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Description of a new species, *Pseudodiscocotyla mikiae* n. sp. (Monogenea: Discocotylidae) parasitic on gills of *Pristipomoides filamentosus* from off Okinawa-jima island in Japan, with redescription of *Pseudodiscocotyla opakapaka*

Yusuke Kamio · Aina Inoue · Masato Nitta

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Abstract Two species of *Pseudodiscocotyla* Yamaguti, 1965 (Monogenea: Discocotylidae) were collected from crimson jobfish *Pristipomoides filamentosus* (Valenciennes) (Perciformes: Lutjanidae) off Okinawa-jima island, southern Japan. *Pseudodiscocotyla opakapaka* is redescribed and represents the first Japanese record. A new species, *Pseudodiscocotyla mikiae* **n. sp.**, differs from *Ps. opakapaka* in the absence of spines around the male genital pore, the shape of the vaginal pore, the presence of spines inside the vaginal pore, and the shape of the clamp. The locations of the male genital atrium and the vaginal pore in both species were similar, and the observed armament differences of the male copulatory organ are therefore presumed to establish reproductive isolation. The phylogenetic trees for the Mazocraeidea based on the partial 28S rDNA sequences were created using new sequences of *Pseudodiscocotyla mikiae* **n. sp.**, and Discocotylidae formed a sister group with the species Diclidophoridae, Macrovalvitrematidae, and Plectanocotylidae. *Pristipomoides filamentosus* is widely distributed across the Indo-Pacific, and *Pseudodiscocotyla mikiae* **n. sp.** could share the distribution of the host.

Introduction

Yamaguti (1965) established a monotypic genus for *Pseudodiscocotyla opakapaka* Yamaguti, 1965 (Monogenea: Discocotylidae) on the basis of specimens found on the gills of crimson jobfish *Pristipomoides filamentosus* (Valenciennes) (as *Pristipomoides microlepis*) (Perciformes: Lutjanidae) and *Aphareus rutilans* Cuvier in Hawaii, USA. *Pseudodiscocotyla* is distinguished from the other genera belonging to Discocotylidae by the following characters: the intestinal caeca do not unite, the shape of the male copulatory organ is elliptical and bulbus, and the genital atrium is armed (Yamaguti, 1965, 1968).

Pristipomoides filamentosus is a deep-water etelinae snapper inhabiting the tropical and subtropical Indo-Pacific, ranging from Southern Japan to the South China Sea (Nakabo, 2013) and is very important for commercial fishes in Japan and Hawaii (Taki, 2000). Three species of monogeneans have been

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Y. Kamio (✉) · A. Inoue
Hakuryo Junior and Senior High School, 2260 Amida, Amida-cho, Takasago, Hyogo 676-0827, Japan
e-mail: usukekamio@gmail.com

M. Nitta
Pathology Division, Nansei Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, 422-1, Nakatsuhamaura, Minami-Ise, Watarai, Mie 516-0193, Japan

reported in *Pr. filamentosus* from Hawaii: *Pseudallobenedenia opakapaka* Yamaguti, 1966 (Monopisthocotylea: Capsalidae), *Oliveriplectanum opakapaka* (Yamaguti, 1968) (Monopisthocotylea: Diplectanidae) (as *Diplectanum opakapaka*), and *Pseudodiscocotyla opakapaka* (Yamaguti, 1965, 1968).

During a survey of monogeneans from marine fishes around Okinawa-jima island, specimens of *Pseudodiscocotyla* were collected from *Pr. filamentosus* off the coasts of Okinawa City and Yomitan Town on Okinawa-jima island, southern Japan. We present *Ps. opakapaka* along with its redescription as the first record of this monogenean in Japan and describe a new *Pseudodiscocotyla* species.

Materials and methods

One specimen of *Pr. filamentosus* was purchased at the Awase Fish Market in Okinawa City on 21 December 2018 and another at the Yomitan Fishery Port in Yomitan Town on 23 May 2019. The former fish was captured off Okinawa-jima island, and the latter one was captured using a set-net off Yomitan Town (26°21′51.4″N, 127°43′09.2″E). The fish were transported on ice to the laboratory of Ryukyu University Museum (Fujukan), identified following Nakabo (2013) and examined for parasites. Fifteen monogeneans were removed from the gills using forceps under a dissecting microscope (Olympus SZ), then flattened under coverslip pressure and fixed in 99% ethanol or acetic acid–formalin–alcohol (AFA). For molecular analysis, the bodies of two ethanol-fixed specimens were used: a small piece was cut from the right lateral side of the main body using a razor, and was preserved in 99% ethanol. Thirteen specimens fixed in AFA and one specimen fixed in 99% ethanol (the hologenophore) were stained with Heidenhain's iron hematoxylin. The remaining haptor of the ethanol-fixed specimen (the hologenophore) was not stained. Then, the 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. The following voucher specimens from the parasite collection at the National Museum of Natural History (USNM) and Meguro Parasitological Museum (MPM) were examined for comparative purposes: the holotype of *Pseudodiscocotyla opakapaka* (USNM 1359220) and the paratypes of *Ps. opakapaka* (15550, 15551) described by Yamaguti (1965). Voucher specimens were deposited in the collection of the Meguro Parasitological Museum (21967–21969), Tokyo, Japan.

DNA was extracted from the isolated body of two specimens using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) and sequencing protocols followed Kamio & Nitta (2022). The edited sequences were submitted to the DNA Data Bank of Japan (DDBJ). The partial fragments of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox1*) region were compared with the available sequences for related species in the International Nucleotide Sequence Databases (INSD) using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) software on 14 March 2022.

For phylogenetic analysis, the 28S rDNA region was used. Sequences obtained for the 28S rDNA region in the present study were aligned with 39 other monogenean sequences retrieved from INSD (Table 1). The sequences of the 28S rDNA were aligned using MAFFT version 7 (Katoh et al., 2019) using the "unalignlevel: 0.0" and "Try to align gappy regions anyway" options under the G-INS-i strategy. Ambiguous sites in the aligned datasets were removed with Gblocks ver. 0.91b (Castresana, 2000) using the "Allow gap positions within the final blocks" option. Nucleotide substitution models were analyzed for each molecular marker based on the Bayesian information criterion using IQ-TREE version 2.0.4. (Kalyaanamoorthy et al., 2017; Minh et al., 2020). The maximum likelihood (ML) phylogeny was constructed under the GTR+F+I+G4 model using IQ-TREE version 2.0.4 with 1,000 bootstrap (BS) repeats. Bayesian inference (BI) and Bayesian posterior probabilities (PP) were estimated using MrBayes 3.2.6 (Ronquist et al., 2012) under the GTR+G+I model. Two independent runs of four Markov chains were conducted for 1,000,000 generations and the tree was sampled every 100 generations. Parameter estimates and convergence were checked using Tracer v. 1.6.0 (Rambaut & Drummond, 2013); the first 25,000 samples from each run were discarded as burn-in and the remaining were analyzed.

