

Article

Fluorescent Anemones in Japan—Comprehensive Revision of Japanese Actinernoidea (Cnidaria: Anthozoa: Actiniaria: Anenthemonae) with Rearrangements of the Classification [†]

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Abstract: Actinernoidea is a superfamily of the suborder Anenthemonae of the order Actiniaria, subclass Hexacorallia. This superfamily has been diagnosed by a peculiar endocoelomic mesenterial arrangement and included two families, Actinernidae and Halcuriidae. Although the monophyly of this superfamily is anticipated based on preceding molecular phylogenetic works, the relationship between these two families was not certain because of the rarity of actinernoidean anemones. We conducted comprehensive sampling in Japan, where the highest diversity of actinernoideans is known, and conducted phylogenetic analyses using nuclear and mitochondrial gene markers. According to the comprehensive analyses, both Actinernidae and Halcuriidae were not indicated as monophyletic but rather as poly- or paraphyletic. Based on our phylogeny reconstruction, we propose a new classification for Actinernoidea composed of three families, including Isactinernidae fam. nov., and seven genera, including *Isohalcurias* gen. nov. We also describe four new species, *Halcurias hiroomii* sp. nov., *H. fragum* sp. nov., *Isohalcurias citreum* sp. nov., and *I. malum* sp. nov.; and propose a new combination, *Isohalcurias carlgreni* comb. nov. This work is the first study of Actinernoidea that comprehensively analyzes its phylogeny and rearranges its classification, showing that there is highly divergent fauna in the seas around Japan.

Keywords: systematics; new family; new genus; new species; redescription; aquarium; deep sea; monograph; actinernid; halcuriid

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1. Introduction

Sea anemones are well-known marine animals that belong to the order Actiniaria Hertwig, 1882, of the class Anthozoa of the phylum Cnidaria. Actiniaria currently contains approximately 1100 valid species from 264 genera of 50 families [1]. All sea anemones consist of solitary individual polyps, whereas other anthozoans often form colonies of zooids. Sea anemones have only a few reliable taxonomic characters, and therefore, researchers have often been puzzled by their taxonomy and have struggled to correctly classify them by morphology [2]. Their taxonomy is mainly based on the arrangement of mesenteries, which are prominent structures in the bodies of sea anemones and develop in the direction of the distal–proximal axis and separate the actinarian coelenteron into partitions. Mesenteries are distinguished into two types: macrocnemes, which are usually distinct and develop several structures such as retractor muscles, gonads, and filaments,

and microcnemes, which are small and without these structures [2,3]. The mesenteries usually form pairs with one another and develop in a particular cyclic pattern (Figure 1): first, eight macrocnemes are formed (Figure 1A), and later, another four macrocnemes develop (Figure 1B). These 12 macrocnemes (mesenteries indicated as d, ld, lv, and v in Figure 1B) are called “mesenteries in the first cycle”. Subsequently, mesenteries of the second cycle develop between the mesenteries in the first cycle (Figure 1C,D), and younger mesenteries continue to develop. These arrangements of mesenteries differ depending on species and genera and are thus considered as among the most important taxonomic characters of sea anemones. In general, the thicker and larger the column of sea anemones, the more numerous the macrocnemes [4].

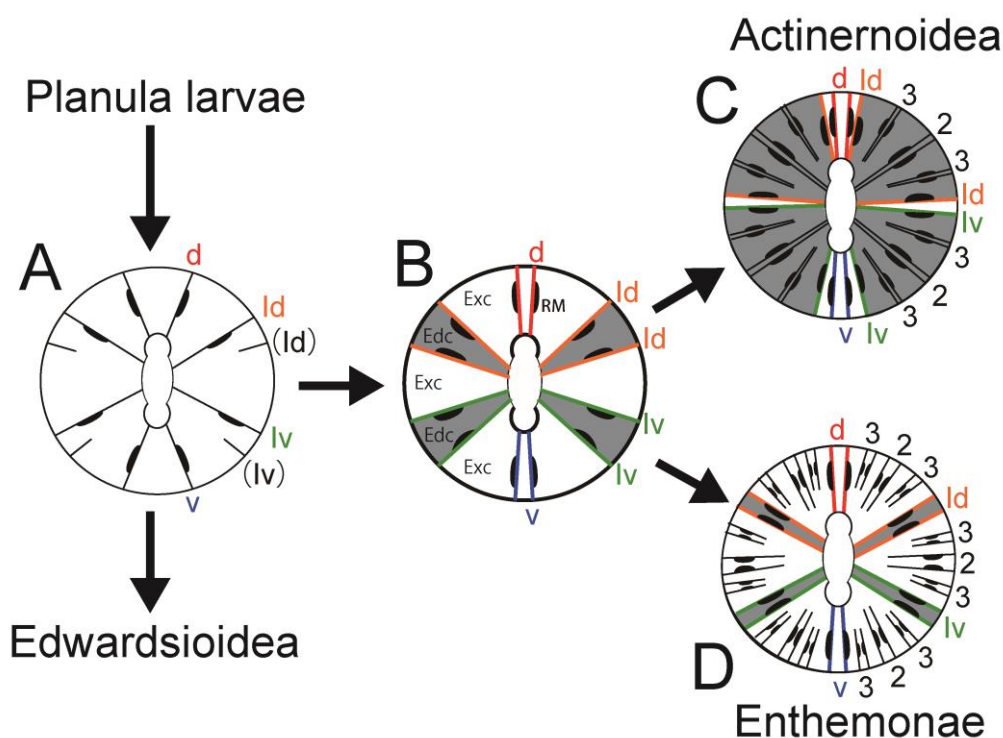


Figure 1. Schematic diagram of the development of mesenterial arrangement. All diagrams show the mesenterial arrangements in transversal sections. Arrows show the order of development. (A) Stage with eight macrocnemes in the first mesenterial cycle. Species of Edwardsiidae have this arrangement even in adults; (B) stage with twelve macrocnemes in six pairs. All macrocnemes of this stage are in the first mesenterial cycle; (C,D) arrangements at more developed stages. (C) Arrangement of Actinernoidea. (D) Arrangement of general Enthemonae. Abbreviations: d, dorsal directive (indicated in red); Edc, endocoel (in gray); Exc, exocoel (in white); ld, dorso-lateral mesentery (in orange); lv, ventro-lateral mesentery (in green); RM, retractor muscle; v, ventral directive (in blue); 2, mesenteries in the second cycle; 3, mesenteries in the third cycle. Revised from Yanagi (2017) [2].

In this study, we mainly focused on the superfamily Actinernoidea. This superfamily is characterized by a peculiar mesenterial arrangement: mesenteries of the second and younger mesenterial cycles originate in the endocoels in the first cycle (Figure 1C). In contrast, in all other anemones (except anemones in which mesenteries in the first cycle are less than twelve, such as Edwardsiidae Andres, 1881, or Halcampulactinidae, Gusmão et al., 2019; Figure 1B), these mesenteries develop in exocoels (Figure 1D) [2,5,6]. In addition, some species are characterized by the presence of nematocyst batteries and spirocysts on their column. At present, Actinernoidea includes two families: Actinernidae Stephenson, 1922, and Halcuriidae Carlgren, 1918 [7,8]. They are distinguished by the number of siphonoglyphs attached to the actinopharynx (two siphonoglyphs for Actinernidae and only one for Halcuriidae) and the number of macrocnemes (twenty for Halcuriidae and more than twenty for Actinernidae).

Originally, in the classification by Carlgren [4,9], the first comprehensive classification system of the order Actiniaria, Actinernidae and Halcuriidae were accommodated in the independent suborder Endocoelanthae Carlgren, 1925, because of the peculiar mesenterial arrangements. This classification was used in subsequent studies [10–12], until recently. However, Rodríguez et al. (2014) [13], in the most comprehensive analysis of Actiniaria so far, included all higher taxa in the classification system of Carlgren and revealed that Endocoelanthae was closely related to the family Edwardsiidae of the suborder Anenthemonae on the basis of a molecular phylogeny reconstruction. The conclusion was that Endocoelanthae was a part of the suborder Anenthemonae Rodríguez and Daly, 2014. Thus, Rodríguez et al. maintained the lower taxon of Anenthemonae, accommodating Actinernidae and Halcuriidae, by reviving the superfamily Actinernoidea Stephenson, 1922 [13]. At present, both families are treated as members of the superfamily Actinernoidea of the suborder Anenthemonae. However, this classification system also has some taxonomic problems. The taxon sampling in Rodríguez et al. (2014) was still not complete. Their phylogenetic analyses contained few species of Actinernoidea: only three actinerniid species in three genera and only one halcuriid species. After that, Izumi et al. [14] added two more species to the phylogenetic tree, but there was still a lack of genetic information that would be needed to conduct a comprehensive revision of Actinernoidea. Thus, phylogenetic studies on this superfamily have not been sufficient yet. Hence, comprehensive taxon sampling of each lower taxa is required to obtain a better phylogeny reconstruction of Anenthemonae.

Actinernoidea has a relatively rich diversity in Japan: six species of four genera of Actinernidae and three species of *Halcurias* McMurrich, 1893, of Halcuriidae (Table 1) [11,12,14,15]. However, two of the three species of *Halcurias* newly described in Uchida (2004) [11] are regarded as invalid nomina nuda indicated by * in Table 1) [1,2], and additionally, there have been several anemones of Actinernoidea considered to be undescribed species collected from the oceans around Japan.

Table 1. Nominal genera and species of Actinernidae in the world and distribution records in Japan. Species with * indicates that the name is a nomen nudum.

Family	Species	Distribution
Actinernidae		
	<i>Actinernus antarcticus</i> (Carlgren, 1914)	Antarctica
	<i>Actinernus elongatus</i> (Hertwig, 1882)	Antarctica, Chile, New Zealand, Argentina
	<i>Actinernus mercedae</i> Gusmão, (López-González & Rodríguez, 2021)	South Atlantic
	<i>Actinernus michaelsarsi</i> Carlgren, 1918	North Atlantic (Spanish Waters)
	<i>Actinernus nobilis</i> Verrill, 1879	Northwest Atlantic
	<i>Actinernus robustus</i> (Hertwig, 1882)	Northwest Pacific (Japanese Waters)
	<i>Isactinernus quadrilobatus</i> Carlgren, 1918	West Pacific (including Japanese Waters)
	<i>Synactinernus churaumi</i> Izumi & Fujii in [14]	Northwest Pacific (Japanese Waters)
	<i>Synactinernus flavus</i> Carlgren, 1918	Northwest Pacific (Japanese Waters)
	<i>Synhalcurias elegans</i> (Wassilieff, 1908)	Northwest Pacific (Japanese Waters)
	<i>Synhalcurias kahakui</i> Izumi & Yanagi, 2021	Northwest Pacific (Japanese Waters)
Halcuriidae		
	<i>Halcurias carlgreni</i> McMurrich, 1901	Northwest Pacific (Japanese Waters)
	<i>Halcurias capensis</i> Carlgren, 1928	South Africa
	<i>Halcurias endocoelactis</i> Stephenson, 1918	West Pacific (New Zealand)
	<i>Halcurias japonicus</i> Uchida, 2004	Northwest Pacific (Japanese Waters) *
	<i>Halcurias levis</i> Uchida, 2004	Northwest Pacific (Japanese Waters) *
	<i>Halcurias minimus</i> Carlgren, 1928	South Indian Ocean (Kercuelen Islands)
	<i>Halcurias pilatus</i> McMurrich, 1893	Northwest Atlantic

<i>Halcurias sudanensis</i> Riemann-Zürneck, 1983	The Red Sea
<i>Halcurias uchidai</i> Rodríguez & Lauretta, 2023	Northwest Atlantic
<i>Carlgrenia desiderata</i> Stephenson, 1918	Northeast Atlantic

In this study, we conducted comprehensive sampling of Actinernoidea anemones from Japan. We observed the detailed morphology of the collected specimens, specifically identified them, and provided detailed descriptions. In addition, we established new systematics of Actinernoidea by using molecular phylogeny with sufficient taxon sampling and sufficient DNA markers. These analyses resulted in a comprehensive revision of superfamily Actinernoidea: the new family Isactinernidae fam. nov. and the new genus *Isohalcurias* gen. nov. are established; four new species, *Halcurias hiroomii* sp. nov., *Halcurias fragum* sp. nov., *Isohalcurias citreum* sp. nov., and *Isohalcurias malum* sp. nov. are formally described. Concerning existing taxa, genus *Synactinernus* Carlgren, 1918, is moved from Actinernidae to Halcuriidae and genera *Isactinernus* Carlgren, 1918, and *Synhalcurias* Carlgren, 1914, have been moved to Isactinernidae fam. nov.; some species of *Halcurias* are moved to *Isohalcurias* gen. nov.; and the nomina nuda of two species of *Halcurias* are completely resolved by their redescription as new species. From the present study, it is confirmed that there are eleven species in six genera of three families of Actinernoidea in the waters around Japan.

2. Materials and Methods

2.1. Sample Collection and Preservation

We collected all specimens from Japanese waters. All sampling localities are shown in Figure 2. The specimens in this study were obtained by scuba diving, dredging, or trawling by research vessels (R/V), fishing boats, or remotely operated vehicles (ROV). Some specimens attached to substrates were usually detached from substrates using fingers or spatulas, but occasionally collected with pieces of substrates. The collected specimens were preserved using several methods: sufficiently healthy specimens were kept alive in calm water in cases, aquaria, or tanks until they elongated their tentacles. Images in vitro were captured to record their external forms, colors, and sizes in vivo. Subsequently, they were anesthetized using a magnesium chloride solution. After they were completely anesthetized, small pieces of tissue for DNA analyses were dissected from their tentacles and preserved in 99% ethanol. Remaining polyps were fixed in 10–20% (*v/v*) formalin sea water solution for morphological analyses. Occasionally, specimens were directly preserved in ethanol or formalin solution if they were weakened, damaged, or almost dead. Polyps fixed in formalin solution were immersed into 70% ethanol after they were completely preserved. For DNA analyses, we also obtained tissues of tentacles from some living polyps from Okinawa Churaumi Aquarium (Motobu, Okinawa) and preserved them in 99% ethanol. Specimens have been deposited at the National Museum of Nature and Science (NSMT), Tokyo, and the Coastal Branch of Natural History Museum and Institute (CMNH), Chiba. In addition, some type specimens were observed in the museums in which they had been deposited: Museum of Evolution Zoology, Uppsala University (UUZM), The Swedish Museum of Natural History (SMNH), Biological Museum, Lund University (MZLU), Natural History Museum of Denmark (ZMUC), and the Natural History Museum, London (BM). The type specimens were photographed, and their external and gross internal morphological characters were examined.

2.2. Morphological Observation and Preparation of Histological Sections

Morphological observations were performed on external and internal characters. Preserved polyps were dissected into pieces to obtain some tissues for histological sections after external morphological characters were observed. The dissected tissues were then dehydrated by 80–100% ethanol, 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 (HE). The method of HE staining was based on Presnell and Schreibman (1997) [16], but the staining or washing times were slightly altered for sea anemones: the times were suitably extended for the samples in which the mesoglea was thick. Sections were mounted on slide glasses and enclosed by slide glasses. Finally, anatomies of several internal features were observed using conventional microscopes. In this study, we refer to the terminology by Carlgren (1949), Daly (2015), and Yanagi (2017) for the morphology of sea anemones [2,4,17].

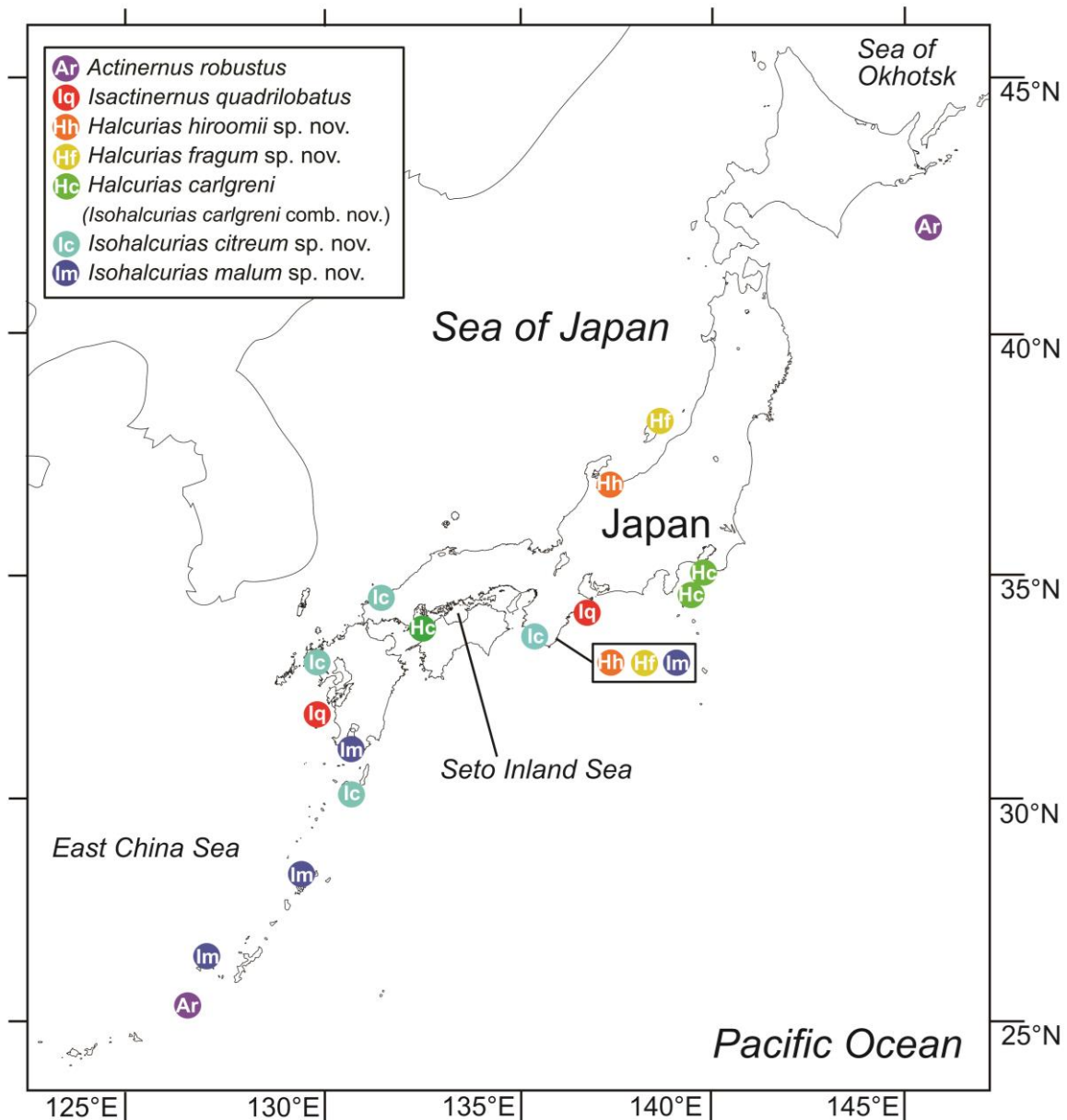


Figure 2. The localities of actinernoidean anemone specimens examined in this study.

