

# Description of a New Species, *Microcotyle pacinkar* n. sp. (Monogenea: Microcotylidae), Parasitic on Gills of *Sebastes taczanowskii* (Sebastidae) from off Usujiri, Hokkaido, Northern Japan

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*Microcotyle pacinkar* n. sp. (Monogenea: Microcotylidae) is described from the gills of *Sebastes taczanowskii* Steindachner, 1880 (Scorpionfishes: Sebastidae) (Japanese name: Ezo-mebaru) collected from off Usujiri in Hokkaido, northern Japan in the North Pacific Ocean. The new species is morphologically most similar to *M. ditrematis* Yamaguti, 1940 in having an elongated body and an inverted pear-shaped genital atrium but differs from the congener in the arrangement of the spines in the genital atrium and the shapes of the anterolateral sclerites of the clamps. The phylogenetic trees for species of *Microcotyle* van Beneden and Hesse, 1863 based on the partial cytochrome *c* oxidase subunit I region were estimated using new sequences of *M. pacinkar* n. sp.; the new species formed a sister group with *M. sebastis* Goto, 1894.

**Key Words:** *Microcotyle pacinkar* n. sp., Microcotylidae, monogenea, *Sebastes taczanowskii*, northern Japan.

## Introduction

The Microcotylidae is one of the largest families of polyopisthocotylean monogeneans, and within the family, *Microcotyle* van Beneden and Hesse, 1863 is the largest genus and all are parasites of marine fishes. In total, 17 valid species of *Microcotyle* have been recorded from Japanese coasts (Goto 1894; Ishii and Sawada 1937, 1938a, b; Yamaguti 1937a, b, 1940, 1958; Kihara 1960; Machida et al. 1972; Ono et al. 2020).

The white-edged rockfish *Sebastes taczanowskii* Steindachner, 1880 (Scorpionfishes: Sebastidae) is common in the coastal waters of northern Japan and is important to the coastal fisheries around Hokkaido (Takahashi et al. 1991). During the parasitological survey of marine fishes in Hokkaido, we collected an undescribed microcotylid species parasitic on *S. taczanowskii* in Usujiri Port, Hokkaido, northern Japan. In this study, we describe the specimens as a new *Microcotyle* species, providing a partial sequence of the cytochrome *c* oxidase subunit I gene as a DNA barcode.

## Materials and Methods

In total 56 specimens of *S. taczanowskii* were collected from Usujiri Port (41°56'10.6"N, 140°56'39.4"E) in Usujiri-

cho, Hakodate City, Hokkaido on 11 February 2020 and 29 August 2022. The fish were frozen and transported on ice to the laboratory, identified following Nakabo and Kai (2013) and examined for parasites. Monogeneans were removed from the gills using forceps under a dissecting microscope, then flattened under coverslip pressure and fixed in 99% ethanol or acetic acid-formalin-alcohol. All specimens except those used in the molecular study were stained with Heidenhain's iron hematoxylin. The anterior parts of some specimens that were fixed in 99% ethanol were excised using a razor for molecular analysis. Stained specimens and remaining bodies and haptors of the ethanol-fixed specimens were dehydrated in an ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube fitted on an Olympus BX50 compound microscope. Measurements are in micrometers, expressed as the range followed by mean values and the number of specimens measured shown in parentheses. Voucher specimens were deposited in the collection of the Meguro Parasitological Museum (MPM), Tokyo, Japan. The scientific names of fishes used in this paper follow Froese and Pauly (2023).

DNA was extracted from the whole body or the anterior portions of the monogenean bodies using a Wizard Genomic DNA Purification Kit (Promega, Madison, USA) in accordance with the manufacturer's protocol. Because the portions of the monogenean bodies are very small, Ethachinamate (NIPPON GENE, Tokyo, Japan) was added when

transferring supernatant to a tube containing isopropanol. The partial cytochrome *c* oxidase subunit I (*cox1*) gene was amplified by polymerase chain reaction (PCR) using the primers JB3 (5'-TTT TTT GGG CAT CCT GAG GTT TAT-3') and CO1-R trema (5'-CAA CAA ATC ATG ATG CAA AAG G-3') (Bowles et al. 1993; Miura et al. 2005). PCR amplification was performed in a 15 µL reaction mixture containing 7.5 µL of PCR buffer (Toyobo, Osaka, Japan), 3.0 µL of dNTPs, 1.4 µL of MilliQ water, 0.3 µL of KOD Fx Neo (Toyobo), 0.9 µL of forward and reverse primers (5 pmol/µL) and 1.0 µL of template DNA using a thermal cycler (Gene Atlas 322). The thermocycling profile was as follows: 35 cycles of 10 s at 98°C, 30 s at 48–50°C, and 60 s at 68°C. PCR products were checked on 1.0% agarose gel and bands were confirmed under a UV illuminator. Then, the bands were extracted from the gel and sequenced (SolGent Co., Ltd., Daejeon, South Korea). Sequence data and electropherograms were inspected and edited manually using MEGA7 (Kumar et al. 2016).