Table 1 List of species used in the present study to construct the phylogenetic trees based on 28S rDNA data.

Species	Host	Accession number	Reference
Suborder **Mazocraeinea** (Mazocraeidea)			
Family **Mazocraeidae**			
Pseudomazocraes selene Hargis, 1957	*Caranx latus* (Agassiz)	MG984594	Camargo & Santos (2020)
Family **Plectanocotylidae**			
Plectanocotyle gurnardi (Van Beneden & Hesse, 1863)	*Eutrigla gurnardus* (Linnaeus)	AF382045	Olson & Littlewood (2002)
Octoplectanocotyla trichiuri Yamaguti, 1937	*Trichiurus japonicus* (Temminck & Schlegel)	MT890142	Unpublished (Direct submission)
Suborder **Gastrocotylinea**			
Family **Gastrocotylidae**			
Gastrocotyle kurra Unnithan, 1968	*Decapterus russelli* (Rüppell)	KF804030	Tambireddy et al. (2016)
Allopseudaxine katsuwonis (Ishii, 1936)	*Katsuwonus pelamis* (Linnaeus)	MT890130	Unpublished (Direct submission)
Pseudaxine trachuri Parona & Perugia, 1890	*Trachurus trachurus* (Linnaeus)	AM157222	Badets et al. (2011)
Family **Thoracocotylidae**			
Pricea multae Chauhan, 1945	*Scomberomorus commerson* (Lacepède)	ON032998	Baghdadi et al. (2022)
Family **Gotocotylidae**			
Gotocotyla bivaginalis (Ramalingam, 1961)	*Scomberomorus commerson* (Lacepède)	AF382039	Olson & Littlewood (2002)
Family **Protomicrocotylidae**			
Bilaterocotyle madrasensis (Radha, 1966)	*Megalaspis cordyla* (Linnaeus)	KF804029	Tambireddy et al. (2016)
Bilaterocotyloides carangis Ramalingam, 1961	*Megalaspis cordyla* (Linnaeus)	KF804032	Tambireddy et al. (2016)
Neomicrocotyle pacifica (Meserve, 1938)	*Caranx hippos* (Linnaeus)	AF382043	Olson & Littlewood (2002)
Family **Allodiscocotylidae**			
Allodiscocotyla diacanthi Unnithan, 1962	*Scomberoides commersonnianus* (Lacepède)	KF804038	Tambireddy et al. (2016)
Metacamopia oligoplites Takemoto, Amato & Luque, 1996	*Oligoplites* sp.	AF382038	Olson & Littlewood (2002)
Family **Chauhaneidae**			
Gemmaecaputia corrugata Tripathi, 1959	*Sphyraena forsteri* (Cuvier)	LC623879	Kamio & Nitta (2022)
Suborder **Discocotylinea**			
Family **Discocotylidae**			
Discocotyle sagittate (Leuckart, 1842)	*Salmo trutta* (Linnaeus)	AF382036	Olson & Littlewood (2002)
Anthocotyle americana (MacCallum, 1916)	*Merluccius gayi* (Guichenot)	MT890120	Unpublished (Direct submission)
Anthocotyle merlucci Van Beneden & Hesse, 1863	*Merluccius merluccius* (Linnaeus)	MT890118	Unpublished (Direct submission)
Pseudodiscocotyla mikiae **n. sp.**	*Pristipomoides filamentosus* (Valenciennes)	LC732579	This study
Family **Diplozoidae**			

Table 1 (continued)

Species	Host	Accession number	Reference
Eudiplozoon nipponicum (Goto, 1891)	*Cyprinus carpio* (Linnaeus)	AF382037	Olson & Littlewood (2002)
Paradiplozoon hemiculteri (Ling, 1973)	*Hemiculter leucisculus* (Basilewsky)	MN545903	Unpublished (Direct submission)
Family **Octomacridae**			
Octomacrum europaeum Roman & Bychowsky, 1956	*Alburnoides bipunctatus* (Bloch)	MT441500	Benovics et al. (2021)
Suborder **Hexostomatinea**			
Family **Hexostomatidae**			
Hexostoma thynni (Delaroche, 1811)	*Thunnus thynnus* (Linnaeus)	OM731590	Ayadi et al. (2022)
Leptohexostoma gymnosarda Li, Zhu, Ding, & Yuan, 2018	*Gymnosarda unicolor* (Rüppell)	MN242399	Al-Nabati et al. (2021)
Suborder **Microcotylinea**			
Family **Microcotylidae**			
Microcotyle erythrini Van Beneden & Hesse, 1863	*Pagrus pagrus* (Linnaeus)	MN814849	Víllora-Montero et al. (2020)
Bivagina pagrosomi (Murray, 1931)	*Sparus auratus* (Linnaeus)	Z83002	Littlewood et al. (1997)
Cynoscionicola sp.*	*Umbrina xanti* (Gill)	AF382050	Olson & Littlewood (2002)
Family **Diclidophoridae**			
Choricotyle australiensis Roubal, Armitage, & Rohde, 1983	*Rhabdosargus sarba* (Forsskål)	AF382046	Olson & Littlewood (2002)
Diclidophora minor (Olsson, 1876)	*Micromesistius poutassou* (Risso)	AF382048	Olson & Littlewood (2002)
Diclidophora denticulata (Olsson, 1876)	*Pollachius virens* (Linnaeus)	AY157169	Lockyer et al. (2003)
Heterobothrium victorwepeneri Acosta & Smit, 2021	*Amblyrhynchotes honckenii* (Bloch)	MW115856	Acosta & Smit (2021)
Urocotyle nibae Zhang & Xiao in Zhang, Yang, & Liu, 2001	*Johnius belengerii* (Cuvier)	FJ432588	Su (2009)
Family **Heteraxinidae**			
Heteraxinoides atlanticus Gayevskaya & Kovaljova, 1979	*Nemipterus japonicus* (Bloch)	KU245366	Verma & Verma (2021)
Cemocotyle carangis (MacCallum, 1913)	*Caranx latus* (Agassiz)	MG984598	Al-Nabati et al. (2021)
Probursata brasiliensis Takemoto, Amato, & Luque, 1993	*Oligoplites* sp.	AF382049	Olson & Littlewood (2002)
Kannaphallus leptosomu Nitta, Kondo, Ohtsuka, Kamarudin, & Ismail, 2022	*Scyris indica* (Rüppell)	LC664021	Nitta et al. (2022)
Zeuxapta seriolae (Meserve, 1938)	*Seriola lalandi* (Valenciennes)	EF653384	Aiken et al. (2007)
Family **Heteromicrocotylidae**			
Heterapta heterapta Unnithan, 1961	–	KF804036	Al-Nabati et al. (2021)
Family **Macrovalvitrematidae**			
Nicolasia canosoroum Suriano, 1975	*Umbrina canosai* (Berg)	MT890128	Unpublished (Direct submission)
Pseudotagia rubri Luque, Amato, & Takemoto, 1993	*Orthopristis ruber* (Cuvier)	MT890129	Unpublished (Direct submission)
Order **Diclybothriidea**			
Family **Hexabothriidae**			

