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Three species of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) collected from Japanese rays

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Abstract Eighteen species of *Dendromonocotyle* Hargis, 1955 (Monogenea: Monocotylidae) have so far been described from elasmobranchs worldwide. In this paper, two new species are described; *Dendromonocotyle tsutsumii* n. sp. from the skin of the Japanese eagle ray, *Myliobatis tobijei* Bleeker from Tokyo Bay and the pitted stingray, *Dasyatis matsubarai* Miyosi, from Ooarai, Ibaraki Prefecture, Japan, and *Dendromonocotyle fukushimaensis* n. sp. from the skin of the cow stingray, *Dasyatis ushiei* (Jordan & Hubbs) reared at an aquarium in Fukushima Prefecture, Japan. *Dendromonocotyle tsutsumii* is distinguished from the congeners by the presence of a sclerotised duct connecting the vagina with the seminal receptacle, and *De. fukushimaensis* by the large body size and the presence of a donut-shaped structure encircling the male copulatory organ near its distal end. Additionally, the reproductive system of *Dendromonocotyle akajeii* Ho & Perkins, 1980 is redescribed, based on specimens from the skin of the whip stingray, *Hemitrygon akajei* (Müller & Henle) (syn. *Dasyatis akajei*) caught in Hamana Lake, Shizuoka Prefecture, Japan. A key to the 20 species of *Dendromonocotyle* including the present new species is provided.

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This article is part of the Topical Collection Monogenea.

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Introduction

More than 200 species of elasmobranchs are known from Japanese waters (Nakabo, 2013), but only a few species have been examined for parasites. Among the parasites recorded so far, the majority are cestodes and copepods (Yamaguti, 1934, 1952; Shiino, 1954a, b, 1957a, b; Izawa, 2010; Nagasawa & Uyeno, 2015). As for monogeneans, only twelve species are known to infect elasmobranchs from Japanese waters. They comprise six species of monocotylids, i.e. *Triloculotrema japanicae* Kearn, 1993, *Monocotyle ijimae* Goto, 1894, *Calicotyle mitsukurii* Goto 1894, *Calicotyle japonica* Kitamura, Ogawa, Shimizu, Kurashima, Mano, Taniuchi & Hirose, 2010, *Heterocotyle chinensis* Nitta & Nagasawa, 2015 and *Dendromonocotyle akajeii* Ho & Perkins, 1980, five species of hexabothriids, i.e. *Rajonchocotyle kenojei* Yamaguti, 1938, *Squalonchocotyle laymani* Yamaguti, 1958, *Onchocotyle spinacis* Goto, 1894 [now assigned as an unconfirmed species of

Squalonchocotyle Cerfontaine, 1899 by Boeger & Kritsky (1989)], *Erpocotyle modama* Iwata, 1991, and *Squalonchocotyle mitsukurii* Kitamra, Ogawa, Taniuchi & Hirose, 2006, and one species of microbothriid, *Haplocotyle japonica* Nitta & Nagasawa, 2017.

Species of *Dendromonocotyle* Hargis, 1955 are parasites of the skin of rays of the families Dasyatidae, Myliobatidae and Urolophidae (see Chisholm et al., 2004). Irigoitia et al. (2016) reported a new *Dendromonocotyle* from *Zearaja chilensis* (Guichenot) (Rajidae) and the genus is known to infect rays of four families, currently comprising 18 species (Irigoitia et al., 2016). Among the nine species of rays of the genus *Dasyatis* distributed in Japanese waters (Nakabo, 2013), *Dasyatis akajei* (Müller & Henle) [now reclassified as *Hemitrygon akajei* (Müller & Henle) by Last et al. (2016)] is the only species from which species of *Dendromonocotyle* have been recorded. Ho & Perkins (1980) described this species collected from *H. akajei* (as *Da. akajei*), caught in Toyama Bay, Sea of Japan. In the present study, we had a chance to examine *Dendromonocotyle* specimens which had been collected from three species of rays caught on the Pacific coast of Japan, the Japanese eagle ray *Myliobatis tobijei* Bleeker, the pitted stingray *Dasyatis matsubarai* Miyosi and the cow stingray *Dasyatis ushiei* (Jordan & Hubbs), all of which have been poorly examined parasitologically. From *M. tobijei*, the nematode *Raphidascaroides myliobatum* Yin & Zhang, 1983 [now a synonym of *Mawsonascaris myliobatum* according to Li et al. (2012)], and the cestode *Echeneibothrium tobijei* Yamaguti, 1934 (see Yamaguti, 1934, 1952), from *Da. matsubarai*, the parasitic copepod *Trebius akajei* Shiino, 1954 (see Kido et al., 2016), the parasitic isopod *Gnathia capillata* Nunomura & Honma, 2004 and from *Da. ushiei*, and the parasitic copepod *Pseudocharopinus markewitschi* (Gusev, 1951) (see Nagasawa & Uyeno, 2015).

