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Haplocotyle japonica n. gen., n. sp. (Monogenea: Microbothriidae) Parasitic on *Rhinobatos hynnicephalus* (Elasmobranchii: Rajiformes: Rhinobatidae) in Japanese Waters

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Haplocotyle japonica n. gen., n. sp. (Monogenea: Microbothriidae) is described from the skin and gill cavity of *Rhinobatos hynnicephalus* Richardson, 1846 (Elasmobranchii: Rajiformes: Rhinobatidae) in the Seto Inland Sea off Hiroshima Prefecture and the southern Sea of Japan off Fukuoka Prefecture, Japan. The new genus is closely related to *Dermophthirioides* Cheung and Nigrelli, 1983, *Dermophthirius* MacCallum, 1926, *Dermopristis* Kearn, Whittington, and Evans-Gowing, 2010, and *Pseudoleptobothrium* Young, 1967 in common morphological features (*i.e.*, the anterior extremity of the oötype having a tetrahedral shape, the anterior aperture of a bell-shaped pharynx, and the structure of the male copulatory organ) but differs from the latter four genera by the presence of a single testis and an ovary not looping the intestinal caeca. The phylogenetic analysis based on 28S rDNA sequences suggests that the new species shows affinity with *Dermophthirius* and *Dermopristis*. All currently known species of Microbothriidae Price, 1936 are listed, and a key to 12 genera, including *Haplocotyle* n. gen., of the family is provided.

Key Words: Monogenea, Microbothriidae, *Haplocotyle japonica* n. gen., n. sp., *Rhinobatos hynnicephalus*, the Seto Inland Sea, the Sea of Japan, Japan.

Introduction

Rhinobatos hynnicephalus Richardson, 1846 (Elasmobranchii: Rajiformes: Rhinobatidae) is a demersal elasmobranch found in the coastal waters of Far East Asia from South Japan to South China (Hatooka *et al.* 2013). In Japan, it is an important trawl fishery target species (Ishihara 1997; Hirai and Nishinokubi 2003; Ide *et al.* 2003). The parasite fauna of *R. hynnicephalus* has been understudied: the only two species of metazoan parasite recorded from this fish are *Neoheterocotyle forficata* Timofeeva, 1981, a monocotylid monogenean (Timofeeva 1981; Chisholm and Whittington 1997) and *Dangoka japonica* Izawa, 2011, an eudactylinid copepod (Izawa 2011).

The family Microbothriidae Price, 1936 parasitize the skin of elasmobranchs using the haptor lacking sclerotized structures, and it has been suggested that the members consist of a monophyletic group based on the 28S rDNA analysis (Perkins *et al.* 2009; Whittington and Kearn 2011). In this paper, a new species representing a new genus of Microbothriidae is described with a phylogenetic evidence based on the analysis of partial 28S rDNA sequence data along with available 28S rDNA data for various microbothriid species from GenBank. Yamaguti (1963) and Price (1963) independently provided a key of the family, but six genera have since been added to the family (Robinson 1961; Price 1963; Dillon and Hargis 1965; Young 1967; Cheung and Nigrelli 1983; Kearn *et al.* 2010). In this paper, all known species of this family including a new species are listed (Table 1), and an emended key to genera of the family is also given.

Anoplodiscus spari (Yamaguti, 1958) (originally as *Pseudomicrobothrium spari*) was described from *Acanthopagrus schlegelii* (Bleeker 1854) (Actinopterygii: Perciformes: Sparidae) as a member of Microbothriidae Price, 1936 by Yamaguti (1958), but the parasite has been currently replaced in Anoplodiscidae Tagliani, 1912 by Ogawa and Egusa (1981). Thus, this study is actually represented by the first record of a microbothriid monogenean from Japan.

Materials and Methods

Four specimens of *Rhinobatos hynnicephalus* caught by commercial trawl fishing were examined: two specimens were individually from the central Seto Inland Sea off Ōsaki-kami-jima Island (33°14′N, 132°48′E), Hiroshima Prefecture on 24 June 2013 and 30 August 2015, and another two from the southern Sea of Japan off Tsuyazaki Port (33°47′N, 130°24′E), Fukuoka Prefecture, on 3 July 2016. The fish were brought alive to the laboratory at Hiroshima

Table 1. List of valid microbothriid species with original description and redescription.