Table 1 (continued)

Species	Host	Accession number	Reference
Narcinecotyle longifilamentus Torres-Carrera, Ruiz-Escobar, García-Prieto, & Oceguera-Figueroa, 2020	*Narcine entemedor* (Jordan & Starks)	MN367805	Torres-Carrera et al. (2020)
Pseudohexabothrium taeniurae Agrawal, Chisholm, & Whittington, 1996	*Taeniura lymna* (Forsskål)	AF382035	Olson & Littlewood (2002)

*This species name is registered as “*Cynoscionicola branquialis*” by Olson & Littlewood (2002), but the name regarded as *Cynoscionicola branquialis* (taxon inquirendum) by Gibson (2022). In our literature review, we found no references to these specific names used for Microcotylidae species prior to 2002, and it is likely that they are nomen nuda. Therefore, this species is treated here as *Cynoscionicola* sp.

Family Discocotylidae Price, 1936

Subfamily Pseudodiscotylinae Yamaguti, 1965

Genus *Pseudodiscocotyla* Yamaguti, 1965

Pseudodiscocotyla opakapaka Yamaguti, 1965

Type-host: *Pristipomoides filamentosus* (Valenciennes) (Perciformes: Lutjanidae) (as *Pristipomoides microlepis*: see Yamaguti, 1965).

Other-host: *Aphareus rutilans* Cuvier (see Yamaguti, 1965).

Type-locality: Hawaii, USA (see Yamaguti, 1965).

Other-locality: East China Sea, off Yomitan, Okinawa City, Okinawa Prefecture, Japan (this study).

Newly collected material examined: 4 specimens from off Yomitan Town (21967).

Comparative museum material examined: Holotype and 13 paratypes (USNM 1359220) deposited in the Smithsonian US National Parasite Collection; 27 paratypes of *Pseudodiscocotyla opakapaka* (15550, 15551) deposited in the Meguro Parasitological Museum.

Site on host: Gills.

Redescription (Figs. 1, 2, 3, 4)

Body elongated, total length 3,200–4,375 (3,875, n = 3), width at level of posterior end of germarium 375–500 (425, n = 3). Haptor ginkgo-leaf-shaped, symmetrical, with 4 pairs of clamps. Clamps (Fig. 2A) of equal structure, typically *Discocotyle*-type, each clamp 100–145 × 100–205 (118 × 159, n = 3). Clamp consisting of pair of antero-lateral sclerites and postero-lateral sclerites, ventral mid-sclerite and dorsal mid-sclerite. Antero-lateral sclerites and postero-lateral sclerites thin and long. Small sclerites from proximal end of postero-lateral sclerites toward

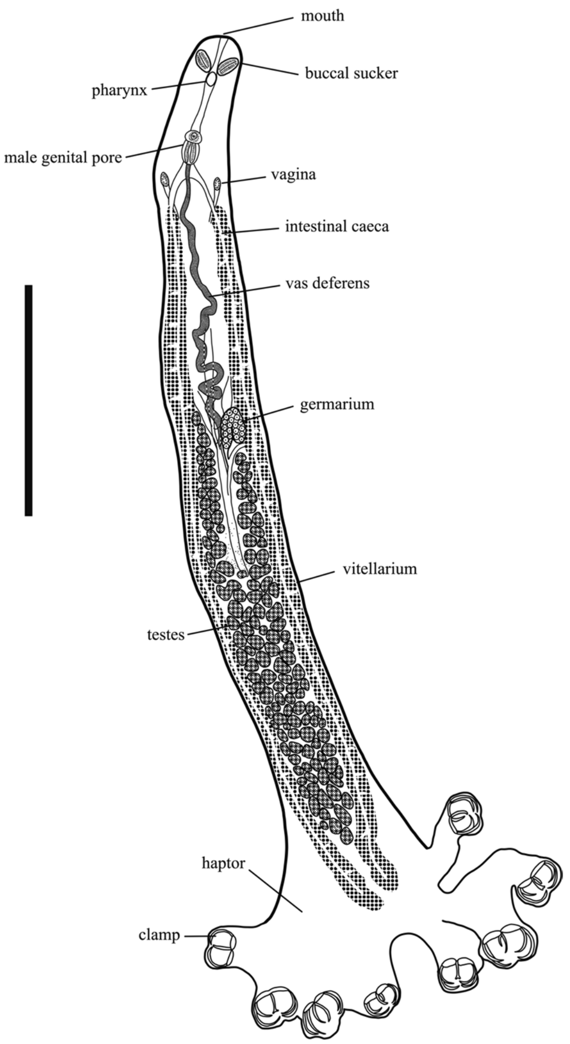

Fig. 1 *Pseudodiscocotyla opakapaka* Yamaguti, 1965 from *Pristipomoides filamentosus* (Valenciennes) off Okinawa-jima island, Japan. Whole body (ventral view, 21967). Scale bar: 1 mm.

