Two New Species of *Taeniogyrus* and *Scoliorhapis* (Echinodermata: Holothuroidea: Apodida: Chiridotidae: Taeniogyrinae) from Wakayama and Okinawa, Japan

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Abstract: Two new taeniogyrinid sea cucumbers collected in the intertidal zone of southern Japan, *Taeniogyrus verruculosus* sp. nov. and *Scoliorhapis sesokoensis* sp. nov. are described. Both species possess a small body size (approximately 30–40 mm), light body color (translucent white in preserved specimens), ten tentacles, and numerous verrucous surfaces on the body, within which small (mostly less than 70 μ m in length) sigmoid-hook ossicles are present. The two new species have a very similar external appearance to each other, however they do differ in the number of tentacle digits: *T. verruculosus* possesses up to eight digits, whereas *S. sesokoensis* possesses up to six. In both species, wheel papillae are not present. *T. verruculosus* possesses only a few small (mostly less than 30 μ m in diameter) wheel ossicles in the anterior dorsal skin, which are not present in *S. sesokoensis*. Additionally, the DNA barcode sequences of two new species were determined and are available in the DDBJ/NCBI/GenBank databases under the accession numbers.

Key words: Chiridotidae, DNA barcode, *Scoliorhapis sesokoensis* sp. nov., *Taeniogyrus verruculosus* sp. nov.

Introduction

Recently, many intertidal sea cucumbers of the order Apodida [or Synaptida; see Smirnov (2012) regarding their higher-level classification] have been collected in southern Japan, including some species that appear distinct from all others reported in Japan. In this report, two of them are described and compared with their congeners using careful survey of the literature. We determine these two species to be a new discovery.

Regarding their ossicle morphologies, one species possesses sigmoid-hook ossicles, and the other one possesses sigmoid-hooks as well as wheel ossicles in the outer skin. The former feature is identical to the genus *Scoliorhapis* Clark, 1946, and the latter feature is identical to the genus *Taeniogyrus* Semper, 1868. Although our two new species are different in ossicle morphology, they possess many similar features in the external and internal morphologies. Therefore, we also determined the DNA sequence data of the mitochondrial *cytochrome c oxidase subunit 1* (*COI*)

gene for each new species in order to aid future efforts in species identification.

Materials and Methods

Animals were collected from the lower intertidal zone of sandy-gravel shores at the following sites in southern Japan: (MOT) Motoshima Islet, Mera, Tanabe, Wakayama (33°43'56.0"N, 135°20'58.0"E), 17 June 2015, 7 July 2016 (coll. Y. Yamana); (SES) Sesoko Island, Motobu, Okinawa (26°39'02.6"N, 127°51'24.1"E), 7 October 2014 (coll. H. Tanaka), 10–12 February 2016 (coll. Y. Yamana).

Large specimens were collected by hand net, from sediments approximately 10 cm below the sea floor. Small specimens were collected using the following method: several scoops of sediment were taken approximately 30 cm below the sea floor, and were quickly washed with seawater in a bucket to suspend interstitial animals before the water was filtered through a 40–100 μ m mesh sieve. The specimens from these filtered samples were sorted live under a binocular dissecting microscope (Nikon SMZ). Sorted specimens were fixed and preserved in 80% ethanol. Some specimens were fixed after anaesthetization in a

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menthol solution (0.37 g of menthol dissolved into 100 mL of 99% ethanol, this then being diluted to 3.0% with seawater) for 30 min.

All specimens in the type series were dissected, with the tentacles, Polian vesicles, ciliated funnels, and calcareous rings then examined under a binocular dissecting microscope (Nikon SMZ); drawings of the tentacles and calcareous rings were made with a camera lucida. Ossicles from four regions of the body wall (dorsal anterior, dorsal posterior, ventral anterior, and ventral posterior) and the lateral tentacles were characterized. To extract ossicles, small pieces of tissue were isolated using sharpened ophthalmologic scissors under the dissecting microscope, and dissolved using sodium hypochlorite solution (NaClO, 5%). Ossicles extracted from all five parts were mounted on glass slides in polyester resin (Showadenko RIGOLAC) and characterized under a compound microscope (Nikon Optiphot). Several ossicles from the tentacles and the anterior dorsal body-wall were rinsed with deionized water, dehydrated in 99% ethanol, mounted on aluminium stubs using conductive tape (Nisshin NEM Tape), dried at room temperature, and characterized using a scanning electron microscope (SEM) (Nippondenshi JEOL JSM-6480LV).