Monogenean species	Junior synonym	Host species	Locality	Reference
Asthenocotyle Robinson, 1961				
A. kaikourensis Robinson, 1961		*Centroscymnus plunketi* (Waite, 1910) (as *Scymnodon plunketi*)	New Zealand	Robinson (1961)
		C. plunketi (as *S. plunketi*)	New Zealand	Beverley-Burton *et al.* (1987)
		C. plunketi	New Zealand	Kearn *et al.* (2012)
A. taranakiensis Beverley-Burton, Klassen, and Lester, 1987		*Oxynotus bruniensis* (Ogilby, 1893)	New Zealand	Beverley-Burton *et al.* (1987); Kearn *et al.* (2012)
A. azorensis Kearn, Whittington and Thomas, 2012		*Etmopterus princeps* Collett, 1904	Azores, Portugal	Kearn *et al.* (2012)
Dermophthirius MacCallum, 1926				
D. carcharhini MacCallum, 1926,		*Carcharhinus leucas* (Müller and Henle, 1839)	Wood's Hole, USA	MacCallum (1926a)
D. maccallumi Watson and Thorson, 1976		*C. leucas*	Costa Rica; Nicaragua	Watson and Thorson (1976)
D. melanopteri Cheung, Nigrelli, Ruggieri, and Crow, 1988		*Carcharhinus melanopterus* (Quoy and Gaimard, 1824)	Christmas Island, Australia	Cheung *et al.* (1988)
D. nigrellii Cheung and Ruggieri, 1983		*Negaprion brevirostris* (Poey, 1868)	Florida, USA	Cheung and Ruggieri (1983)
D. penneri Benz, 1987		*Carcharhinus brevipinna* (Müller and Henle, 1839)	Gulf of Mexico, USA	Benz (1987)
		Carcharhinus limbatus (Müller and Henle, 1839)	Florida, USA	Benz (1987)
Dermopristis Kearn, Whittington and Evans-Gowing, 2010				
D. cairae Whittington and Kearn, 2011		*Glaucostegus typus* (Anonymous [Bennett], 1830)	Australia	Whittington and Kearn (2011)
D. paradoxus Kearn, Whittington, and Evans-Gowing, 2010		*Pristis microdon* Latham, 1794	Australia	Kearn *et al.* (2010)
Dermophthirioides Cheung and Nigrelli, 1983				
D. pristidis Cheung and Nigrelli, 1983		*Pristis pectinata* Latham, 1794	Florida, USA	Cheung and Nigrelli (1983)
Haplocotyle n. gen.				
H. japonica n. sp.		*Rhinobatos hynnicephalus*	Japan	This study
Leptobothrium Gallien, 1937				
L. pristiuri (Gallien, 1937)	*Pseudobothrium pristiuri* Gallien, 1937			Gallien (1937a, b); Dawes (1946)
Leptocotyle Monticelli, 1905*				
L. minor (Monticelli, 1888)	*Pseudocotyle minor* Monticelli, 1888	*Scyliorhinus canicula* (Linnaeus, 1758)	Italy	Monticelli (1888)
	Paracotyle caniculae Johnstone, 1911	*S. canicula* (Linnaeus, 1758)	UK	Johnstone (1911)
	Microbothrium caniculae (Johnstone, 1911)	*S. canicula*	UK	Jones (1933); Dawes (1946)
		S. canicula	Norway	Brinkmann (1952b)
		S. canicula	UK	Palombi (1949); Kearn (1965)
Leptomicrobothrium Dillon and Hargis, 1965				
L. longiphallus Dillon and Hargis, 1965		*Cephaloscyllium laticeps* (Duméril, 1853) (as *C. isabella* Whitley, 1932)	New Zealand	Dillon and Hargis (1965)
Pseudocotyle van Beneden and Hesse, 1865				
P. squatinae van Beneden and Hesse, 1865		*Squatina squatina* (Linnaeus, 1758)	Belgium	van Beneden and Hesse (1865); Taschenberg (1879); Braun (1890)