For phylogenetic analysis, *cox1* region was used. The *cox1* sequences obtained in the present study were aligned with other monogenean sequences retrieved from International Nucleotide Sequence Databases (INSD) (Table 1). They were aligned using MAFFT ver. 7 (Katoh et al. 2019) using the “unalignlevel: 0.8” and “Leave gappy regions” options under the G-INS-i strategy. Ambiguous sites in the aligned datasets were removed with Gblocks ver. 0.91b (Castresana 2000, 2002) using the “Allow smaller final blocks” option. The final sequence matrices consisting of 379 bp were subjected to maximum likelihood (ML) analyses using MEGA7 (Kumar et al. 2016). The best-fit substitution models were selected using MEGA7 for the ML analyses, and the Tamura-Nei (TN93) and Gamma distribution (G) models were applied. Statistical support for each node was evaluated using bootstrapping with 1000 bootstrap (BS) repeats. Bayesian Inference (BI) and Bayesian posterior probabilities (PP) were estimated using MrBayes 3.2.6 (Ronquist et al. 2012) under the general time-reversible (GTR) + G model for the data set. Two independent runs of four Markov chains were conducted for 1000000 generations and the tree was sampled every 100 generations. Parameter estimates and convergence were checked using Tracer ver. 1.6.0 (Rambaut and Drummond 2013); the first 250000 samples from each run were discarded as burn-in and the remaining were analyzed. Genetic distances [Kimura 2-parameter distance (Kimura 1980)] were estimated with MEGA7. A total of 376 positions were used.

## Results

Family **Microcotylidae** Taschenberg, 1879  
Subfamily **Microcotylinae** Taschenberg, 1879  
Genus **Microcotyle** van Beneden and Hesse, 1863  
**Microcotyle pacinkar** n. sp.  
(Figs 1, 2)

**Type material.** Holotype (MPM Coll.-No. 25226) and 21 paratypes (MPM Coll.-Nos 25227, 25228).

Table 1. Species of the Microcotylidae used in the present molecular study.

Monogenean species	Host species	Locality	INSD accession number	Reference
<i>Microcotyle pacinkar</i> n. sp.	<i>Sebastes taczanowskii</i> Steindachner, 1880	Japan, off Usujiri	LC753264, LC753264	Present study
<i>Microcotyle sebastis</i> Goto, 1894	<i>Sebastes schlegelii</i> Hilgendorf, 1880	South Korea, coasts of Tongyeong	MT876115, MT876116	Song et al. (2021)
<i>Microcotyle kasago</i> Ono, Matsumoto, Nitta, and Kamio, 2020	<i>Sebastiscus marmoratus</i> (Cuvier, 1829)	Japan, off Hiraio	LC472525	Ono et al. (2020)
<i>Microcotyle caudata</i> Goto, 1894	<i>Sebastes inermis</i> Cuvier, 1829	Japan, off Tarumi	LC472527	Ono et al. (2020)
	<i>Sebastes ventriosus</i> Temminck and Schlegel, 1843	Japan, off Tarumi	LC472528	Ono et al. (2020)
<i>Microcotyle visa</i> Bouguerche, Gey, Justine, and Tazerouti, 2019	<i>Pagrus caeruleostictus</i> (Valenciennes, 1830)	Off Algeria	MK275653, MK275654	Bouguerche et al. (2019)
<i>Microcotyle isyebi</i> Bouguerche, Gey, Justine, and Tazerouti, 2019	<i>Boops boops</i> (Linnaeus, 1758)	Off Algeria	MN816020, MN816021	Villora-Montero et al. (2020)
<i>Microcotyle algeriensis</i> Ayadi, Gey, Justine, and Tazerouti, 2017	<i>Scorpaena notata</i> Rafinesque, 1810	Off Algeria	KX926443	Ayadi et al. (2017)
<i>Microcotyle merche</i> Villora-Montero, Pérez-del-Olmo, Valmaseda-Angulo, Raga, and Montero, 2023	<i>Helicolenus dactylopterus</i> (Delaroche, 1809)	Off Guardamar del Segura	OQ243286, OQ243287	Villora-Montero et al. (2023)
<i>Microcotyle</i> sp. DG-2016	<i>Helicolenus dactylopterus</i>	Off Algeria	KX926446	Ayadi et al. (2017)
<i>Paramicrocotyle</i> sp. FAS-2014	<i>Pinguipes chilensis</i> Valenciennes, 1833	Chile	KJ794215	Oliva et al. (2014)
<i>Microcotyle erythrinus</i> van Beneden & Hesse, 1863	<i>Pagellus erythrinus</i> (Linnaeus, 1758)	Spain, off Guardamar del Segura	MN816012, MN816017	Villora-Montero et al. (2020)
<i>Microcotyle whittingtoni</i> Villora-Montero, Pérez-del-Olmo, Georgieva, Raga, and Montero, 2020	<i>Dentex dentex</i> (Linnaeus, 1758)	Spain, off Guardamar del Segura	MN816010, MN816011	Villora-Montero et al. (2020)
<i>Bivagina pagrosomi</i> (Murray, 1931)	<i>Sparus auratus</i> Linnaeus, 1758	Australia, Coff's Harbour	Z83003	Littlewood et al. (1997)