The ossicles were classified into 'wheels' (i.e. wheel-like ossicles with spokes and a series of teeth on the inner margin of the rim), 'sigmoid-hooks' (fishhook-like ossicles, with an open pointed end and closed blunt end, with the point of the hook turned outwards), and 'rods' (rod-like ossicles, slightly curved, sometimes distally branching or spreading). Measurements of 11–21 ossicles were taken to the nearest micrometer for every tissue sample. To compare these measurements among individuals and among parts of the body, Kruskal–Wallis tests were performed. The dissected specimens, glass slides, and SEM material were deposited in the Invertebrate Collection (INV) of the Wakayama Prefectural Museum of Natural History (WMNH) in Kainan, Wakayama, Japan.

DNA extractions from small pieces of ethanol-preserved tissue were performed using the DNeasy Blood and Tissue Kit (Qiagen, USA) following the manufacturer's protocol. Partial sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (*COI*) gene were PCR-amplified using the following primer set: forward (LCO1490; Folmer et al. 1994) and reverse (CO1e-R; Arndt et al. 1996). The 25 μ L reaction contained 0.125 μ L of *TaKaRa Ex Taq* HS (TA-KARA BIO Inc., Japan), 2.5 μ L of 10 $\times$ *Ex Taq* buffer, 2 μ L of dNTP mix, 1 μ L of each primer (5 pmol each), 1 μ L of template DNA, and 17.375 μ L of sterilized distilled water. The PCR protocol included an initial denaturation step at 95°C for 2 min, followed by 40 cycles of denaturation at 98°C for 10 s, annealing at 45°C for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. Quantity and length of the PCR products were checked by 1% agarose S (Nippon Gene, Japan) gel electrophoresis, stained with ethidium bromide. The products were purified for sequencing using a FastGene Gel/PCR Extraction

Kit (NIPPON Genetics Co, Ltd, Japan), according to the manufacturer's protocol. Sequencing (of both the forward and reverse reads) was performed by the MacroGen Japan Corp. (Tokyo) with the same primers used for PCR amplification. A homology search of *COI* sequences was performed by BLAST (Altschul et al. 1990, 1997) with the Discontiguous MegaBLAST program from the National Center for Biotechnology Information (NCBI, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The evolutionary distances of the nucleotide sequences were estimated with MEGA6 (Tamura et al. 2013) using Kimura's two parameter model (Kimura 1980). Standard error estimates were obtained by a bootstrap procedure (1000 replicates).

Taxonomy

Order **Apodida** Brandt, 1835 (sensu Östergren, 1907)

Family **Chiridotidae** Östergren, 1898

Subfamily **Taeniogyrinae** Smirnov, 1998

Genus **Taeniogyrus** Semper, 1868

Taeniogyrus verruculosus sp. nov.

(Figs 1A, B, 2A, C, 3A, B, C)

Material examined Holotype: WMNH-INV-2015-154 (MOT, 17 June 2015) (length 23.9 mm, width 3.0 mm). Paratypes: WMNH-INV-2016-205 (MOT, 7 July 2016) (length 27.8 mm, width 2.7 mm); WMNH-INV-2016-206 (MOT, 7 July 2016) (anterior half only: length 8.2 mm, width 3.2 mm).

Other material: 1 specimen, WMNH-INV-2016-207 (MOT, 7 July 2016) (posterior half only: length 6.4 mm, width 1.9 mm).

Description Body small, anesthetized length of largest specimen (WMNH-INV-2016-205) 27.8 mm (after preservation), cylindrical, slightly tapered toward posterior end (Table 1). Body color, translucent pale yellowish white to pale orange in living specimens (Fig. 1A), and translucent white in preserved specimens (Fig. 1B). Numerous verrucous surfaces present in anterior body skin, sparse posterior. Mouth anterior; anus posterior. Oral disc slightly inclined toward ventral side.

Verrucous surfaces, high, hollow, with a blunt conical form, dense in inter-radius, sparse in radius. Color of verrucous surfaces same as adjacent body skin (preserved and living specimens). Size and shape of verrucous surfaces almost uniform, height approximately 0.2–0.4 mm and width of basal part approximately 0.2–0.3 mm in largest specimen (paratype, WMNH-INV-2016-205), becoming gradually smaller as body size decreases.

Calcareous ring composed of five radial and five inter-radial plates, these all being centrally contracting, and distally spreading ends (Fig. 2A). Thin retractor muscles, combined with longitudinal muscles by mesentery. Ten tentacles non-retractile, each with up to four pairs of digits

Table 1. Summary of measurements, and counts for type specimens of *Taeniogyrus verruculosus* sp. nov. and *Scoliorhapis sesokoensis* sp. nov.

