Zootaxa 4344 (2): 277–290
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4344.2.4
http://zoobank.org/urn:lsid:zoobank.org:pub:91059357-3476-415F-B534-D20F8319E05F

Scolionema sanshin sp. n., a new species (Hydrozoa, Limnomedusae, Olindiidae) from the Ryukyu Archipelago, southern Japan

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Abstract

A new species of hydrozoan jellyfish belonging to the order Limnomedusae is reported from the Ryukyu Archipelago, Southern Japan. The species belongs to the genus *Scolionema*, which prior to this study includes just a single valid species, *S. suvaense*. This name, however, has several junior synonyms and considerable diversity has been reported for different populations from Mediterranean, Indian ocean and Pacific ocean localities, including Central Japan. The species described in this paper, *Scolionema sanshin* **sp. n.**, can be differentiated from all other described populations of *Scolionema* based on shape of gonad, number of tentacles of its medusa stage, and/or genetic sequences. A discussion of the diversity presently united under the name *S. suvaense* suggests that additional work is necessary to clarify the true number of *Scolionema* species.

Key words: Development, envenomation, hydromedusae, medusa budding, Okinawa, polyp

Introduction

The order Limnomedusae Kramp, 1938 so far comprises about 50 described species in four families: Armorhydridae Swedmark & Teissier, 1958, Microhydrulidae Bouillon & Deroux, 1967; Monobrachiidae Mereschkowsky, 1877 and Olindiidae Haeckel, 1879 (Bouillion *et al.* 2006). Limnomedusae are characterized by having the following characters: 1) medusae with gonads along radial canals or unusually on manubrium, 2) marginal tentacles peripheral, hollow, without true basal bulb, tentacle base usually with a parenchymatic endodermal core embedded in the umbrellar mesoglea, 3) marginal sense organs as internal enclosed ecto-endodermal statocysts embedded in the mesoglea near the ring canal or in the velum (Bouillon and Boero 2000). The first identified species of Limnomedusae, *Maeotias marginata* (Modeer, 1791) was described by Modeer (1791) (as *Medusa marginata*). Kramp (1938) erected the order Limnomedusae to accommodate three families Moerisiidae Poche, 1914, Olindiidae, and Williidae Forbes, 1846. Hand & Hendrickson (1950), Swedmark and Teissier (1958) and Bouillon and Deroux (1967) erected the three families, Proboscidactylidae, Armorhydridae and Microhydrulidae, respectively. Naumov (1960) regarded Monobrachiidae as a family in the order. Bouillon (1985) classified only two families, Monobrachiidae and Olindiidae within the Limnomedusae, not including Armorhydridae or Microhydrulidae. Additionally, molecular phylogenetic analyses have suggested that Moerisiidae and Proboscidactylidae are more closely related to anthoathecate species than they are to Limnomedusae (Collins 2002; Collins *et al.* 2006).

Limnomedusae are widely distributed in the Pacific, Atlantic and Indian Oceans (Kramp 1961). Although most limnomedusan species occur in salt waters, *Astrohydra japonica* Hashimoto, 1981, *Calpasoma dactylopterum* Fuhrmann, 1939, *Craspedacusta* species and *Limnocnida* species appear in freshwaters such as lakes, ponds and wells (Oka and Hara 1922; Hashimoto 1981; Jankowski 2001; Jankowski *et al.* 2008, Lewis *et al.* 2012). Several limnomedusan species are known to cause societal problems. *Maeotias marginata* (Modeer, 1791) and *Gonionemus vertens* A. Agassiz, 1862 are well known non-indigenous medusae that have been transported through ship's ballast water or as a part of the fouling community on ships (Edwards 1976; Bakker 1980; Rees and

Accepted by A. Collins: 2 Oct. 2017; published: 7 Nov. 2017

Gershwin 2000; Miglietta and Lessios, 2009; Rodriguez *et al.* 2014; Toyokawa and Fujii 2015). Envenomation by *G. vertens* and *Olindias sambaquiensis* Müller, 1861 poses a serious problem to public health (Pigulevsky and Michaleff 1969; Arai and Brinkmann-Voss 1980; Mianzan and Ramirez 1996; Resgalla *et al.* 2011).

The genus *Scolionema* Kishinouye, 1910 is a monospecific taxon comprising *Scolionema suvaense* (A. Agassiz & Mayer, 1899) in the family Olindiidae (Bouillion *et al.* 2006). *Scolionema* has been confused with the genus *Gonionemus* (Kramp 1965; Goy 1973). The two genera share the following characters: 1) four simple radial canals, 2) no centripetal canals, 3) evenly distributed marginal tentacles of a single kind, with rudimentary organs of adhesion (Bouillion *et al.* 2006). However, *Scolionema* can be distinguished from *Gonionemus* by the number of statocysts and distribution of gonads (*Scolionema* with 16 or less statocysts and gonads on outer half of radial canals).

Nine species of Limnomedusae have been reported in Japanese waters (Kubota and Gravili 2007; Toyokawa and Fujii 2015) (Table 1). In this study, ten specimens of an unknown species of Limnomedusae were collected from southern Japan. These morphological and molecular phylogenetic analyses suggest that the limnomedusan species should be regarded as a new species within the genus *Scolionema* Kishinouye, 1910.

TABLE 1. List of Japanese Limnomedusae. a Hashimoto 1981; b Kubota & Gravili 2007; c Oka & Hara 1922; d Lewis *et al.* 2012; e Nagao 1973; f Kakinuma 1971; g Toyokawa & Fujii 2015; h Kishinouye 1910; i Goy 1973.

Scientific name	Japanese name	Reference
Astrohydra japonica Hashimoto, 1981	Yume-no-kurage	a, b
Craspedacusta iseana (Oka & Hara, 1922)	Ise-mamizu-kurage	b, c
Craspedacusta sowerbii Lankester, 1880	Mamizu-kurage	b, d
Eperetmus typus Bigelow, 1915	Kita-kurage	b, e
Gonionemus vertens A. Agassiz, 1862	Kaginote-kurage	b, f
Maeotias marginata (Modeer, 1791)	Kisui-kurage	g
Monobrachium parasitum Mereshkovsky, 1877	Katate-ningyo	b
Olindias formosus Goto, 1903	Hanagasa-kurage	b
Scolionema suvaense (A. Agassiz & Mayer, 1899)	Komochi-kaginote-kurage	b, h, i

Methods

Collection and Fixing. Specimens were collected using an underwater fish-luring lamp (YF-500, Hapyson, Japan) and a reflector floodlight (T-3, Nikko Electric, Japan) at Ginowan, Motobu, Nago, Aka Island and Ishigaki Island, southern Japan between 4 July 1999 and 10 June 2014 (Fig. 1). Medusae were taken by a scoop, 170 mm in diameter. The medusae were fixed in 5% formalin in seawater, and deposited in the National Museum of Nature and Science, Tsukuba, Japan (NSMT). Some specimens were preserved in 99.5 % ethanol for DNA extraction.