Table 2. Measurements of *Microcotyle pacinkar* n. sp. from *Sebastes taczanowskii* off Japan, compared with *Microcotyle* spp. from different hosts.

Species	<i>Microcotyle pacinkar</i> n. sp.		<i>M. sebastis</i>		<i>M. ditrematis</i>		<i>M. caudata</i>		<i>M. kasago</i>	
Host	<i>Sebastes taczanowskii</i>	<i>Sebastes</i> sp.	<i>Sebastes schlegelii</i>	<i>Sebastes maliger</i> (Jordan and Gilbert, 1880), <i>Sebastodes caurinus</i> Richardson, 1844	<i>Ditrema temminckii</i> Bleeker, 1853	<i>Sebastes inermis</i>	<i>Sebastes inermis</i>	<i>Sebastes inermis</i> , <i>Sebastes ventricosus</i> , <i>Sebastes cheni</i> Barsukov, 1988	<i>Sebastes marmoratus</i> , <i>Epinephelus akaara</i> (Temminck and Schlegel, 1842), <i>Sebastes ventricosus</i> , <i>Sebastichthys pachycephalus</i> Temminck and Schlegel, 1843	<i>Sebastes marmoratus</i>
Locality	Off Usujiri, Japan	Off Hakodate, Japan	Mutu Bay, Japan	Puget Sound, USA	Hamazima, Japan	Ise Bay, Japan	Off Tarumi, Japan	Seto Inland Sea, Japan	Off Tarumi, Japan	Off Hiroshima, Japan
Reference	This study	Goto (1894)	Yamaguti (1934)	Bonham and Guberlet (1937)	Yamaguti (1940)	Yamaguti (1934)	Yamaguti (1938)	Ono et al. (2020)	Yamaguti (1958)	Ono et al. (2020)
Body length	2690–6250 (mean: 4788)	5500	3900–4100	3100–4600 (3900)	3400–4500	2440, 2830	1800–2700	945–5550 (2769)	1700–4400	1900–3550 (2669)
Body width	300–774 (472)		560–670	640–1050 (860)	420–530	670, 870	440–750	180–1400 (819)	700–1100	360–720 (599)
No. of clamps	34–67 (49)	58	52, 56	62	78–88	60	32–52	27–69 (53)	22–62	32–43 (39)
Clamp length	48–76 (58)				60–80			40–100 (67)	80	35–58 (44)
Clamp width	68–100 (84)	68–128			33–42			25–90 (44)		50–80 (69)
Buccal sucker length	50–80 (68)				39–42			30–80 (46)		70–100 (79)
Buccal sucker width	45–90 (64)				30–32			20–90 (53)		63–98 (77)
Pharynx length	45–95 (66)		45–48	52–81 (70)						53–75 (69)
Pharynx width	37.5–85 (61)				33–35			20–100 (60)		23–75 (63)
Genital atrium length	45–175 (124)							10–210 (65)	100–240	63–113 (93)
Genital atrium width	60–160 (106)				90 (longest)			40–250 (145)		65–108 (92)
Length of genital spines	4–7 (5)	17 (longest)		12 (longest)				5–15 (9.1)		2.5–10 (6.6)
No. of testes	14–28 (21)	40	36, 43	21–48 (32)	22–25					10–14 (12)
Egg length	120–180 (150)			240	180					
Egg width	50–65 (58)			65	60					