Registration ^a	Type status	Locality ^b	Date	Body state	Body size (mm)		Counts				Gonad state
					Length	Width	Ten- tacles	Tentacle digits	Madre- porite	Polian vesicles	
<i>T. verruculosus</i> sp. nov.											
WMNH- INV-2015-154	holotype	MOT	17 June 2015	anaesthetized	23.9	3.0	10	5–8	1	1	testis
WMNH- INV-2016-205	paratype	MOT	7 July 2016	anaesthetized	27.8	2.7	10	6–7	1	1	testis
WMNH- INV-2016-206	paratype	MOT	7 July 2016	anterior half	8.2	3.2	10	6–7	1	1	ovary
<i>S. sesokoensis</i> sp. nov.											
WMNH- INV-2015-721	paratype	SES	7 October 2014	unanaesthe- tized	2.4	1.0	8	4	1	1	immature
WMNH- INV-2015-722	paratype	SES	7 October 2014	unanaesthe- tized	3.3	0.6	9	3–4	1	1	immature
WMNH- INV-2016-53	holotype	SES	10–12 February 2016	anaesthetized	36.5	3.0	10	6	1	1	testis
WMNH- INV-2016-54	paratype	SES	10–12 February 2016	anaesthetized	31.7	2.9	10	6	1	1	testis
WMNH- INV-2016-55	paratype	SES	10–12 February 2016	anaesthetized	14.9	1.8	10	4–6	1	1	immature

^a WMNH-INV: Invertebrate Collection of the Wakayama Prefectural Museum of Natural History^b MOT: Motoshima Islet, Mera, Tanabe, Wakayama, SES: Sesoko Island, Motobu, Okinawa

(sometimes unpaired digit present), tip pair largest; sensory cups absent (Fig. 2C). Color of tentacles translucent white (preserved and living specimens). Polian vesicle single, medioventral, and madreporite single (Table 1). Intestine has no loop. Two long bundles of ovaries or testis present, each with approximately 3–4 tubules, mostly unbranched except in basal part. One row of ciliated funnels situated in inter-radius of anterior to posterior body cavity, in left lateral side, dense in anterior, sparse in posterior. One narrow row of ciliated funnels situated in inter-radius of posterior body cavity, in mid-dorsal side, sparse. Sometimes 5–15 ciliated funnels clustered in a bundle.

Body wall containing sigmoid-hook ossicles and wheel ossicles (Figs 1B, 3B, C, Table 2). Sigmoid-hook ossicles, small, scattered around body surface in both radii and inter-radii, approximately 15–25 hooks arranged in one direction on verrucous surface, similar in appearance to strawberry seeds. Pointed end facing toward tip of verrucous surface, and blunt end facing toward base of verrucous surface. Length of shank, 39–68 μm (means of 51–62 μm in different specimens) in anterior dorsal side, 36–66 μm (means 53 and 58 μm) in anterior ventral side, 38–71 μm (means 48–64 μm) in posterior dorsal side, and 42–64 μm (means 48 and 59 μm) in posterior ventral side. Measurements significantly different among four parts in two complete specimens (WMNH-INV-2015-154 and 2016-205) (Kruskal–Wallis test, $P_s < 0.05$), and measurements of anterior dorsal and ventral parts significantly dif-

ferent among three counted specimens (Kruskal–Wallis test, $P_s < 0.01$).

Wheel ossicles rounded-hexagonal with six spokes (sometimes five or seven). Inner margin of rim with 6–8 continuous teeth per radiant. Spokes broad, their breadth comprising 18–24% of wheel ossicle diameter (Fig. 3B). Wheel ossicle aggregations (wheel papillae) absent. Wheel ossicle diameter 25–32 μm , only observed in anterior dorsal body-wall. Wheels very sparse, and statistical analysis not applied.

Tentacles containing rod ossicles (Fig. 3A, Table 2). Rod ossicles arched; distally branched. Distal branches curved inward considerably. Sometimes having spinous processes distally, however, less so centrally. Rod ossicle length 39–66 μm , means of 54–58 μm in different specimens, significantly different among three counted specimens (Kruskal–Wallis test, $P < 0.01$).

Distribution In sandy-gravel sediment in the lower intertidal zone, Motoshima Islet, Wakayama Prefecture, Japan.

Etymology The specific name *verruculosus* is derived from the Latin word meaning “verrucous”.

DNA barcode sequence The sequences of paratypes (WMNH-INV-2016-205, accession number LC203483 and WMNH-INV-2016-206, accession number LC203484)

Fig. 1. A and B, lateral view of *Taeniogyrus verruculosus* sp. nov. A, living state of WMNH-INV-2016-205 (paratype); B, preserved state of WMNH-INV-2015-154 (holotype). B (av) and B (ad), microphotograph of anterior ventral (av) and dorsal (ad) skin containing ossicles of WMNH-INV-2015-154. C and D, lateral view of *Scoliorhapis sesokoensis* sp. nov. C, living state of WMNH-INV-2016-54 (paratype); D, preserved state of WMNH-INV-2016-53 (holotype). D (av) and D (ad), microphotograph of anterior ventral (av) and dorsal (ad) skin containing ossicles of WMNH-INV-2016-53. Abbreviations: ad, anterior dorsal; av, anterior ventral; pd, posterior dorsal; pv, posterior ventral; sh, sigmoid-hook ossicle; te, tentacle; wh, wheel ossicle.