Morphological investigation. Taxonomic observation and measurements were made on living or preserved specimens. Measurements were made with Image J (NIH, USA) to the nearest 0.1 mm. For nematocyst identification in the medusae, squashes prepared from fresh tissues were examined under a compound microscope (CH 40, OLYMPUS, Japan). Nematocysts were identified according to Kubota (1976) and Östman (2000). For determination of the abundance of nematocyst types in medusae, roughly 400 nematocysts were identified, measured and counted from cultured specimens.

Molecular phylogenetic analysis. Near complete sequences of the nuclear 18S rDNA genes (approximately 1400 bp) were used for molecular phylogenetic analysis. Genomic DNA was extracted from the 99.5 % ethanol-preserved tissue of cultured specimens using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. 18S rDNA was PCR amplified and sequenced using primers and protocols outlined in Collins *et al.* (2008). The new sequences were aligned using MEGA 6.06 with built in ClustalW. Phylogenetic analysis and pairwise distance measurements were determined using the maximum likelihood method with 1000 bootstrap replications in MEGA 6.06. All sequences have been deposited in DDBJ under accession numbers LC320028-LC320029 for the new species (Table 2).

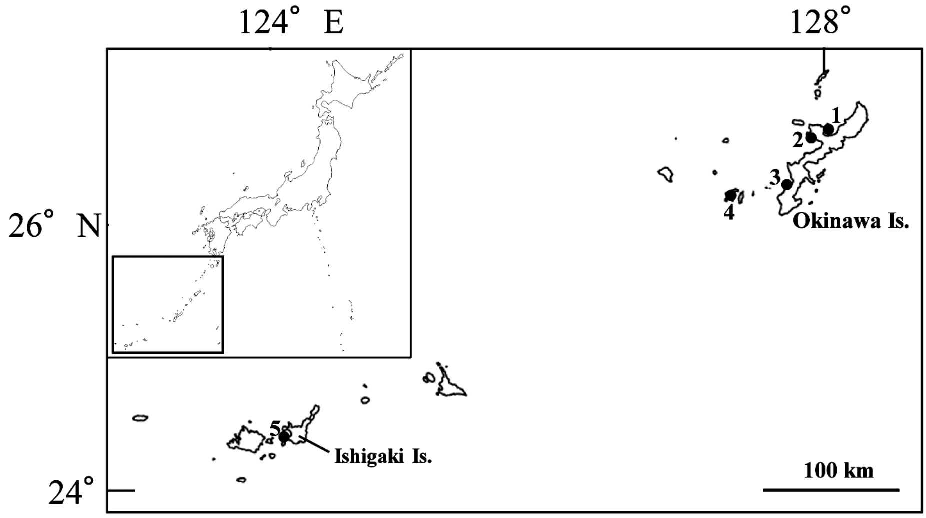


FIGURE 1. Map of the sampling sites. 1 Yagaji Is., Nago; 2 Motobu port, Motobu; 3 Ginowan Marina, Ginowan; 4 Hizushihama, Aka Is.; 5 Fusaki Beach, Ishigaki Is.

TABLE 2. Taxa included in the phylogenetic analyses and accession numbers for sequences. Sequences obtained in this study are in bold.

Species	Database	Accession No.
Craspedacusta sowerbii	GenBank	FJ897537
Craspedacusta sinensis	GenBank	EU247815
Maeotias marginata	GenBank	AF358056
Maeotias marginata	GenBank	AF358057
Limnocnida tanganyicae	GenBank	AY920755
Aglauropsis aeora	GenBank	AY920754
Olindias phosphorica	GenBank	AY920753
Olindias formosus	GenBank	KF184030
Olindias sambaquiensis	GenBank	EU247814
Scolionema suvaense	GenBank	AB231884
Scolionema sanshin **sp. n.**	DDBJ	LC320028
Scolionema sanshin **sp. n.**	DDBJ	LC320029
Monobrachium parasiticum	GenBank	AY920752
Solmundella bitentaculata	GenBank	EU247811

Observation of life cycle. Male and female medusae collected from Nago were kept in a deep petri-dish (diameter 149 mm, height 91 mm, water volume 1500 mL) with filtered seawater (1 µm) at about 30°C in the laboratory at Kitasato University. Different developmental stages (medusa, primary, and mature polyps) were maintained, following procedures described by Toshino *et al.* (2013). Primary polyps were transferred to petri-dishes (diameter 78 mm, height 24 mm) filled with filtered seawater (1 µm) and kept at 30°C. *Artemia* nauplii were chopped on the slide glass by scalpel, and fed directly to primary and secondary polyps using a fine needle, twice or thrice a week. Rearing water was completely replaced with filtered seawater (1 µm) about three hours after feeding.

Newly detached medusae were kept in petri-dishes (diameter 149 mm, height 91 mm) filled with filtered seawater (1 µm) at 30°C. *Artemia* nauplii were fed to the medusae daily. Culture water was replaced with fresh seawater about three hours after feeding. Several medusae were separated and exposed to a wide range of temperatures (20 to 30°C) to observe medusa budding.

Results

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Peterson, 1979

Class Hydrozoa Owen, 1843

Order Limnomedusae Kramp, 1938

Family Olindiidae Haeckel, 1879

Genus *Scolionema* Kishinouye, 1910

***Scolionema sanshin* sp. n.**
New Japanese name: Komochi-kaginote-kurage-modoki
Figure 2–6

Material examined. Holotype: NSMT-Co1597. Southern Japan, Okinawa Prefecture, Aka Island, Hizushihama, 26°11'22.8"N, 127°16'32.2"E, 6 July 1999, collector: Kazuyuki Shimoji, one adult female (Fig. 2A–C). Paratypes. NSMT-Co1598. Same locality as Holotype, 6 July 1999, collector: Kazuyuki Shimoji, one adult male. NSMT-Co1599. Same locality as Holotype, 4 July 1999, collector: Kazuyuki Shimoji, one adult female. NSMT-Co1600. Southern Japan, Okinawa Prefecture, Ginowan, Ginowan Marina, 26°16'38.4"N, 127°43'47.0"E, 12 June 2003, collector: unknown, one adult female. NSMT-Co1601. Southern Japan, Okinawa Prefecture, Ishigaki Island, Fusaki, 24°22'21.5"N, 124°6'50.4"E, 4 June 2002, collector: unknown, one adult male.