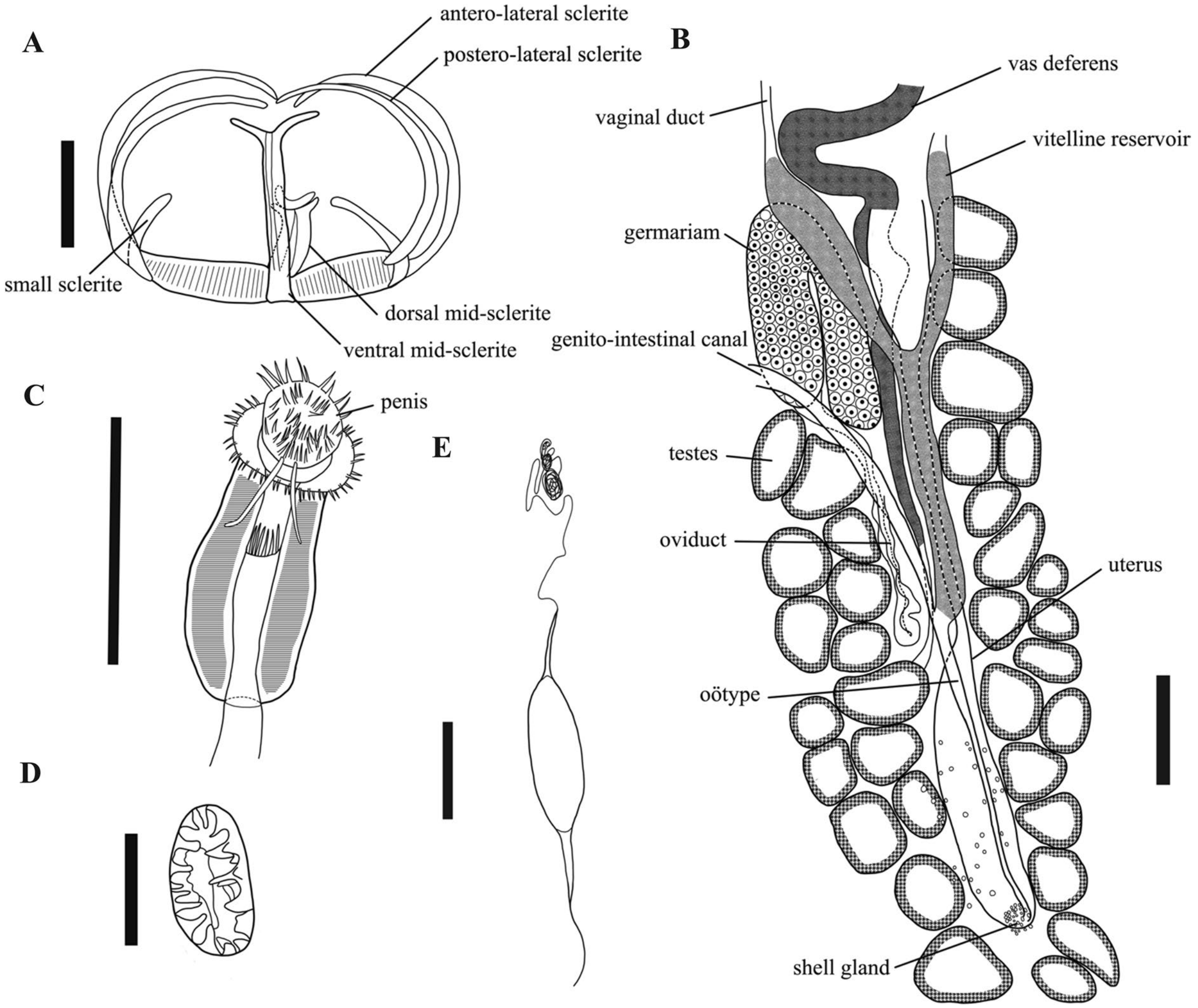

Fig. 2 *Pseudodiscocotyla opakapaka* Yamaguti, 1965. A, Clamp (ventral view, 21967); B, Reproductive organs (ventral view, 21967); C, Male copulatory organ (21967); D, Vaginal pore (21967); E, Egg (21967). Scale bars: A, C, D, 50 μm; B, E, 100 μm.

inside. Ventral mid-sclerite long, bifurcated on top. Dorsal mid-sclerite short, bifurcated on top, armed with accessory skeletal piece at its distal end. Accessory skeletal piece small, V-shaped.

Mouth opening anterior terminal. Pair of buccal suckers elliptical, 50–70 × 40–110 (58 × 97, n = 3). Pharynx globular, lying on body midline immediately posterior to buccal suckers, 50–60 × 40–50 (53 × 43, n = 3). Oesophagus short without diverticula, bifurcating anterior or posterior to the male genital pore. Intestinal caeca with numerous lateral diverticula, extending into haptor, not united posteriorly.

Testes (Fig. 2B) with irregular shape, 99–127 (108, n = 3) in number, postgermarial, arranged in the posterior half of body and confined to intercrural field, divided into two lateral groups from posterior end of germarium to level of the shell gland. Vas deferens (Fig. 2B) long, conspicuous, coming from anterior testes on right side of body at level of oötype, ventral to germarium, running forward along body midline, entering base of male genital pore. Male copulatory organ (Fig. 2C), consisting of thick-walled duct and penis, length 99–127 (108, n = 3) from base to tip. Penis bearing numerous minute spines: lateral surface with numerous spines; distal end of penis with minute

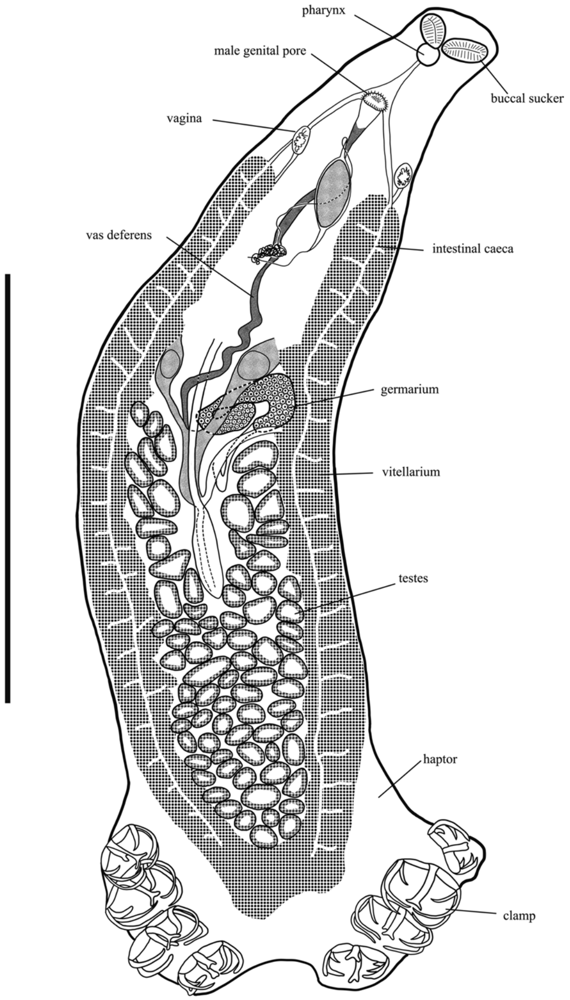

Fig. 3 Holotype (USNM 1359220) of *Pseudodiscocotyla opakapaka* Yamaguti, 1965 from *Pristipomoides filamentosus* (Valenciennes). Whole body (dorsal view). Scale bar: 1 mm.

acicular spines and a pair of long and slender spines. Male genital pore ventral, opening anterior to bifurcating of oesophagus, with muscular rim, armed with two alternating rows of minute spines.

Germarium (Fig. 2B) pretesticular 180–200 × 240–260 (190 × 150, n = 2), inverted U-shaped. Oviduct (Fig. 1) arising from the posterior end on the right side of germarium and opening into genito-intestinal canal. Genito-intestinal canal (Fig. 2B) originating from right intestinal cecum, bifurcating into vitelline reservoir, ventral to testes. Oötype (Fig. 2B) extending from vitelline reservoir to uterus. Uterus (Fig. 2B) originating from oötype, running anteriorly along body midline, ventral to vas deferens, opening anterior to the male genital pore. Vaginal pore (Fig. 2D) elliptical, cuticle uneven, with many peaks and valleys, no spines, paired, parallel, located posterior to level of male copulatory organ, ventrolateral 55–67.5 × 20–35 (62.1 × 29.2, n = 3); length/width ratio 1.83–3.13 (2.19, n = 3). Vaginal duct (Fig. 2B) long, narrow, arising from vaginal pore, connecting vitelline reservoir, ventral to germarium, traveling anteriorly, parallel to the intestinal caeca on its ventral side. Eggs (Fig. 2E) fusiform, 150–175 × 60–70 (162.5 × 65, n = 2) excluding filaments, with filaments at anterior and posterior ends. Vitelline follicles (Fig. 1) coextensive with intestinal branches, extended from behind vaginal pore to haptor. Vitelline duct not observed.