2.3. Observation of Cnidae

The cnidae of the tentacle, actinopharynx, column, and mesenterial filament were observed. Tissues from every part were placed on glass slides and mounted using 50% (*v/v*) glycerin–sea water. Images of the cnidae were obtained using a differential interference contrast microscope, generally following the method of Yanagi et al. (2015) [18]. The lengths and widths of the cnidae were measured using the software ImageJ ver. 1.49 (Rasband,

1997–2012) [19]. We followed Mariscal (1974) [20] for the nomenclature of the cnidom. We evaluated the number of cnidae as follows: <5: rare; 5≤ and <10: few; and 10≤: numerous.

2.4. PCR and DNA Sequences

DNA was extracted from each tissue that was preserved in 99% ethanol by using a Charge Switch gDNA Micro Tissue Kit (Invitrogen). In addition, some tissue samples for DNA were processed following the guanidine extraction protocol (Sinniger et al., 2010) [21]. PCR amplifications were performed in 10 µL reaction volume, consisting of 0.4 µL of 25 µM forward and reverse primers, 5.0 µL of Emerald Amp PCR Master Mix (TaKaRa), and 3.4 µL of distilled water. For PCR amplifications, three mitochondrial markers—12S, 16S rDNA—three nuclear markers—18S and 28S rDNA—and internal transcribed spacers (ITS) 1 and 2 with 5.8S rDNA were used. The primers and amplification conditions are shown in Table 2. Amplifications were performed using five traditional molecular markers of Actiniaria: 12S, 16S, 18S, and 28S rDNA, adopted in Rodríguez et al. (2014) [13], following the methods of preceding phylogenetic studies on sea anemones (Apakupakul et al., 1999; Geller and Walton, 2001; Medina et al., 2001; Meldin et al., 1988; Sinniger et al., 2005) [22–26]. We used another set of primers for 28S rDNA during some amplifications (Littlewood et al., 2000; Williams et al., 2003; Williams and Ozawa, 2006) [27–29] if the primers of Medina et al. (2001) [24] did not work effectively. We newly adopted the ITS1/ITS2 marker with 5.8S rDNA and used the method of amplification from preceding studies for another phylum (Heath et al., 1995) [30]. The PCR products were processed using exonuclease I and shrimp alkaline phosphate (ExoSAP-IT; Thermo Fisher) before sequencing. The sequencing reaction was performed using BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems) and using just PCR primers (12S, 16S, COXIII, and ITS1/2) or both PCR primers and internal primers (18S and 28S; Table 2). We used four internal primers (two forward and two reverse) for 18S (Apakupakul et al., 1999) [22] and three primers (two forward and one reverse) for 28S (Williams and Ozawa, 2005) [29]. Sequencing was performed using an ABI 3500xL Genetic Analyzer (Applied Biosystems). The sequence of each marker was individually assembled using GeneStudio ver. 2.2.0.0 (<http://genestudio.com>; accessed on 7 January 2021).

Table 2. Primers and protocols of polymerase chain reactions of every molecular marker.

Marker	Primer	Sequences (5'-3')	PCR protocol	Reference	
12S	12S1a	TAAGTGCCAGCAGACGCGGT	(95 °C for 4 min) + 4 × [(94°C for 30 s) → (50°C for 1 min) → (72°C for 2 min)] + 30 × [(94°C for 30 s) → (55°C for 1 min) → (72°C for 2 min)] + (72°C for 4 min)	[26]	
	12S3r	ACGGGCAATTTGTACTAACA			
16S	ANEM16SA	CACTGACCGTGATAATGTAGCGT	(95°C for 4 min) + 30 × [(95°C for 30 s) → (46°C for 45 s) → (72°C for 1 min)] + (72°C for 5 min)	[23]	
	ANEM16SB	CCCCATGGTAGCTTTTATTCG			
	16Sant1a 16SbmoH	GCCATGAGTATAGACGCACA CGAACAGCCAACCCCTGG	(95°C for 4 min) + 30 × [(95°C for 30 s) → (46°C for 45 s) → (72°C for 1 min)] + (72°C for 5 min)	[26]	
18S	PCR	18SA 18SB	AACCTGGTGTATCCTGCCAGT TGATCCTTCCGCAGGTTACCT	(94°C for 4 min) + 35 × [(94°C for 20 s) → (57°C for 20 s) → (72°C for 1 min 45 s)] + (72°C for 7 min)	[22,25]
	Only Sequence	18SL	CCAACACTACGAGCTTTTAACTG		
		18SC	CGGTAATTCAGCTCCAATAG		
		18SY	CAGACAAATCGCTCCACCAAC		
		18SO	AAGGGCACCACCAGGAGTGGAG		
PCR1	F63sq R2077sq	AATAAGCGGAGGAAAAGAAAC GAGCCAATCCTTWTCCCGARGTT	(94°C for 5 min) + 30 × [(94°C for 30 s) → (45°C for 1 min) → (72°C for 3 min)] + (72°C for 10 min)	[24]	
	PCR2	LSU5 LSU1600R	TAGGTCGACCCGCTGAAYTTAAGCA AGCGCCATCCATTTTCAGG	(94°C for 5 min) + 30 × [(94°C for 30 s) → (45°C for 1 min) → (72°C for 3 min)] + (72°C for 10 min)	[27–29]
Only Sequence	LSU330F	CAAGTACCGTGAGGGAAAGTTG			
	LSU900F	CCGTCTTGAAACACGGACCAAG			
	ECD2S	CTTGGTCCGTGTTTCAAGACGG			
ITS1/2	ITSF ITSR	GTTTCCGTAGGTGAACCTG CTCGTCTGATCTGAGGTCG	(94°C for 5 min) + 30 × [(94°C for 35 s) → (55°C for 45 s) → (72°C for 2 min)] + (72°C for 1 min)	[30]	

2.5. Phylogenetic Analyses

The base sequences used in phylogenetic analyses are shown in Table 3. The sequence data of five species were obtained from GenBank (registration numbers are shown in Table 3). Each dataset was aligned using MAFFT ver. 7.402 (Katoh and Standley, 2013) [31] under the default settings. Ambiguously aligned areas were eliminated using Gblocks ver. 0.91b (Castresana, 2002) [32] with the type of DNA sequence and using default parameters, except allowing small final blocks and gap positions within the final blocks. The obtained data were processed using Kakusan 4 (Tanabe, 2011) [33] to concatenate every alignment to one file and select substitution models for the RAxML and MrBayes analyses (models are shown in Table 4). In the combined dataset, substitution parameters were estimated separately for each gene partition. The maximum likelihood (ML) analysis was performed using RAxML-VI-HPC (Stamatakis, 2006) [34], with substitution models recommended by Kakusan 4 and evaluated using 100 bootstrap replicates. Bayesian inference (BI) was conducted using MrBayes ver. 3.2.6 (Ronquist and Huelsenbeck, 2003) [35] with substitution models recommended by Kakusan 4. Two independent runs of the Markov Chain Monte Carlo were performed simultaneously for 5,000,000 generations; trees were sampled every 100 generations, and the average standard deviations of split frequencies (ASDSF) every 100,000 generations were calculated. As the ASDSF was calculated on the basis of the last 75% of the samples, the initial 25% of the sampled trees were discarded as burn-in. Constructed ML and Bayesian trees were rooted and combined using FigTree ver. 1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>; accessed on 7 January 2021).

Table 3. Base sequences in the phylogenetic analyses. Sequences indicated in bold were deposited to GenBank in the present study. The names of OTUs are based on the previous classification of Actinernoidea. *Edwardsia timida* was chosen as the outgroup of phylogenetic analysis of Actinernoidea. OTUs indicated in gray are obtained from GenBank. Note that the names of OTUs are based on the previous classification of Actinernoidea: *Halcurias japonicus* is described in this article as *Halcurias hiroomii* sp. nov., *Halcurias levis* as *Isohalcurias citreum* sp. nov., *Halcurias* sp. 1 as *Halcurias fragrum* sp. nov., *Halcurias* sp. 2 as *Isohalcurias malum* sp. nov., and *Halcurias carlgreni* is renamed to *Isohalcurias carlgreni* comb. nov.

Higher Taxon	Family	Genus	Species	Localities	Voucher Numbers	12S	16S	18S	28S	5.8S/ITS1/ITS2
Actiniaria										
Anenthemonae										
Actinernoidea	Actinerni- dae	<i>Actinernus</i>	<i>robustus</i>	Ryukyu-Trough	-	LC768522	-	LC484632	LC768597	-
		<i>Isactinernus</i>	<i>quadrilobatus</i>	Kumano-nada	NSMT-Co 1662	-	LC484643	LC484638	LC768598	LC768624
		<i>Synhalcurias</i>	<i>elegans</i>	Kumano-nada	NSMT-Co 1693	-	-	LC768573	LC768599	LC768625
		<i>Synhalcurias</i>	<i>elegans</i>	Okinawa	-	LC768523	LC768547	LC768574	LC768600	LC768626
		<i>Synhalcurias</i>	<i>kahakui</i>	Ogasawara	NSMT-Co 1695	LC768524	LC768548	-	LC768601	LC768627
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	NSMT-Co 1660	LC768525	LC484639	LC484633	LC768602	LC768628
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	-	LC768526	LC484640	LC484634	LC768603	LC768629
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	-	LC768527	LC768549	LC768575	LC768604	-
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	-	LC768528	LC768550	LC768576	-	-
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	-	LC768529	LC768551	LC768577	-	-
		<i>Synactinernus</i>	<i>flavus</i>	Goto-nada	-	LC768530	LC768552	LC768578	-	-
		<i>Synactinernus</i>	<i>flavus</i>	Wakayama	CMNH-ZG 09732	LC768531	LC768553	LC484635	LC768605	-
		<i>Synactinernus</i>	<i>churaumi</i>	Off Ishigaki Island	NSMT-Co 1661	LC768532	LC484641	LC484636	LC768606	LC768630
		<i>Synactinernus</i>	<i>churaumi</i>	Off Ishigaki Island	-	LC768533	LC484642	LC484637	LC768607	LC768631
		<i>Synactinernus</i>	<i>churaumi</i>	Off Ishigaki Island	-	-	LC768554	LC768579	-	-
		<i>Synactinernus</i>	<i>churaumi</i>	Off Ishigaki Island	-	LC768534	LC768555	LC768580	-	-
Actinerni- dae	<i>Actinernus</i>	<i>antarcticus</i>	-	-	-	KJ482930	KJ482966	KJ483023	KJ483126	-
	<i>Isactinernus</i>	<i>quadrilobatus</i>	-	-	-	KJ482932	KJ482968	KJ483024	KJ483105	-
	<i>Synhalcurias</i>	<i>elegans</i>	-	-	-	KJ482942	-	KJ483021	KJ483120	-
Halcuriidae	<i>Halcurias</i>	<i>japonicus</i>	Toyama-Bay	CMNH-ZG 10212	-	-	LC768556	LC768581	LC768608	LC768632
	<i>Halcurias</i>	<i>japonicus</i>	Toyama-Bay	NSMT-Co 1824	-	-	LC768557	LC768582	LC768609	LC768633

	<i>Halcurias</i>	<i>japonicus</i>	Toyama-Bay	CMNH-ZG 10213	-	LC768558	LC768583	LC768610	LC768634
	<i>Halcurias</i>	<i>japonicus</i>	Ise-Bay	CMNH-ZG 10214	-	LC768559	LC768584	LC768611	LC768635
	<i>Halcurias</i>	<i>japonicus</i>	Wakayama	CMNH-ZG 10215	LC768535	LC768560	LC768585	LC768612	LC768636
	<i>Halcurias</i>	sp. 1	Sado Island	-	LC768536	LC768561	LC768586	LC768613	LC768637
	<i>Halcurias</i>	sp. 1	Sado Island	CMNH-ZG 10216	LC768537	LC768562	LC768587	LC768614	-
	<i>Halcurias</i>	sp. 1	Wakayama	CMNH-ZG 10217	LC768538	LC768563	LC768588	LC768615	LC768638
	<i>Halcurias</i>	<i>carlgreni</i>	Suo-Oshima	NSMT-Co 1697	LC768539	LC768564	LC768589	LC768616	LC768639
	<i>Halcurias</i>	<i>carlgreni</i>	Suo-Oshima	NSMT-Co 1698	LC768540	LC768565	LC768590	LC768617	LC768640
	<i>Halcurias</i>	<i>carlgreni</i>	Kumano-nada	CMNH-ZG 10218	LC768541	LC768566	LC768591	LC768618	-
	<i>Halcurias</i>	<i>carlgreni</i>	Ukishima Island	CMNH-ZG 10219	-	LC768567	LC768592	LC768619	LC768641
	<i>Halcurias</i>	<i>carlgreni</i>	Ukishima Island	CMNH-ZG 10220	-	LC768568	-	-	-
	<i>Halcurias</i>	<i>levis</i>	Kuju-kushima	CMNH-ZG 10221	LC768542	LC768569	LC768593	LC768620	LC768642
	<i>Halcurias</i>	<i>levis</i>	Wakayama	-	LC768543	LC768570	LC768594	-	LC768643
	<i>Halcurias</i>	<i>levis</i>	Off Yaku Island	CMNH-ZG 10222	LC768544	-	LC768595	LC768621	LC768644
	<i>Halcurias</i>	sp. 2	Off Cape-Sata	NSMT-Co 1699	LC768545	LC768571	-	LC768622	LC768645
	<i>Halcurias</i>	sp. 2	Wakayama	CMNH-ZG 10225	LC768546	LC768572	LC768596	LC768623	LC768646
	Halcuriidae	<i>Halcurias</i>	<i>pilatus</i>	-	KJ482931	KJ482967	KJ483020	KJ483109	-
Edwardsioidea	Edwardsiidae	<i>Edwardsia</i>	<i>timida</i>	-	GU473281	-	GU473315	KJ483088	KT852241

Table 4. The substitution models of phylogenetic analyses on each marker.

	Mitochondrial			Nuclear	
	12S rDNA	16S rDNA	18S rDNA	28S rDNA	5.8S rDNA/ITS1/ITS2
ML analysis	GTR + Gamma	GTR + Gamma	GTR + Gamma	GTR + Gamma	GTR + Gamma
Bayesian inference	HKY85 + Gamma	K80 + Gamma	K80 + Gamma	GTR + Gamma	K80 + Gamma

3. Results and Discussions

3.1. Phylogeny

We analyzed the phylogeny of Actinernoidea using 34 specimens of 11 species of all genera except *Carlgrenia* Stephenson, 1918 [36], including both families, Actinernidae and Halcuriidae [4,14]. Two large clades were formed in the phylogenetic tree (Figure 3). One clade included three genera, *Actinernus* Verrill, 1879, *Isactinernus*, and *Synhalcurias* of Actinernidae, and the other included *Synactinernus* of Actinernidae and *Halcurias* of Halcuriidae. Actinernidae (the lineages indicated in pink in Figure 3) thus became polyphyletic, and Halcuriidae (in orange in Figure 3) was paraphyletic with regards to the genus *Synactinernus*. The monophyly of every node (Figure 4A–D) was supported with bootstrap values of 88–100% and posterior probability of 1. We also tried the analysis independently using mitochondrial and nuclear markers: in the results, the topology was strongly supported with nuclear markers (with bootstrap values/posterior probabilities of 98%/1 on node A, 100%/1 on node B, 87%/0.95 on node C, and 100%/1 on node D; Supplementary Figures S1 and S2) but not supported well with mitochondrial markers (node A was not supported, and bootstrap values/posterior probabilities were 98%/1 on node B, 51%/0.95 on node C, and 66%/0.99 on node D; Supplementary Figures S3 and S4). The support rates were comparatively low despite the topologies being not so different from each other, so we decided to analyze using whole markers.