Fig. 2. A and B, one radial and one inter-radial plate each of the calcareous ring on the right side. A, *Taeniogyrus verruculosus* sp. nov. (holotype, WMNH-INV-2015-154); B, *Scoliorhapis sesokoensis* sp. nov. (holotype, WMNH-INV-2016-53); viewed from right side with dorsal side above. C and D, tentacles of left side. C, *T. verruculosus* sp. nov. (holotype, WMNH-INV-2015-154); D, *S. sesokoensis* sp. nov. (holotype, WMNH-INV-2016-53); viewed from right side with dorsal side above. Abbreviations: ip, inter-radial plate; rp, radial plate.

Table 2. Counts and measurements (Mean $\pm$ sd) of ossicles from body skin for type specimens of *Taeniogyrus verruculosus* sp. nov.

Registration ^a	Body skin	Rod			Sigmoid-hook			Wheel ^b			
		(<i>n</i>)	Length (μ m)	Breadth (μ m)	(<i>n</i>)	Length (μ m)	Breadth (μ m)	(<i>n</i>)	Diameter (μ m) ^c	Number of teeth ^d	Breadth of spoke (%) ^e
WMNH- INV-2015-154	Tentacle	(12)	55.4±5.4	4.8±0.5	(0)			(0)			
	(Range)		(46–62)	(4–5)							
	Anterior dorsal	(0)			(14)	62.0±4.5	8.1±0.5	(4)	27.0±1.8	6.8±1.0	21.2±2.2
	(Range)					(53–68)	(7–9)		(46–75)	(6–8)	(19–24)
	Posterior dorsal	(0)			(19)	63.7±4.0	8.8±0.9	(0)			
	(Range)					(55–71)	(8–11)				
Anterior ventral	(0)			(20)	57.8±4.9	8.3±0.7	(0)				
	(Range)					(47–66)	(7–9)				
Posterior ventral	(0)				(16)	59.3±3.6	8.0±0.7	(0)			
	(Range)					(54–64)	(7–10)				
WMNH- INV-2016-205	Tentacle	(15)	53.8±5.2	4.7±0.8	(0)			(0)			
	(Range)		(39–60)	(4–6)							
	Anterior dorsal	(0)			(20)	50.8±5.8	7.0±0.6	(1)	32	7	21.9
	(Range)					(39–62)	(6–8)		(32)	(7)	(21.9)
	Posterior dorsal	(0)			(16)	47.9±5.3	6.1±0.6	(0)			
	(Range)					(38–56)	(5–7)				
Anterior ventral	(0)			(18)	52.6±8.1	7.2±0.9	(0)				
	(Range)					(36–63)	(5–8)				
Posterior ventral	(0)				(16)	48.4±5.2	6.3±0.6	(0)			
	(Range)					(42–56)	(5–7)				
WMNH- INV-2016-206	Tentacle	(15)	58.2±6.0	5.3±0.6	(0)			(0)			
	(Range)		(45–66)	(4–6)							
	Anterior dorsal	(0)			(17)	58.5±6.8	7.2±0.5	(2)	29.5	6.5	18.6
	(Range)					(44–67)	(6–8)		(28 and 31)	(6 and 7)	(17.9 and 19.4)
Anterior ventral	(0)			(20)	57.3±8.8	6.9±0.4	(0)				
	(Range)					(38–70)	(6–8)				

^a WMNH-INV: Invertebrate Collection of the Wakayama Prefectural Museum of Natural History^b Wheels with six spokes were only measured.^c Values were measured at the vertices of a hexagon.^d Values were counted at a radian of a hexagon.^e Values were represented by percentage of breadth of a spoke in a diameter.Genus *Scoliorhapis* Clark, 1946*Scoliorhapis sesokoensis* sp. nov.
(Figs 1C, D, 2B, D, 3D, E)

Material examined Holotype: WMNH-INV-2016-53 (SES, 10–12 February 2016) (length 36.5 mm, width 3.0 mm). Paratypes: WMNH-INV-2015-721 (SES, 7 October 2014) (length 2.4 mm, width 1.0 mm); WMNH-INV-2015-722 (SES, 7 October 2014) (length 3.3 mm, width 0.6 mm); WMNH-INV-2016-54 (SES, 10–12 February 2016) (length 31.7 mm, width 2.9 mm); WMNH-INV-2016-55 (SES, 10–12 February 2016) (length 14.9 mm, width 1.8 mm).

Other material: 1 specimen, WMNH-INV-2015-720 (SES-1, 7 October 2014); 11 specimens, WMNH-INV-2015-1459–1469 (SES, 13 September 2015); 43 speci-

mens, WMNH-INV-2016-10–52 (SES, 10–12 February 2016).