Table 1. continued

Monogenean species	Junior synonym	Host species	Locality	Reference
Pseudoleptobothrium Young, 1967				
P. aptychotremae Young, 1967		*Aptychotrema rostrata* (Shaw, 1794) [as *Rhinobatus banksii* (Müller and Henle, 1841)]	Australia	Young (1967)
		Trygonorrhina fasciata Müller and Henle, 1841	Australia	Glennon *et al.* (2006); Vaughan and Chisholm (2011)
P. christisoni Vaughan and Chisholm, 2011		*Rhinobatos annulatus* Müller and Henle, 1841	South Africa	Vaughan and Chisholm (2011)
Microbothrium Olsson, 1869				
M. apiculatum Olsson, 1869		*Squalus acanthias* Linnaeus, 1758 (as *Acanthias vulgaris* Bonaparte, 1846)	Skagerrack, Norway	Olsson (1869)
		S. acanthias (as *A. vulgaris*)	France	Saint-Remy (1891)
	Pseudocotyle apiculatum (Olsoon, 1869)	*S. acanthia*	Canada	Stafford (1904)
	Philura orata MacCallum, 1926	*Carcharhinus leucas* (Müller and Henle, 1839)	Wood's Hole, USA	MacCallum (1926a)
	Dermopllagus squali MacCallum, 1926	*S. acanthia*	Wood's Hole, USA	MacCallum (1926b); Price (1938); Dawes (1946)
M. tolloi Brinkmann, 1952		*Mustelus mento* Cope, 1877 (as *M. edulis* Pérez Canto, 1886)	Chili	Brinkmann (1952a)
M. lepidorhini (Guiart, 1938)	*Pseudocotyle lepidorhini* Guiart, 1938	*Centrophorus squamosus* (Bonnaterre, 1788) [as *Lepidorhinus squamosus*]	Finistère, France	Guiart (1938)
	Microbothrium centrophori Brinkmann, 1940	*C. squamosus*	Norway	Brinkmann (1940, 1952b)
Neodermophthirius Price, 1963				
N. harkemai Price, 1963		*Negaprion brevirostris* (Poey, 1868)	North Carolina, USA	Price (1963)

*The year when *Leptocotyle* Monticelli, 1905 was erected has been reported erroneously as 1904 by some authors (*e.g.*, Sproston 1946; Robinson 1961; Yamaguti 1963; Kearn *et al.* 2012). However, the publication date printed on the inside cover of the journal is "10 febbraio 1905", and like in Price (1938, 1963) and Dawes (1946), the present paper adopts "1905".

Table 2. List of the monogenean species used in this study with their host, host family, locality, and GenBank accession numbers.

Monogenean species	Host species	Host family	Locality	GenBank ID
Microbothriidae Price, 1936				
Asthenocotyle kaikourensis Robinson, 1961	*Centroscymnus plunketi*	Somniosidae	New Zealand	FJ971965
Dermophthirius penneri Benz, 1987	*Carcharhinus limbatus*	Carcharhinidae	USA	FJ971987
Dermopristis cairae Whittington and Kearn, 2011	*Glaucostegus typus*	Rhinobatidae	Australia	FJ971988*
Haplocotyle japonica n. gen., n. sp.	*Rhinobatos hynnicephalus*	Rhinobatidae	Japan (Hiroshima)	LC150819
	R. hynnicephalus	Rhinobatidae	Japan (Fukuoka)	LC228581
Leptocotyle minor (Monticelli, 1888)	*Scyliorhinus canicula*	Scyliorhinidae	UK	AF382063
Pseudoleptobothrium sp.	*Aptychotrema rostrata*	Rhinobatidae	Australia	FJ972012
Monocotylidae Taschenberg, 1879				
Calicotyle japonica Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi, and Hirose, 2010	*Squalus mitsukurii* Jordan and Snyder, 1903	Squalidae	Japan	AB485996
CapsalidaeBaird, 1853				
Benedenia seriolae (Yamaguti, 1934)	*Seriola quinqueradiata* Temminck and Schlegel, 1845	Carangidae	Japan	AY033941
Capsala pricei Hidalgo-Escalente, 1959	*Istiophorus platypterus* (Shaw, 1792)	Istiophoridae	China	JN980397

*This record deposited as *Dermophthirius* sp. was identified as *Dermopristis cairae* by Whittington and Kearn (2011).