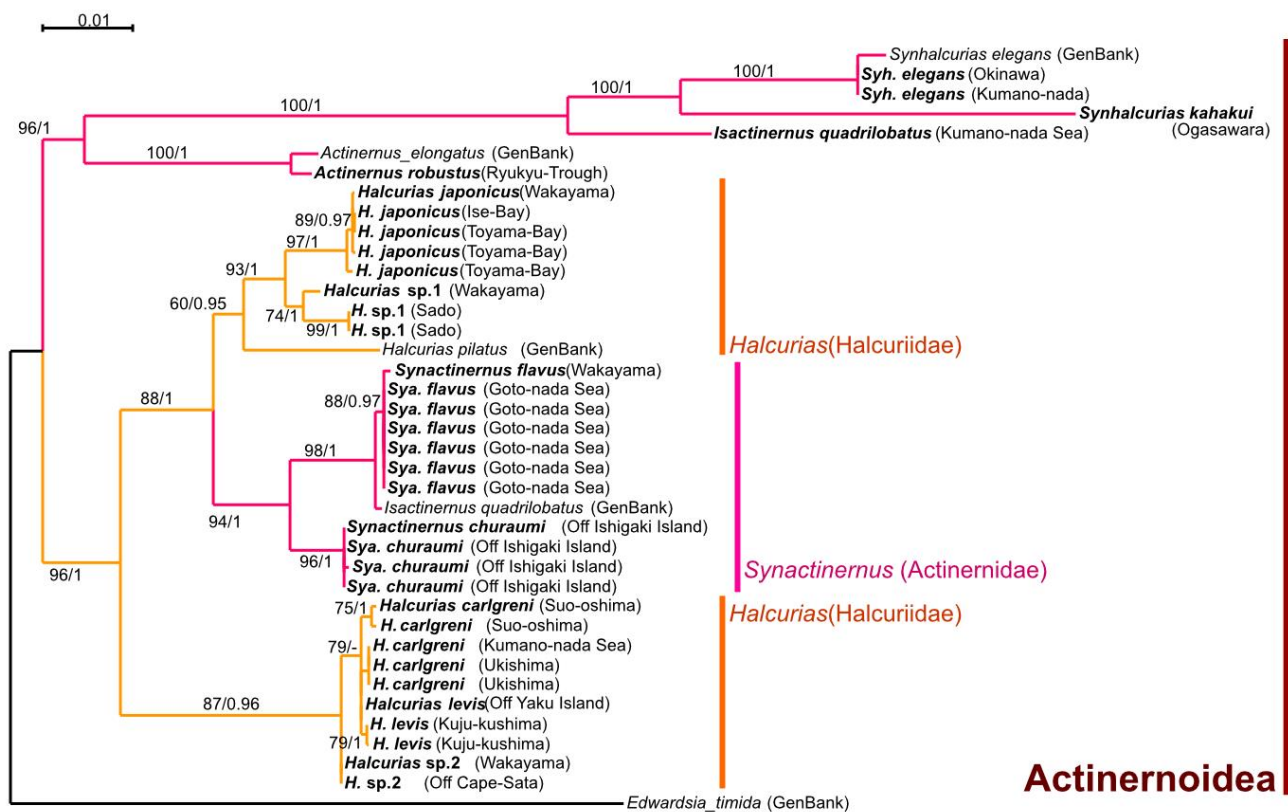


Figure 3. Maximum likelihood tree of the superfamily Actinernoidea based on the combined dataset of mitochondrial 12S and 16S; nuclear 18S, 28S, and 5.8S rDNA; and ITS1/ITS2 (total 4614 bp). The lineages of the species that had been classified in the family Actinernidae are colored in pink, and the lineages of species of the family Halcuriidae are in orange. The bar in pink indicates the genus *Synactinernus* (Actinernidae), and two orange bars indicate the genus *Halcurias* (Halcuriidae). The numbers above or below branches indicate ML bootstrap support values, followed by BI posterior probabilities of the nodes (bootstrap values of $\geq 50\%$ and posterior probabilities of ≥ 0.9 are shown). Note that the names of OTUs are based on the previous classification of Actinernoidea; compare with Figure 4 for the new species names.

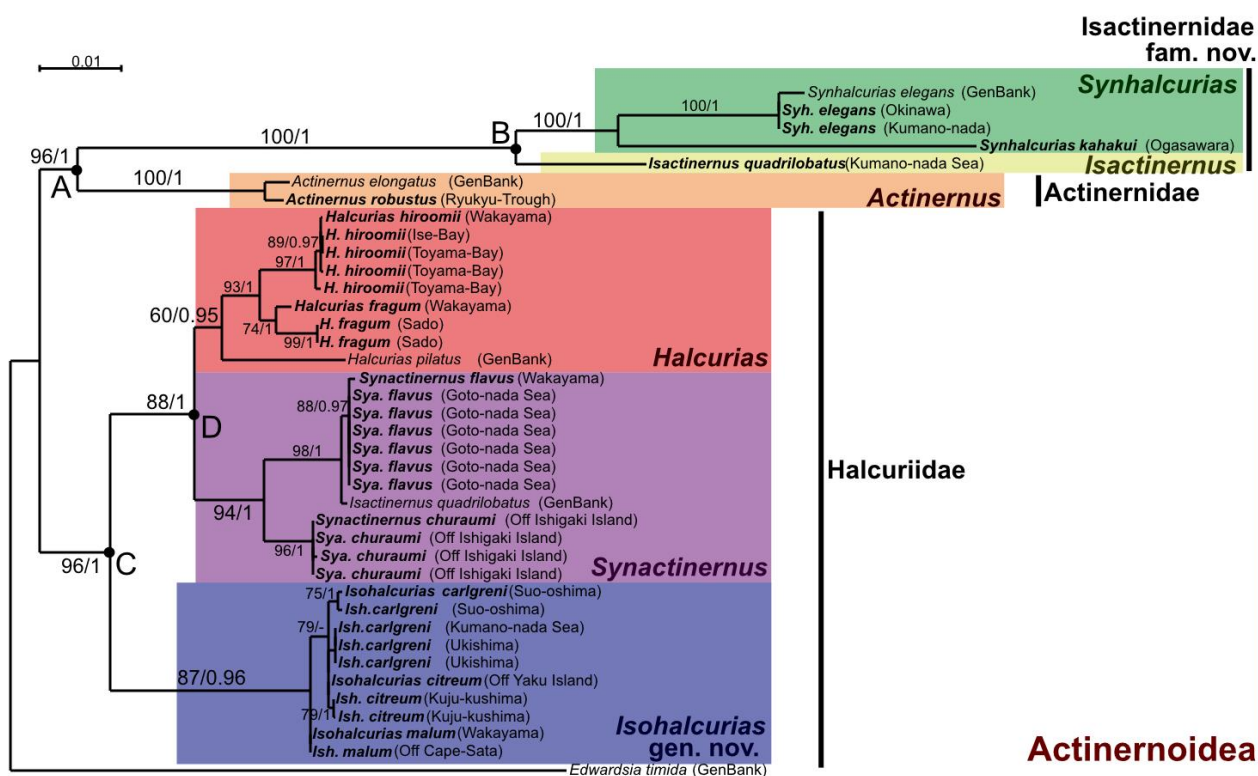


Figure 4. Maximum likelihood tree of the superfamily Actinernoidea based on the combined dataset of mitochondrial 12S and 16S; nuclear 18S, 28S, and 5.8S rDNA; and ITS1/ITS2 (total 4614 bp) following the new actinernoidean classification system. All clades of a genus rearranged in the present study are indicated in boxes in different colors, and those of families are indicated by bars. The numbers above or below branches indicate ML bootstrap support values, followed by BI posterior probabilities of the nodes (bootstrap values of $\geq 50\%$ and posterior probabilities of ≥ 0.9 are shown). Node in A indicates the base of the clade of families Actinernidae and Isactinernidae; node B indicates that of the clade of family Isactinernidae; node C indicates that of the clade of family Halcuriidae; and node D indicates that of genera *Halcurias* and *Synactinernus* in Halcuriidae.

Remarks. The two families Actinernidae and Halcuriidae of the superfamily Actinernoidea have peculiar mesenterial arrangements. Only these two families develop their mesenteries of the second mesenterial cycle in the endocoels of the mesenteries of the first cycle (Figure 1C). Conversely, almost all species of actinarians except for these two families develop mesenteries in the exocoels, out of the mesenterial pairs of the first cycle (Figure 1D). Therefore, the classification system in Carlgren (1949) accommodated Actinernidae and Halcuriidae in the independent suborder Endocoelanthae and distinguished them from the other families (Table 1 in [14]). Carlgren (1949) [4] stated that these two families could be distinguished from each other by the number of cycles of mesenteries and the number of siphonoglyphs (see introduction). This classification system of these families has been accepted up until the present study [10–12,14]. This is mainly because Actinernoidea anemones are scarcely collected owing to their rarity. Even Rodríguez et al. [13], who conducted the most comprehensive phylogenetic analysis of Actinernoidea to date, could obtain only four species of this suborder; hence, they only changed the taxonomic rank of the suborder to superfamily and did not discuss the inner classifications of these two families.

In contrast, numerous specimens representing eleven species covering five out of six genera of Actinernoidea were analyzed in the molecular analyses in this study. Thus, we demonstrate that Actinernidae as previously defined is polyphyletic and Halcuriidae as previously defined is paraphyletic (Figure 3). Thus, based on our phylogeny, we comprehensively revise Actinernoidea (Figure 4): the genus *Synactinernus* (indicated in the purple

box in Figure 4) is transferred from Actinernidae to Halcuriidae; *Isactinernus* and *Synhalcurias* are accommodated into the newly established family Isactinernidae fam. nov. (indicated in the upper black bar in Figure 4) as there were few common features between *Actinernus* (in the orange box) and *Isactinernus/Synhalcurias* (in the green and yellow boxes; see remarks of family Isactinernidae fam. nov.); and some species of *Halcurias* are moved to the new genus *Isohalcurias* gen. nov. (in the blue box) to solve the paraphyly of the genus *Halcurias* (in the red box). This study further demonstrates that Japan has an extraordinarily rich fauna of the superfamily Actinernoidea: six out of seven genera and over half of the species of the world are known from this region

3.2. Descriptions

Order ACTINIARIA Hertwig, 1882.

Suborder ANENTHEMONAE Rodríguez and Daly, 2014.

Superfamily ACTINERNOIDEA Stephenson, 1922.

(Japanese name: yatsuba-kawari-ginchaku-jouka [37].)

Diagnosis. Anenthemonae with a well-developed **basal disc** but without basilar muscles. Column smooth, or with nematocyst batteries, nearly always with spirocysts. Margin tentaculate. Sphincter absent or weak mesogleal. Tentacles in variable number, with **or without** thickening aboral side, either in two alternating cycles or, although usually arranged in cycles, in a peculiar way related to the development of mesenteries. Longitudinal muscles of tentacles and radial muscles of oral disc ectodermal, with a slight mesogleal tendency. Oral disc sometimes lobed. One or two siphonoglyphs. Usually more mesenteries than directives attached to siphonoglyphs. Unique mesenterial arrangement: after the first twelve mesenteries (six couples) are developed, all subsequent pairs appear in lateral endocoels with longitudinal muscles oriented as in directives. Cnidom: spirocysts, basitrichs, holotrichs, and microbasic *p*-mastigophores. (Revised points from Rodríguez et al. (2014) [13] are indicated in **bold**.)

Remarks. Actinernidae Verrill, 1879, and Halcuriidae Carlgren, 1918, have peculiar mesenterial arrangement [7,38]. Despite that almost all sea anemones develop their second cycle of mesenteries out of first cycle of mesenterial pairs (at exocoels), only species belonging to these two families develop them in the first cycle pairs (at endocoels). So, in the classification system of Carlgren [4], they were accommodated in the suborder Endocoelanthae Carlgren, 1925 [9]. However, the phylogenetic research of Rodríguez et al. (2014) revealed that species of Endocoelanthae were related to Edwardsiidae, and thus, they established a new suborder, Anenthemonae Rodríguez and Daly, 2014, for Actinernidae, Halcuriidae, and Edwardsiidae [13]. Thus, Endocoelanthae disappeared, and they used the superfamily Actinernoidea Stephenson, 1922, instead to accommodate those two families.

There are two families and six genera of actinernoideans confirmed from Japan. According to our phylogenetic and morphological analyses (Figures 3 and 4), the monophyly was strongly supported as a superfamily, and the new family Isactinernidae and a new genus *Isohalcurias* were established. See Table 5 for comparison of Isactinernidae fam. nov. and the other families of Actinernoidea. The new taxonomic key of this superfamily to each genus, including *Isohalcurias* gen. nov., is as below.

Taxonomic key of the suborder Actinernoidea

- | | |
|-----------------------------------------------------------|-------------------------------------|
| 1A. Mesenteries distinguished macrocnemes and microcnemes | See 2. |
| 2A. Base of tentacles thickening in aboral side | <i>Actinernus</i> Verrill, 1879 |
| 2B. Tentacle simple, no thickening | See 3. |
| 3A. Edge of oral disc developed into lobes | <i>Synactinernus</i> Carlgren, 1918 |
| 3B. Edge of oral disc simple, not lobed | See 4. |
| 4A. 12 macrocnemes in column | <i>Carlgrenia</i> Stephenson, 1918 |
| 4B. 20 macrocnemes in column | See 5. |

- 5A. Spirocysts absent on column. Muscular processes simple and unified *Halcurias* McMurrich, 1893
 5B. Spirocysts present on column. Part of retractor muscles clustered *Isohalcurias* gen. nov.
 1B. All mesenteries perfect and macrocnemes See 6.
 6A. Edge of oral disc developing into lobes *Isactinernus* Carlgren, 1918
 6B. Edge of oral disc simple, not lobed *Synhalcurias* Carlgren, 1914

Family ACTINERNIDAE Stephenson, 1922.

(Japanese name: yatsuba-kawari-ginchaku-ka [39].)

Actinernidae Stephenson, 1922: 258; Carlgren, 1949: 20.

Diagnosis. Actinernoidea with an elongated body. Distal margin of column usually expanded and drawn into lobes but sometimes not expanded nor lobed. Column with or without nematocyst batteries. Tentacles numerous, with basal **thickening** on their aboral side. With two siphonoglyphs. Mesenteries numerous, divisible into macro- and microcnemes, **and develop bilaterally**. Retractors rather weak. All stronger mesenteries fertile. **Cnidom: basitrichs, spirocysts, and microbasic p-mastigophores.**

(The revised points from Uchida (2007) [12] are indicated in **bold**.)

Type genus. *Actinernus* Verrill, 1879.

Remarks. This family until now contained four genera: *Actinernus* Verrill, 1879, *Synhalcurias* Carlgren, 1914, *Isactinernus* Carlgren, 1918, and *Synactinernus* Carlgren, 1918, and have been characterized by having two siphonoglyphs on their actinopharynx and lacking the deviation between macrocnemes and microcnemes [7,39,40]. However, though there was apparent deviation between macrocnemes and microcnemes only in *Actinernus* (see Uchida, 2007 [12]; Figure 5) and *Synactinernus* (see Figure 4 in Izumi et al. (2019) [14]), no apparent deviation was observed in *Isactinernus* (see Figure 8) and *Synhalcurias* (see Figure 3 in Izumi and Yanagi (2021) [15]), and it is doubtful that these four genera should be accommodated in same family.

Table 5. Comparison of Isactinernidae fam. nov. and the other two families in superfamily Actinernoidea.

	Isactinernidae fam. nov.	Actinernidae Stephenson, 1922	Halcuriidae Carlgren, 1918
Type genus	<i>Isactinernus</i> Carlgren, 1918	<i>Actinernus</i> Verrill, 1879	<i>Halcurias</i> McMurrich, 1893 <i>Synactinernus</i> Carlgren, 1918
The other genera	<i>Synhalcurias</i> Carlgren, 1914	none	<i>Carlgrenia</i> Stephenson, 1918 <i>Isohalcurias</i> gen. nov.
Characters			
Mesenterial arrangement	Cyclic	Bilateral	Cyclic
Microcnemes	Absent	Present	Present
Number of macrocnemes	Unfixed (more than 68)	Unfixed (more than 20)	Fixed (12, 20, or 36)
References	[7,12,40] The present study	[8,12]	[7,8,11,12] The present study

In this study, our phylogenetic analyses (Figures 3 and 4) revealed that *Synactinernus* was not monophyletic with the other three genera of Actinernidae but instead within the clade of Halcuriidae. Thus, it was necessary to remove this genus from Actinernidae. Moreover, there was no synapomorphy between *Actinernus* and the other two genera, and rather, there were apparent differences between these genera in mesenterial arrangement and cnidom (see remarks part of Isactinernidae fam. nov.). These are the reasons why we have established a new family for *Isactinernus* and *Synhalcurias* and removed them from Actinernidae.

Finally, this family became monotypic to type genus *Actinernus*. The most characteristic feature of this genus is developing their mesenteries laterally: this arrangement is only observed in the species of *Actinernus* in Actinernoidea. In addition, the species of this family are distinguished from the species of the other two families by a combination of “lacking spirocysts in tentacle and column” and “thickened aboral ends of tentacles”.

Genus *Actinernus* Verrill, 1879.

(Japanese name: Yatsuba-kawari-ginchaku-zoku.)

Actinernus Verrill, 1879: 474; McMurrich, 1893: 165; Carlgren, 1914: 62; Carlgren, 1918: 31; Carlgren, 1921: 14, 184; Stephenson, 1922: 259; Carlgren, 1949: 20.

Porponia Hertwig, 1882, p. 111; Carlgren, 1914, p. 61.

Diagnosis. Column cylindrical, the upper part expanded and forms more or less distinctly eight lobes. **Tentacles numerous, with basal thickening on their aboral side.** Mesenteries many, consist of **24–52 perfect** mesenteries and a half number of imperfect mesenteries. After the formation of ten pairs, first and second cycles, further mesenterial formation takes place at the middle point of each eight lateral endocoel. Then, the formation is not in a radial but in a bilateral way. Furthermore, the size in higher cycles of mesenteries is much different to its partner in each mesenterial pair. **Cnidom: basitrichs (all tissues), spirocysts (actinopharynx and filaments), and microbasic p-mastigophores (actinopharynx and filaments).**

(The revised points from Uchida (2007) [12] are indicated in **bold**.)

Type species. *Actinernus elongatus* (Hertwig, 1882).

Actinernus robustus (Hertwig, 1882).

(Japanese name: yatsuba-kawari-ginchaku.)

Figures 5–7, Table 6.

Porponia robusta Hertwig, 1882: 107, pl. 1 Figure 10a; Carlgren, 1914: 61.

Actinernus robustus: Carlgren, 1918: 12, 34, pl. 1 Figure 9; Stephenson, 1922: 259; Carlgren, 1949: 21.

Material examined. CMNH-ZG 09735 (Figures 5 and 6): specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, corrected on 28 April 2002, from Okinawa Trough (27°02.34' N, 126°58.24' E; St. D-2), 1550 m in depth, during research cruise of R/V Tansei-Marui (KT02-3 reg. 2), by ORE beam trawl, by Kensuke Yanagi; CMNH-ZG 09736: specimen dissected, tissues embedded in paraffin, histological sections prepared, same collector, locality, and method as for CMNH-ZG 09735; CMNH-ZG 09737, 09738: whole specimens, same collector, locality, and method as for CMNH-ZG 09735; CMNH 10211: specimen dissected, corrected on 23 September 2001, from Pacific off Kushiro (42°11.02' N, 146°17.02' E; St. XR-11), 5346–5473 m in depth, collected during research cruise of R/V Hakuho-Marui (KH01-2), by ORE beam trawl, by Kensuke Yanagi; BM 89-11-25-30 (holotype) (Figure 7A,B): dissected specimen, collected on 17 June 1875 southeast off the Boso Peninsula (34°37' N, 140°32' E), depth 1875 fathoms (ca. 3500 m) by trawl, St. 237 of the Challenger Expedition.