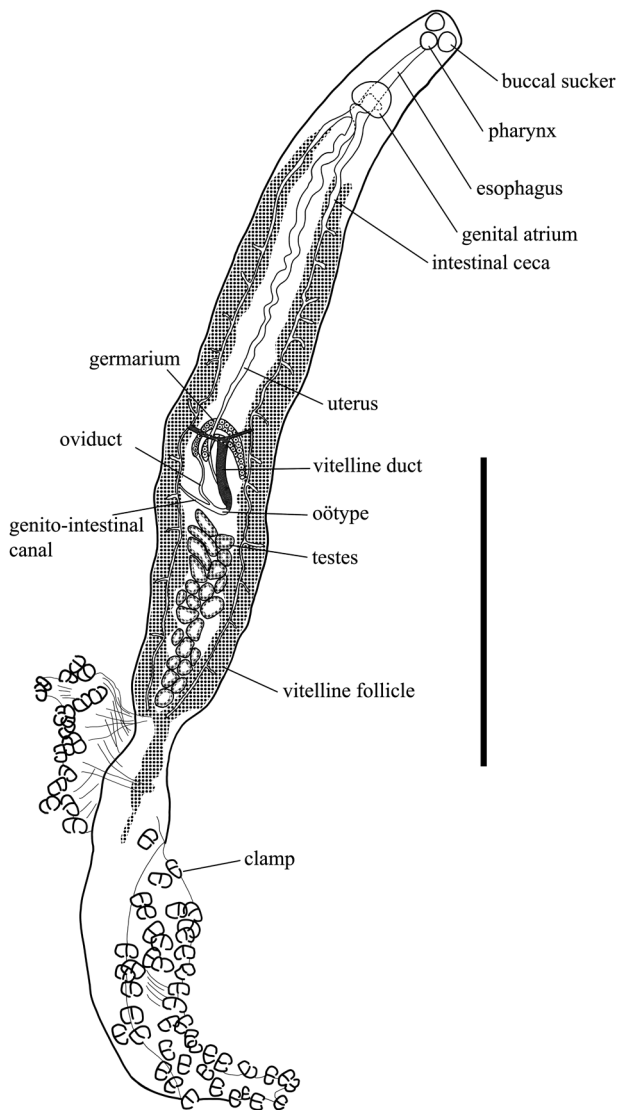


Fig. 1. *Microcotyle pacinkar* n. sp. from *Sebastes taczanowskii*. Whole body (ventral view, MPM Coll.-No. 25226). Scale bar: 500  $\mu$ m.

**Description.** Body (Fig. 1) elongated 2690–6250 (4788,  $n=15$ ) long including haptor, 300–774 (472,  $n=21$ ) wide at level of germarium. Haptor wedge-shaped, sub-symmetrical, with 34–67 (49,  $n=16$ ) clamps, arranged in 2 subequal lateral rows. Clamps (Fig. 2A) of equal structure, each clamp 48–76  $\times$  68–100 (58  $\times$  84,  $n=21$ ) in diameter. Clamps of *Microcotyle*-type, formed by two jaws. Ventral arm of median spring long, thin, ends distally in an inverted T, with short branches. Lateral sclerites of ventral jaw approaching midline distally. Dorsal arm of median spring inverted Y-shaped. Posterolateral sclerite and anterolateral sclerites curved toward inside.

Pair of buccal suckers (Fig. 1) septate, elliptical, 50–80  $\times$  45–90 (68  $\times$  64,  $n=17$ ). Pharynx (Fig. 1) globular, immediately posterior to buccal sucker, sometimes overlapping buccal sucker, 45–95  $\times$  37.5–85 (66  $\times$  61,  $n=18$ ). Esophagus (Fig. 1) short, without lateral diverticula. Intestinal bifurcation (Fig. 1) behind genital atrium. Intestinal ceca (Fig. 1) blind, extending to haptor, with numerous lateral di-

verticula, not united posteriorly.

Testes (Figs 1, 2B) with irregular shape, 14–28 (21,  $n=20$ ) in number, post-ovarian, intercaecal, in posterior half of body. Vas deferens (Figs 1, 2B) long, narrow, coming from anterior of testes, ventral to germarium, extending anteriorly, dorsal to uterus along its dorsal side, entering base of genital atrium. Genital atrium (Figs 1, 2C) inverted heart-shaped, 45–175  $\times$  60–160 (124  $\times$  106,  $n=17$ ), located posterior to pharynx, with lateral expansions, surrounded by muscle. Genital atrium proper bearing numerous small spines. Edge of genital atrium opening and its inner walls armed with numerous conical spines, 4–7 (5,  $n=10$ ) in length. Spines more dense in central anterior region, less dense in lateral pockets; no spines present in center of posterior pockets.