Description Body small, anaesthetized length of largest specimen (WMNH-INV-2016-53) 36.5 mm (after preservation), cylindrical, slightly tapered toward posterior end (Table 1). Body color, translucent pale yellowish white to pale orange in living specimens (Fig. 1C), and translucent white in preserved specimens (Fig. 1D). Numerous verrucous surfaces present in anterior body skin, sparse in posterior body skin. Mouth anterior; anus posterior. Oral disc slightly inclined toward ventral side.

Verrucous surfaces, high, hollow, with a blunt conical form, dense in inter-radius, sparse in radius. Verrucous surfaces undeveloped in small specimens (less than approximately 4 mm). Color of verrucous surfaces same as adjacent body skin (preserved and living specimens). Size and shape of verrucous almost uniform, height approxi-

Fig. 3. A–E, SEM image of ossicles. A, rod ossicles from tentacle; B, wheel ossicles; and C, sigmoid-hook ossicles from anterior dorsal body skin of *Taeniogyrus verruculosus* sp. nov. (holotype, WMNH-INV-2015-154). D, rod ossicles from tentacle; and E, sigmoid-hook ossicles from anterior dorsal body skin of *Scoliorhapis sesokoensis* sp. nov. (holotype, WMNH-INV-2016-53). Abbreviations: sh, sigmoid-hook ossicles; ro, rod ossicles; wh, wheel ossicles; wh', internal side of wheel ossicles.

Table 3. Counts and measurements (Mean $\pm$ sd) of ossicles from body skin for type specimens of *Scoliorhapis sesokoensis* sp. nov.

Registration ^a	Body skin	Rod			Sigmoid-hook			Wheel			
		(n)	Length (μ m)	Breadth (μ m)	(n)	Length (μ m)	Breadth (μ m)	(n)	Diameter (μ m)	Number of teeth	Breadth of spoke (%)
WMNH- INV-2015-721	Tentacle (Range)	(12)	43.3 $\pm$ 4.7 (35–54)	4.8 $\pm$ 0.8 (4–6)	(0)			(0)			
	Anterior dorsal (Range)	(0)			(17)	39.5 $\pm$ 1.8 (37–43)	4.9 $\pm$ 0.3 (4–5)	(0)			
	Posterior dorsal (Range)	(0)			(17)	42.2 $\pm$ 2.5 (37–47)	5.1 $\pm$ 0.6 (4–6)	(0)			
	Anterior ventral (Range)	(0)			(17)	39.5 $\pm$ 1.8 (36–42)	4.8 $\pm$ 0.5 (4–6)	(0)			
	Posterior ventral (Range)	(0)			(17)	41.8 $\pm$ 1.9 (38–45)	5.1 $\pm$ 0.2 (5–6)	(0)			
WMNH- INV-2015-722	Tentacle (Range)	(11)	49.2 $\pm$ 6.1 (36–57)	4.3 $\pm$ 0.6 (3–5)	(0)			(0)			
	Anterior dorsal (Range)	(0)			(18)	40.1 $\pm$ 1.8 (36–43)	5.3 $\pm$ 0.5 (5–6)	(0)			
	Posterior dorsal (Range)	(0)			(18)	41.3 $\pm$ 1.7 (38–44)	5.3 $\pm$ 0.5 (5–6)	(0)			
	Anterior ventral (Range)	(0)			(17)	39.3 $\pm$ 2.2 (35–43)	4.9 $\pm$ 0.4 (4–6)	(0)			
	Posterior ventral (Range)	(0)			(17)	41.5 $\pm$ 2.2 (38–44)	4.9 $\pm$ 0.6 (4–6)	(0)			
WMNH- INV-2016-53	Tentacle (Range)	(16)	61.4 $\pm$ 7.9 (47–72)	5.9 $\pm$ 1.0 (4–7)	(0)			(0)			
	Anterior dorsal (Range)	(0)			(19)	54.8 $\pm$ 6.3 (41–68)	7.3 $\pm$ 0.6 (6–8)	(0)			
	Posterior dorsal (Range)	(0)			(16)	57.6 $\pm$ 2.7 (53–61)	7.0 $\pm$ 0.5 (6–8)	(0)			
	Anterior ventral (Range)	(0)			(19)	53.4 $\pm$ 4.4 (46–64)	7.2 $\pm$ 0.5 (6–8)	(0)			
	Posterior ventral (Range)	(0)			(17)	52.2 $\pm$ 4.1 (40–56)	6.8 $\pm$ 0.6 (6–8)	(0)			
WMNH- INV-2016-54	Tentacle (Range)	(11)	57.6 $\pm$ 6.8 (46–66)	4.9 $\pm$ 0.5 (4–6)	(0)			(0)			
	Anterior dorsal (Range)	(0)			(20)	50.9 $\pm$ 3.6 (43–58)	7.0 $\pm$ 0.6 (6–8)	(0)			
	Posterior dorsal (Range)	(0)			(17)	52.8 $\pm$ 2.1 (48–56)	6.8 $\pm$ 0.4 (6–7)	(0)			
	Anterior ventral (Range)	(0)			(20)	53.1 $\pm$ 4.1 (47–61)	7.2 $\pm$ 0.5 (6–8)	(0)			
	Posterior ventral (Range)	(0)			(17)	52.4 $\pm$ 2.3 (47–55)	6.5 $\pm$ 0.6 (5–7)	(0)			
WMNH- INV-2016-55	Tentacle (Range)	(15)	54.6 $\pm$ 6.8 (41–65)	5.1 $\pm$ 1.0 (4–7)	(0)			(0)			
	Anterior dorsal (Range)	(0)			(19)	55.7 $\pm$ 6.2 (45–64)	6.5 $\pm$ 0.6 (5–7)	(0)			
	Posterior dorsal (Range)	(0)			(20)	51.5 $\pm$ 5.7 (39–61)	6.1 $\pm$ 0.9 (4–7)	(0)			
	Anterior ventral (Range)	(0)			(20)	51.3 $\pm$ 4.2 (44–58)	6.5 $\pm$ 0.7 (5–8)	(0)			
	Posterior ventral (Range)	(0)			(20)	54.7 $\pm$ 4.1 (45–60)	6.2 $\pm$ 0.6 (5–7)	(0)			