Diagnosis. External anatomy. Body cylindrical (Figure 5A), up to ca. 2–6 cm in length and ca. 2.5–5 cm in width on preserved specimens. Column surface smooth, without any structures. Ectoderm of column easily peeled off, without nematocyst batteries, and nematocysts very sparsely distributed. The naked column is opaque milky white in color (Figure 5A). Basal disk in aboral end, thin and fragile but adhesive (Figure 5A). Upper part of column widely expanded and thrown into eight lobes, four larger and four smaller ones (Figure 5B,C). Upper margin continued to tentacle bases, and the bases have a spine-like shape (Figure 5A). Tentacles ca. 60–90 in number on oral disk, all marginal. All tentacles ca. 7–10 mm in length, pale white to brown in color, simple shape but with strong thickenings at their aboral base. Oral disc pale brown, with radial streaks corresponding to every tentacle. Mouth at center of oral disk, apparently swelled, lip-like, brown in color.

Internal anatomy. Circa 24–52 macrocnemes (Figure 5D) on actinopharynx: 12, including 4 directives, in the 1st cycle; 8 in the 2nd cycle; maximum 16 in the 3rd cycle; and maximum 16 in the 4th cycle. Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, and fourth ones in the endocoels of third ones (bilateral arrangement). Judged by the numbers of tentacles, 20–40 more microcnemes on distal end. Each tentacle either exo- or endocoelic. Tentacular longitudinal muscle and tentacular circular muscle both too weak to observe in histological sections. On aboral ends of tentacles, mesoglea apparently thickened. Retractor muscles extremely weak and diffused (Figure 5D). Muscle processes very short, simple or a little branched, around 30–50 in each muscle

pennon. Parietal muscles of macrocnemes very weak and indistinct, with a few muscle fibers. Mesoglea thickest in body wall and actinopharynx (Figure 5D), reaching 2–2.5 mm in thickness, and far thicker than the ectoderm and endoderm. However, mesoglea thinner in mesenteries and basal disc (Figure 5D,E) and thinnest in tentacles. Actinopharynx, with siphonoglyphs on dorsal and ventral sides (Figure 5D), always connected to actinopharynx, and with ca. 8–12 longitudinal grooves as deep as siphonoglyphs. Sphincter muscle absent. On the aboral end, basilar muscle absent (Figure 5E). Dioecious.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores (Figure 6, Table 6).

Remarks. *Actinernus robustus* is the only species of this genus in Japan. Our specimens completely correspond to the description of Hertwig (1882) and Uchida (2007) [12,41]. In contrast to all Japanese Actinernoidea species, which live between 20 and 500 m, this species inhabits waters deeper than 1000 m.

The approximate morphology and cnidom of our specimens corresponded to the descriptions of Hertwig (1882) and Uchida (2007), and these specimens can be identified as *A. robustus*. However, there were no holotrichs in filaments, though Uchida (2007) [12] observed a few.

Table 6. Cnidoms of the species of *Actinernus* and *Isactinernus*.

	<i>Actinernus robustus</i>			<i>Isactinernus quadrilobatus</i>		
	CMNH-ZG 9735			NSMT-Co 1662		
	Length × Width (µm)	frequency	N	Length × Width (µm)	frequency	N
Tentacle						
basitrichs	S 18.2–28.8 × 3.7–5.9	numerous	53	9.1–21.5 × 2.0–3.5	numerous	15
	L 40.0–53.6 × 4.3–4.8	few	8	31.1–42.3 × 3.0–4.4	numerous	24
spirocysts	25.2–54.7 × 5.2–8.7	few	5	20.9–49.5 × 3.1–9.8	numerous	78
microbasic <i>p</i> -mastigophores						
microbasic <i>b</i> -mastigophores				29.9–31.7 × 4.4–5.2	rare	3
Actinopharynx						
basitrichs	S 20.2–25.1 × 4.0–5.5	numerous	10	8.3–13.0 × 2.2–3.3	numerous	36
	L 38.8–49.3 × 3.9–5.4	few	9	36.1–43.4 × 3.5–5.2	numerous	27
spirocysts				25.4–36.4 × 4.7–6.3	few	4
microbasic <i>p</i> -mastigophores	11.7–14.0 × 3.9–5.6	few	5	34.9–44.5 × 6.3–8.6	numerous	23
Column						
basitrichs	20.4–22.5 × 4.3–5.4	rare	3	Column damaged		
Filament						
basitrichs	15.8–23.8 × 3.8–5.6	numerous	35	17.8–47.2 × 2.9–4.2	few	9
	27.7–52.8 × 3.4–5.4	numerous	27			
spirocysts	41.6 × 7.2	rare	1	28.1–42.5 × 4.8–7.6	numerous	55
microbasic <i>p</i> -mastigophores	38.8–42.3 × 8.1–9.8	rare	4	35.3–42.4 × 6.0–7.2	numerous	25

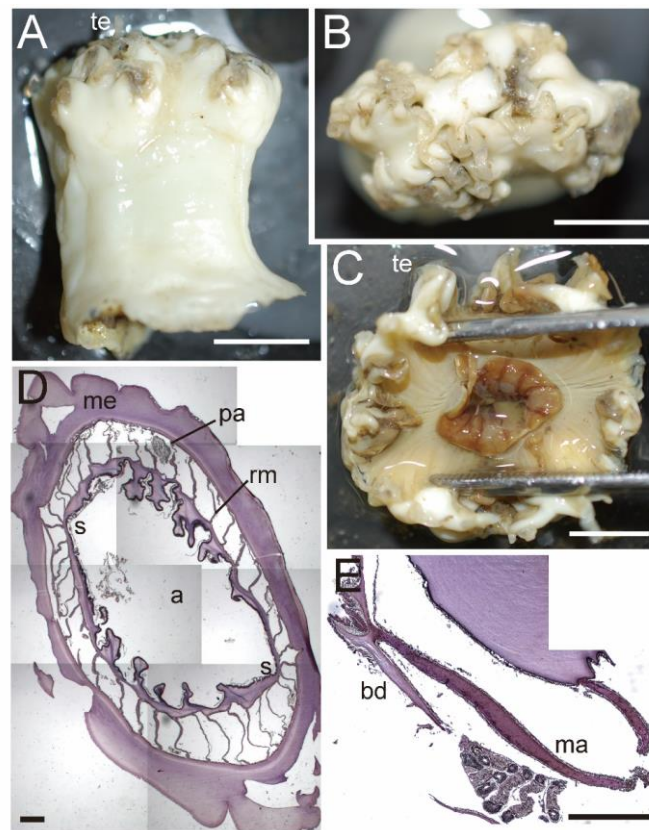


Figure 5. External and internal morphology of *Actinernus robustus* (CMNH-ZG 9735). (A) Lateral view of a fixed specimen; (B) oral view; (C) bared oral disc; (D) transverse section of column; (E) longitudinal section of basal disc. Basilar muscle absent; abbreviations: a, actinopharynx; bd, basal disc; ma, macrocneme; me, mesoglea; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; te, tentacle. Scale bars indicate 1 cm in (A–C), 1 mm in (B,E), and 500 μ m in (D,E).

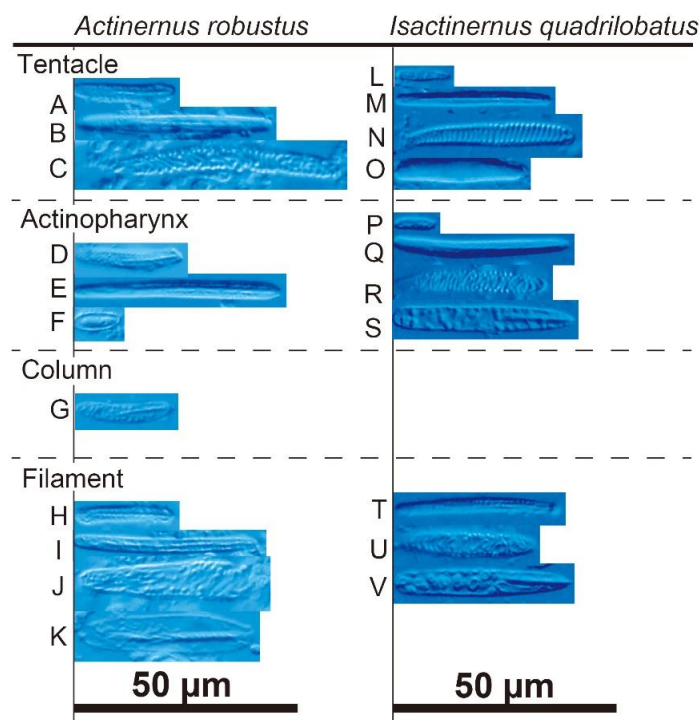


Figure 6. Cnidoms of *Actinernus robustus* and *Isactinernus quadrilobatus*. A–K: *A. robustus*. A–C: tentacle; A, small basitrich; B, large basitrich; C, spirocyst. D–F: actinopharynx; D, small basitrich; E,

large basitrich; F, microbasic *p*-mastigophore. G, basitrich in column. H–K: filament; H, small basitrich; I, large basitrich; J, spirocyst; K, microbasic *p*-mastigophore. L–V: *I. quadrilobatus*. L–O: tentacle; L, small basitrich; M, large basitrich; N, spirocyst; O, microbasic *b*-mastigophore. P–S: actinopharynx; P, small basitrich; Q, large basitrich; R, spirocyst; S, microbasic *p*-mastigophore. T–V: filament; T, small basitrich; U, large basitrich; V, microbasic *p*-mastigophore.

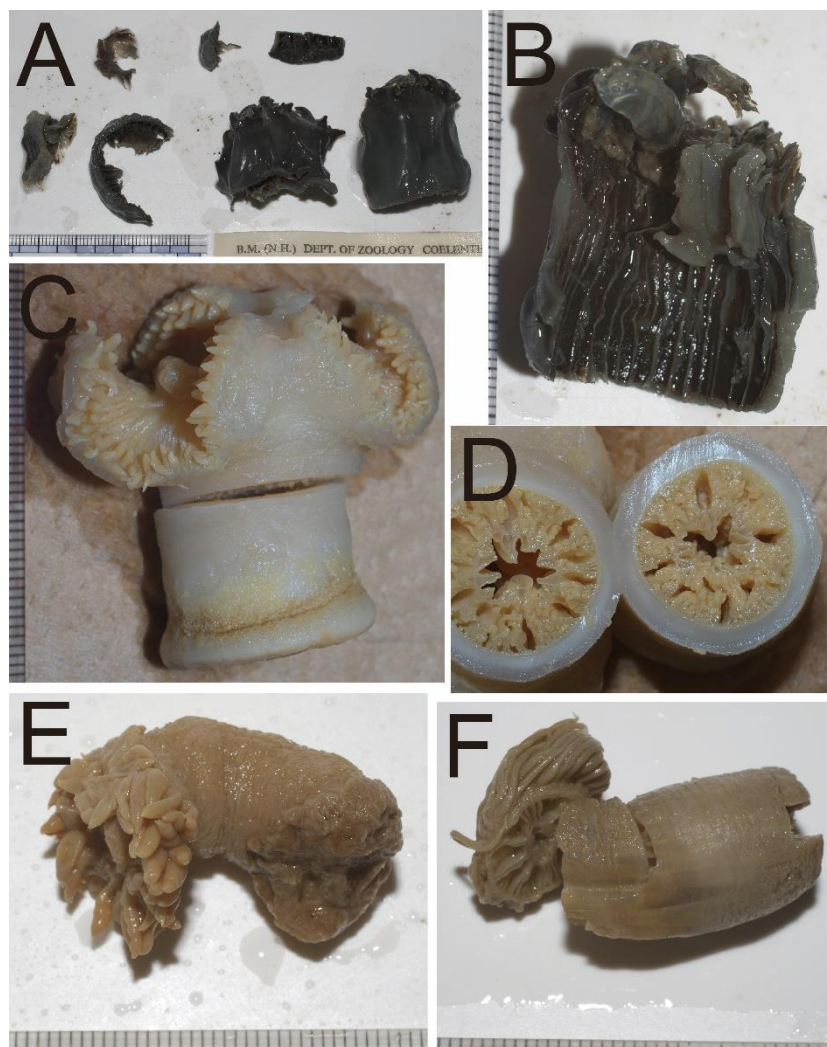


Figure 7. Type specimens of actinernoidean anemones. The type specimen of *Actinernus robustus* ((A,B): BM 89-11-25-30, holotype), *Isactinernus quadrilobatus* ((C,D): ZMUC-ANT-000098, one of the syntypes), and *Isohalcurias carlgreni* ((E): UUZM 705b (Anthozoa), one of the syntypes, (F): SMNH-TYPE-1200, one of the syntypes).

Family ISACTINERNIDAE fam. nov.

(New Japanese name: yotsuba-kawari-ginchaku-ka.)

urn:lsid:zoobank.org:act:E3DEAB20-FDE6-4E96-A2E1-A0E892DD485A

Diagnosis. Actinernoidea with an elongated body. Distal margin of column usually expanded and drawn into lobes but sometimes not expanded nor lobed. Column with or without nematocyst batteries. Tentacles numerous, simple or with basal thickening or on their aboral side. With two siphonoglyphs. Mesenteries all macrocnemes, numerous, and developing cyclically. Retractors rather weak. All mesenteries fertile. Cnidom: basitrichs, spirocysts, and microbasic *p*-mastigophores (each in all tissue).

Type genus. *Isactinernus* Carlgren, 1918.

Etymology. Derived from the name of type genus, *Isactinernus* Carlgren, 1918.

Remarks. In this study, our phylogenetic analyses (Figure 4) revealed that *Isactinernus* and *Synhalcurias* were the most closely related genera and formed a monophyly with

Actinernus. However, there were no synapomorphies between *Actinernus* and *Isactinernus/Synhalcurias*: *Synactinernus* has almost all the general features of Actinernidae, and we could not find any accurate common feature shared among the remaining three families. In addition, there are apparent differences between *Actinernus* and *Isactinernus/Synhalcurias* in mesenterial arrangement and cnidom: in Actinernoidea, only the latter two genera have microbasic *p*-mastigophores in their tentacles and column, and their mesenteries are all macrocnemes, the same in number as tentacles [10,12,15]. For these reasons, we have established this new family for *Isactinernus* and *Synhalcurias* and removed them from Actinernidae (Figure 4). See Table 5 for the comparison with the other families of Actinernoidea. This family is characterized by possessing microbasic *p*-mastigophores in their tentacles and column and all mesenteries being perfect and fertile.

Genus *Isactinernus* Carlgren, 1918.

(Japanese name: yotsuba-kawari-ginchaku-zoku.)

Isactinernus Carlgren, 1918: 29; Stephenson, 1922: 260; Carlgren, 1949: 20; Fautin and den Hartog, 2003: 107.

Diagnosis. Column cylindrical, the upper part expanded, and forms four large lobes. With many tentacles, **thickening on aboral side**. Mesenteries ca. 34–70 pairs of large and many small ones, **all mesenteries** perfect and **almost all** fertile. After the formation of ten pairs of the oldest mesenteries, in the first and second cycles, further mesenterial formation by cyclic in each eight lateral endocoel, in the same manner as those of *Halcurias*. With two siphonoglyphs. Column ectoderm with minute nematocyst batteries. **Cnidom: basitrichs, spirocysts, and microbasic *p*-mastigophores (each in all tissue).**

(The revised points from Uchida (2007) [12] are indicated in **bold**.)

Type species. *Isactinernus quadrilobatus* Carlgren, 1918.

Remarks. This genus had been monotypic with *I. quadrilobatus* Carlgren, 1918, for a century. However, accompanied by Fautin and Hartog (2003), the work which synonymized *Synactinernus flavus* Carlgren, 1918, into *I. quadrilobatus*, genus *Synactinernus* became a junior synonym of this genus. However, our phylogenetic analyses indicated that these genera are less related phylogenetically (Figure 3): *I. quadrilobatus* and *S. flavus* are polyphyletic, not closely related in the phylogenetic tree, and thus, the species should be accommodated into a different family (see also remarks of *Synactinernus*).

Thus, *Isactinernus* should remain as a monotypic genus, the same status as Carlgren (1918) [7]. However, it turned out to be appropriate that this genus should not be accommodated into Actinernidae, but into a new family (see the remarks of Isactinernidae).

***Isactinernus quadrilobatus* Carlgren, 1918.**

(Japanese name: yotsuba-kawari-ginchaku.)

Figures 6–8, Table 6.

Isactinernus 4-lobatus Carlgren, 1918: 7, 29, textfigs. 5 and 6, pl. 1 Figures 4 and 5; Carlgren, 1940: 22; Carlgren, 1949: 20.

Isactinernus quadrilobatus: Stephenson, 1922: 260; Fautin and den Hartog, 2003: 108–113, Figures 1–5.

Material examined. NSMT-Co 1662: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared and collected on 8 March 2016, from the Kumano-nada Sea off Hamajima Island, Mie Prefecture, around a depth of 350 m, by trawling of fishing boat *Kiei-Marui*, kept in Toba Aquarium for a while, preserved on 19 March 2016 by Takeya Moritaki in 70% ethanol, and re-preserved on 19 April 2016 by Takuma Fujii in 10% formalin–seawater solution (*v/v*) for histological section; CMNH-ZG 09734: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared and 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-Marui*, kept in Kagoshima City Aquarium for a while, and preserved on 6 May 2018 by Takato Izumi. ZMUC-ANT-000098 (syntypes): three specimens, 14 May 1914, off the Goto Islands (32°17' N, 128°11' E), depth 207 m, collected by Theodor Mortensen (Figure 7C,D). MZLU-L14/3033 (syntype): collection data same as above syntypes.