Description. Mature medusae with smooth, transparent, plate-like exumbrella (Fig. 2A, D). UH about 4 mm and UD about 7 mm (Table 3). Exumbrella lacking nematocyst warts or centripetal canals (Fig. 2A, B, D, E). Cock's-comb shaped gonads attached along edge of distal part of four radial canals only, and light red to orange in color (Fig. 2B, C, D, E, 3A). Testes were full of sperm (Fig. 3A), while ovaries contained fully mature eggs (Fig. 3B). Marginal tentacles 60 to 70, with nematocyst rings and evenly spaced adhesive pads (Fig. 3C). Statocysts 16, enclosed in mesoglea (Fig. 3D). The endoderm cells at the base of tentacles, radial canals and gonads were fluorescent green in color under LED light (Fig. 3A, D, E). The portion of the tentacle proximal from the adhesive pad is nearly straight, while the distal portion is bent in a hook-like manner (Fig. 3B, C). The distal portion of tentacle was light red in living specimens (Fig. 3C). The manubrium had a quadrate base, and was light red in color, folded, and with a short peduncle and fluorescent at each corner (Fig. 3E). Medusa-buds were not produced from the radial canals in the collected specimens (Fig. 3A, B).

TABLE 3. Size (mm) of *Scolionema sanshin* **sp. n.**. *: The holotype. Nos. Co1598-1601 are paratypes. UH = umbrella height; UD = umbrella width.

Specimen No.	UH	UD	No. of tentacles	Sex	Sampling site	Date	Lat. Long
NSMT-Co1597*	2.9	6.4	66	Male	Aka Island, Hizushihama	1999/7/6	26°40'22.8"N, 128°1'26.5"E
NSMT-Co1598	2.4	4.0	49	Male	Aka Island, Hizushihama	1999/7/6	26°40'22.8"N, 128°1'26.5"E
NSMT-Co1599	3.5	6.7	61	Female	Aka Island, Hizushihama	1999/7/4	26°11'22.8"N, 127°16'32.2"E
NSMT-Co1600	3.2	5.8	68	Female	Ginowan, Ginowan Marina	2003/6/12	26°16'38.4"N, 127°43'47.0"E
NSMT-Co1601	2.5	4.2	68	Male	Ishigaki Island, Fusaki	2002/6/4	24°22'21.5"N, 124°6'50.4"E

Life cycle. Planulae settled on the bottom of petri-dishes and developed into tiny primary polyps within six days (Fig. 4A). The shape of the primary polyps resembles a pouch, with three to four tentacles. At a body length of about 0.09 mm the mouth disc diameter of the polyps was about 0.08 mm. The primary polyps developed into mature polyps within 24 days (Fig. 4A–C). The shape of the mature polyps was almost identical to the primary polyp stage, but they were larger (mean body length 1.8 mm, mean mouth disc diameter 0.47 mm) and had more tentacles (five to eight). The basal disc was enveloped by periderm over about half of the total length of its body. Frustule and medusa budding were observed when the polyps were at the mature stage (Fig. 4D, E). Both buddings occurred from the middle part of the calyx. The frustules were about 0.5 mm in length and had no tentacles. They settled and grew out into mature polyps within 60 days. Medusa buds developed into free swimming young medusae within three days.

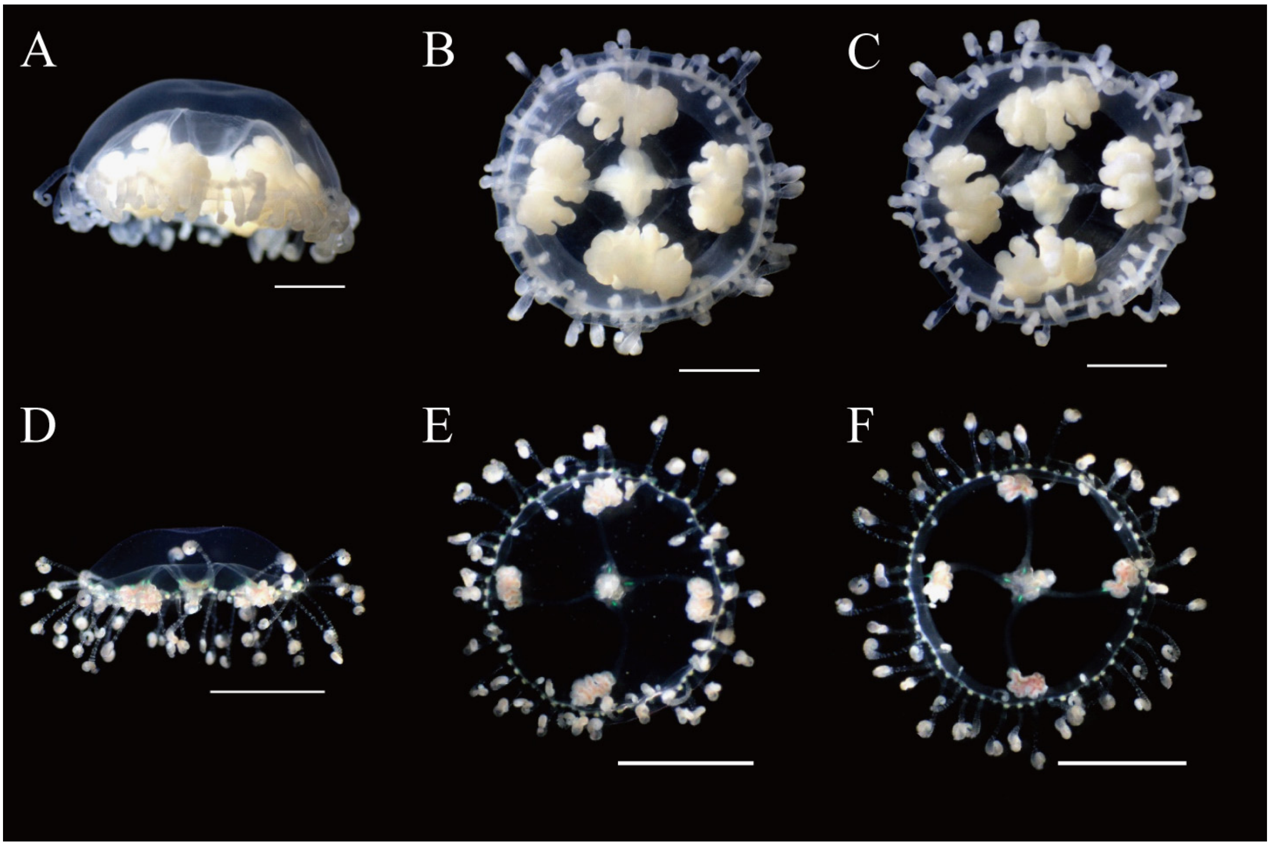


FIGURE 2. *Scolionema sanshin* **sp. n.**, (A–C fixed Holotype, D–F live specimen) A, lateral view B, apical view C, oral view D, lateral view E, apical view F, oral view. Scale bars: 2 mm (A–C), 5 mm (D–F).