Remarks

The holotype and paratypes are in good state but did not appear to be stained (Figs. 3, 4). The details of male copulatory organ could not be observed and number of minute acicular spines on penis could not be counted in all examined specimens. However, they possess several minute acicular spines and a pair of long spines on penis and male genital pore with a crown of two alternating rows of minute spines (Fig. 4C).

The newly collected specimens from *Pr. filamentosus* in Japanese waters show the diagnostic morphological characteristics of *Pseudodiscocotyla* provided by Yamaguti (1965) and agree approximately with the descriptions of *Ps. opakapaka* by Yamaguti (1965, 1968). We could distinguish differences between the newly collected specimens and the original description in clamp structure. Yamaguti (1965) indicated that the distal ends of both mid-sclerites bifurcated. In the newly collected specimens, the holotype and paratypes, an accessory skeletal piece represented by a projecting V-shaped process was observed on the distal end of the dorsal mid-sclerite that was not mentioned nor illustrated in the original description.

Some differences between the original description and our observation justify the need to emend the generic diagnosis: intestine bifurcation at anterior or posterior end of male copulatory organ, the arms of vitelline reservoir are not absolutely distended with sperm at anterior end.

Pseudodiscocotyla mikiae n. sp.

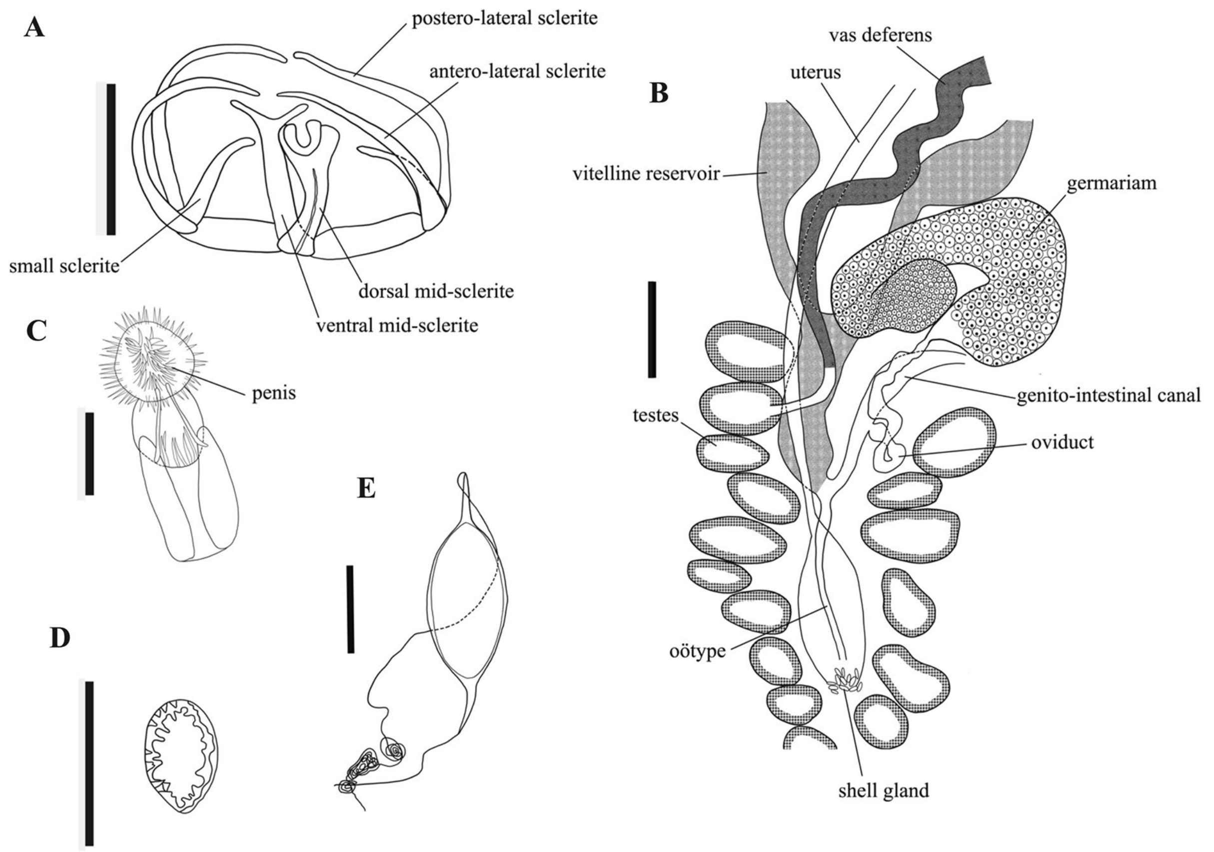

Fig. 4 Holotype (USNM 1359220) of *Pseudodiscocotyla opakapaka* Yamaguti, 1965. A, Clamp (dorsal view); B, Reproductive organs (dorsal view); C, Male copulatory organ; D, Vaginal pore; E, Egg. Scale bars: C, D, 50 μm; A, B, E, 100 μm.

Type-host: *Pristipomoides filamentosus* (Valenciennes) (Perciformes: Lutjanidae).

Type-locality: East China Sea, off Awase, Okinawa City, Okinawa Prefecture, Japan.

Type-materials: Holotype (21968) and 10 paratypes (21969).

Site of host: Gills.

Representative DNA sequences: Partial 28S rDNA gene and partial *cox*1 gene sequences obtained from a paratype (21969) were submitted to the DNA Data Bank of Japan (DDBJ) under the accession numbers LC732579 and LC732580, respectively.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Pseudodiscocotyla mikiae* n. sp. is urn:lsid:zoobank.org:act: EC9D29B3-F0EC-429E-BDA6-774E82C24DEB.

Etymology: The species is named in condolence to Miki Haruna. She was a student of the first author and had conducted research on parasites with the authors during her school years, but died four years ago at a young age due to a sudden illness.