Description. External anatomy. Body cylindrical (Figure 8A), up to ca. 4.5–7 cm in length and ca. 5 cm in width on preserved specimens. Column surface comparatively smooth, without any structures or with small tenaculi. Ectoderm of column is pale white (Figure 8A), with small nematocyst batteries. The naked column is opaque milky white in color. Basal disk in aboral end, robust and adhesive (Figure 8A). Upper part of column widely expanded and thrown into four large lobes (Figure 8B). Upper margin continued to tentacle bases, and the bases have numerous spine-like shapes (Figure 8B). Tentacles ca. 72–140 in number on oral disk, all marginal. All tentacles ca. 3–5 mm in length, pale white in color, simple shape, but with strong thickenings at their aboral base. Oral disc is pale white, with radial streaks corresponding to every tentacle. Mouth at center of oral disk, apparently swelled, lip-like, cross-like shape, white in color.

Internal anatomy. Circa 72–140 macrocnemes on actinopharynx; 12, including 4 directives, in the 1st cycle; 8 in the 2nd cycle; 16 in the 3rd cycle; and 32 in 4th cycle. Judged by the numbers of tentacles, the mesenterial cycle reaching to sixth. Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, same as the general arrangement of Actinernoidea. Microcnemes absent (Figure 8C). Each tentacle either exo- or endocoelic. Tentacular longitudinal muscle and tentacular circular muscle both too weak to observe in histological sections. On aboral ends of tentacles, mesoglea apparently thickened. Retractor muscles extremely weak and diffused (Figure 8C,D). Muscle processes very short, simple or a little branched, around 40–70 in each muscle pennon. Parietal muscles of macrocnemes very weak and indistinct, with a few muscle fibers. Mesoglea thickest in body wall and actinopharynx (Figure 8C,D), reaching 2.5–3 mm in thickness, and far thicker than the ectoderm and endoderm. Mesoglea evenly thick in mesenteries (Figure 8C) and basal disc but far thinner in tentacles. Actinopharynx, with siphonoglyphs on dorsal and ventral sides, always connected to actinopharynx, and with 10 longitudinal grooves as deep as siphonoglyphs. Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious: almost all mesenteries fertile and matured oocytes in CMNH-ZG 09734.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores (Figure 6, Table 6).

Remarks. This species has been the only species of genus *Isactinernus* for a century. According to our research, *Synactinernus flavus* Carlgren, 1918, once synonymized into *I. quadrilobatus* by Fautin and den Hartog (2003), is not only a different species but also a species in a different family (see remarks of genus *Synactinernus* [14]).

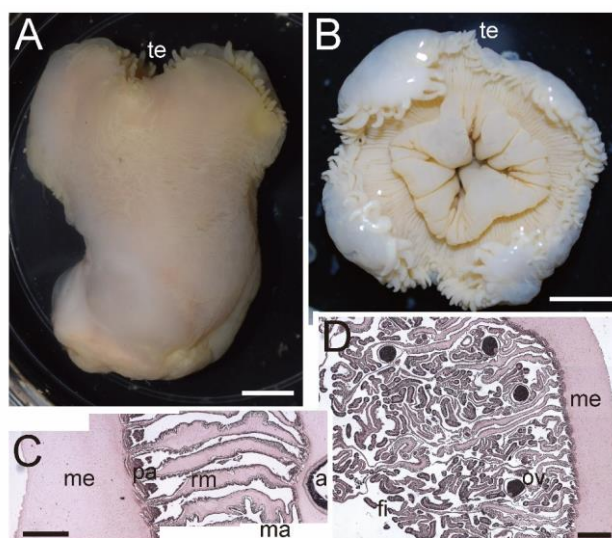


Figure 8. External and internal morphology of *Isactinernus quadrilobatus* (CMNH-ZG 9734). (A) Lateral view of a fixed specimen; (B) oral view; (C) enlarged transverse section of upper column; (D) enlarged transverse section of lower column. Abbreviations: a, actinopharynx; fi, filament; ma,

macrocneme; me, mesoglea; ov, ovary; pa, parietal muscle; rm, retractor muscle; te, tentacle. Scale bars indicate 1 cm in (A,B) and 1 mm in (C,D).

Genus *Synhalcurias* Carlgren, 1914.

(Japanese name: seitaka-kawari-ginchaku-zoku.)

Synhalcurias Carlgren, 1914: 53; Carlgren, 1918: 27; Stephenson, 1922: 260; Carlgren, 1949: 19.

Diagnosis. Column cylindrical, the upper part not expanded nor forms lobes. **With many tentacles in a simple shape.** Mesenteries **number 68** to ca. 100, and **all** mesenteries are perfect and fertile. After the formation of ten pairs of the oldest mesenteries, in the first and second cycles, further mesenterial formation by cyclic in each eight lateral endocoel in the same manner as those in *Halcurias*. With two siphonoglyphs. Column ectoderm with **or without** small nematocyst batteries. **Cnidom: basitrichs, spirocysts, and microbasic p-mastigophores (each in all tissue).**

(The revised points from Uchida (2007) [12] are indicated in **bold**.)

Type species. *Synactinernus elegans* (Wassilieff, 1908).

Remarks. Different from *Isactinernus*, *Synhalcurias* has been continuously monotypic since the foundation of the genus. However, a different species of *Synhalcurias* were discovered recently: they are apparently smaller than *S. elegans*, and there were several differences between *S. elegans* and these recently discovered specimens, although their morphological features satisfied the diagnosis of *Synhalcurias*. Based on this, Izumi and Yanagi (2021) [15] determined these specimens as a new species of *Synhalcurias* and described them as *Synhalcurias kahakui* Izumi and Yanagi, 2021.

Synhalcurias has long belonged to Actinernidae, but it is appropriate that *Synhalcurias* and *Isactinernus* should not be accommodated into the family by our morphological and phylogenetic analyses, and so, we have established a new family for these genera (see the remarks of family Isactinernidae).

***Synhalcurias elegans* (Wassilieff, 1908).**

(Japanese name: seitaka-kawari-ginchaku.)

Figures 2–4 and Table 1 in Izumi and Yanagi (2021) [15].

Ilyanthopsis elegans Wassilieff, 1908: 8, textfigs. 2–5, pl. 1 Figure 2, pl. 3 Figure 38, pl. 4 Figures 39, 40a, and 40b.

Synhalcurias elegans: Carlgren, 1914: 50–53, pl. Figures 1–4; Carlgren, 1918: 6, 27, textfigs. 2–4; Stephenson, 1922: 260; Carlgren, 1940: 22; Carlgren, 1949: 20; Uchida, 1992: 129, pl. 29 Figure 5; Uchida and Soyama, 2001: 21.

Material examined/description. See Izumi and Yanagi (2021) [15].

***Synhalcurias kahakui* Izumi, 2021.**

(New Japanese name: kobito-seitaka-kawari-ginchaku.)

Figures 4 and 5 and Table 1 in Izumi and Yanagi (2021) [15].

Synhalcurias kahakui Izumi and Yanagi, 2021: 567–573.

Material examined/description. See Izumi and Yanagi (2021) [15].

Family HALCURIIDAE Carlgren, 1918 (1897).

(Japanese name: kawari-ginchaku-ka.)

Halcuriidae Carlgren, 1918: 24; Carlgren, 1921: 13; Stephenson, 1922: 257; Carlgren, 1938: 17; Carlgren, 1949: 18.

Diagnosis. (Revised parts are shown in **bold**). **Actinernoidea** with an elongated body. Distal margin of column **lobed or** not lobed. Column with or without nematocyst batteries. Tentacles without basal **thickening** on their aboral side. With a single **or double** siphonoglyph. Mesenteries divisible into macro- and microcnemes. Macrocnemes six, ten **or eighteen** pairs. Some of the microcnemes, however, are perfect in many species. Retractors of the macrocnemes restricted. Macrocnemes fertile with filaments but microcnemes sterile without filaments.

(The revised points from Uchida (2004) [11] are indicated in **bold**.)

Type genus. *Halcurias* McMurrich, 1893.

Remarks. Regarding the nomenclature of the family, refer to Sanamyan and Sanamyan (2020) [42]. This family has accommodated two genera: *Halcurias* McMurrich, 1893, and *Carlgrenia*, Stephenson, 1918 (the latter genus never confirmed from Japan, including in our research) [7,43]. However, our phylogenetic analyses revealed that *Synactinernus* Carlgren, 1918, one of the genera of Actinernidae, was in the clade of Halcuriidae (Figure 3). However, species of this genus have almost the same morphology as Actinernidae, and there was no synapomorphy between *Halcurias* and *Synactinernus*. Thus, it was unnatural to classify the synactinernid species into *Halcurias*.

Thus, we divided the previous *Halcurias* into “true” *Halcurias* and a new genus, *Isohalcurias* gen. nov., following the phylogeny (Figure 4). According to the tree, there were only two species from Japan, *Halcurias hiroomii* sp. nov. and *Halcurias fragum* sp. nov., in the same clade as *Halcurias pilatus* McMurrich, 1893, the type species of the genus. On the other hand, the other three *Halcurias* species were in the other clade, which was paraphyletic to “true” *Halcurias* and *Synactinernus*. Thus, these three species, *Isohalcurias carlgreni* (McMurrich, 1901) comb. nov., *Isohalcurias citreum* sp. nov., and *Isohalcurias malum* sp. nov., were accommodated into the new genus, *Isohalcurias*. See Table 7 for a comparison of the four genera included in the suborder Anenthemonae.

Table 7. Comparison of *Isohalcurias* gen. nov. and the other genera of Halcuriidae.

	<i>Isohalcurias</i> gen. nov.	<i>Halcurias</i> McMurrich, 1893	<i>Synactinernus</i> Carlgren, 1918	<i>Carlgrenia</i> Stephenson, 1918
Type species	<i>Isohalcurias carlgreni</i> (McMurrich, 1901) comb. nov.	<i>Halcurias pilatus</i> McMurrich, 1893	<i>Synactinernus flavus</i> Carlgren, 1918	<i>Synhalcurias elegans</i> (Was-silieff, 1908)
Characters				
Edge of oral disc	Simple, not forming any lobes	Simple, not forming any lobes	Forming eight lobes	Simple, not forming any lobes
Number of macrocnemes	20 (10 pairs)	20 (10 pairs)	36 (18 pairs)	12 (6 pairs)
Shape of retractor muscle	Pinnate	Simple	Simple	Strongly restricted
Spirocysts of column	Present	Absent	Present	Unknown
References	The present study	[4,11,43]	[7,10,12]	[12,36]

Genus *Halcurias* McMurrich, 1893.

(Japanese name: kawari-ginchaku-zoku.)

Halcurias McMurrich 1893: 142; McMurrich, 1901: 155, 158; Carlgren, 1914: 60; Carlgren, 1918: 25; Stephenson, 1918: 14; Carlgren 1938: 18; Carlgren, 1949: 18.

Halcuriopsis Carlgren 1921: 93.

Diagnosis. Mesenteries up to 34 pairs arranged in 4 cycles, 6 + 4 + 8 + 16. Macrocnemes, ten pairs, fertile, filamented and with restricted retractors. Microcnemes in only the upper part of the body, some of them perfect. **Retractor muscles strong and diffused.** **Parietal** muscles rather well developed to fairly weak. Tentacles, up to 68. Dioecious. Cnidom: basitrichs (**in all tissues**), spirocysts (**in tentacles, actinopharynx, and filaments**), and microbasic *p*-mastigophores (**in actinopharynx and filaments**).

(The revised points from Uchida (2004) [11] are indicated in **bold**.)

Type species. *Halcurias pilatus* McMurrich, 1893.

Remarks. Halcuriidae Carlgren, 1918, contained two genera, *Halcurias* McMurrich, 1893, and *Carlgrenia* Stephenson, 1918 [7,43]. The latter genus has been monotypic, so almost all species of this family have been accommodated in *Halcurias*. However, according to our phylogenetic analyses, *Halcurias* became paraphyletic (Figure 3). Thus, we established the new genus *Isohalcurias* gen. nov. and moved some species into this genus (Figure 4). Compared to *Isohalcurias*, *Halcurias* is characterized by two features: lacking spirocysts in their column (compare *Halcurias* species in Table 8 and *Isohalcurias* species in Table

10) and retractor muscles with comparatively simple, not clustered muscular processes (Figures 9D and 11D; Table 7).

The two species of Halcuriidae from Japan remain in *Halcurias*: *Halcurias hiroonii* sp. nov. and *Halcurias fragum* sp. nov. Across the world, *Halcurias pilatus* McMurrich, 1893, was confirmed to belong to this genus by phylogenetic analyses (Figure 4). Concerning the other described species, classification was conjectured by the condition of the column, as below.

The following species possibly remain in *Halcurias*: *Halcurias uchidai*, Rodríguez and Lauretta, 2023 (according to Rodríguez et al. (2013); this species, described as *Halcurias macmurrich* Uchida, 2004, does not contain spirocysts in its column [44]; recently, Rodríguez and Lauretta (2023) gave a new name for this species [45]); *Halcurias endocoelactis* Stephenson, 1918 (it was described that spirocysts were absent in the body wall [36]); *Halcurias capensis* Carlgren, 1928 (same as *H. endocoelactis* [36]).

Species possibly assigned to *Isohalcurias*: *Halcurias minimus* Carlgren, 1928; *Halcurias sudanensis* Riemann-Zürneck, 1983 (it was described that spirocysts were present on the body walls of both species [46,47]).

***Halcurias hiroonii* sp. nov.**

(Japanese name: abata-kawari-ginchaku.)

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Figures 9 and 10; Table 8.

Halcurias japonicus (nomen nudum) Uchida, 2004: 13–15, Figure 2. pl. 1C–1E.

Material examined. Holotype. CMNH–ZG 10212: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 2016 (date unknown) from Toyama Bay, Toyama Prefecture, around a depth of 50–100 m, gill net of fishing boat *Koei-Marui*, provided by Itaru Kobayashi. **Paratype.** NSMT–Co 1824: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected from Toyama Bay, off Toyama Prefecture, by gill net (anonymous collector, and date unknown), kept in a tank of Uozu Aquarium by Tomoharu Kimura, and fixed on 28 February 2015 by Takato Izumi. **Other specimens.** CMNH–ZG 10213: whole specimen, same date, collector, and method as for CMNH–ZG 10212; NSMT–Co 1825–1827, same date, collector, and method as for NSMT–Co 1824: whole specimens; CMNH–ZG 10214: whole specimen; originally collected from Ise Bay by trawling of fishing boat *Kiei-Marui*, kept in Takeshima Aquarium, and fixed by Takato Izumi on 29 June 2017. CMNH–ZG 10215: whole specimen; originally collected in January 2018 at Urugami, Wakayama Prefecture, at 100–130 m by Isao Hirabayashi, and fixed by Takuma Fujii and Kensuke Yanagi from the tank at the Kushimoto Marine Park on 22 May 2018.

Description. External anatomy. Body cylindrical (Figure 9A,B), up to ca. 40 mm in height and ca. 15 mm in width in a living specimen and 15–30 mm in height and 5–22 mm in width in a preserved specimen. Column surface comparatively rough, reddish-orange ectoderm layer with white tenaculi-like nematocyst batteries. Nematocysts densely distributed, especially in nematocyst batteries. Upper part of column a little narrower, pale yellow in color, simple and not thrown into any lobes. Tentacles simple, conical, all marginal, 6–8 mm in length, no thickenings including their aboral base, pale yellow in color, and 68 in number on oral disk; inner and outer ones alternately bared (Figure 9C). The tip of tentacles pointed. Basal disk in aboral end, opaque and mesenterial insertion invisible (Figure 9A,B). Oral disk diameter as broad as column, hemi-transparent pale yellow. Mouth at center of oral disk, highly swelled, lip-like, smooth, bright yellow and orange (Figure 9C).

Internal anatomy. Twenty macrocnemes on actinopharynx (our specimens, all twenty): twelve, including four directives, in the first cycle and eight in the second 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. Mesenteries in third and fourth cycle microcnemes. Each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle exocoelotic and circular muscle too weak to observe in

histological section; retractor muscles restricted at the center of mesenteries, comparatively weak but distinct (Figure 9D). Muscle processes simple or slightly branched, around 25–41 in each muscle pennon (Figure 9D). Parietal muscles of macrocnemes quite weak with 10–13 simple processes (Figure 9D). Mesoglea thickest in body wall and actinopharynx, far thicker than ectoderm and endoderm (Figure 9G). Mesoglea thinner in mesoglea (Figure 9D,E). Actinopharynx, with siphonoglyphs on one side, always connected to actinopharynx, and with the other eleven longitudinal grooves less deep than siphonoglyphs. Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, immature testis in our specimen (Figure 9E). Mesenteries in second cycle fertile.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores. Spirocysts are absent in the column (Figure 10, Table 8).

Etymology. This name is derived from Hiro'omi Uchida, the author of Uchida (2004) [11] in which *Halcurias hiroomii* sp. nov. was originally described. He defined this anemone as a new species. Thus, we renamed this species to make it valid and used his first name.

Table 8. Cnidoms of the species of *Halcurias*.