University, Higashi-Hiroshima city, Hiroshima Prefecture or the Fishery Research Laboratory of Kyushu University, Fukutsu city, Fukuoka Prefecture, and examined for parasites. Monogeneans were picked up from the ventral skin and gill cavities using forceps. Specimens collected from the Seto Inland Sea were flattened under slight coverslip pressure and fixed in 70% ethanol except one specimen identified under an Olympus BX51 light microscope and preserved in 99% ethanol for molecular analysis. Specimens collected from the Sea of Japan were fixed in 99% ethanol.

All specimens, except the one for molecular study, from the Seto Inland Sea and four specimens from the Sea of

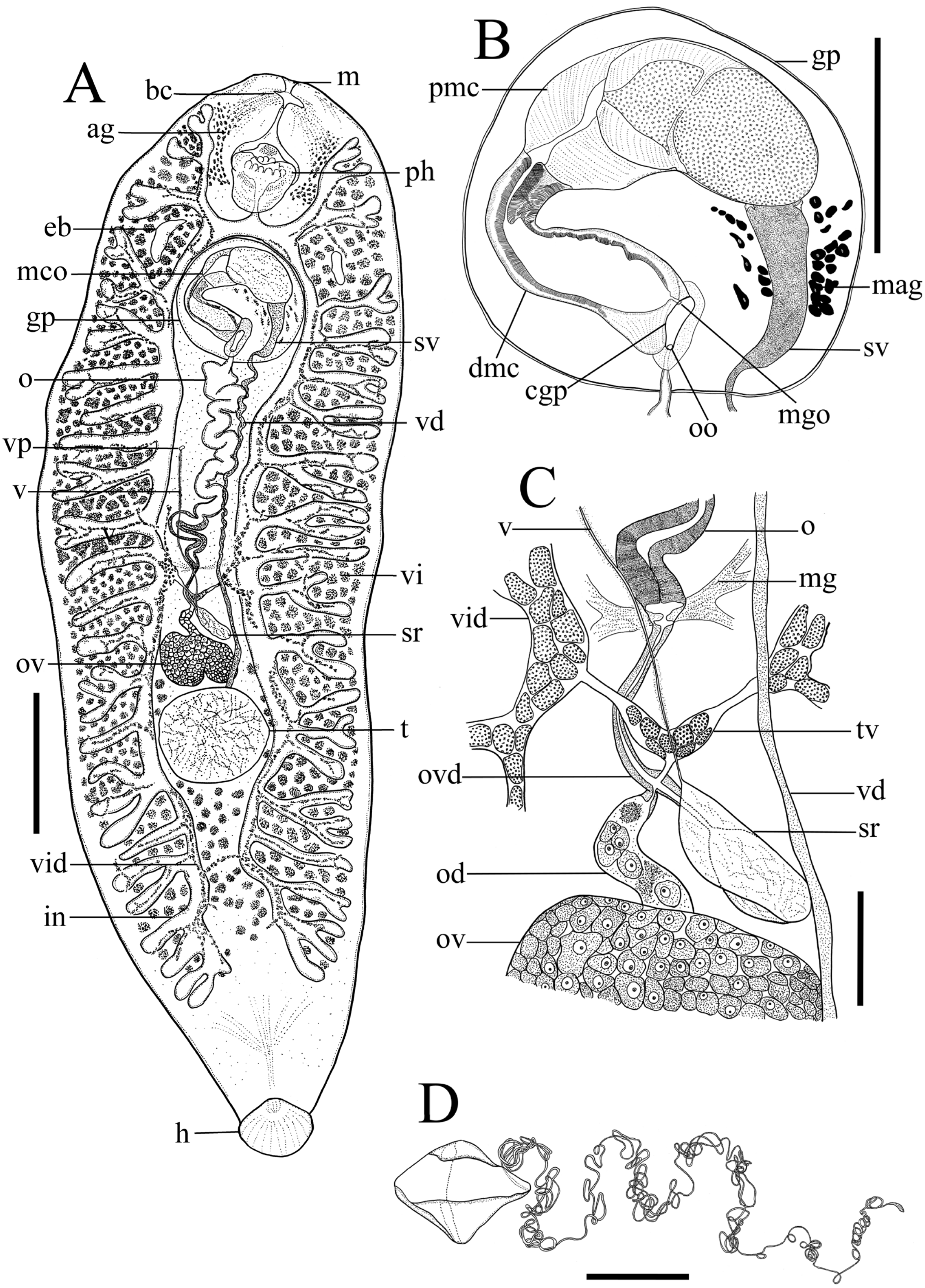

Fig. 1. *Haplocotyle japonica* n. gen., n. sp. from *Rhinobatos hynnicephalus*. A–C, holotype (NSMT-Pl 6167); D, paratype (NSMT-Pl 6168). A, whole mount (ventral view); B, male copulatory organ; C, reproductive system; D, egg in oötype. Scale bars: A, 200 µm; B–D, 100 µm. Abbreviations: ag, anterior gland; bc, buccal cavity; cgp, common genital pore; dmc, distal part of male copulatory organ; eb, excretory bladder; gp, genital pouch; h, haptor; in, intestinal caeca; m, mouth; mag, male accessory gland; mco, male copulatory organ; mg, Mehlis' gland; mgo, male genital opening; o, oötype; od, oviduct; oo, opening of oötype; ov, ovary; ovd, ovovitelline