Young medusae just released from polyps had a spherical exumbrella (Fig. 5A–C). UH about 1.0 mm and UD about 1.3 mm. Exumbrella with very small nematocyst warts along entire umbrella (Fig. 5D). Four simple radial canals. Gonads not developed (Fig. 5D). Statocysts eight, enclosed in mesoglea (Fig. 5E, F). Marginal tentacles 19 to 25, with nematocyst rings and adhesive pads (Fig. 5G). Manubrium length about 30% of umbrella height (Fig. 5A). Peduncle not developed (Fig. 5A).

Seven-day-old medusae were about 3.4 mm in width (Table 4). Umbrella bowl-shaped. Oval gonads appeared on middle part of radial canals. Marginal tentacles about 40. Fifteen-day-old medusae were about 2.9 mm in height and about 7.3 mm in diameter. The gonads became cock's comb-shaped on the distal part of the canals. Sixteen statocysts were observed. Marginal tentacles about 50. Twenty-two-day-old medusae were about 3.6 mm in height and about 10.6 mm in diameter. Gonads were enlarged. Marginal tentacles about 65. Asexual production of medusa buds was not observed at temperatures between 20 to 30°C.

Cnidome. Three different nematocyst types were identified and measured in the adult medusa, young medusae and mature polyps (Table 5, Fig. 6). Adult medusae had two nematocyst types. Microbasic euryteles were found on both the tentacles and the manubrium while basitrichous isorhizas were only found on the tentacles The young medusae had three nematocyst types. Tentacle: microbasic euryteles, Umbrella: basitrichous isorhizas and ovoid isorhizas, Manubrium: microbasic euryteles. The mature polyps had one nematocyst type; microbasic euryteles.

Molecular phylogenetics. The Maximum Likelihood tree (Fig. 7) revealed the present samples and *Scolionema suvaense* within a monophyletic group within Limnomedusae. There was substantial divergence between sequences from *S. sanshin* and *S. suvaense*—greater than the sequence divergence observed between various species of Olindias Müller, 1861 (Table 7).

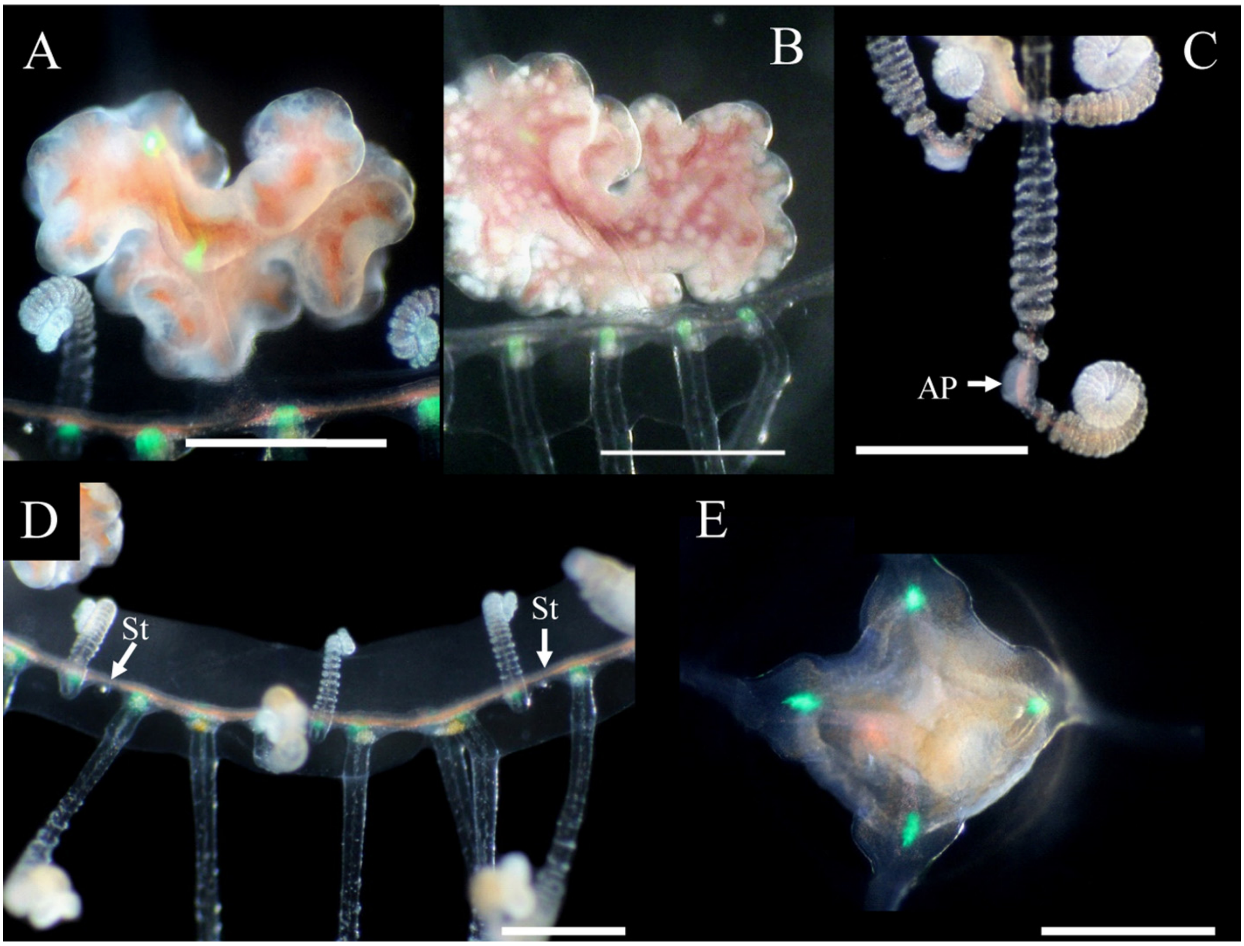


FIGURE 3. *Scolionema sanshin* **sp. n.** live, in laboratory. A testis B ovary C tentacle D velum E manubrium. AP: Adhesive pad; St: Statocyst. All bars represent 1 mm.