^a WMNH-INV: Invertebrate Collection of the Wakayama Prefectural Museum of Natural History

mately 0.2–0.4 mm and width of basal part approximately 0.2–0.3 mm in largest specimen (holotype, WMNH-INV-2016-53), becoming gradually smaller as body size decreases.

Calcareous ring composed of five radial and five inter-radial plates, these all being centrally contracting, and distally spreading ends (Fig. 2B). Thin retractor muscles, combined with longitudinal muscles by mesentery. Ten tentacles non-retractile, each with up to three pairs of digits (sometimes unpaired digit present), tip pair largest; sensory cups absent (Fig. 2D). Color of tentacles translucent white (preserved and living specimens). Polian vesicle single, medioventral, and madreporite single (Table 1). Intestine has no loop. Two long bundles of ovaries or testis present, each with approximately 2–4 tubules, mostly unbranched except in basal part. One wide band of ciliated funnels situated in inter-radius of anterior to posterior body cavity, in left lateral side, dense in anterior, sparse in posterior. One narrow band of ciliated funnels situated in inter-radius of posterior body cavity, in mid-dorsal side, sparse. Sometimes 5–15 ciliated funnels clustered in a bundle.

Body wall containing sigmoid-hook ossicles (Figs 1D, 3E, Table 3), small, scattered in body surface in both radii and inter-radii, approximately 15–30 hooks arranged in one direction on verrucous surfaces similar in appearance to strawberry seeds. Pointed end facing toward tip of verrucous surfaces, and blunt end facing toward base of verrucous surfaces. Length of shank, 36–68 μm (means of 39–56 μm in different specimens) in anterior dorsal side, 35–64 μm (means 39–53 μm) in anterior ventral side, 37–61 μm (means 39–53 μm) in posterior dorsal side, and 38–60 μm (means 41–55 μm) in posterior ventral side. Measurements mostly significantly different among four parts (Kruskal–Wallis test, $P_s < 0.05$), except for one specimen (WMNH-INV-2016-54), and measurements of four parts significantly different among five counted specimens (Kruskal–Wallis test, $P_s < 0.05$).

Tentacles containing rod ossicles (Fig. 3D, Table 3). Rod ossicles arched; distally branched. Distal branches curved inward considerably. Sometimes having spinous processes distally, however, poorly in centrally. Rod ossicle length 35–72 μm , means of 43–61 μm in different specimens, significantly different among five counted specimens (Kruskal–Wallis test, $P < 0.01$).

Distribution In sandy-gravel sediment in the lower in-

tertidal zone, Sesoko Island, Okinawa Prefecture, Japan.

Etymology The specific name *sesokoensis* is derived from the name of the type locality of an island, Sesoko.

DNA barcode sequence The sequences of holotype (WMNH-INV-2016-53, accession number LC203485) and paratype (WMNH-INV-2016-55, accession number LC203486).

Evolutionary distance of nucleotide sequences based on *COI* gene between two new species

The length of the obtained *COI* sequences were 819 bp and the alignment of each sequence contained no insertions and deletions. In *Taeniogyrus verruculosus* sp. nov., two identical sequences were obtained. Base frequency was A=18.9%, C=14.9%, G=23.2%, T=43.0%. In *Scoliorhapis sesokoensis* sp. nov., two identical sequences are obtained. Base frequency was A=21.9%, C=13.9%, G=21.7%, T=42.5%. The evolutionary distance between two new species was 0.11 ± 0.01 (Table 4).