Description (Fig. 5)

Body elongated, total length 4,675–6,600 (5,807, n = 7), width at level of posterior end of germarium 750–1,300 (1,115, n = 8). Haptor ginkgo-leaf-shaped, symmetrical, with 4 pairs of clamps. Clamps (Fig. 6A) of equal structure, typically *Discocotyle*-type, each clamp 65–260 × 147.5–300 (157 × 228, n = 7). Clamp consisting of pair of antero-lateral sclerites and postero-lateral sclerites, ventral mid-sclerite and dorsal mid-sclerite. Antero-lateral sclerites and

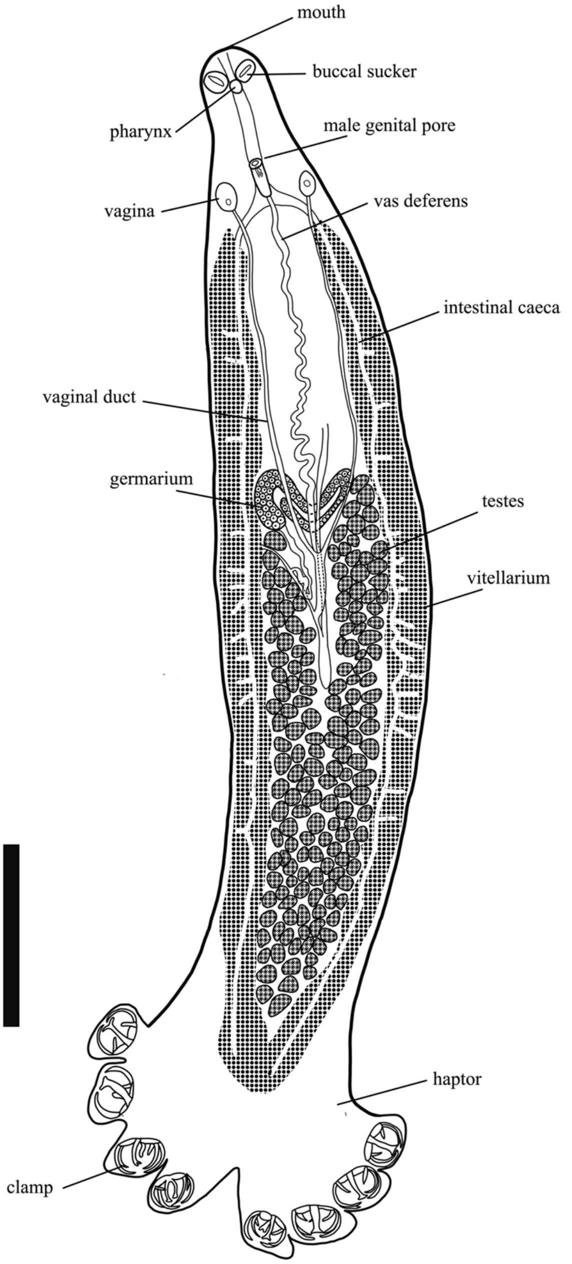

Fig. 5 *Pseudodiscocotyla mikiae* **n. sp.** from *Pristipomoides filamentosus* (Valenciennes) off Okinawa-jima island, Okinawa Prefecture. 1830. Whole body (ventral view, 21968). Scale bar: 1 mm.

postero-lateral sclerites thin and long. Small sclerites from proximal end of postero-lateral sclerites toward inside. Ventral mid-sclerite long, bifurcated on top. Dorsal mid-sclerite short, spanner-shaped.

Mouth opening anterior terminal. Pair of buccal suckers elliptical 70–120 × 50–150 (100 × 133, n = 10). Pharynx globular, lying on body midline immediately posterior to buccal suckers, 70–90 × 50–70 (79 × 59, n = 10). Oesophagus short without diverticula, bifurcating anterior or posterior to the male genital pore. Intestinal caeca with numerous lateral diverticula, extending into haptor, not united posteriorly.

Testes (Fig. 6B) with irregular shape, 93–146 (127, n = 9) in number, postgermarial, arranged in the posterior half of body and confirmed to intercrural field, divided into two lateral groups from posterior end of germarium to level of the shell gland. Vas deferens (Fig. 6B) long, conspicuous, coming from anterior testes on right side of body at level of oötype, ventral to germarium, running forward along body midline, entering base of male genital pore. Male copulatory organ (Fig. 6C), consisting of thin-walled duct and penis, length 50–62.5 × 17.5–32.5 (54 × 25, n = 7) from base to tip. Penis bearing numerous slender spines. Male genital pore ventral, opening anterior to bifurcating of oesophagus, with muscular rim.

Germarium (Fig. 6B) pretesticular 150–350 × 230–570 (303 × 467, n = 7), beginning on right side of body, extending from right to left side, turned anterior to right side of body and then extended toward posterior. Oviduct (Fig. 6B) long, twisted, arising from distal end of germarium and opening into genito-intestinal canal. Genito-intestinal canal (Fig. 6B) originating from right intestinal cecum, bifurcating into vitelline reservoir, ventral to testes. Oötype (Fig. 6B) extending from vitelline reservoir to uterus. Uterus (Fig. 6B) originating from oötype, running anteriorly along body midline, ventral to vas deferens, opening anterior to the male genital pore. Vaginal pore (Fig. 6D) circular, with numerous small spines, paired, parallel, located posterior to level of male copulatory organ, ventral, lateral 25–37.5 × 22.5–32.5 (30.5 × 25.3, n = 10), surrounded by radiate short muscle fibers; length/width ratio 0.83–1.63 (1.23, n = 10). Vaginal duct (Fig. 6B) long, narrow, arising from vaginal pore, connecting vitelline reservoir, ventral to germarium, traveling anteriorly, parallel to the intestinal caeca on its ventral side. Eggs fusiform, 120–192.5 × 60–80 (156.3 × 70, n = 2) excluding filaments, with filaments at anterior and posterior ends.

Vitelline follicles coextensive with intestinal branches, extended from behind vaginal pore to