This paper reports two new species of *Dendromonocotyle*, one from *M. tobijei* and *Da. matsubarai* and the other from *Da. ushiei* in the Pacific and provides a redescription of *De. akajeii* newly collected from *H. akajei* in Lake Hamana. The new species represent a 14th and 15th monogeneans from Japanese elasmobranchs and *Dendromonocotyle* comprises 20 species worldwide.

Materials and methods

Dendromonocotylids were sampled from four species of rays in Japan: Japanese eagle ray *M. tobijei* and pitted stingray *Da. matsubarai* Miyosi caught in Tokyo Bay in June, 1996 and off Ooarai between March, 1993 and July, 1997, respectively; cow stingray, *Da. ushiei* reared at an aquarium in Fukushima Prefecture, Japan (dissected in August 2006); whip stingray, *H. akajei* caught in Hamana Lake in July 2003. Monogeneans were collected from the skin of rays, flattened between a coverslip and slide glass and fixed in AFA. They were stained with Heidenhain's iron hematoxylin or alum carmine, dehydrated in a graded alcohol series and mounted in Canada balsam. Figures were drawn with the aid of a drawing tube. Measurements were made using Nikon Distal Sight DS-L2 measurement system and given in micrometres as the range followed by the mean and the number of specimens measured in parentheses (only when all of the specimens could not be measured).

Paratypes of the following material were examined for comparison: *Dendromonocotyle pipinna* Chisholm & Whittington, 2004 (South Australian Museum 28452–28456, 28536) and *De. akajeii* Ho & Perkins, 1980 (U.S. National Parasite Collection 077682.00)

Fish scientific names follow Nakabo (2013) and Eschmeyer et al. (2018).

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 all new taxa have been submitted to ZooBank. For each new taxon, the Life Science Identifier (LSID) is reported in the taxonomic summary.

Family Monocotylidae Taschenberg, 1879
Genus *Dendromonocotyle* Hargis, 1955

Dendromonocotyle tsutsumii n. sp.

Type-host: *Myliobatis tobijei* Bleeker (Myliobatiformes: Myliobatidae), Japanese eagle ray; Japanese name: tobi-ei.
Other host: *Dasyatis matsubarai* Miyosi (Myliobatiformes: Myliobatidae), pitted stingray; Japanese name: hoshi-ei.
Type-locality: Off Nakanose (35°38′N, 139°46′E), Tokyo Bay, Tokyo Metropolis, Japan (18.vi.1996).

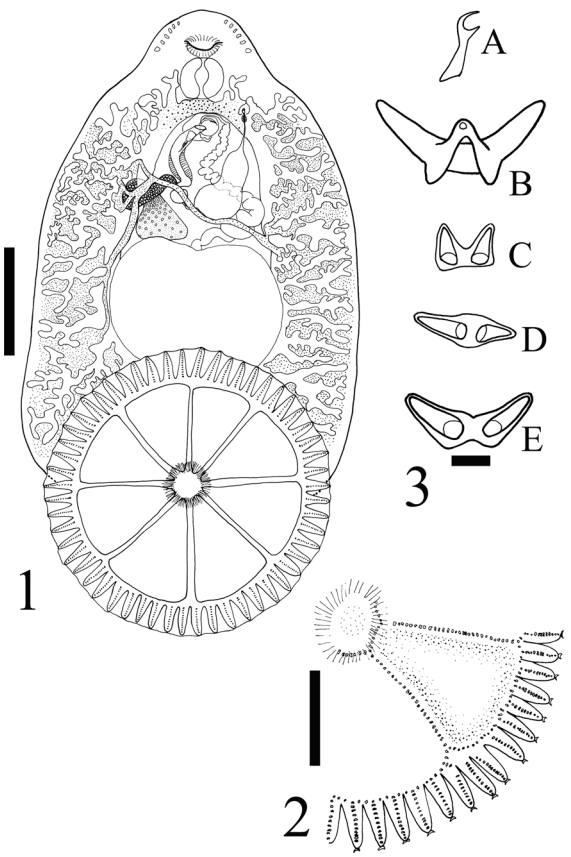
Other localities: Ex *M. tobijei*: off Ooarai (36°19′N, 140°35′E), Ibaraki Prefecture, Japan (iii.1997; exact date not specified). Ex *D. matsubarai*: off Ooarai (36°19'N, 140°35'E), Ibaraki Prefecture, Japan (3.iii.1993; iii.1997; exact date not specified; 3.vii.1997).

Type-material: The holotype and paratypes are deposited in the Meguro Parasitological Museum (MPM coll. nos 21002–21006).

Site on host: Skin.

