sea in Japanese.

**Derivation of new Japanese name.** Same as the species epithet.

**Taxonomic remarks.** *Synactinernus churaumi* sp. nov. is the second species of this genus; this species is clearly distinguished from *S. flavus*. Comparing the two species in adult individuals which have matured gametes (Figs. 3E, 5E), *S. churaumi* is approximately 3–5 times larger than *S. flavus* in body length (*S. churaumi* is one of the largest species of Actinernidae), and *S. churaumi* has over 350 tentacles while *S. flavus* has around half the number. The margin of the oral disk of *S. churaumi* develops into eight same sized lobes, while that of *S. flavus* has four larger and four smaller lobes. The molecular phylogenetic tree (Fig. 8) indicated that these two species formed apparently separate clades. These evidences indicate that *S. churaumi* is not conspecific with *S. flavus* but is an independent species. In addition, there is possibly a difference in the presence of asexual fission (see Ecological remarks).

The eight-lobed oral disk of *S. churaumi* sp. nov. resembles that of *Actinernus Verrill*, 1879. However, *S. churaumi* does not correspond to the diagnosis of *Actinernus* of
Actinernidae Stephenson, 1922. Actinernus species have mesogleal thickening at the tentacle base, and we confirmed this in both the holotype of *Actinernus robustus* Hertwig, 1882, the only *Actinernus* species from the North Pacific, (Natural History Museum, London, BM 89-11-25-30; Fig. 6E, F) and our specimens (CMNH-ZG 09735). However, tentacles of *S. churaumi* were not thickened at any part. Moreover, mesenterial arrangement of *Actinernus* is bilateral, and the fourth cycle mesenteries are born in the endocoel of the third cycle (Carlgren, 1918), and Uchida (2007) confirmed this for *A. robustus*. The mesenterial arrangement of *S. churaumi* is completely different from that of *Actinernus* (Fig. 4).

**Ecological remarks.** *Synactinernus churaumi* sp. nov. is distributed around depths of 320–350 m off Okinawa Island. This species forms small aggregations on the top of hill-like submarine topography as recorded by the ROV (Fig. 2H). Incidentally, no asexual fission of *S. churaumi* in the tank at the Okinawa Churaumi Aquarium was observed for 15 years, while *S. flavus* has self-divided several times over seven years (Fig. 2D).

**Phylogenetic analyses**

Regarding the phylogenetic tree of 18S rDNA (1623 bp), all *Synactinernus* specimens significantly formed a clade (ML boot strap value = 79; BI posterior probability = 0.99), which was separated from the other genera of Actinernidae (Fig. 8). The *Synactinernus* clade was separated into two clades at high support (ML bootstrap value = 100; BI posterior probability = 1.0 for both clades), and they corresponded to respectively *S. flavus* and *S. churaumi* specimens. All three sequences of *S. flavus* were completely identical, and *S. churaumi* showed only a 2-base-pair difference between the two sequences (this small difference may be intraspecific variation because nuclear 18S marker has higher base pair substitution late than mitochondrial ones in Actiniaria [Daly et al., 2010]). In addition, the sequence of *I. quadrilobatus* deposited in GenBank (KJ483024) was also identical with *S. flavus*. On the other hand, our specimen of *I. quadrilobatus* (NSMT-Co 1662) was nested with *S. elegans* (KJ483021), the most basal clade in Actinernidae, and completely separated from "*I. quadrilobatus*" in GenBank. The relationship between *Actinernus* and *Synactinernus* was unsolved with unreliable node (ML boot strap value = 43; BI posterior probability = 0.64).

Regarding 16S (581 bp), there were no differences in the sequences between *S. flavus* and *S. churaumi* at all. It has been shown that 16S is less useful than 18S in the phylogenetic analyses of sea anemone for phylogeny in species level in previous research (Daly et al., 2010), and the results of this research support that assertion.

**DISCUSSION**

**Taxonomic interpretation of molecular phylogeny**

The phylogenetic tree (Fig. 8) certifies several taxonomic suggestions as below. *Synactinernus flavus* and *S. churaumi* sp. nov. are distinct species, but they belong to the same genus. That the specimens from Uragami are conspecific with *S. flavus* from Goto is clearly shown by the molecular data.

*Isactinernus quadrilobatus* was completely separated from *S. flavus* on the 18S phylogenetic tree (Fig. 8) suggesting these two species are different, contrary to their synonimization by Fautin and Hartog (2003). The 18S sequence of "*Isactinernus quadrilobatus*" deposited in GenBank...
(KJ483024), which was used in recent phylogenetic analyses (Rodríguez et al., 2014), is in fact the sequence of *S. flavus* specimen, which was mistakenly identified as *I. quadrilobatus*. Rodríguez et al. (2014) showed other sequences of four markers, 12S, 16S, 28S, and COXIII, as well as an 18S sequence from the same specimen for their phylogenetic analyses. The sequences of these markers should be inspected using our *I. quadrilobatus* specimen (NSMT-Co 1662) in the future. The locality of "*I. quadrilobatus*" (PNG 9032) is Papua New Guinea, so it is possible that the distribution of *Synactinernus flavus* is far broader than we realize.

**Higher diversity of Japanese Actinernidae**

Yanagi (2006) and Uchida (2007) reported three valid species of Actinernidae from Japan: *Actinernus robustus*, *Isactinernus quadrilobatus*, and *Synhalcurias elegans*. The present study adds two *Synactinernus* species; thus, five species of Actinernidae, covering all four genera of the family, are distributed in Japanese waters. Of the four genera, *Synhalcurias* Carlgren, 1914 and *Synactinernus* are endemic to Japan (Fautin, 2013). The key to the species currently found in Japan modified from Uchida (2007) is shown below.

A1. Margin of the oral disk not developing into any lobes ....................... *Synhalcurias elegans* (Wassilieff, 1908)
A2. Margin of the oral disk developing four or eight lobes ............................ B
B1. Aboral side of the tentacles not thickening .......... C
   C1. Four larger and four smaller lobes on the margin of oral disk. Tentacle number ca. 150–200. Body size around 3–5 cm .........................
   .............. *Synactinernus flavus* Carlgren, 1918
   C2. Eight lobes of almost the same size on the margin of oral disk. Tentacle number over 350. Body size over 10 cm ........................................
   .................. *Synactinernus churaumi* sp. nov.
B2. Aboral side of the tentacles apparently thickening .......................... D
D1. Four lobes on the margin of oral disk .............. *I. quadrilobatus* Carlgren, 1918
D2. Eight lobes on the margin of oral disk .............. *A. robustus* (Hertwig, 1882)

**CONCLUSIONS**

1. *Synactinernus* Carlgren, 1918 is a valid genus which includes two species: *S. flavus* Carlgren, 1918 and *S. churaumi* sp. nov.

2. *Isactinernus* Carlgren, 1918 is independent and less closely related genus from *Synactinernus*; *Isactinernus quadrilobatus* Carlgren, 1918 is distinguished from *S. flavus* by several features.

3. This is the first report of the transversal fission of *S. flavus*, and of the aggregations of *S. churaumi* sp. nov. in situ for actinernid sea anemones.

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