	<i>Halcurias hiroomii</i> sp. nov.		<i>Halcurias fragum</i> sp. nov.				
	CMNH-ZG 10212		CMNH-ZG 10216				
	Length × Width (µm)	frequency	N	Length × Width (µm)	frequency	N	
Tentacle							
basitrichs	S	10.3–12.4 × 2.0–3.0	rare	2	9.7–13.7 × 2.0–3.1	numerous	13
	L	24.0–34.5 × 2.3–4.6	numerous	60	21.1–27.5 × 2.5–4.8	numerous	44
spirocysts		13.6–39.4 × 2.6–6.7	numerous	67	20.8–31.0 × 3.5–7.0	numerous	68
Actinopharynx							
basitrichs	S	8.6–12.3 × 2.2–3.1	few	8	22.8–32.6 × 2.4–4.0	numerous	45
	L	20.3–31.5 × 2.3–4.2	numerous	61	19.6–27.8 × 4.2–5.3	few	6
spirocysts		26.2–36.8 × 5.4–7.4	numerous	64	20.4–29.2 × 4.5–7.7	numerous	43
microbasic <i>p</i> -mastigophores		23.8–31.9 × 4.5–6.7	numerous	63			
Column							
basitrichs		26.7–36.1 × 2.0–4.2	numerous	84	31.2–39.9 × 2.3–4.2	numerous	70
Filament							
basitrichs		25.3–33.6 × 2.9–4.2	numerous	31	23.9–33.5 × 2.4–4.3	numerous	28
spirocysts		20.4–38.5 × 4.9–7.1	numerous	30	24.8 × 45.3	rare	1
microbasic <i>p</i> -mastigophores		12.2–33.5 × 3.6–8.7	numerous	61	13.4–29.6 × 3.5–10.5	numerous	79

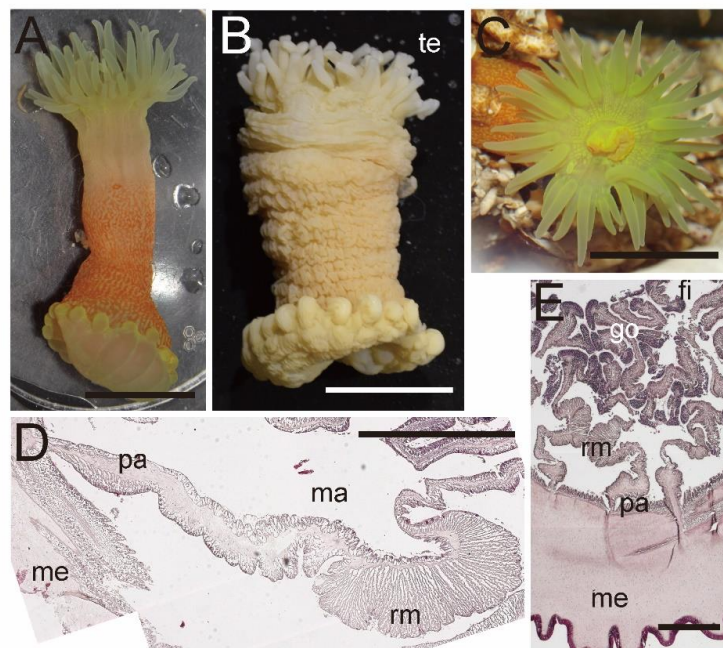


Figure 9. External and internal morphology of *Halcurias hiroonii* sp. nov. (CMNH-ZG 10212). (A) Lateral view of a living specimen; (B) lateral view of a fixed specimen; (C) oral view of a living specimen; (D) enlarged transverse section of upper column; (E) enlarged transverse section of lower column. Abbreviations: fi, filament; go, gonad; ma, macrocneme; me, mesoglea; pa, parietal muscle; rm, retractor muscle; te, tentacle. Scale bars indicate 1 cm in (A–C) and 500 µm in (D,E).

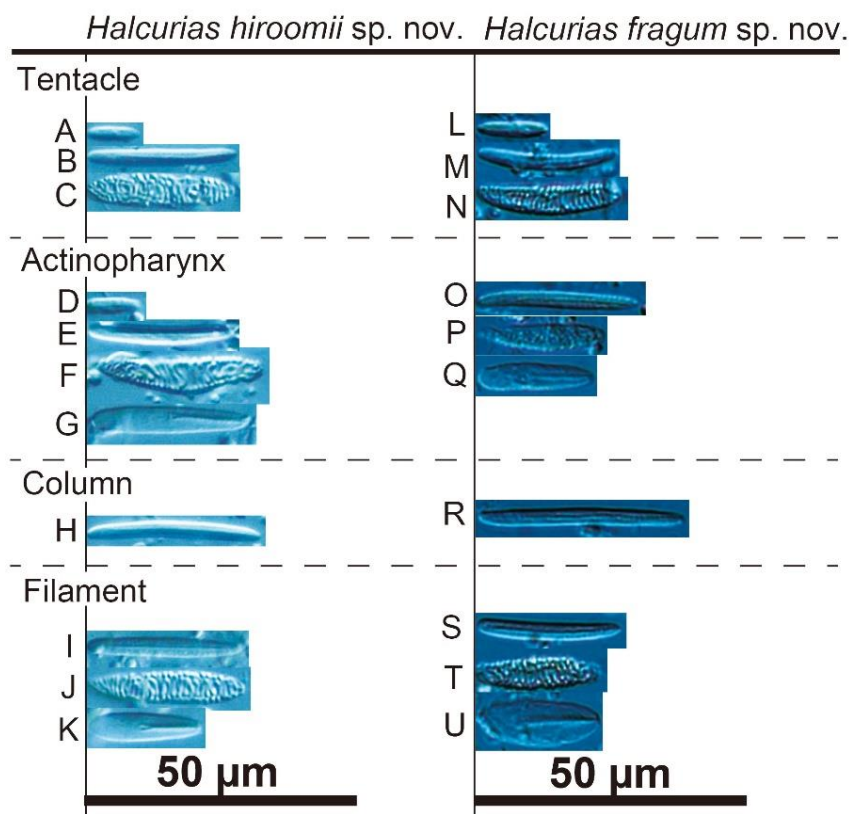


Figure 10. Cnidoms of *Halcurias hiroonii* sp. nov. and *H. fragum* sp. nov. A–K: *H. hiroonii*. A–C: tentacle; A, small basitrich; B, large basitrich; C, spirocyst. D–F: actinopharynx; D, small basitrich; E, large basitrich; F, spirocyst. G, microbasic *p*-mastigophore. H, basitrich in column. I–K: filament;

I, small basitrich; J, large basitrich; K, microbasic *p*-mastigophore. L–U: *H. fragum*. L–N: tentacle; L, small basitrich; M, large basitrich; N, spirocyst. O–Q: actinopharynx; O, basitrich; P, spirocyst; Q, microbasic *p*-mastigophore. R, basitrich in column. S–U: filament; S, basitrich; T, spirocyst; U, microbasic *p*-mastigophore.

Remarks. See Table 9 for comparison to the other species of Halcuriidae. Uchida (2004) [11] described this species as *Halcurias japonicus*. However, this species name is nomen nudum as Uchida’s description in 2004 did not designate any type specimens. Even though Uchida indicated a specimen as the type belatedly in Uchida (2007), there is no description together, and the species name is not valid currently [48]. In the present study, we collected halcuriids which almost corresponded to the description of Uchida (2004) [11]. He only reported this species in the Japanese Sea off the Hokuriku region, but we recorded this species also from Kumano-nada Sea, the Pacific Ocean.

According to our phylogenetic tree (Figure 4), this halcuriid species was in the clade of *Halcurias* and lacks spirocysts in their column, and *H. hiroomii* should therefore remain in this genus.

Table 9. Comparison of *Halcurias fragum* sp. Nov. and the other species of *Halcurias*, including species possibly assignable to the genus.

	<i>Halcurias fragum</i> sp. nov.	<i>Halcurias hiroomii</i> sp. nov.	<i>Halcurias pila-</i> <i>tus</i> McMurrich, 1893	* <i>Halcurias</i> <i>capensis</i> Carlgren, 1928	* <i>Halcurias endo-</i> <i>coelactis</i> Stephenson, 1918	* <i>Halcurias uchidai</i> Uchida, 2004
Characters						
Size (height)	9–10 mm	15–30 mm	14–15 mm	12–22 mm	48 mm	40 mm
Basitrichs of tentacles	2 types	1 type	1 type	1 type	1 type	1 type
Spirocysts in actinopharynx	Present	Present	Present	Absent	Absent	Present
Long basitrichs in filaments	Absent	Absent	Present	Unknown	Unknown	Absent
References	The present study	[11] The present study	[43,44]	[11,46]	[11,36]	[11,45]

* Species without molecular phylogenetic information.

***Halcurias fragum* sp. nov.**

(New Japanese name: ichigo-kawari-ginchaku.)

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Figures 10 and 11; Table 8.

Material examined. Holotype. CMNH–ZG 10216: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected by scuba diving on 22 March 2018 at Mushizaki, Sado Island, Niigata Pref., 15 m in depth, by Akihito Omori. **Paratypes.** NSMT–Co 1828–1829: whole specimen, same date, collector, and method as for CMNH–ZG 10216. Other specimens; CMNH–ZG 10217: whole specimen; originally collected in January 2018 at Urugami, Wakayama Prefecture, at 100–130 m, by Isao Hirabayashi, and fixed by Takuma Fujii and Kensuke Yanagi from the tank at the Kushimoto Marine Park on 22 May 2018.

Description. External anatomy. Body cylindrical (Figure 11A,B), up to ca. 10 mm in height and ca. 5 mm in width in a living specimen and 9 mm in height and 4 mm in width in a preserved specimen. Column surface comparatively smooth, reddish-orange ectoderm layer and sometimes patchy pattern with dark red and light orange, and with white tenaculi-like nematocyst batteries. Nematocysts densely distributed, especially in nematocyst batteries. Upper part of column a little narrower, white in color, simple and not thrown into lobes. Tentacles simple, all marginal, 3–6 mm in length, no thickenings

including their aboral base, bright red to orange in color and pale white in roots, and 68 in number on oral disk; inner and outer ones alternatingly bared (Figure 11C). The tip of tentacles pointed. Basal disk in aboral end, opaque and mesenterial insertion invisible. Oral disk diameter broader than column, hemi-transparent, pale white. Mouth at center of oral disk, highly swelled, lip-like, groove on surface, bright orange (Figure 11A).

Internal anatomy. Twenty (ten pairs of) macrocnemes on actinopharynx: twelve, including four directives, in the first cycle and eight in the second cycle (Figure 11G,H). Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, an arrangement obeying the rule of mesenterial arrangement of Actinernidae. Mesenteries in third and fourth cycle microcnemes. Each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle exocoelotic (Figure 11F) and circular muscle too weak to observe in histological section (Figure 11F); retractor muscles restricted near actinopharynx or filaments, obviously developed (Figure 11D). Muscle processes simple or slightly branched, around 15–25 in each muscle pennon (Figure 11D). Parietal muscles of macrocnemes comparatively developed and distinct (Figure 11D). Parietal muscle processes are simple, four to seven on each side. Mesoglea thickest in body wall and actinopharynx, generally thicker than ectoderm and endoderm (Figure 11G). Mesoglea thinner than the other parts, especially in the tentacle: far thinner than ectoderm (Figure 11E,F). Actinopharynx, with siphonoglyphs on one side, always connected to actinopharynx, and the other 11 longitudinal grooves are less deep than siphonoglyphs (Figure 11G). Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, immature testis in our specimen. Mesenteries in second cycle fertile.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores. Spirocysts are absent in the column (Figure 10, Table 8).

Etymology. *Fragum* means strawberry in Latin. This name is derived from the nematocyst batteries on the red surface of columns of this species, which look like the seeds of strawberries.

Remarks. See Table 9 for comparison to the other species of *Halcurias*. This species can be easily distinguished from any other species of *Halcurias* as they possess two types of basitrichs in their tentacles.

This halcuriid most resembles *H. pilatus* McMurrich, 1893, in external morphology, but there are no long basitrichs in the filaments of this species. The long basitrichs strongly characterize *H. pilatus* [44]. Moreover, phylogenetic analyses indicated that this halcuriid was monophyletic with *Halcurias hiroomii* sp. nov., and this anemone and *H. pilatus* were paraphyletic. Thus, we determined this sea anemone is not *H. pilatus* but a new species described here.

Genus *Synactinernus* Carlgren, 1918.

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

Synactinernus Carlgren, 1918: 30; Carlgren, 1949: 21.

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

Type species. *Synactinernus flavus* Carlgren, 1918.

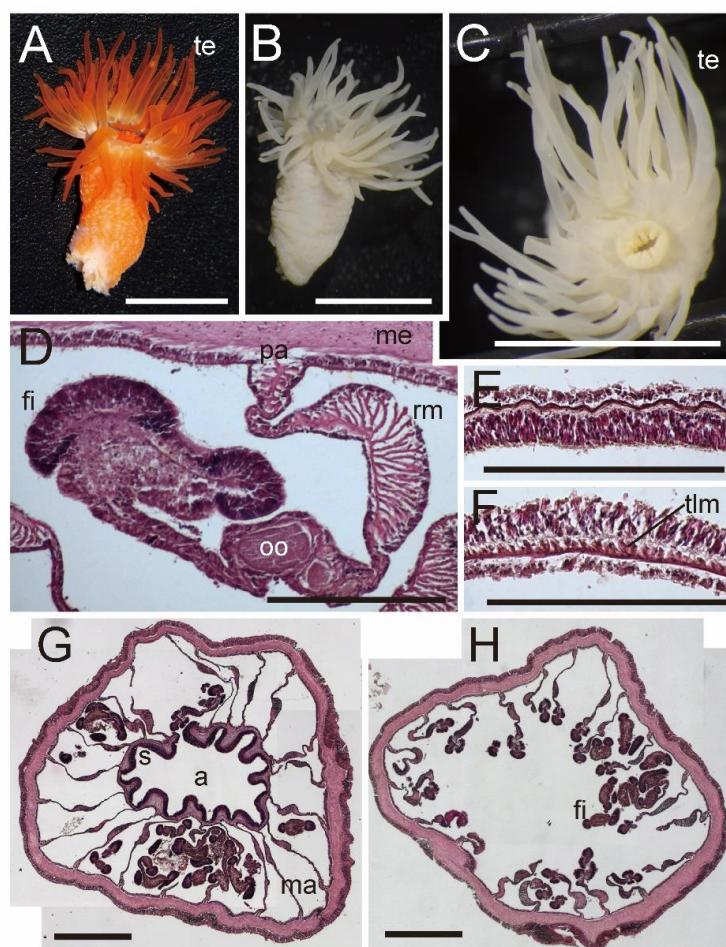


Figure 11. External and internal morphology of *Halcurias fragum* sp. nov. (CMNH-ZG 10206). (A) Lateral view of a living specimen; (B) lateral view of a fixed specimen; (C) oral view of a fixed specimen; (D) enlarged transverse section of upper column; (E) enlarged longitudinal section of tentacle; (F) enlarged transverse section of tentacle; (G) enlarged transverse section of upper column; (H) enlarged transverse section of lower column. Abbreviations: a, actinopharynx; fi, filament; ma, macrocneme; me, mesoglea; oo, oocyte; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; te, tentacle. Scale bars indicate 1 cm in (A–C), 1 mm in (G,H), 500 μ m in (D), and 100 μ m in (E,F). Picture A was taken by Akihito Omori.

Remarks. The genus *Synactinernus* was established in the family Actinernidae by Carlgren (1918) [4] monotypically for *Synactinernus flavus* Carlgren, 1918. Later, Carlgren (1949) [7] transferred this genus to Family Actinernidae Stephenson, 1922, of suborder Endocoelanthae Carlgren, 1925. Recently, Fautin and den Hartog (2003) [10] argued that *Synactinernus flavus*, the only species of *Synactinernus*, cannot be distinguished from *Isactinernus quadrilobatus* Carlgren, 1918, and so, they synonymized *Synactinernus* with *Isactinernus*. However, other studies treated this genus as valid [1]. Thus, the actual status of *S. flavus* and the validity of *Synactinernus* has remained unclear.

One century after the original description of Carlgren, Izumi et al. (2019) [14] newly collected specimens of two *Synactinernus* species and *Isactinernus quadrilobatus*. According to their morphological and phylogenetic analyses, it was suggested that the *Synactinernus* species can be distinguished from *Isactinernus quadrilobatus* both by morphology and phylogeny—*Synactinernus* species lack the thickening on the edges of their oral disc that *Isactinernus* have, and they only have 36 macrocnemes, while *Isactinernus* have over 100; *S. flavus* and *S. churaumi* formed a clade separate from *I. quadrilobatus* [14]. Thus, they re-described *Synactinernus* as a valid genus of the family Actinernidae and revised the diagnosis to accommodate *S. churaumi* Izumi and Fujii, 2019.

However, the molecular phylogeny of the present study showed that the genus *Synactinernus* is an independent clade from the other three genera of Actinernidae (Figure 3). Moreover, two Halcuriidae clades were paraphyletic to *Synactinernus*, so this genus should not be accommodated in Actinernidae but should be in Halcuriidae.

This genus is endemic in Japan and is distributed at around 300 m in depth in the East China Sea and the Pacific Ocean.

***Synactinernus flavus* Carlgren, 1918.**

(Japanese name: Kuroba-kawari-ginchaku.)

Figures 2, 3, 5, and 6 in Izumi et al. (2019).

Synactinernus flavus Carlgren, 1918: 31.

Synactinernus flavus: Izumi et al., 2019b: 531–534, Figures 2, 3, 5, and 6.

Material examined/Description. See Izumi et al. (2019) [14].

***Synactinernus churaumi* Izumi and Fujii, 2019.**

(Japanese name: Churaumi-kawari-ginchaku.)

Figures 2, 5, and 7 in Izumi et al. (2019).

Synactinernus churaumi Izumi and Fujii, 2019: Izumi et al., 2019b: 534–537; Figures 2, 5, and 7.

Material examined/description. See Izumi et al. (2019) [14].

Genus *Isohalcurias* gen. nov.

(New Japanese name: kawari-ginntyaku-modoki-zoku.)

urn:lsid:zoobank.org:act:E16FF13C-6711-4822-9F54-0803733AC956

Diagnosis. Mesenteries up to 34 pairs arranged in 4 cycles, 6 + 4 + 8 + 16. Macrocnemes, 10 pairs, fertile, filamented, and with restricted retractors. Microcnemes in only the upper part of the body, some of them perfect. Retractor muscles diffused and pinnate. Parietal muscles rather well developed to fairly weak. Tentacles, up to 68. Dioecious. Cnidom: spirocysts and basitrichs (in all tissues) and microbasic *p*-mastigophores (in actinopharynx and filaments).

Type species. *Isohalcurias carlgreni* (McMurrich, 1901).

Etymology. *Isohalcurias* is derived from “*Iso* (=Greek isos, equal)” + “*halcurias* (the divided genus)”. *Isohalcurias* species resemble species of *Halcurias* so much that they cannot be distinguished unless making sections or inspecting cnidae.