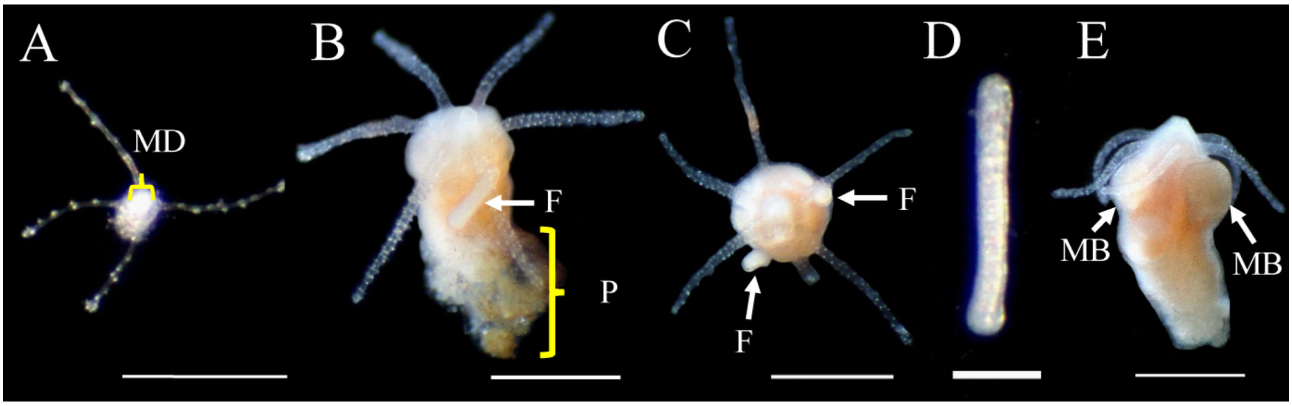


FIGURE 4. *Scolionema sanshin* **sp. n.**, polyp, live, in laboratory. A, primary polyp, oral view. B, adult polyp, lateral view. C, adult polyp, oral view. D, frustule, lateral view. E, medusa budding polyp, lateral view. F: frustule; MB: medusa bud; MD: mouth disc; P: periderm. Scale bars: 0.5 mm (A), 1 mm (B–C, E), 0.2 mm (D).

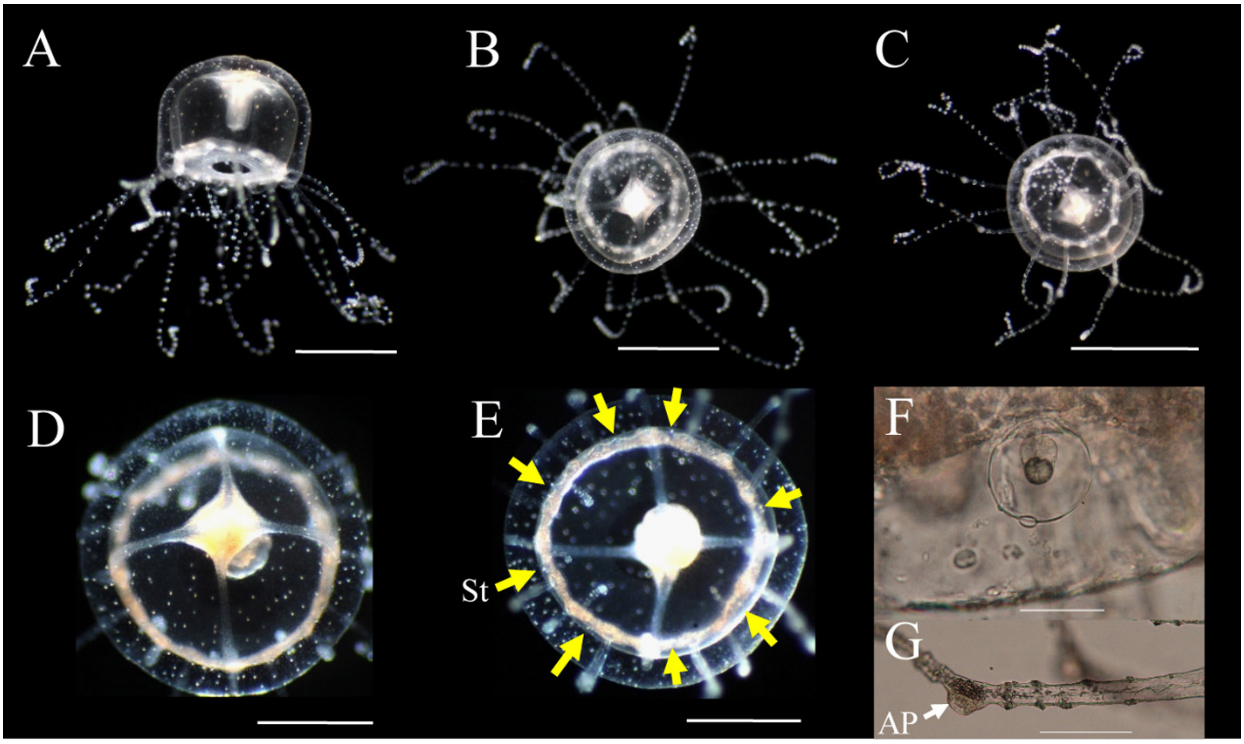


FIGURE 5. *Scolionema sanshin* **sp. n.**, young medusa, live, in laboratory. A, lateral view. B, aboral view. C, oral view. D, exumbrella, aboral view. E–F, statocysts. G, tentacle. AP: Adhesive pad; St: Statocyst. Scale bars: 1 mm (A–C), 0.5 mm (D, E), 50 µm (F), 200 µm (G).