Discussion

The diagnostic characters of Taeniogyrinae Smirnov, 1998 are as follows: 10 or 12 tentacles; body wall ossicles comprise wheels of chiridotid type and sigmoid-hooks or sigmoid-hooks only. To date, the features of sigmoid-hook ossicles has only been observed in the congener of this group, with the following four genera having sigmoid-hook ossicles: *Sigmodota* Studer, 1876; *Scoliorhapis*; *Taeniogyrus*; *Rowedota* O'Loughlin & VandenSpiegel, 2010. Among them, *Sigmodota* has 12 tentacles, and *Rowedota* has wheels with teeth in six discrete groups, therefore they are not agreeable to the present new species, and only two genera *Scoliorhapis* and *Taeniogyrus* can be agreeable.

The genus *Scoliorhapis* Clark, 1946 (q.v.) replaces the rejected genus *Scoliodota* Clark, 1907 (q.v.), which was originally erected through the description of *Scoliodota japonica* Clark, 1907, based on the description of Australian specimens of *Taeniogyrus japonicus* (Marenzeller, 1881, as *Chirodota japonica*) (q.v.) of Théel's report (Théel 1886). However, Ohshima (1914) reported that *T. japonicus* had small wheels in very low frequency, and therefore *T. japonicus* was omitted from the genus *Scoliodota* (and temporarily belonged to the genus *Trochodota* Ludwig, 1891), and Clark (1921) had abandoned the genus without taking into consideration the Australian specimens. There-

Table 4. Evolutionary distances of *COI* nucleotide sequences between two new species of *Taeniogyrus* and *Scoliorhapis*.

	1	2	3	4
1 <i>T. verruculosus</i> sp. nov. (WMNH-INV-2016-205)		0*	0.01*	0.01*
2 <i>T. verruculosus</i> sp. nov. (WMNH-INV-2016-206)	0		0.01*	0.01*
3 <i>S. sesokoensis</i> sp. nov. (WMNH-INV-2016-53)	0.11	0.11		0*
4 <i>S. sesokoensis</i> sp. nov. (WMNH-INV-2016-55)	0.11	0.11	0	

*Estimated standard error

fore, Heding had erected *Scoliorhapis theelii* (Heding, 1928, as *Scoliodota theelii*) (q.v.) based on the description of these Australian specimens. Heding's genus *Scoliodota* had been then a monotypic genus, and a junior homonym of *Scoliodota* Clark, 1907 contained only *S. theelii*. After that, due to the belief "*Scoliodota* is a dead synonym of *Trochodota*", Clark (1946) replaced *Scoliodota* with *Scoliorhapis*. Furthermore, Oguro (1961) had described a new genus *Scoliodotella* Oguro, 1961, to replace *Scoliodota*, because he was not aware of Clark's report (1946). Recently, O'Loughlin & VandenSpiegel (2010) had synonymized *Scoliodotella* with *Scoliorhapis* (O'Loughlin & VandenSpiegel 2010) and had amended diagnosis of *Scoliorhapis* as follows: taeniogyrinae with 10 or 12 peltato-digitate tentacles, each with up to eight pairs of digits; single polian vesicle; ciliated funnels present; body wall ossicles sigmoid hooks only, hooks scattered or clustered or aligned (clustering of ossicles was rejected as a reliable generic diagnostic character); lacking wheels in body wall; tentacle ossicles bracket-shaped or rods.

The genus *Taeniogyrus* Semper, 1868 (q.v.) was originally separated from *Chirodota* Eschscholtz, 1829, defining *Taeniogyrus* based on the primary features of the wheel ossicles and sigmoid-hook ossicles in the skin of the body: wheel ossicles aggregated and forming papillae, and sigmoid-hook ossicles scattered uniformly. However, aggregations of wheel ossicles have been observed in only a few species besides the type species [e.g. *Taeniogyrus cidaridis* Ohshima, 1914; *T. heterosigmus* Heding, 1931 (q.v.)], and not observed in others. Therefore, some *Taeniogyrus* species that lack wheel papillae were thought to be better classified in the genus *Trochodota* Ludwig, 1891, which differs from *Taeniogyrus* primarily in the lack of aggregations of wheel ossicles (Ludwig 1898). Both *Taeniogyrus* and *Trochodota* were considered valid for a long time, although it was difficult to draw a definite line of demarcation between them. Recently, O'Loughlin and VandenSpiegel (2010) revised them and concluded that *Trochodota studeri* (Theel, 1886), the type species of *Trochodota*, is a junior synonym of *Holothuria* (*Fistularia*) *purpurea* Lesson, 1830. Furthermore, they placed *H. (F.) purpurea* into *Taeniogyrus*, as *T. purpureus* (Lesson, 1830). At the same time, O'Loughlin & VandenSpiegel (2010) had amended diagnosis of *Taeniogyrus* as follows: taeniogyrinae with 10 peltato-digitate tentacles, each with up to eight pairs of digits, terminal pair longest; body wall ossicles sigmoid hooks and wheels; teeth on the inner rim of wheels in continuous series; wheels and hooks variably grouped or scattered in body wall (wheels aggregated in body wall, "wheel papilla" were rejected as a reliable generic diagnostic character); no miliary granules; 1–12 polian vesicles; ciliated funnels present.