duct; ph, pharynx; pmc, proximal part of male copulatory organ; sr, seminal receptacle; sv, seminal vesicle; t, testis; tv, transverse vitelline duct; v, vagina; vd, vas deferens; vi, vitellarium; vid, vitelline duct; vp, vaginal pore.

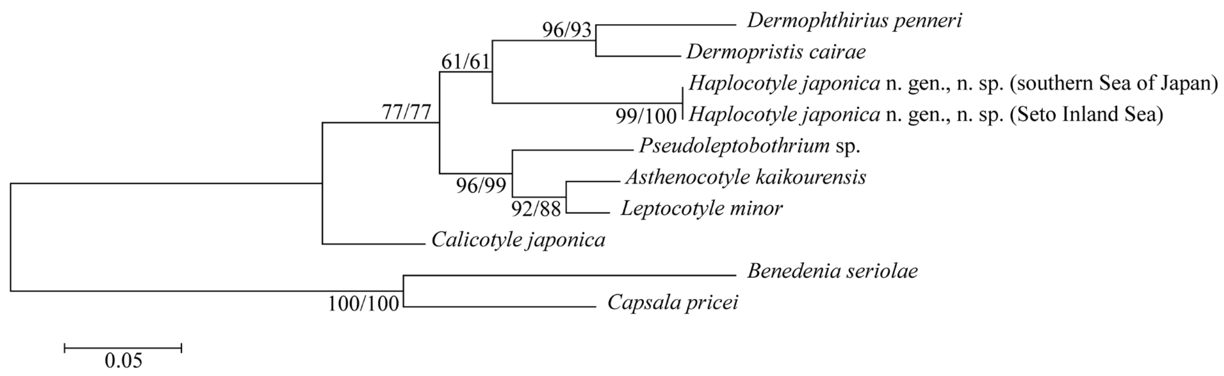

Fig. 2. Maximum likelihood (ML) tree for the Microbothriidae obtained using partial 28S rDNA sequences with *Calicotyle japonica* Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi, and Hirose, 2010 (Monocotylidae), *Benedenia seriolae* (Yamaguti, 1934) and *Capsala pricei* Hidalgo-Escalente, 1959 (Capsalidae) as outgroups. Bootstrap values shown along the branches are based on 1,000 replicates for the ML and NJ analysis.

Japan were stained in Heidenhain's iron hematoxylin or alum carmine, dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Drawings were made with the aid of a drawing tube fitted on an Olympus BX51 light microscope. Measurements, in micrometers, are expressed as the range followed in parentheses by the mean and the number (*n*) of specimens examined. Fish identification was based on Hatooka *et al.* (2013), and the scientific names of fishes used in this paper follow Froese and Pauly (2016). Specimens are deposited in the Platyhelminthes collection of the National Museum of Nature and Science, Tsukuba city, Ibaraki Prefecture, Japan (NSMT-Pl).