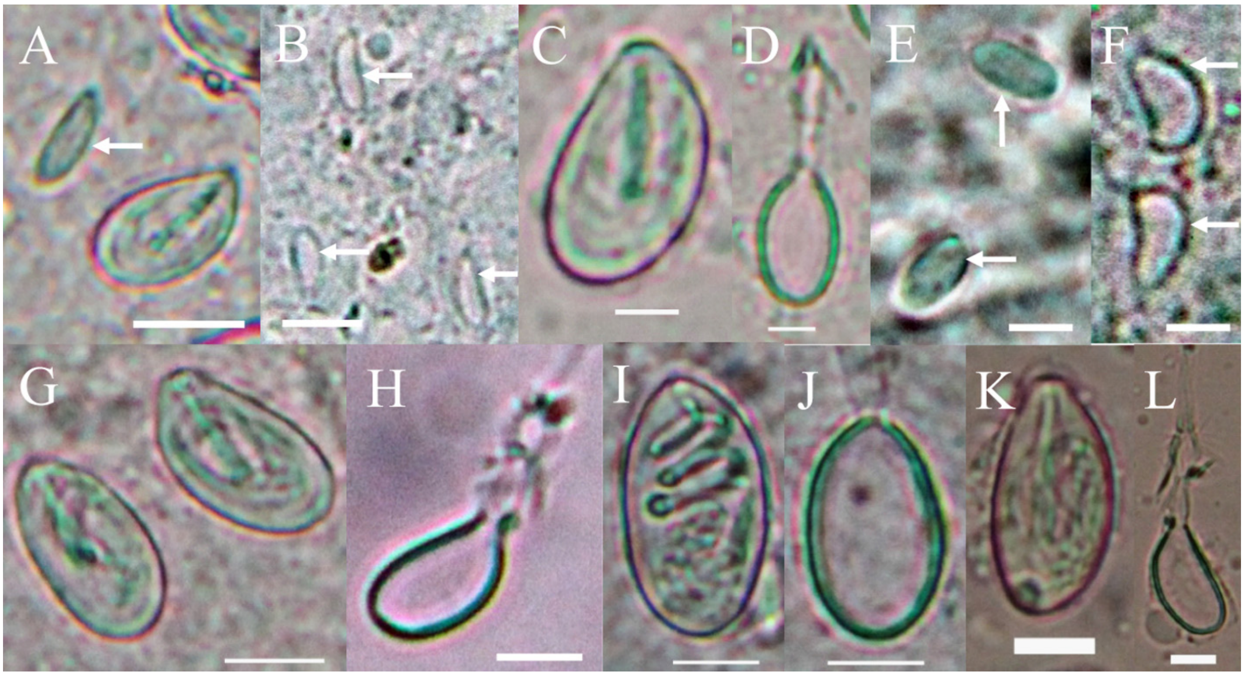
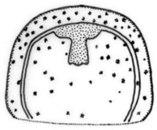
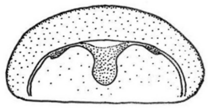
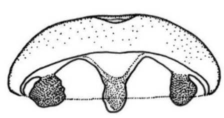
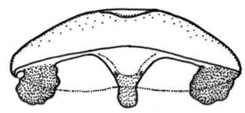
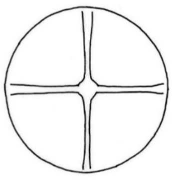
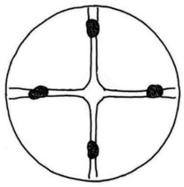
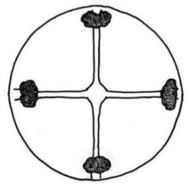
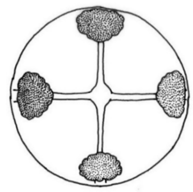
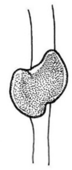
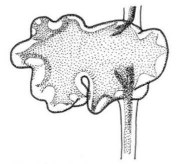
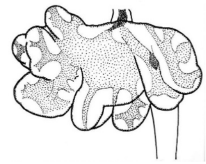
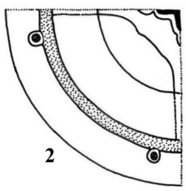
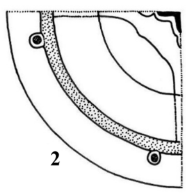
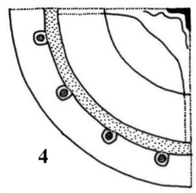
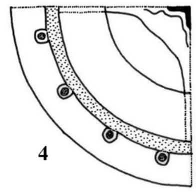


FIGURE 6. Nematocysts of *Scolionema sanshin* **sp. n.**. A, B Basitrichous isorhizas, adult medusae. Intact (A), discharged (B) C, D Microbasic euryteles, adult medusae. Intact (C), discharged (D) E, F Basitrichous isorhizas, young medusae. Intact (E), discharged (F) G, H Microbasic euryteles, young medusae. Intact (G), discharged (H) Ovoid isorhizas, young medusae. Intact (I), discharged (J) Microbasic euryteles, mature polyp. Intact (K), discharged (L). Scale bars: 5 µm (E–F), 10 µm (A–D, G–L).

TABLE 4. The process of development in the medusa of *Scolionema sanshin* **sp. n.**.

	0 day	7 days	14 days	22 days
Exumbrella (lateral view)				
Exumbrella (apical view)				
Gonad				
No. of Statocysts (per quadrant)	 2	 2	 4	 4
UH/ UD	1.0/ 1.3	1.3/ 3.4	2.9/ 7.3	3.6/ 10.6
No. of tentacles	19-25	40	50	65
	·Bell spherical, light brown to brownish ·Tiny nematocyst warts scattered along whole bell ·Gonads & peduncle not developed	·Bell bowl-shaped ·Development of oval gonad at middle part of the canals	·Development of cock's comb-shaped gonad at distal part of the canals	·Enlargement of gonads ·Lacking of asexual medusa budding

Habitat and ecology. *Scolionema sanshin* medusae appear during summer in a range of subtropical temperature localities in the Ryukyu Archipelago, southern Japan. Medusae from various populations, united under the name *S. suvaense*, have been reported from summer to fall from Indo Pacific, Mediterranean, and Japanese localities (Agassiz & Mayer 1899; Goy 1973; Uchida & Sugiura 1976).

The medusae of *S. sanshin* were collected near shore on a beach and in a fishing port during the night, but were not observed during daytime. The medusae seem to be nocturnal in behaviour. Moreover, young and adult medusae showed resting behavior using their tentacles with their adhesive pads under lighted conditions. In the field, they may rest on seaweed or sea grass during the day, as with *Scolionema suvaense* and *Gonionemus vertens* (Uchida and Sugiura 1976; Singla 1977). Young and adult medusae appeared between June to August. Polyps of *S. sanshin* may metamorphose into medusae during summer, when temperatures rise from approximately 25 to over 30°C (Japan Meteorological Agency 2016). Stinging events attributable to *S. sanshin* are presently unknown.