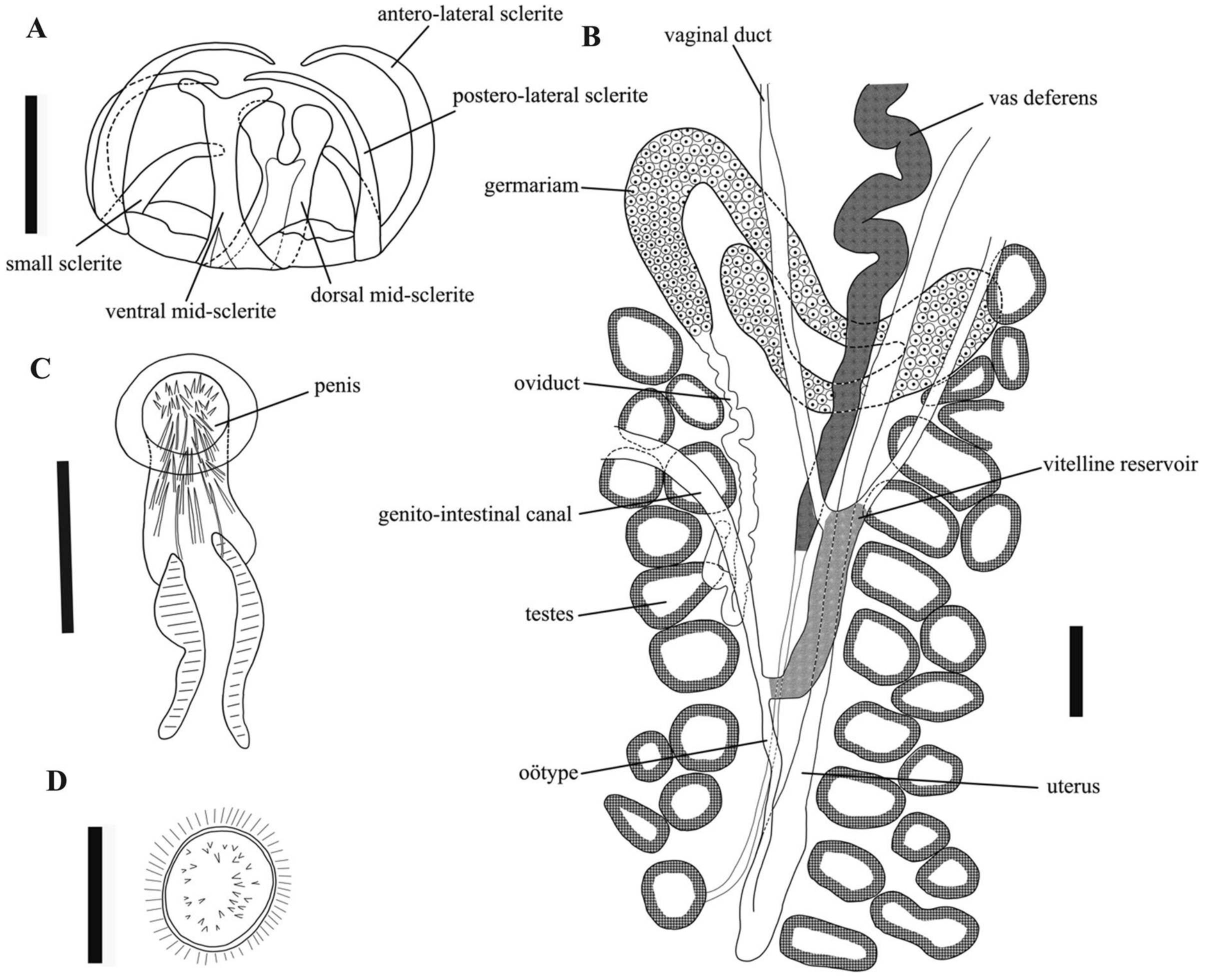

Fig. 6 *Pseudodiscocotyla mikiae* **n. sp.** A, Clamp (ventral view, 21968); B, Reproductive organs (ventral view, 21969b); C, Male copulatory organ (21969c); D, Vaginal pore (21969c). Scale bars: C, D, 50 µm; A, B, E, 100 µm.

haptor, fused posterior to testes. Vitelline duct not observed.

Remarks

Pseudodiscocotyla mikiae **n. sp.** shows the diagnostic morphological characteristics of the genus (see Yamaguti, 1965). Currently, only *Pseudodiscocotyla opakapaka* Yamaguti, 1965 is assigned to this genus (Yamaguti, 1965). This new species is distinguished from *Ps. opakapaka* by the following characters of the new species: (i) the absence of spines around the male genital pore, (ii) the circular vaginal pore (length/width ratio: 0.83–1.63 vs. 1.83–3.13), (iii) bearing numerous small spines inside, and (iv) the spanner-shaped dorsal mid-sclerite. Morphological measurements of *Pseudodiscocotyla* species are presented in Table 2.

Molecular data analysis

The result of the BLAST search for the sequences of *cox1* gene of *Ps. mikiae* obtained in this study are shown in Table 3. The close hits were *Microcotyle caudata* Goto, 1894, *Microcotyle sebastis* Goto, 1894 (Microcotylidae), and *Heterobothrium okamotoi* Ogawa, 1991 (Diclidophoridae).

The trimmed multiple sequence alignment length of the 28S rDNA fragments consisted of 980 base pairs including gaps. The topologies

Table 2 Measurements and counts of *Pseudodiscocotyla* species.

Species	*Ps. mikiae* **n. sp.**	*Ps. opakapaka*	*Ps. opakapaka*
Host	*Pr. filamentosus*	*Pr. filamentosus*	*Pr. microlepis* *A. rutilans*
Locality	Okinawa-jima island, Japan	Okinawa-jima island, Japan	Hawaii, USA
Source	This study	This study	Yamaguti (1965, 1968), this study*
Body length	4675–6600 (5807, n = 7)	3200–4375 (3875, n = 3)	1600–2700
Body width	750–1300 (1115, n = 8)	375–500 (425, n = 3)	220–600
Clamp	65–260 × 147.5–300 (157 × 228, n = 7)	100–145 × 100–205 (118 × 159, n = 3)	100–180
Buccal sucker	70–120 × 50–150 (100 × 133, n = 10)	50–70 × 40–110 (58 × 97, n = 3)	40–70 × 60–110
Pharynx	70–90 × 50–70 (79 × 59, n = 10)	50–60 × 40–50 (53 × 43, n = 3)	30–60 × 30–45
No. testes	93–146 (127, n = 9)	99–127 (108, n = 3)	50–100
Testes	70–110 × 80–130 (88.5 × 105, n = 10)	50–60 × 40–70 (57 × 53, n = 3)	21.9–100 × 34.4–84.4*
Male copulatory organ	50–62.5 × 17.5–32.5 (54 × 25, n = 7)	99–127 (108, n = 3)	75×60
Germarium	150–350 × 230–570 (303 × 467, n = 7)	180–200 × 240–260 (190 × 150, n = 2)	100–170 × 60–112
Vagina	25–37.5 × 22.5–32.5 (30.5 × 25.3, n = 10)	55–67.5 × 20–35 (62.1 × 29.2, n = 3)	42.6–82 × 26.2–42.6*
Egg	120–192.5 × 60–80 (156.3 × 70, n = 2)	150–175 × 60–70 (162.5 × 65, n = 2)	160–170 × 60–70

[*] The values were measured in the type specimen in this study because Yamaguti (1965, 1968) did not measure testis and vaginal pore

Table 3 The result of the BLAST search for the *cox1* gene of *Ps. mikiae*.

INSD accession No.	Species	Family	Max score	Query cover (%)	Identity (%)	Reference
LC472531	*Microcotyle caudata* Goto, 1894	Microcotylidae	507	80	74.71	Ono et al. (2020)
LC472530	*Microcotyle caudata* Goto, 1894	Microcotylidae	507	80	74.71	Ono et al. (2020)
MT180126	*Microcotyle caudata* Goto, 1894	Microcotylidae	500	83	74.05	Nam et al. (2020)
LC472529	*Microcotyle caudata* Goto, 1894	Microcotylidae	494	76	75.03	Ono et al. (2020)
LC472527	*Microcotyle caudata* Goto, 1894	Microcotylidae	493	80	74.33	Ono et al. (2020)
LC472528	*Microcotyle caudata* Goto, 1894	Microcotylidae	483	74	74.16	Ono et al. (2020)
MT876116	*Microcotyle sebastis* Goto, 1894	Microcotylidae	483	75	74.8	unpublished
NC057207	*Heterobothrium okamotoi* Ogawa, 1991	Diclidophoridae	480	75	74.73	Li et al. (2019)

of each constructed by ML and BI analysis were almost identical, and the phylogenetic trees based on BI analysis are shown in Fig. 7. The Mazocraeidea are divided into two major clades. The clade includes the Hexostomatidae, Allodiscocotylidae, Mazocreaeidae, Protomicrocotylidae, Chauhaneidae, Gastorocotylidae, Axinidae, Gotocotylidae and Thoracocotylidae. The other clade separates two groups, one consists of the Discocotylidae, Diclidophoridae, Macrovalvitrematidae, and Plectanocotylidae; and the other is comprised of the Heteraxinidae, Microcotylidae, Octomacridae, Heteromicrocotylidae, and Diplozoidae. Each of the families for which multiple species were used in the