Remarks. According to our phylogenetic analyses (Figures 9 and 10), the species which were originally identified as genus *Halcurias* were paraphyletic. The outer clade, which contained *H. carlgreni*, *H. citreum* sp. nov., and *H. malum* sp. nov., did not contain the type species, *Halcurias pilatus*. Thus, this group cannot be named *Halcurias*. This is why we established the new genus *Isohalcurias* gen. nov. for these three species.

See Table 7 for a comparison with the other genera of Halcuriidae. This genus can be identified by having spirocysts in the column (comparison of *Isohalcurias* gen. nov. in Tables 8 and 10) and partly pinnate retractor muscles (e.g., Figures 12D, 14D, and 15C). In addition to the three species, *Halcurias minimus* Carlgren, 1928, and *Halcurias sudanensis* Riemann-Zürneck, 1983, are possibly in this new genus as these species have spirocysts in the body wall [11,46,47]. It is hoped to obtain molecular information on these species for phylogenetic analyses in future.

***Isohalcurias carlgreni* (McMurrich, 1901) comb. nov.**

(Japanese name: kawari-ginchaku.)

urn:lsid:zoobank.org:act:71D07E54-D6C4-4888-8FD5-4A3F5301CE7A

Figures 12 and 13; Table 10.

Halcurias Carlgreni McMurrich 1901: 159.

Halcurias carlgreni: Carlgren, 1914: 69; Uchida 2004: 9–13, Figure 1, pl. 1A, B.

Material examined. NSMT-Co 1697: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 19 September 2011, collected from Suo-Oshima, Yamaguchi Pref., Japan, at 10 m in depth, by scuba diving, by Takuma Fujii; NSMT-Co 1698: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, same collector, locality, and method as for NSMT-Co 1697; NSMT-Co 1830: whole specimen, same collector, locality,

and method as for NSMT-Co 1697; CMNH-ZG 10218: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared; collected by trawling of fishing boat Jinsho-Maruo from Kumano-nada Sea off Mie Pref. (depth and date unknown), by Takeya Moritaki, kept in Toba Aquarium for a while, and preserved on 18 June 2017 by Takato Izumi; CMNH-ZG 10219: juvenile specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 19 September 2015, collected from west-off Uki-shima, Chiba Pref., Japan, at 20 m in depth, by scuba diving, by Junji Okuno; CMNH-ZG 10220: whole specimen, same collector, locality, and method as for CMNH-ZG 10219; UUZM 705b, 705c (syntypes): on 14 March 1890, Hirado Strait (33°5' N, 129°16' E); UUZM 705a (syntype): in 1893, off Hirado (33°15' N, 129°15' E), 45 fathoms (ca. 82 m) depth; UUZM 705d (syntype; Figure 7E): Korea Strait 65 fathoms (ca. 120 m) depth; ZMNH-TYPE-4908 (syntype): in 1893, China sea, 30 fathoms (ca. 55 m) depth; ZMNH-TYPE-1200 (syntype; Figure 7F): in 1893, off Hirado (33°15' N, 129°15' E), 45 fathoms (ca. 82 m) depth.

Description. External anatomy. Body cylindrical (Figure 12A,B), up to 17–70 mm in height and ca. 11–50 mm in width. Column surface comparatively smooth, with reddish-orange ectoderm layer and sparse small nematocyst batteries. Nematocysts densely distributed, especially in nematocyst batteries. Tentacles simple, all marginal, 6–40 mm in length, no thickenings including their aboral base, pale white in color, and 68 in number on oral disk; inner and outer ones alternatingly bared (Figure 12A). The tip of tentacles pointed. Basal disc distinct and adhesive (Figure 12A), opaque, and mesenterial insertion invisible. Oral disk diameter as broad as column, hemi-transparent, pale white. Mouth at center of oral disk, highly swelled, lip-like, groove on surface, white in color.

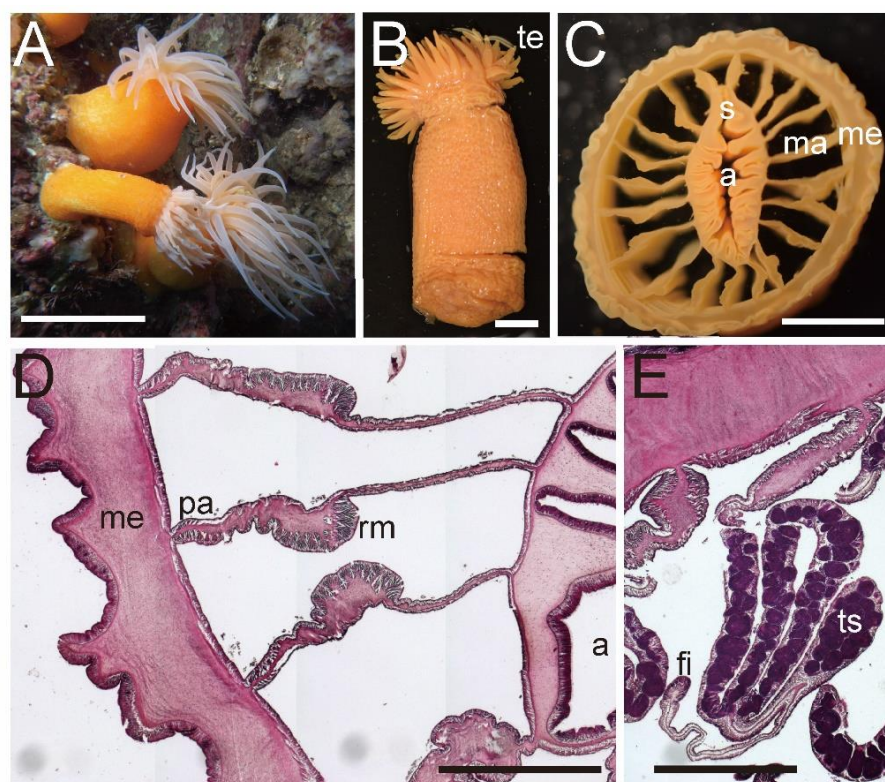


Figure 12. External and internal morphology of *Isohalcurias carlgreni* comb. nov. (NSMT-Co 1697). (A) Living individuals in nature; (B) lateral view of a fixed specimen; (C) transverse section of upper column; (D) enlarged transverse section of upper column; (E) enlarged transverse section of lower column. Abbreviations: a, actinopharynx; fi, filament; ma, macrocneme; me, mesoglea; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; te, tentacle; ts, testis. Scale bars indicate 5 cm in (A), 1 cm in (B,C), 1 mm in (D), and 500 µm in (E). Picture A was taken by Takuma Fujii.

Internal anatomy. Twenty (ten pairs of) macrocnemes on actinopharynx: twelve, including four directives, in the first cycle and eight in the second cycle (Figure 12C). Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, an arrangement obeying the rule of mesenterial arrangement of Actinernidae. Mesenteries in third and fourth cycle microcnemes. Each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle exocoelotic and circular muscle endocoelotic; retractor muscles restricted near parietal muscles, obviously developed and pinnate (Figure 12D). Muscle processes well-branched, around 8–15 in each muscle pennon (Figure 12D). Parietal muscles of macrocnemes comparatively developed and distinct with simple or slightly branched 4–11 processes in each side. Mesoglea thickest in body wall and actinopharynx, generally thicker than ectoderm and endoderm (Figure 12D). Mesoglea thinner than the other parts, especially in the tentacle: far thinner than ectoderm. Actinopharynx, with siphonoglyphs on one side, always connected to actinopharynx, and the other 15–19 longitudinal grooves are less deep than siphonoglyphs (Figure 12C). Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, immature testis in our specimen (Figure 12E). Mesenteries in second cycle fertile.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores. Spirocysts are numerous in the column (Figure 13, Table 10).

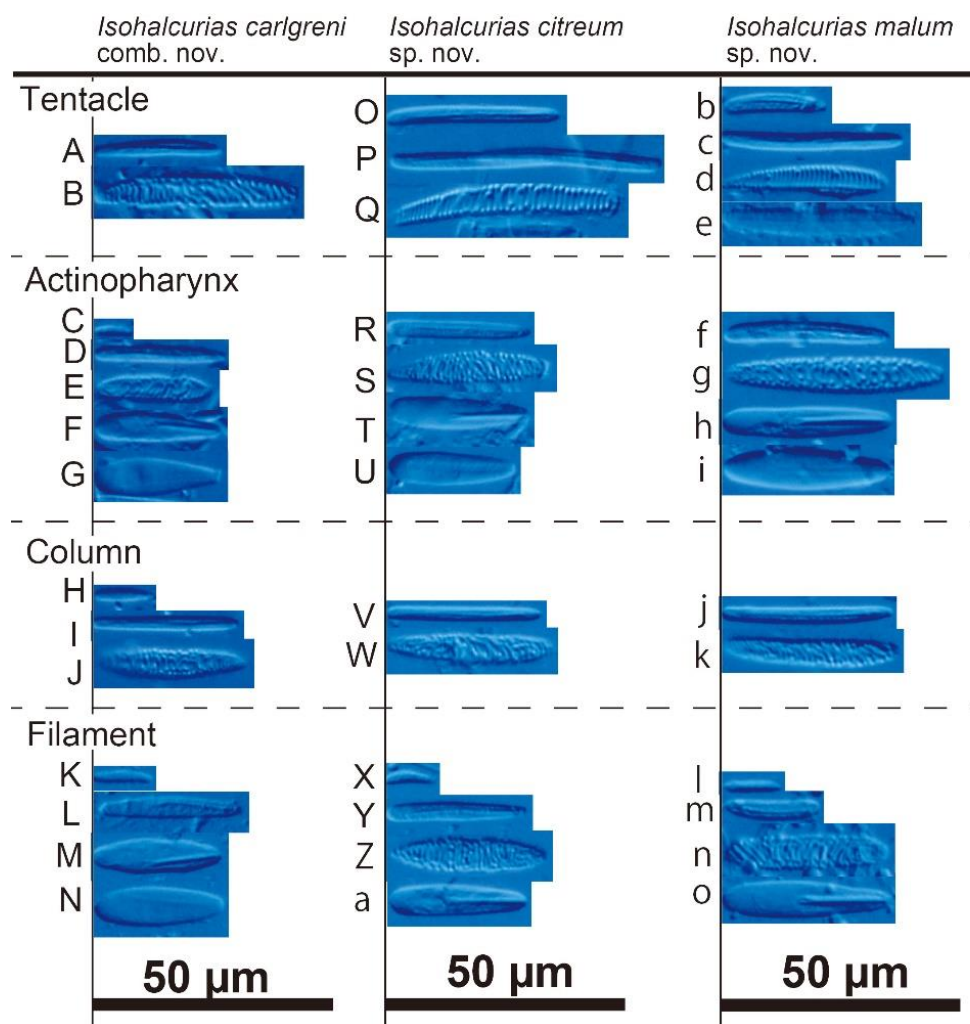


Figure 13. Cnidoms of *Isohalcurias carlgreni* comb. nov., *I. citreum* sp. nov., and *I. malum* sp. nov. A–N: *I. carlgreni*. A–B: tentacle; A, basitrich; B, spirocyst. C–G: actinopharynx; C, small basitrich; D, large basitrich; E, spirocyst; F, microbasic *p*-mastigophore; G, microbasic *b*-mastigophore. H–J: column; H, small basitrich; I, large basitrich; J, spirocyst. K–N: filament; K, small basitrich; L, large basitrich; M, microbasic *p*-mastigophore; N, microbasic *b*-mastigophore. O–a: *I. carlgreni*. O–Q:

tentacle; O, small basitrich; P, large basitrich; Q, spirocyst. R–U: actinopharynx; R, basitrich; S, spirocyst; T, microbasic *p*-mastigophore; U, microbasic *b*-mastigophore. V–W: column; V, basitrich; W, spirocyst. X–a: filament; X, small basitrich; Y, large basitrich; Z, spirocyst; a, microbasic *p*-mastigophore. b–o: *I. malum*. b–e: tentacle; b, small basitrich; c, small basitrich; d, spirocyst; e, microbasic *b*-mastigophore. f–i: actinopharynx; f, basitrich; g, spirocyst; h, microbasic *p*-mastigophore; i, microbasic *b*-mastigophore. j–k: column; j, basitrich; k, spirocyst. l–o: filament; l, small basitrich; m, large basitrich; n, microbasic *p*-mastigophore; o, microbasic *p*-mastigophore.

Table 10. Cnidoms of the species of *Isohalcurias* gen. nov.

	<i>Isohalcurias carlgreni</i> comb. nov. NSMT–Co 1697			<i>Isohalcurias citreum</i> sp. nov. CMNH–ZG 10221			<i>Isohalcurias malum</i> sp. nov. NSMT–Co 1699		
	Length × Width (µm)	frequency	N	Length × Width (µm)	frequency	N	Length × Width (µm)	frequency	N
Tentacle									
basitrichs	13.1–38.4 × 2.4–7.8	numerous	30	27.0–34.8 × 2.6–4.1 47.4 × 5.2	numerous rare	61 1	15.8–19.9 × 3.0–3.5 28.9–39.1 × 2.7–4.4	rare numerous	3 37
spirocysts	20.6–26.3 × 2.6–3.7	numerous	101	22.5–42.9 × 2.6–5.9	numerous	43	21.8–41.2 × 4.0–7.3	numerous	25
microbasic <i>b</i> -mastigophores							30.1–36.3 × 3.4–6.0	numerous	11
Actinopharynx									
basitrichs	S 8.4–12.9 × 1.5–2.4 L 20.6–27.8 × 2.7–3.9	numerous numerous	19 13	22.6–30.8 × 3.0–4.3	numerous	62	25.7–36.2 × 3.2–4.5	numerous	37
spirocysts	21.0–25.5 × 4.3–5.9	few	5	22.6–33.9 × 4.1–7.9	numerous	51	31.5–52.0 × 5.8–9.6	numerous	64
microbasic <i>p</i> -mastigophores	19.3–23.7 × 4.5–6.3	numerous	13	23.4–29.2 × 5.2–7.0	numerous	39	29.0–35.2 × 5.3–7.2	numerous	16
microbasic <i>b</i> -mastigophores	20.6–23.9 × 5.4–6.4	rare	3	25.6–28.6 × 4.3–5.4	rare	4	30.1–37.1 × 5.6–7.4	few	6
Column									
basitrichs	S 7.3–11.3 × 1.5–2.6 L 23.4–31.6 × 2.2–3.8	numerous numerous	23 57	23.6–34.1 × 2.3–3.5	numerous	24	23.8–34.2 × 2.7–4.1	numerous	37
spirocysts	15.7–27.5 × 3.9–5.2	numerous	13	24.7–32.3 × 4.2–7.0	numerous	69	27.8–37.1 × 4.2–6.1	numerous	27
Filament									
basitrichs	S 7.3–11.9 × 1.5–2.8 L 24.5–25.3 × 3.9–4.2	numerous rare	38 2	7.0–12.5 × 1.5–2.8 25.9–30.1 × 2.7–4.2	numerous numerous	47 24	9.9–19.8 × 1.7–4.4 26.7–28.0 × 3.8–5.0	numerous rare	48 2
spirocysts				7.5–31.4 × 1.6–6.9	numerous	71	28.3–33.3 × 5.4–7.1	rare	4
microbasic <i>p</i> -mastigophores	14.4–26.3 × 5.0–8.4	numerous	41	23.2–27.7 × 5.4–7.6	numerous	28	25.2–34.8 × 5.6–9.2	numerous	52
microbasic <i>b</i> -mastigophores	23.6–25.3 × 6.2–7.8	rare	3						

Remarks. This species was described as *Halcurias carlgreni* in McMurrich (1901) [49] and reported from Japan in 2004 [11]. *H. carlgreni* was characterized by the ectoderm of the column containing numerous spirocysts [11]. Our specimens corresponded well with these descriptions, so we identified them as *H. carlgreni*.

However, as mentioned above, *Halcurias* became paraphyletic to *Synactinernus*. The clade, including *Halcurias carlgreni*, *H. citreum* sp. nov., and *H. malum* sp. nov., did not contain *Halcurias pilatus*, the type species (Figure 4). Thus, these species should be moved to the new genus *Isohalcurias*, and thus, *H. carlgreni* becomes *Isohalcurias carlgreni* comb. nov.

***Isohalcurias citreum* sp. nov.**

(Japanese name: oo-kawari-ginchaku.)

urn:lsid:zoobank.org:act:AB02934A-BFDF-400B-8432-E33092D96DE9

Figures 13 and 14; Table 10.

Halcurias levis (nomen nudum) Uchida, 2004: 16, Figures 3 and 4, pl. 1F.

Material examined. Holotype. NSMT–Co 1831: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 28 January 2017 from Kuju-kushima (off Kuroshima), Nagasaki Pref., 35 m in depth, bycatch with fishes by gill net, kept in the tank of Saikai National Park Kuju-kushima Aquarium, and preserved on 14 April 2017 by Takato Izumi. **Paratype.** CMNH 10221: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 7 October 2008 from Aishima Island, Hagi, Yamaguchi Pref., 47 m in depth, collected by scuba diving by Kensuke Yanagi. **Other specimen.** CMNH–ZG10222:

specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, collected on 17 May 2018 off Yaku-shima Island (30°09.39' N, 130°38.03' E; St. 3) at 200–255 m in depth, using beam trawl, during research cruise of R/V Toyoshio-Maruru, by Itaru Kobayashi.

Description. External anatomy. Body cylindrical (Figure 14A,B), up to ca. 150 mm in height and ca. 100 mm in width in a living specimen and 34–90 mm in height and 20–35 mm in width in a preserved specimen. Column surface smooth and without nematocyst batteries. Whole body bright yellow in color. Nematocysts sparsely distributed on column. Upper part of column narrower, same color as column, simple and not thrown into any lobes. Tentacles simple, all marginal, 30–50 mm in length, no thickenings including their aboral base, bright yellow in color, and 68 in number; inner and outer ones alternately bared (Figure 14A). The tips of tentacles pointed. Basal disk in aboral end (Figure 14A), yellowish opaque and mesenterial insertion inapparently visible. Oral disk diameter narrower than column, hemi-transparent, bright yellow. Mouth at center of oral disk, highly swelled, lip-like, groove on surface, bright yellow.