Two specimens individually from the Seto Inland Sea and the Sea of Japan for molecular analysis were cut by a razor under the genital pouch, and the upper half was preserved in 99% ethanol. DNA was extracted from the lower half part using the DNeasy blood and tissue kit (Qiagen) in accordance with the manufacturer's instructions. The DNA was amplified by polymerase chain reaction (PCR) using the primer pair C1 (5′-ACC CGC TGA ATT TAA GC A T-3′) and D2 (5′-TGG TCC GTG TTT CAA GAC-3′) to amplify partial 28S rDNA (Hassouna *et al.* 1984). A total of 25 μL PCR reaction consisted of 1 μL of DNA template, 1×ExTaq Buffer (TaKaRa), 0.2 mM of each dNTP, 1 μM of each primer, and 2.5 units of TaKaRa Ex Taq DNA Polymerase (TaKaRa). PCR was carried out with the following protocol: 94°C for 30 sec followed by 35 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 2 min, and 10 min of final hold at 72°C. PCR products were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and sequenced with a 3130X Genetic Analyzer (Applied Biosystems) with the same primers that generated the PCR products.

The 28S rDNA sequences of five species of microbothriid, three species as outgroups [Monocotylidae: *Calicotyle japonica* Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi, and Hirose, 2010, and Capsalidae: *Benedenia seriolae* (Yamaguti, 1934); and *Capsala pricei* Hidalgo-Escalente, 1959] from GenBank (Table 2), and the new species were edited and aligned with ClustalW using the default parameter, verified/edited visually using Mega6 (Tamura *et al.* 2013). Phylogenetic trees were constructed using the maximum likelihood (ML) and neighbor-joining (NJ) methods using Mega6. The Tamura 3-parameter+G model, which was determined to be the best-fit model using the Bayesian information, and the Kimura 2-parameter model was used to estimate distances for the ML and NJ analyses, respectively, and the robustness of the inferred phylogeny was assessed using a bootstrap procedure with 1,000 replications for the ML and NJ analyses.

Family **Microbothriidae** Price, 1936
[New Japanese name: kagi-nashi-hada-mushi-ka]
Genus ***Haplocotyle*** n. gen.
[New Japanese name: yamato-kagi-nashi-hada-mushi-zoku]

Diagnosis. Body elliptical. Haptor without sclerotized armature. Mouth opening sub-terminal; buccal cavity with anterior glands; pharynx present; bifurcate intestinal caecum not uniting posteriorly, with numerous diverticula leading laterally. Testis single; vas deferens running anteriorly on left and ventral side of body; seminal vesicle expanded in genital pouch. Male copulatory organ without sclerotized structures in genital pouch, consisting of proximal and distal part of male copulatory tract; male genital opening on ventral in common genital pore. Ovary lobate to round. Vaginal pore ventral, away from common genital pore; vaginal tube thin; oötype meandering, thick-walled, top formed tetrahedral shape, opening posterior to male genital opening in common genital pore. Ectoparasite of Rhinobatidae.

Type species. *Haplocotyle japonica* n. sp.

Etymology. *Haplocotyle* is from haplos (Greek), simple, and cotyle (Greek), a cup, referring to the haptor lacking any sclerotized armature.

Japanese name. The new Japanese family name, "kagi-nashi-hada-mushi" ("ka" means a family) refers to the opisthohaptor without anchors nor hooks, and "hada-mushi" means skin flukes. The new Japanese generic name is a combination of "yamato" meaning Japan and new Japanese family name ("zoku" means a genus).

Haplocotyle japonica **n. sp.**
(Fig. 1)
[New Japanese name: yamato-kagi-nashi-hada-mushi]

Holotype. Adult (NSMT-Pl 6167 collected on 24 June 2013).

Paratypes. Nine adults (NSMT-Pl 6168, 6289 collected on 24 June 2013) and four adults (NSMT-Pl 6290 collected on 6 July 2016).

Description. Body (Fig. 1A) elliptical, 2452–3983 (3253; $n=14$) long (including haptor), 1058–1471 (1301; $n=14$) wide. Haptor oval to fan-shaped, without sclerotized armature, 203–318 (248; $n=13$) long, 211–440 (307; $n=13$) wide. Eyes absent. Mouth opening sub-terminal of body; prepharynx with small buccal cavity connected anterior glands on each side; pharynx bell-shaped, 171–261 (214; $n=13$) long, 199–292 (248; $n=13$) wide with anterior aperture surrounded by papillae; esophagus short; bifurcate intestinal caecum with numerous diverticula leading laterally. Pair of excretory bladders located at same level as upper part of genital pouch.