The diagnosis of *Scoliorhapis* and *Taeniogyrus* can apply to each one of the present new species: *S. sesokoensis* which lacks wheels (an observation for more than 50 specimens, including young and adult) and *T. verruculosus*

which has wheels. Presently, *Scoliorhapis* consists of five species (Paulay 2015) and *Taeniogyrus* consists of 26 species (Paulay 2013). Presently, two species of *Scoliorhapis* and four species of *Taeniogyrus* have been reported in Japan: *S. lindbergi* (D'yakonov in D'yakonov, Baranova, & Savael'eva, 1958) from Hokkaido (Oguro 1961, as *Scoliodotella uchidai* Oguro, 1961); *S. dianthus* Solis-Marin, Komatsu, Soliman, Uchida, Shimotani, Nozaki, 2014 from the Sea of Japan (Solis-Marin et al. 2014); *T. cidaridis* Ohshima, 1915 from Goto Islands, off Sagami Bay, and Uraga Strait (Ohshima 1914, 1915); *T. misakiensis* (Ohshima, 1919) from off. Sagami Bay (Ohshima 1914, 1919); *T. japonicus* (Marenzeller, 1881) from Sagami Bay (Marenzeller 1881; Ohshima 1914) and from several regions of middle and southern Japan (Utinomi 1965; Imaoka 1995); and *T. roseus* (Ohshima, 1914) from Okinawa (Ohshima 1914). In the present observation, none of the congeners were agreeable to the two new species.

For the "clustering of ossicles" in both the genera, a kind of clustering of sigmoid-hooks had been reported in the nominal three species: *Scoliorhapis theelii*, *Taeniogyrus japonicus* and *T. dendyi* (Mortensen, 1925), as "hook papilla", in which sigmoid-hooks were arranged radially in a single circle. The length and the number of sigmoid-hooks are 100–140 μm , 63–120 μm , and 80–140 μm , numbering approximately 10 but up to 16, and 3–6 in each papillae of *S. theelii*, *T. japonicus* and *T. dendyi*, respectively (Theel 1886; Ohshima 1914; Mortensen 1925; Heding 1928). In the two new species, however, 15–30 very small sigmoid-hooks (mostly less than 70 μm) are arranged in the verrucous surface in one direction, similar in appearance to strawberry seeds, and the structure is completely different from that observed in the congeners of the respective genera. Furthermore, according to Ohshima's (1913) illustrations and photographs, there is a possibility that the hook papilla of *T. japonicus* is not hollow, and it may have a completely different function from that of the two new species. An accurate knowledge of the structures of hook papillae in both the genera will be necessary in future studies.

Although DNA analysis and ossicle morphology clearly demonstrate that *Scoliorhapis sesokoensis* and *Taeniogyrus verruculosus* are separate species, they are not easily distinguished from each other based on the external appearance of their body shape and color. In external appearance, only the number of tentacle digits serves as a useful distinguishing feature in the adult forms. The number of tentacle digits of *T. verruculosus* is up to eight (seven digits in two paratype specimens, eight digits in the holotype), while *S. sesokoensis*, having up to six digits (in five type specimens, and more than 50 specimens of other materials, including young and adult). In the ossicle morphology, the most reliable distinguishing feature is the existence of wheels in the anterior dorsal skin of *T. verruculosus*. Another rough distinguishing feature is the size range of the shank length of sigmoid-hooks. The sigmoid-hooks of *T.*

verruculosus are comparatively larger than that of *S. sesokoensis* in animals of similar body size. However, the size ranges of the shank length of sigmoid-hooks differ significantly among specimens and parts, and became smaller in small sized animals. Furthermore, in the apodid sea cucumbers, the apparent dimensions of the animals are easily reduced by autotomy and shrinkage. Therefore, it may be difficult to judge whether the sigmoid-hooks are large or small if there is not a complete specimen or anaesthetized specimen.

Based on our observations, wheel ossicles can reliably be detected in *T. verruculosus*. However, wheel ossicles were very sparse in all specimens, and nearly lacking in a paratype specimen (WMNH-INV-2016-205, in which we could detect one wheel only). If we ignore the presence of wheel ossicles, then the two new species closely resemble each other, and it would seem that they could be phylogenetically related. However, the results of DNA analysis demonstrated the dichotomy between the two species. However, we still question whether the amount of evolutionary distance between them justifies a level of difference that would occur between two genera, and further analyses we conduct will address this question.

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