Etymology. The species name *sanshin* (noun in apposition) was derived from the Okinawan traditional instrument "Sanshin"—a snakeskin-adorned banjo-like instrument. Hook-like tentacles of the jellyfish resemble a sanshin performer's fingers when they play the instrument.

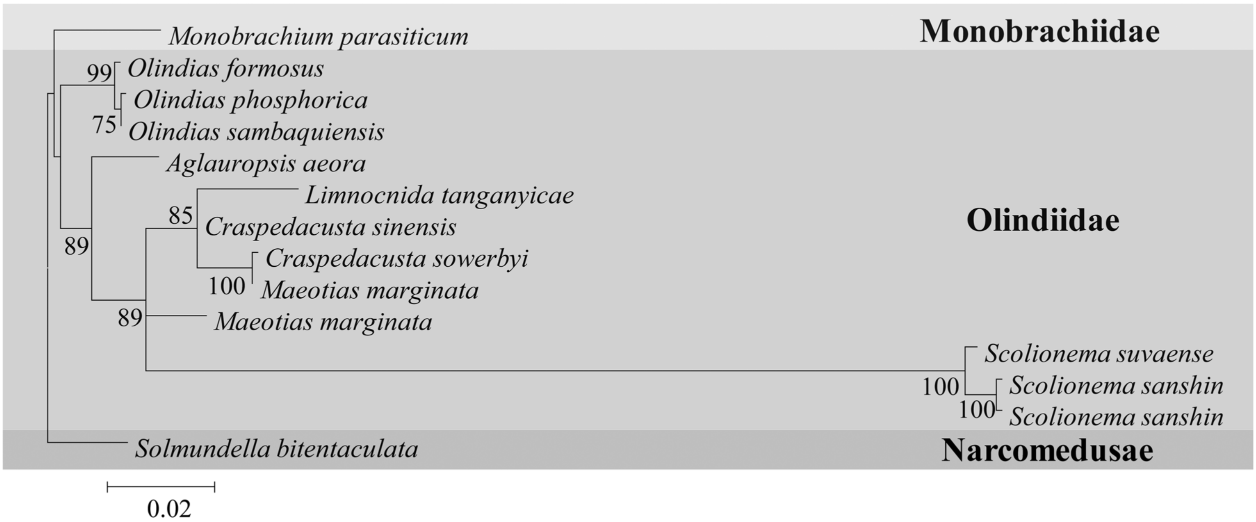


FIGURE 7. Maximum-likelihood tree for 11 limnomedusan taxa based on the nuclear 18S rDNA data set. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value; only values >50% are shown.

TABLE 5. Cnidomes of *Scolionema sanshin* sp.n., cultured specimens. D, L represent capsule diameter and length, respectively, in µm.

	Part	Type		Min	Max	Mean	SD	N
Adult medusae	Tentacle	Basitrichous isorhiza	D	2.60	5.22	3.81	0.54	100
			L	6.87	12.67	9.76	1.00	
		Microbasic eurytele	D	8.36	12.08	9.76	0.72	100
			L	14.67	18.86	16.59	0.82	
	Umbrella	N/A	D	—	—	—	—	—
			L	—	—	—	—	—
	Manubrium	Microbasic eurytele	D	6.32	9.17	7.63	0.78	50
			L	9.10	14.89	12.65	1.16	
Young medusae	Tentacle	Microbasic eurytele	D	7.87	10.21	8.92	0.66	30
			L	14.25	18.81	16.50	1.17	
	Umbrella	Ovoid isorhiza	D	14.48	17.74	15.88	0.86	30
			L	24.48	30.91	27.61	1.38	
		Basitrichous isorhiza	D	2.78	4.90	3.75	0.51	30
			L	7.53	9.75	8.82	0.51	
	Manubrium	Microbasic eurytele	D	7.24	9.41	7.94	0.45	30
			L	11.37	14.88	13.46	0.98	
Mature polyps	Body	Microbasic eurytele	D	11.75	16.79	15.24	1.23	30
			L	22.60	33.25	28.32	2.68	

Differential diagnosis. A comparison of key features of the species of *Scolionema* is presented in Table 6. *Scolionema sanshin* can be distinguished from *S. suvaense* by the shape of the gonads and number of tentacles in adult medusae. Gonads of *S. sanshin* are cock's-comb shaped, while those of *S. suvaense* are sinusoidal, papilliform, folded and lobed or ribbon-shaped. Medusa buds are produced in *S. suvaense* from Japan and Mediterranean Sea, while *S. sanshin* apparently lacks buds. The number of tentacles of *S. sanshin* is much more than that of *S. suvaense* from Japan, Mediterranean Sea and Maldives. All populations of *Scolionema* have 16 statocysts, adhesive pads on tentacles, and gonads located in the distal 1/3 to 1/2, or edge of distal part of the radial canals.

TABLE 6. Morphology of adult and juvenile medusae and mature polyps in previous and the present study. Bars represent a lack of data. BI = Basitrichous isorhiza, ME = Microbasic eurytele.

		S. sanshin n. sp.	*S. suvaense* (as *G. suvaensis*)	*S. suvaense* (as *G. pelagicus*)	*S. suvaense* (as *G. hornelli*)	*S. suvaense* (as *S. gemmifera*)	*S. suvaense*	*S. suvaense* (as *G. suvaense*)
Adult medusae	Umbrella diameter (mm)	4.0-6.7 (mean: 5.4)	8	20	6	4	4.5	7
	No. of tentacles	49-68 (mean: 62)	70	50	70	32	36-48	48
	Nematocyst types (tentacles)	BI, ME	-	-	-	-	-	-
	No. of statocysts	16	16	16	16	8	16	16
	Shape of gonads	Cock's-comb shaped	Sinusoidal curve alternately to the right and left	Papilliform	Folded and lobed	-	Ribbon-shaped	Ribbon-shaped
	Distribution of gonads	Edge of distal part of radial canals	Edge of distal part of radial canals	Distal 1/3 of radial canals	Edge of distal part of radial canals	Middle part of radial canals	Edge of distal part of radial canals	Distal 1/3 of radial canals
	Budding of medusa buds	Absent	Absent	Absent	Absent	Present	Present	Present
Juvenile medusae	Umbrella diameter	1.0-1.9 (mean: 1.3)	-	-	-	0.7	0.8-1.3	0.6
	No. of tentacles	19-25 (mean: 22)	-	-	-	8	24-28	16
	Nematocyst types (tentacles)	ME	-	-	-	-	-	-
	No. of statocysts	8	-	-	-	4	8	8
Mature polyps	Body diameter	0.7	-	-	-	-	-	-
	Boby length	1.8	-	-	-	-	-	-
	Nematocyst types	ME	-	-	-	-	-	ME
	No. of tentacles	5-8	-	-	-	-	-	2-5
	Asexual reproduction	Frustule	-	-	-	-	-	Frustule
Distribution		Ryukyu Archpelago, Southern Japan	Suva Harbor, Fiji	Gadu island, Suvadiva atoll, Maldives	Pearl Banks, Gulf of Manaar, Indian Ocean	Misaki, Sagami Bay, Central Japan	Misaki, Sagami Bay, Central Japan	Bay of Villefranche-sur-mer, Mediterranean Sea, France
References		This study	Agassiz & Mayer (1899)	Bigelow 1904	Browne 1905	Kishinouye 1910	Uchida & Sugiura 1976	Goy 1973