Internal anatomy. Twenty (ten pairs of) macrocnemes on actinopharynx: twelve, including four directives, in the first cycle and eight in the second cycle (Figure 14C). Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, an arrangement obeying the rule of mesenterial arrangement of Actinernidae. Mesenteries in third and fourth cycle microcnemes. Each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle and circular muscle too weak to observe in histological section (Figure 14E); retractor muscles diffused, weak, and pinnate (Figure 14D,F). Muscle processes well branched, around 9–17 in each muscle pennon (Figure 14D). Parietal muscles of macrocnemes well developed and distinct. Parietal muscle weak, with simple or slightly branched processes, around 10 in each side (Figure 14D,F). Mesoglea thickest in body wall, and even thick in mesenteries and actinopharynx, far thicker than ectoderm and endoderm (Figure 14C,D). Actinopharynx, with siphonoglyphs on one side, always connected to actinopharynx, and the other 19 longitudinal grooves are as deep as siphonoglyphs (Figure 14C). Sphincter muscle absent. On the aboral end, basilar muscle absent. Dioecious, matured ovaries in NSMT-Co 1831 (Figure 14F). Mesenteries in second to third cycle fertile.

Cnidom. Basitrichs, spirocysts, and microbasic *p*-mastigophores. Spirocysts are numerous in the column (Figure 13, Table 10).

Etymology. Citreum means lemon in Latin. This name is derived from the fluorescent lemon-yellow color of this species.

Remarks. Uchida (2004) [11] described this species as *Halcurias levis*. However, this species name became nomen nudum for the same reason as *H. japonicus*. The specimens we collected correspond well to the description of Uchida [11] morphologically, so we report them as this species provisionally.

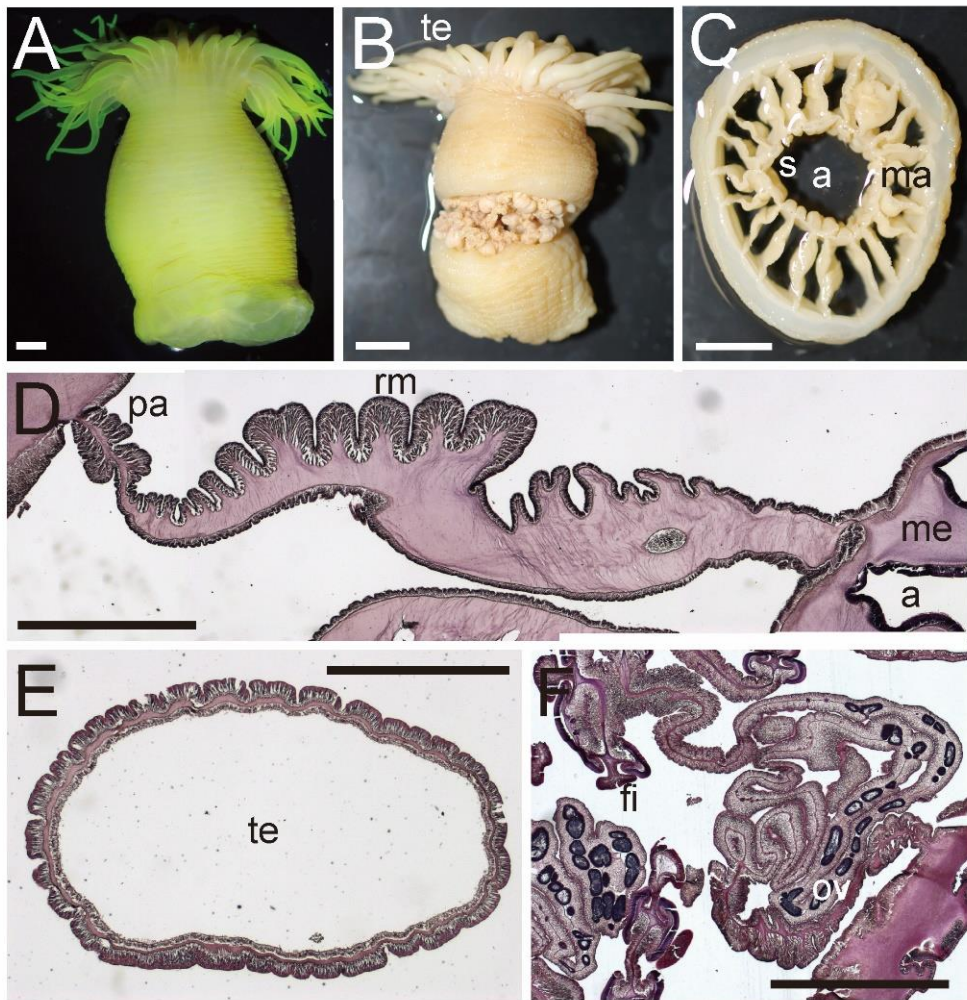


Figure 14. External and internal morphology of *Isohalcurias citreum* sp. nov. (NSMT-Co 1831). (A) Lateral view of a living specimen; (B) lateral view of a fixed specimen; (C) transverse section of dissected specimen; (D) enlarged transverse section of upper column; (E) enlarged longitudinal section of lower column. (F) enlarged transverse section of lower column; Abbreviations: a, actinopharynx; fi, filament; ma, macrocneme; me, mesoglea; ov, ovary; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; te, tentacle. Scale bars indicate 1 cm in (A–C) and 1 mm in (D,E).

This species was accommodated in the clade of *Isohalcurias* but cannot be obviously separated from *I. carlgregeni* comb. nov. in the phylogenetic tree (Figure 10). However, there were several differences between these halcuriids, e.g., body sizes, presence or absence of nematocyst batteries, development of retractor muscles, and so forth (see Table 11). Judging by the common view on phylogenetic analyses of sea anemones with the combination of several molecular markers [13,50,51], it is probable that any simple DNA marker phylogenetic analysis method of the present day cannot distinguish *I. carlgregeni* and *I. citreum* sp. nov. in a phylogenetic tree. In conclusion, this study described this species as *Isohalcurias citreum* sp. nov. by morphology.

This species had been discovered only in Syoga-se, off Wakayama Pref. [11,52,53], but we newly confirmed it in the Sea of Japan (CMNH-ZG 10221), Goto-Nada Sea (NSMT-Co 1831), and the East China Sea (CMNH-ZG 10222).

***Isohalcurias malum* sp. nov.**

(New Japanese name: ringo-kawari-ginchaku.)

urn:lsid:zoobank.org:act:6E2B09F5-3C94-4BAD-B690-E12C13E9711B

Figures 13 and 15; Table 10.

Material examined. Holotype. CMNH-ZG 10223: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, originally collected on 15 May 2005 from Ryukyu Trough off Kume-jima Island (25°30.96' N, 126°29.21' E; St. OT-14), at 372–375 m in depth, using beam trawl, during research cruise of R/V Hakuho-Maru by Kensuke Yanagi. **Paratypes.** NSMT-Co 1699: specimen dissected, tissues embedded in paraffin, histological sections prepared, nematocysts prepared, originally collected on 24 May 2015, west off Cape Sata (31°02.06' N, 130°33.37' E; St. 7), at 202 m in depth, using biological dredge, during research cruise of R/V Toyoshio-Maru by Mikihito Arai and Akito Ogawa; CMNH-ZG 10224: specimen dissected, tissues embedded in paraffin, nematocysts prepared, collected on 23 May 2019, west off Amami-Oshima Island (28°22.42' N, 129°15.14' E), at 315 m in depth, using beam trawl, during research cruise of R/V Toyoshio-Maru by Itaru Kobayashi. Other specimen: CMNH-ZG 10225: whole specimen, originally collected on January 2018 at Uragami, Wakayama Prefecture, at 100–130 m by Isao Hirabayashi, and fixed by Takuma Fujii and Kensuke Yanagi from the tank at the Kushimoto Marine Park on 22 May 2018.

Description. External anatomy. Body cylindrical (Figure 15A), up to ca. 50 mm in height and ca. 30 mm in width in a living specimen and 25–40 mm in height and 10–20 mm in width in a preserved specimen. Column surface smooth and without nematocysts batteries, pale red to pale orange ectoderm layer, and sometimes fine dark red or orange patches on the middle column. Aboral end of column pale yellow. Nematocysts sparsely distributed on column. Upper part of column a little expanded, same color as column, simple and not thrown into lobes. Tentacles simple, all marginal, 10–20 mm in length, no thickenings including their aboral base, bright red to orange in color (more blight color on distal side), and 68 in number on oral disk; inner and outer ones alternatingly bared. The tips of tentacles pointed. Basal disk in aboral end (Figure 15A), yellowish opaque and mesenterial insertion inapparently visible. Oral disk diameter broader than column, hemi-transparent, pale white. Mouth at center of oral disk, highly swelled, lip-like, groove on surface, bright orange.

Internal anatomy. Twenty (ten pairs of) macrocnemes on actinopharynx: twelve, including four directives, in the first cycle and eight in the second cycle (Figure 15B). Macrocnemes in the second cycle born in the endocoel of the first cycle mesenteries, an arrangement obeying the rule of mesenterial arrangement of Actinernidae. Mesenteries in third and fourth cycle microcnemes (Figure 15B). Each tentacle between either exo- or endocoelic. Tentacular longitudinal muscle and circular muscle too weak to observe in histological section (Figure 15D); retractor muscles diffused but obviously developed and pinnate (Figure 15C, E). Muscle processes well branched, around 10–20 in each muscle pennon (Figure 15C). Parietal muscles of macrocnemes well developed and distinct. Parietal muscle processes well branched, around 10 in each side (Figure 15E). Mesoglea thickest in body wall and actinopharynx (Figure 15B,E), far thicker than ectoderm and endoderm. Mesoglea a little thinner than the other parts, but generally thicker than ectoderm and endoderm (Figure 15C,D). Actinopharynx, with siphonoglyphs on one side, always connected to actinopharynx, and the other 11 longitudinal grooves are as deep as siphonoglyphs (Figure 15B). Sphincter muscle absent. On the aboral end, basilar muscle absent (Figure 15G). Dioecious, matured ovaries in NSMT-Co 1699 (Figure 15F). Mesenteries in second to third cycle fertile.

Cnidom. Basitrichs, spirocysts, microbasic *p*-mastigophores, and microbasic *b*-mastigophores. Spirocysts are numerous in the column (Figure 13, Table 10).

Etymology. *Malum* means apple in Latin. This name is derived from the red and yellow color pattern of this species, which resembles apples.

Remarks. See Table 11 for a comparison with the other species of *Isohalcurias* and some species possibly assignable to this genus; since they have spirocysts on their columns [46,47], these species of *Halcurias* are possibly assignable to the genus *Isohalcurias*. However, molecular phylogenetic analyses of them have not been carried out. Thus, new combinations were not proposed for these species in this study.

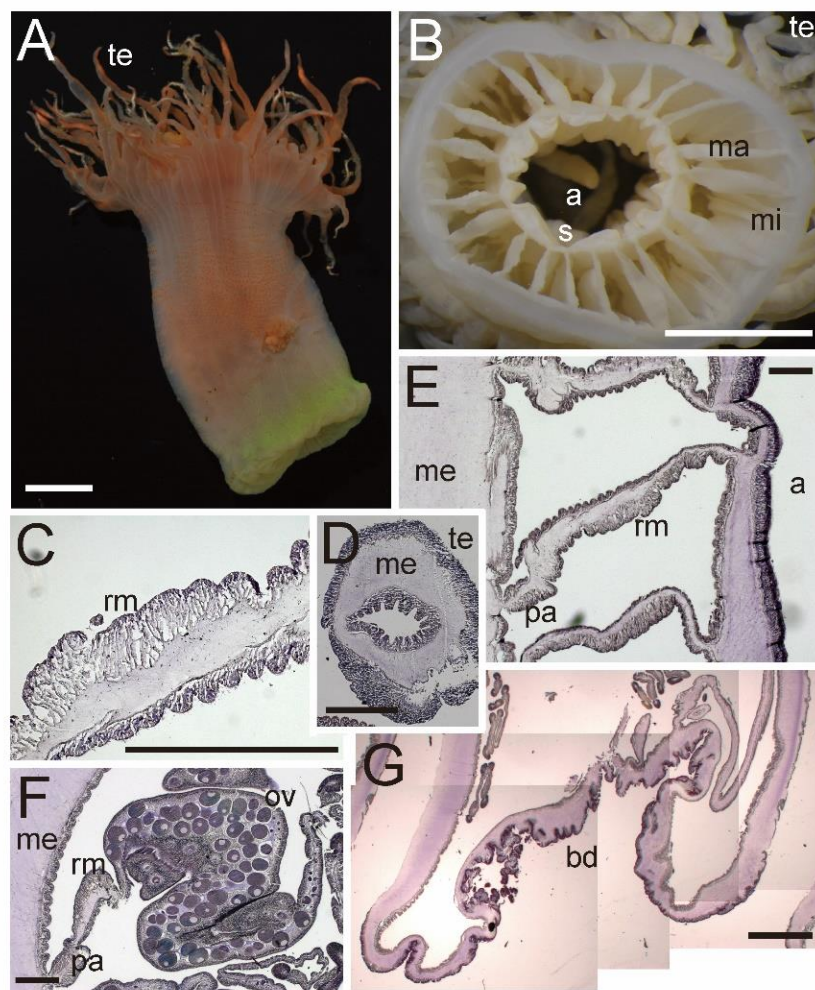


Figure 15. External and internal morphology of *Isohalcurias malum* sp. nov. (NSMT–Co 1699). (A) Lateral view of a living specimen (photographed by Mikihito Arai); (B) transverse section of dissected specimen; (C) enlarged view of retractor muscle; (D) transverse section of tentacle; (E) enlarged transverse section of upper column; (F) enlarged transverse section of lower column; (G) longitudinal section of basal disc. Abbreviations: a, actinopharynx; bd, basal disc; ma, macrocneme; me, mesoglea; mi, microcneme; ov, ovary; pa, parietal muscle; rm, retractor muscle; s, siphonoglyph; te, tentacle. Scale bars indicate 1 cm in (A,B), 1 mm in (G), 500 μ m in (C,E,F), and 200 μ m in (D).

According to the Uchida's taxonomic key [11], these sea anemones are most similar to *Halcurias minimus* Carlgren, 1928, because they have a column without longitudinal muscles, an actinopharynx containing large spirocysts, a column containing spirocysts (though no nematocyst batteries), and tentacles with two types of basitrichs. However, there are several differences between these anemones and *H. minimus*: there are no nematocyst batteries on this species, while they are sparsely distributed on the column of *H. minimus* [11,46]; the size of *H. minimus* is 0.8 cm [46], approximately one-fourth of the size of our specimens. Moreover, *H. minimus* only inhabits the deep sea of the circum-anti-boreal region, far distant from the localities of this anemone, so it is unlikely that the same species lives in Japan, especially in the seas of the temperate zone. Thus, we concluded that these anemones represent a new species, *Isohalcurias malum* sp. nov.

Table 11. Comparison of *Isohalcurias malum* sp. nov. and the other species of *Isohalcurias* gen. nov., including a species possibly assignable to the genus.

	<i>Isohalcurias malum</i> sp. nov.	<i>Isohalcurias carlgreni</i> (McMurrich, 1901) comb. nov.	<i>Isohalcurias citreum</i> sp. nov.	* <i>Halcurias minimus</i> Carlgren, 1928	* <i>Halcurias sudanensis</i> Riemann-Zürneck, 1983
Characters					
Size (height)	25–40 mm	11–50 mm	34–90 mm	8 mm	30 mm
Retractor muscle	Distinct	Distinct	Weak	Distinct	Weak
	Pinnate	Strongly pinnate	Strongly pinnate	Pinnate	Slightly Pinnate
Parietal muscle	Distinct	Weak	Weak	Weak	Weak
	with well-branched muscular processes	with simple muscular processes	with simple muscular processes	with simple muscular processes	with simple muscular processes
Basitrichs of tentacles	2 types	1 type	2 types	2 types	1 type
Spirocysts in actinopharynx	Present	Present	Present	Absent	Present
Spirocysts in column	Present	Present	Present	Present (but sparse)	Present
Long basitrichs in filaments	Absent	Absent	Absent	Unknown	Absent
References	The present study	[11,49] The present study	[11] The present study	[11,46]	[47]

* Species without molecular phylogenetic information.

4. Conclusions

Our taxonomic and phylogenetic study resulted in these conclusions.

1. The superfamily Actinernoidea Stephenson, 1922, is classified into three families, Actinernidae, Halcuriidae, and Isactinernidae fam. nov.
2. Actinernidae Stephenson, 1922, was polyphyletic—the genus *Synactinernus* Carlgren, 1918, was in the clade of Halcuriidae Carlgren, 1918, and thus, it does not belong to the actinernid sea anemones anymore. The other three genera, *Actinernus* Verrill, 1879, *Isactinernus* Carlgren, 1918, and *Synhalcurias* Carlgren, 1914, appeared to be monophyletic but were divided into two families, Actinernidae and Isactinernidae fam. nov., because of a lack of common features.
3. The genus *Halcurias* McMurrich, 1893, was paraphyletic—it was divided into two clades because this genus was paraphyletic to *Synactinernus*. The taxon including the type species remains as *Halcurias*, and the other became a new genus, *Isohalcurias* gen. nov. *Isohalcurias* can be distinguished from *Halcurias* by the presence of spirocysts in columns and developing pinnate retractor muscles.
4. Four new species of Actinernoidea were described, and thus, there are 11 Japanese species of Actinernoidea in total. As a consequence of our study, over a half of Actinernoidea species (11 species out of 23) have been confirmed in Japan.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15060773/s1>, Figure S1: Maximum likelihood tree of the superfamily Actinernoidea based on the combined dataset of nuclear 18S, 28S, and 5.8S rDNA; and ITS1/ITS2; Figure S2: Bayesian tree of the superfamily Actinernoidea based on the combined dataset of nuclear 18S, 28S, and 5.8S rDNA; and ITS1/ITS2; Figure S3: Maximum likelihood tree of the superfamily Actinernoidea based on the combined dataset of mitochondrial 12S and 16S; Figure S4: Bayesian tree of the superfamily Actinernoidea based on the combined dataset of mitochondrial 12S and 16S.

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