Germarium complex (Figs 1, 2B) begins at level of anteriormost testes, continuing anteriorly in midline, reflexing approximately at level of confluence of vitelline ducts, reflexing again toward anterior extremity, forming an equally thin anterior curve and reflexing a last time posteriorly before ending as oviduct, 37.5–200 (101,  $n=21$ ) long, 50–200 (102,  $n=21$ ) wide. Oviduct (Figs 1, 2B) arising from distal end of germarium, extending towards anterior of testes, connected to genito-intestinal canal. Genito-intestinal canal (Figs 1, 2B) originates from right intestinal cecum and connected to oviduct and vitelline duct. Vitelline duct (Figs 1, 2B) Y-shaped, ventral, extending from genito-intestinal canal and bifurcating to either side near the germarium. Oötype (Figs 1, 2B) extending from genito-intestinal canal to uterus. Mehlis' gland not observed. Uterus (Figs 1, 2B) originating from oötype, extending anteriorly along body midline, ventral to vas deferens, to the opening of the genital atrium. Vaginal pore (Fig. 2B) unarmed, ventral in mid-body, posterior to genital atrium. Vaginal duct (Fig. 2B) dorsal to uterus and vas deferens, arising from vaginal pore, connecting to seminal receptacle. Seminal receptacle (Fig. 2B) located ventral to germarium in center of body, oval-shaped. Eggs fusiform 120–180  $\times$  50–65 (150  $\times$  58,  $n=2$ ) excluding filament, with filaments at both ends broken. Vitelline follicles (Fig. 1) coextensive with intestinal branches, extending from behind genital atrium to posterior end of body, fused posterior to testes.

**Type locality.** Usujiri port (41°56'10.6"N, 140°56'39.4"E) in Usujiri-cho, Hakodate City, Hokkaido, northern Japan, the North Pacific Ocean.

**Type host.** *Sebastes taczanowskii* Steindachner, 1880 (Scorpaeniformes: Sebastidae).

**Site of infection.** Gill filament.

**Etymology.** The new specific name "*pacinkar*" refers to the Ainu name of the type host, *S. taczanowskii*, and thus is treated as indeclinable.

**Representative DNA sequences.** INSD accession numbers LC753264 and LC753265 (*cox1*) from two paratypes (MPM Coll.-No. 25228).

**Remarks.** *Microcotyle pacinkar* n. sp. is distinguished from other congeners by the following characters of the new species: the inverted heart-shaped genital atrium, the genital atrium surrounded by muscle, the arrangement of the spines