Testis single, rounded, posterior to ovary, 213–407 (306, $n=14$) long, 316–501 (425, $n=14$) wide. Vas deferens exiting testis anteriorly, traveling medially on ventral side of body, left of oötype entering genital pouch at left posterior side, forming seminal vesicle, and connecting proximal part of male copulatory tract. Unsclerotized male copulatory organ (Fig. 1B) in elliptical genital pouch [219–473 (367, $n=14$) long, 403–520 (455, $n=14$) wide], consisting of muscular proximal part of male copulatory tract and distal part of male copulatory tract. Muscular proximal part of male copulatory tract, 282–413 (343, $n=14$) long, 130–195 (164, $n=14$) wide, divided into three parts; wall of proximal part of male copulatory tract formed to become thicker as closer to distal part of male copulatory tract. Distal part of male copulatory tract, 137–292 (221, $n=14$) long, 54–125 (84, $n=14$) wide, with small caliber at base, curving, widening, and connecting small male genital opening at ventral body surface in common genital pore located posterior of genital pouch. Interior surface of distal part of male copulatory tract covered by small papillae. Seminal vesicle and distal part of male copulatory tract surrounded by male accessory glands.

Ovary (Fig. 1C) lobate to round, in mid-body, 125–233 (187, $n=14$) long, 208–362 (278, $n=14$) wide. Oviduct arising from anterior part of ovary, connecting left side of ovovitelline duct. Unarmed vaginal pore on ventral surface, between right intestinal caecum and midpoint of oötype. Vaginal tube thin, extends to seminal receptacle ventrally. Seminal receptacle anterior of ovary, curving ventro-dorsally, ducting ovovitelline duct directly. Ovovitelline duct extending anteriorly, to base of oötype. Oötype thick-walled, traveling medially, meandering with top formed tetrahedral shape, and opening posterior to male genital opening in common genital pore. Mehlis' glands located base of oötype. Vitellarium co-extensive with intestinal caecum. Transverse vitelline duct crossing laterally, at level of anterior portion of seminal receptacle, connecting right side of ovovitelline duct. Egg with long coiled filament (Fig. 1D), 105–142 (126, $n=13$) long, 98–125 (112, $n=13$) wide without filament, in oötype.

Type host. *Rhinobatos hynnicephalus* (Elasmobranchii: Rajiformes: Rhinobatidae).

Type locality. The central Seto Inland Sea off Ōsaki-kami-jima Island, Hiroshima Prefecture, Japan.

Other locality. The southern Sea of Japan off Tsuyazaki Port, Fukuoka Prefecture, Japan.

Sites of infection. Ventral skin and gill cavities.

Etymology. The specific name is derived from the locality, Japan.

Japanese name. The new Japanese specific name is based on the new Japanese generic name.

Sequence data. Two sequences of the 28S rDNA (842 bp) gene accorded and were submitted to DDBJ (accession no. LC150819, LC228581). The phylogenetic analysis based on 28S rDNA sequences suggests that the new species shows affinity with *Dermophthirius* and *Dermopristis* (Fig. 2).

Discussion

With the inclusion of *Haplocotyle* n. gen., Microbothriidae now contains 12 genera: *Asthenocotyle* Robinson, 1961, *Dermophthirioides*, *Dermophthirius*, *Dermopristis*, *Haplocotyle* n. gen., *Leptobothrium* Gallien, 1937, *Leptocotyle* Monticelli, 1905, *Leptomicrobothrium* Dillon and Hargis, 1965, *Microbothrium* Olsson, 1869, *Neodermophthirius* Price, 1963, *Pseudocotyle* van Beneden and Hesse, 1865, and *Pseudoleptobothrium* Young, 1967. The new genus possessing a single testis differs from *Dermophthirioides*, *Dermophthirius*, and *Dermopristis* which have paired testes (*e.g.*, MacCallum 1926a; Benz 1987; Cheung and Nigrelli 1983; Kearn *et al.* 2010; Whittington and Kearn 2011). The intestinal caecum of *H. japonica* n. gen., n. sp. has diverticula, while that of *Asthenocotyle*, *Leptocotyle*, or *Leptomicrobothrium* is smooth (Jones 1933; Robinson 1961; Yamaguti 1963; Beverley-Burton *et al.* 1987; Kearn *et al.* 2012). In *Pseudoleptobothrium*, the ovary passes around the right intestinal caeca, and the diverticula extend to both sides of the body (Young 1967; Vaughan and Chisholm 2011), but the new species has no such characters. The male copulatory organ of *Microbothrium* and *Pseudocotyle* is sclerotized, and that of *Neodermophthirius* has spines (Price 1938, 1963; Brinkmann 1940, 1952a, b; Yamaguti 1963; Kearn *et al.* 2012), but such spines and a sclerotized male copulatory organ are not present in *H. japonica* n. gen., n. sp. The new genus has a simple vagina and differs from *Leptobothrium* which has a bifurcated vagina (Gallien 1937a; Price 1963; Yamaguti 1963).