TABLE 7. Pairwise genetic distances (K2P) based on 1041 positions of 18S sequences among Limnomedusae. The analysis involved 14 sequences.

	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *Scolionema sanshin* LC320028													
2. *Scolionema sanshin* LC320029	0.002												
3. *Scolionema suvaense* AB231884	0.009	0.009											
4. *Craspedacusta sinensis* EU247815	0.119	0.119	0.115										
5. *Craspedacusta sowerbii* FJ897537	0.118	0.118	0.114	0.011									
6. *Maeotias marginata* AF358056	0.116	0.116	0.115	0.018	0.027								
7. *Maeotias marginata* AF358057	0.116	0.116	0.113	0.010	0.001	0.026							
8. *Limnocnida tanganyicae* AY920755	0.118	0.118	0.113	0.018	0.022	0.035	0.021						
9. *Aglauropsis aeora* AY920754	0.118	0.118	0.115	0.029	0.040	0.029	0.039	0.038					
10. *Olindias phosphorica* AY920753	0.119	0.119	0.116	0.028	0.035	0.030	0.034	0.041	0.026				
11. *Olindias formosus* KF184030	0.119	0.119	0.116	0.028	0.038	0.031	0.036	0.041	0.024	0.003			
12. *Olindias sambaquiensis* EU247814	0.118	0.118	0.115	0.028	0.035	0.031	0.034	0.041	0.025	0.001	0.002		
13. *Monobrachium parasiticum* AY920752	0.132	0.132	0.130	0.038	0.046	0.040	0.045	0.045	0.031	0.025	0.026	0.026	
14. *Solmundella bitentaculata* EU247811	0.127	0.127	0.124	0.037	0.046	0.040	0.045	0.049	0.031	0.025	0.024	0.024	0.032

Discussion and conclusions

The first identified species of the genus *Scolionema*, *S. suvaense* was described by Agassiz and Mayer (1899) based on material collected from Fiji Island (as *Gonionemus suvaensis*). Bigelow (1904) and Browne (1905) described *Scolionema pelagicus* (as *G. pelagicus*) and *S. hornelli* (*G. hornelli*) respectively. Kishinouye (1910) established the genus *Scolionema*, and described *S. gemmifera* collected from Japan. However, Mayer (1910) and Kramp (1959) suggested that *S. pelagicus*, *S. hornelli* and *S. gemmifera* should be regarded as *S. suvaense*. Kramp (1965) reported it again under the genus *Gonionemus* A. Agassiz, 1862 because he believed characters defining *Scolionema* were not significant enough to regard the genus as a separate one. However, Kramp (1968) then used the generic name *Scolionema* when he described specimens from the Indo-Pacific. The genus *Scolionema* is comprised of only one accepted species, *S. suvaense* (Bouillion *et al.* 2006) until this description.

Scolionema suvaense has been reported from the Indo Pacific and Mediterranean Sea (Agassiz and Mayer 1899; Kishinouye 1910, Goy 1973). However, morphological characters differ depending on region. For instance, the number of tentacles of *S. suvaense* from Japan, Mediterranean Sea and Maldives are reported to be less than that of Indo Pacific specimens. Additionally, asexual medusa-buds were not observed in Indo Pacific specimens, but have been reported in Japanese and Mediterranean specimens. *Scolionema sanshin* resembles *S. suvaense* from the Indo Pacific, however, they can be differentiated by the shape of the gonads, being cock's-combed shaped in *S. sanshin* and sinusoidal, papilliform or folded and lobed in *S. suvaense*.

Asexual medusa budding of *S. sanshin* was not observed in collected and cultured specimens. The medusa-buds appear on the radial canals and the gonads will develop when budding is stopped in *S. suvaense* (Goy 1973). While I have not seen evidence that *S. sanshin* produces medusa-buds, it is possible that the budding might occur before the development of gonads in natural conditions.

Sequence divergence from *S. sanshin* and *S. suvaense* from Japan is greater than that of other limnomedusae (Fig. 7). Kimura (1980) 2-parameter (K2P) distance between *Scolionema* spp. and other limnomedusae for 1041 bp of 18S was 0.113–0.132, substantially above the value of interspecific distance 0.009 between *S. sanshin* and *S. suvaense* (Table 7). Therefore, *S. sanshin* and *S. suvaense* represent long branches in the ML tree.

These morphological and molecular phylogenetic analyses provide evidence that *Scolionema* from the Ryukyu Archipelago is a new species. However, morphological variation of the widely-distributed populations of *S. suvaense,* and recognition that many marine species consist of cryptic species complexes, raises the possibility that more than one species is presently united under this name. Future studies are required to clarify Mediterranean, Maldives, Fijian, other Japanese *Scolionema* populations and understand the species richness of *Scolionema*.

Acknowledgements

I would like to express my sincere thanks to Yasuhito Azama for providing the specimens. Thanks are also due to Aki Hashimoto, Junko Fukada, Shu Nakachi, Takuma Mezaki, Kaori Yamashita, Yukimitsu Imahara, Rie Nakano (Kuroshio Biological Research Foundation), Takashi Asahida, Kenichi Hayashizaki, Hiroshi Miyake (Kitasato University) and the staff of the Sesoko Tropical Biosphere Research Center. The manuscript was greatly improved by the constructive comments of Dr. Dhugal J. Lindsay, Dr. Allen G. Collins, Dr. George I. Matsumoto and an anonymous reviewer. The research was financially supported by Showa Seitoku Memorial Foundation.

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