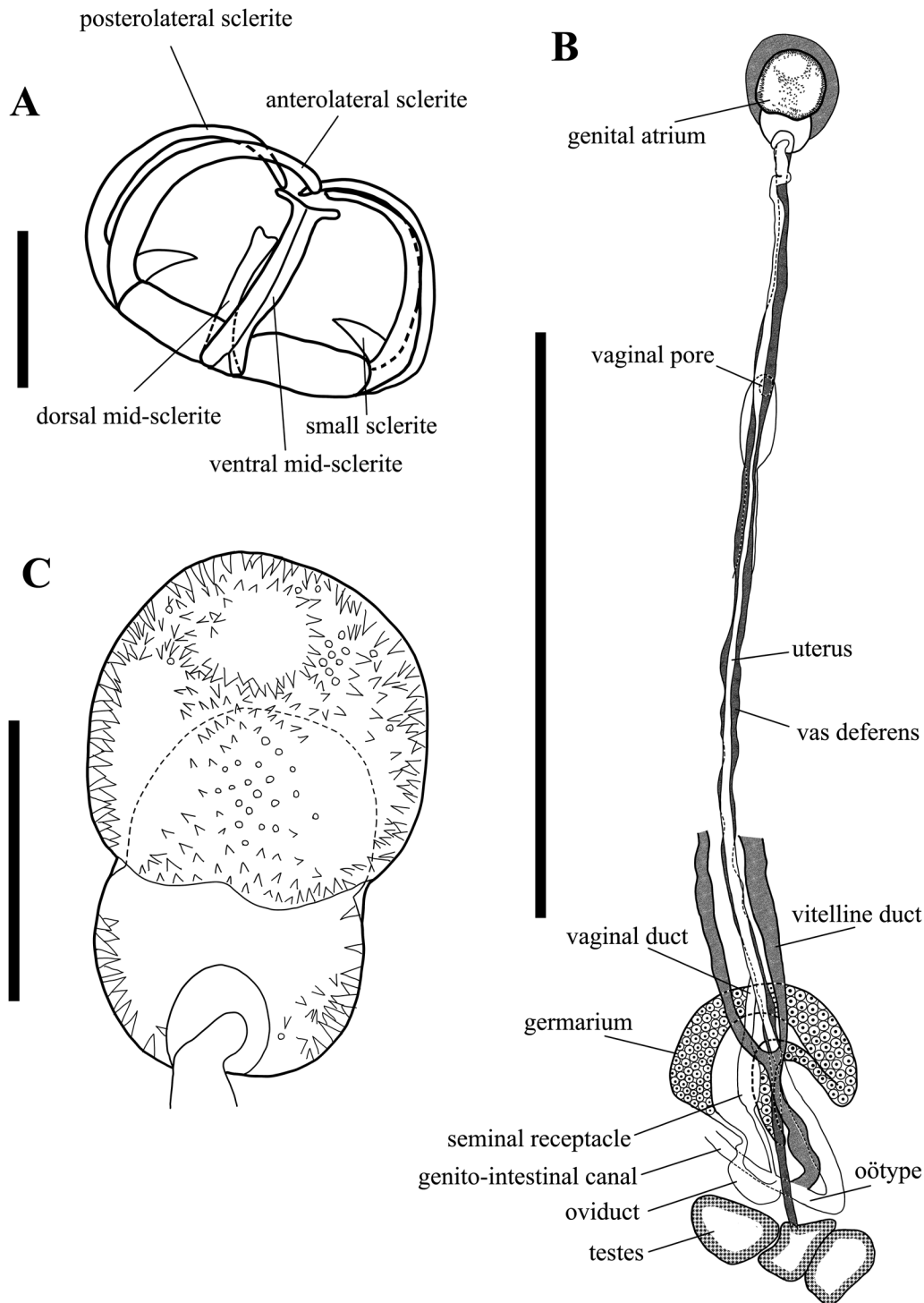


Fig. 2. *Microcotyle pacinkar* n. sp. from *Sebastes taczanowskii*. A, Clamp (ventral view, MPM Coll.-No. 25227); B, reproductive organs (ventral view, MPM Coll.-No. 25227); C, genital atrium (ventral view, MPM Coll.-No. 25227). Scale bars: A, 20  $\mu$ m; B, 1 mm; C, 50  $\mu$ m.

in the genital atrium, the shapes of the posterolateral sclerites of the clamps, the number of testes, and the long and narrow body (length/width ratio: 6.7–16.0).

#### Molecular data analysis

The trimmed multiple sequence alignment of *cox1* fragments consisted of 379 base pairs. A sequence of *Bivagina pagrosomi* (Murray, 1931) (Microcotylidae) was used as the outgroup (Fig. 3). In the phylogenetic tree based on the *cox1*

gene, *M. pacinkar* n. sp. and *M. sebastis* Goto, 1894 formed a clade with a strong support. The intraspecific pairwise sequence difference was 0.8%, and the differences between *M. pacinkar* n. sp. and *M. sebastis* were between 5.8%–7.0%. The pairwise sequence differences of *Microcotyle* species in the *cox1* gene are provided in Table 3.