Haplocotyle n. gen. is closely related to *Dermophthirioides*, *Dermophthirius*, *Dermopristis*, and *Pseudoleptobothrium* because they share the following morphological features: the anterior extremity of the oötype having a tetrahedral shape, the anterior aperture of a bell-shaped pharynx, and the structure of the male copulatory organ (MacCallum 1926a; Young 1967; Watson and Thorson 1976; Cheung and Nigrelli 1983; Cheung and Ruggieri 1983; Benz 1987; Cheung *et al.* 1988; Kearn *et al.* 2010; Vaughan and Chisholm 2011; Whittington and Kearn 2011). In the phylogenetic analysis based on 28S rDNA sequences, the new species is related to *Dermophthirius* and *Dermopristis*, both of which have paired testes (Benz 1987; Whittington and Kearn 2011), and forms a sister group (Fig. 2). *Pseudoleptobothrium* and *Leptocotyle* have a single testis (Jones 1933; Yamaguti 1963; Vaughan and Chisholm 2011) and are included in a sister-group with *Asthenocotyle* bering numerous testes (Robinson 1961). It is thus suggested that paired and numerous testes arise independently from a single testis.

This study is the first legitimate of a microbothriid in Japan. Although more than 200 species of elasmobranchs are known to occur in Japanese waters (Nakabo 2013), much remains unknown about their monogenean fauna. In Japan, three species of *Rhinobatos* (*R. granulatus* Cuvier, 1829, *R. schlegelii* Müller and Henle, 1841, and *R. hynnicephalus*) have been reported (Hatooka *et al.* 2013), and there is the possibility to obtain more species, possibly new, of microbothriids from these elasmobranchs. More studies are needed to clarify the diversity, host range, and geographical distribution of microbothriids in Japan.

All species of microbothriids, including *Haplocotyle japonica* n. gen., n. sp., are listed in Table 1, and a key to 12 genera of the family is provided.

Generic key to Microbothriidae

1. Testes paired 2
   Testis not paired 4
2. Male copulatory organ armed by spines . . . . *Dermophthirius* MacCallum, 1926
   Male copulatory organ not armed by spines 3
3. Obvious copulatory organ present . . . . *Dermophthirioides* Cheung and Nigrelli, 1983
   Obvious copulatory organ absent . . . . *Dermopristis* Kearn, Whittington, and Evans-Gowing, 2010
4. Intestinal caeca smooth 5
   Intestinal caeca diverticulate 7
5. Testes numerous . . . . *Asthenocotyle* Robinson, 1961
   Testis single 6
6. Genital pore postbifurcal . . *Leptocotyle* Monticelli, 1905
   Genital pore not postbifurcal . . . . *Leptomicrobothrium* Dillon and Hargis, 1965
7. Ovary looping intestinal caeca . . . . *Pseudoleptobothrium* Young, 1967
   Ovary not looping intestinal caeca 8
8. Male copulatory organ armed by spines . . . . *Neodermophthirius* Price, 1963
   Male copulatory organ without spines 9
9. Male copulatory organ sclerotized 10
   Male copulatory organ not sclerotized 11
10. Vagina single . . . . *Microbothrium* Olsson, 1869
    Vagina double . . . . *Pseudocotyle* van Beneden and Hesse, 1865
11. Vagina bifurcating to form two branches . . . . *Leptobothrium* Gallien, 1937
    Vagina not bifurcating . . . . *Haplocotyle* n. gen.

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