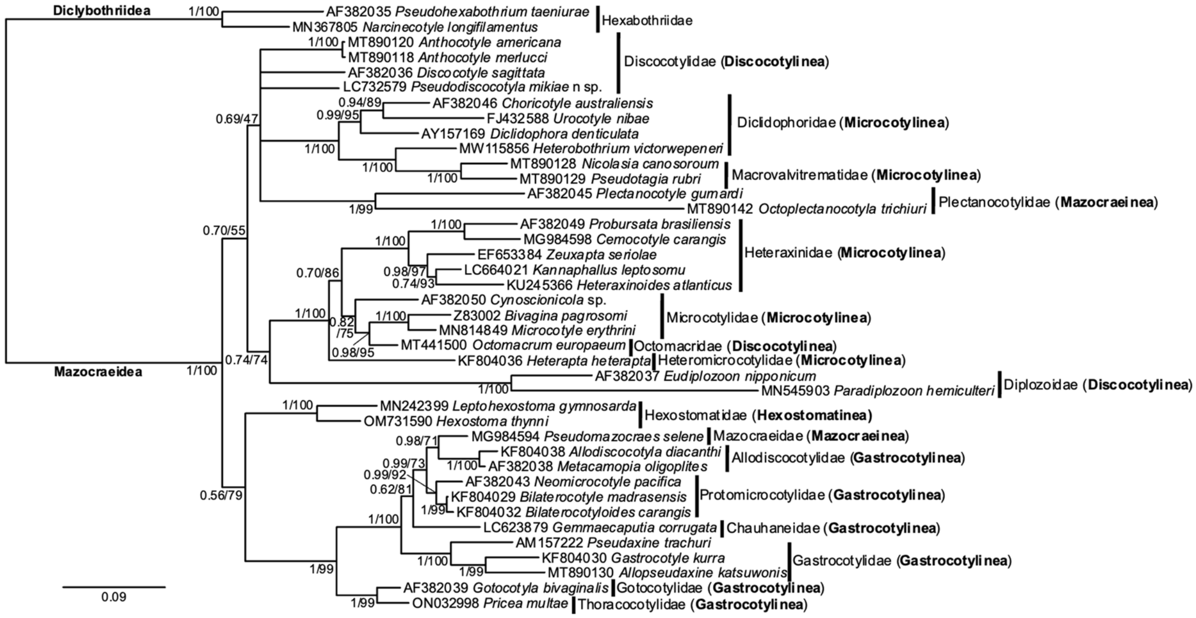

Fig. 7 Bayesian inference (BI) trees for the Mazocraeidea based on partial 28S rDNA (980 bp including gaps) data using two species of Hexabothriidae (Diclybothriidea) as the outgroups. The corresponding INSD accession numbers are shown. The tree includes results for ML and Bayesian inference with BS/PP branch support.

analysis constituted a monophyletic group, with the exception of Discocotylidae. Species of Discocotylidae was a paraphyletic group including Diclidophoridae and Macrovalvitrematidae in the ML analysis, but the BS value of each branch was relatively low (45–66), and those were shown as multibranching in the BI analysis.

Discussion

The *Pristipomoides filamentosus* collected in the waters off Okinawa-jima island was found to be infected with two species of *Pseudodiscocotyla*: *Ps. mikiae* **n. sp.** and *Ps. opakapaka*. The host, *Pr. filamentosus*, is a slow growing and long-lived species and engages in mass spawning of buoyant eggs for five to seven months of the year (Andrews et al., 2011, Meagan et al., 2017, Haight et al., 1993, Leis & Lee, 1994). Additionally, the documented ability of some mature *Pr. filamentosus* to disperse 400 km across deep water channels indicates that adults can contribute to dispersal in this species, at least on an archipelagic scale (Kobayashi, 2008). Therefore, *Pr. filamentosus* is distributed widely in the Indo-Pacific (Nakabo, 2013) and *Ps. opakapaka* and *Ps. mikiae* **n. sp.** could share the distribution of the host. However, *Ps. mikiae* **n. sp.** was not found in previous studies in Hawaii (Yamaguti, 1965, 1968, Kent et al., 2005). However, after Yamaguti (1965, 1968), no detailed morphological examination of the newly collected specimens has been carried out (see Kent et al., 2005). In future studies the distribution rage of those *Pseudodiscocotyla* species will need to be examined.

The locations of the male genital atrium and the vaginal pore in both species were similar. However, the structure of the vagina (circular vaginal pore in *Ps. mikiae* vs. elliptical vaginal pore with corrugated cuticle in *Ps. opakapaka*) and male genital pore (no spines around male genital pore in *Ps. mikiae* vs. two alternating rows of spines around male genital pore in *Ps. opakapaka*) differed. The observed morphological differences are therefore presumed to establish reproductive isolation. Since the sequence of *Ps. opakapaka* was not obtained in the present study, we are not able to test the reproductive isolation. This would require detailed studies of distribution and molecular analysis for these two species.

Boeger & Kritsky (1993, 2001) indicated by morphological analysis that the Mazocraeidea is divided into five suborders (Mazocraeinea, Discocotylinea, Gastrocotylinea, Hexostomatinea, and Microcotylinea). The phylogenetic analysis based on the 28S rDNA did not support the previous phylogenetic tree proposed by Boeger & Kritsky (2001) (Olson & Littlewood 2002, this study). Olson & Littlewood (2002) presented a phylogenetic tree of the whole monogeneans based on the 28S rDNA sequences and supported the monophyly of suborders Gastrocotylinea and Discocotylinea. The present analysis is broadly in agreement except for the position of the Plectanocotylidae, but monophyly was not supported for all suborders. Olson & Littlewood (2002) suggested the 28S rDNA fragment had lost a great deal of resolving power as more taxa were included, and Hebert et al. (2003) reported the ability of *cox*1 gene to discriminate animal taxa. Available *cox*1 sequences of Mazocraeidea species are limited, and the accumulation of molecular studies is needed for the phylogenetic analysis and DNA barcoding of this order.

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Author contributions Kamio and Inoue wrote the main manuscript text and prepared figures 1-6 and Nitta performed a molecular phylogenetic analysis and prepared figures 7. All authors reviewed the manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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