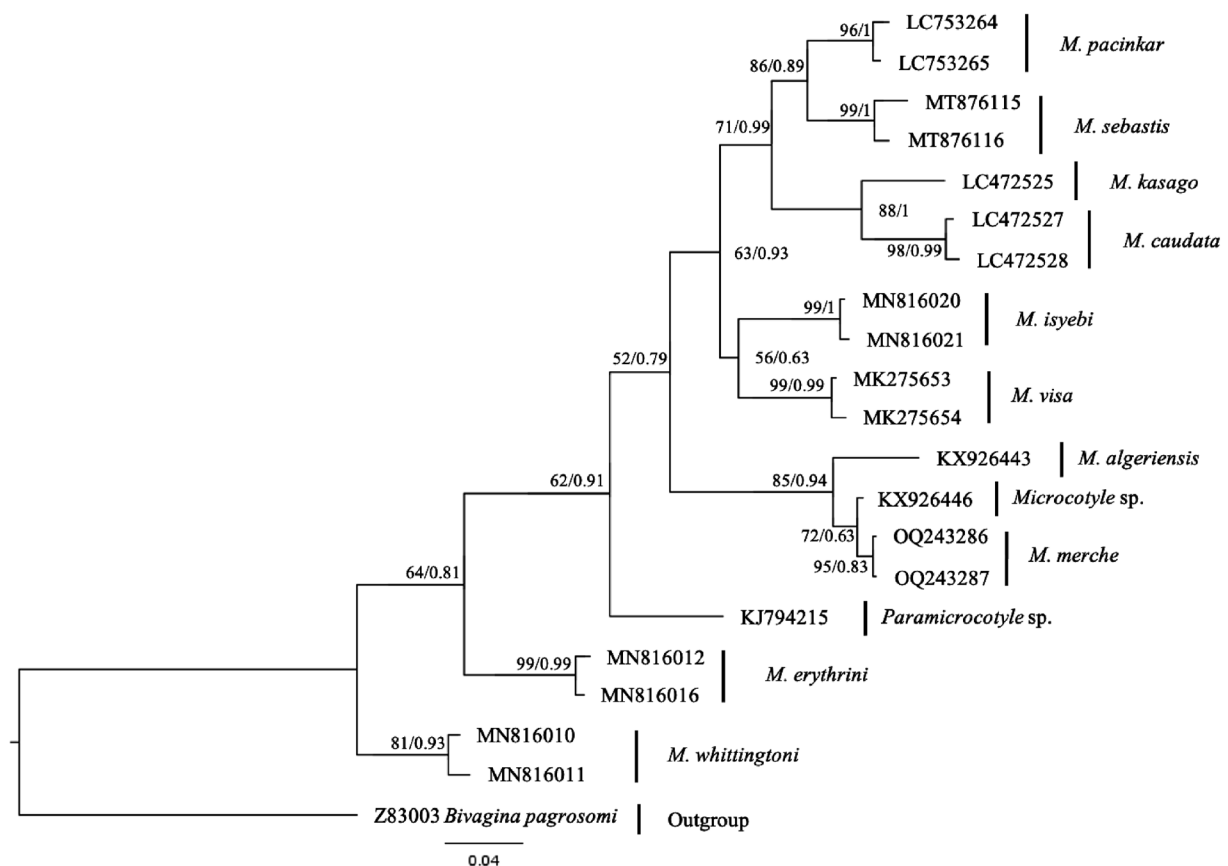


Fig. 3. Bayesian inference (BI) tree for the *Microcotyle* based on partial *cox1* (379 bp) data using *Bivagina pagrosomi* (Microcotylidae) as the outgroup. The corresponding INSD accession numbers are shown. The tree includes results for BI and maximum likelihood with PP/BS branch supports.

## Discussion

*Microcotyle pacinkar* n. sp. most closely resembles *M. ditrematis* Yamaguti, 1940 regarding the long and narrow body and the inverted heart-shaped genital atrium. However, the new species differs in the arrangement of the spines in the genital atrium (spines more dense in central anterior region, less dense in lateral pockets; no spines present in center of posterior pockets vs. entirely covered in spines) and the shapes of the posterolateral sclerites of the clamps (posterolateral sclerite curved toward inside vs. a part of the posterolateral sclerite is indented inward) (Yamaguti 1940). Additionally, the new species differs in the arrangement of number of clamps [34–67 in this study vs. 78–88 (Yamaguti 1940)].

*Microcotyle caudata* Goto, 1894, *M. sebastis*, *M. kasago* Ono, Matsumoto, Nitta, and Kamio, 2020, and *M. pacinkar* n. sp. are both reported from sebastid fishes and collected from coastal waters near Japan (Goto 1894; Yamaguti 1934, 1938, 1958; Bonham and Guberlet 1937; Zhang 2007; Ono et al. 2020). The new species can be differentiated from *M. caudata* and *M. kasago* by the features around the genital atrium (muscle vs. radiate muscle fibers vs nothing). The new species is distinguished from *M. caudata* by genital spines (184–191 small conical spines vs. upper margin

with row of 21–70 longer spines and center of genital atrium armed with 46–172 smaller spines) and from *M. sebastis* and *M. kasago* by the number of testes (14–28 vs. 36–43 vs. 10–14) (see Goto 1894; Yamaguti 1934; Ono et al. 2020) (Table 2). Furthermore, the molecular analyses support that *M. pacinkar* n. sp. is a distinct species compared with *M. caudata*, *M. kasago* and *M. sebastis*.

The *cox1* phylogeny revealed that the four species of *Microcotyle* that parasitize Japanese sebastids are closely related with two distinct clades, one consisting of *M. pacinkar* n. sp. and *M. sebastis*, and the other consisting of *M. caudata* and *M. kasago*. There are similarities in the geographic distribution of these species, as reflected in their *cox1* phylogeny, with *M. pacinkar* n. sp. and *M. sebastis* found in northern Japan [Mutsu Bay (Goto 1894) and Hokkaido (this study)], and *M. caudata* and *M. kasago* distributed around the Japanese archipelago [the Seto Inland Sea, Sea of Japan, North Pacific coast of Japan, and Yellow Sea (see Ono et al. 2020)]. *Microcotyle* species have a widespread global distribution, but available *cox1* data is currently limited to those found in the Mediterranean and from around Japan. Additionally, while 18 species of *Microcotyle* have been recorded in Japan, including the new species, there is currently a lack of sequence data for those found outside of sebastids. Further molecular biological studies are necessary to understand the relationship between phylogenetics, distribution, and hosts for this genus.

Table 3. Estimate of evolutionary divergence between sequences. There were a total of 379 positions in the dataset. Analyses were conducted using the Kimura 2-parameter model, shown as percentages.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. LC753264 <i>Microcotyle pacinkar</i> n. sp.																				
2. LC753265 <i>M. pacinkar</i> n. sp.	0.8																			
3. LC472527 <i>M. caudata</i>	11.3	11.2																		
4. LC472528 <i>M. caudata</i>	11.0	10.9	0.8																	
5. LC472525 <i>M. kasago</i>	8.8	8.8	07.6	8.2																
6. MT876115 <i>M. sebastis</i>	7.0	6.1	12.6	12.3	10.3															
7. MT876116 <i>M. sebastis</i>	6.7	5.8	11.9	12.3	9.7	1.9														
8. KX926443 <i>M. algeriensis</i>	13.5	13.2	15.8	15.8	15.1	14.9	15.2													
9. KX926446 <i>Microcotyle</i> sp.	12.9	12.5	14.8	15.1	13.4	12.5	12.9	4.7												
10. OQ243286 <i>M. merche</i>	12.9	12.5	15.1	15.4	13.1	11.6	11.9	5.6	0.8											
11. OQ243287 <i>M. merche</i>	12.9	12.5	15.1	15.4	13.1	11.6	11.9	5.6	0.8	0										
12. MK275653 <i>M. visa</i>	9.4	9.1	9.4	9.4	11.5	11.3	10.6	13.8	11.2	11.2	11.2									
13. MK275654 <i>M. visa</i>	9.7	9.4	9.7	9.7	11.8	11.6	10.9	14.1	11.5	11.5	11.5	0.3								
14. MN816020 <i>M. isyebi</i>	10.0	9.7	10.9	11.6	11.5	10.6	10.0	13.4	12.2	12.2	12.2	7.9	8.2							
15. MN816021 <i>M. isyebi</i>	10.3	10.0	11.2	11.9	11.8	10.3	9.7	13.8	12.5	12.5	12.5	8.2	8.5	0.3						
16. KJ794215 <i>Paramicrocotyle</i> sp.	10.9	11.2	14.2	13.8	12.5	11.0	11.0	15.2	12.9	12.2	12.2	11.2	11.6	11.9	12.2					
17. MN816010 <i>M. whittingtoni</i>	15.3	15.7	17.4	17.1	17.7	18.8	18.4	17.1	17.5	17.1	17.1	14.1	14.4	16.1	16.4	14.7				
18. MN816011 <i>M. whittingtoni</i>	15.7	16.0	16.7	16.3	17.4	18.8	18.4	17.5	17.1	16.8	16.8	13.4	13.7	16.1	16.4	15.4	1.6			
19. MN816012 <i>M. erythrini</i>	12.7	13.7	17.4	17.1	16.0	14.7	15.4	18.4	17.7	17.3	17.3	12.8	13.1	14.4	14.7	10.9	11.8	12.5		
20. MN816016 <i>M. erythrini</i>	12.4	13.4	17.4	17.1	16.1	14.4	15.1	17.7	16.7	16.3	16.3	11.8	12.2	14.1	14.4	10.6	11.9	12.5	0.8	
21. Z83003 <i>B. pagrosomi</i>	27.5	26.7	27.8	27.4	26.6	27.0	26.6	22.9	20.4	20.4	20.4	25.8	26.2	24.0	24.3	25.5	21.5	20.8	25.5	25.9

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## Authors Contributions

Yusuke Kamio: Conceptualization; Resources; Investigation; Formal analysis; Visualization; Writing – original draft; Funding acquisition. Masato Nitta: Conceptualization; Resources; Investigation; Writing – review & editing; Funding acquisition.

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## Declarations

**Competing interests.** The authors declare no conflicts of interest.

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