ZooBank registration: The Life Science Identifier (LSID) for *Dendromonocotyle tsutsumii* n. sp. is urn:lsid:zoobank.org:act:9FCF746D-7DFF-4805-8D7D-DE2F5342BD8A.

Etymology: The new species is named after the late Mr Toshio Tsutsumi, the collector of this parasite.



Figs. 1–3 *Dendromonocotyle tsutsumii* n. sp. 1, Holotype, ventral view; 2, Paratype, haptor (part), ventral view; 3, Paratypes, haptoral sclerites. *Abbreviations*: A, marginal hooklet; B, terminal papillary sclerite; C, papillary sclerite; D, inner and outer ring sclerite; E, septal. *Scale-bars*: 1, 1 mm; 2, 500 µm; 3, 5 µm

Description (Figs. 1–8)

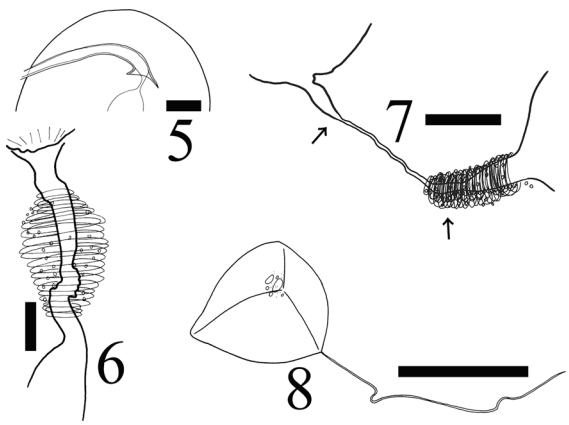
[Based on 10 specimens.] Body, excluding haptor 3,341–6,139 (4,909) long, with maximum width 2,031–4,102 (2,891; n = 9) at level of testis (Fig. 1). Haptor diameter 1,597–2,202 (1,849) (Fig. 1). Haptoral rim with 55–60 marginal haptoral papillae, 121–237 (184) long, 58–116 (89) wide, each armed with 7–9 sclerites (Figs. 1, 2). Hamuli absent. Anterior loculus pair with 6 marginal haptoral papillae each; anterolateral and posterolateral loculus pairs with 7 associated marginal haptoral papillae each; posterior loculus pair with 8 marginal haptoral papillae each, categorised as type A (Vaughan et al., 2008). Marginal hooklets 7–14 (11) long, distributed in marginal valve symmetrically between every 4 papillae (Fig. 3A). Four types of sclerites present (Fig. 3); terminal papillary sclerite 8–16 (11) long, 18–32 (24) wide (Fig. 3B); papillary sclerite 5–9 (7) long, 8–14 (9) wide (Fig. 3C); outer ring sclerite 4–9 (7) long, 8–15 (10) wide (Fig. 3D); inner ring sclerite 6–10 (8, n = 5) long, 9–13 (11, n = 5) wide (Fig. 3D); septal sclerite, outer side 3–12 (7) long, 9–19 (12) wide, inner side 5–11 (8) long, 8–15 (13) wide (Fig. 3E). Number of sclerites in outer ring 190–213 (n = 4), in inner ring 40–51 (n = 5).

Oral sucker ventral, 142–247 (178) long, 214–377 (274) wide. Eye-spots antero-dorsal to pharynx (Fig. 1). Pharynx 297–582 (416, n = 9) long, 302–627 (437) wide. Intestinal caeca bifurcate just posterior to pharynx, extending to posterior end of body proper. Pigment present in caeca.

Testis single, 858–1,251 (1,147) long, 1,274–2,051 (1,660) wide. Vas deferens originating from anterior end of testis, tightly coiled, running antero-sinistrally then antero-dextrally, passing dorsal to vagina, tapering and curving dextrally in front of ejaculatory bulb, delated to form seminal vesicle, 66–91 (79) wide, then entering into ejaculatory bulb posteriorly (Fig. 4). Ejaculatory bulb 182–273 (211) long, 157–245 (189) wide. Male copulatory organ sclerotised, short, 101–149 (121) long in a straight line, 20–33 (26) wide at base, almost constant in width except base, 6–9 (7) wide; sclerotised ridges not observed at distal end (Fig. 5).

Ovary, roughly triangular, 319–588 (461) wide, looping vitelline duct dorsally, leading to seminal receptacle (Fig. 4). Vagina opening in slightly sinistral field of body at level of caecal bifurcation (Fig. 1);

Fig. 4 Reproductive system of *Dendromonocotyle tsutsumii* n. sp. Holotype, ventral view. *Scale-bar*: 500 μm



Figs. 5–8 Reproductive system of *Dendromonocotyle tsutsumii* n. sp. Holotype, ventral view. *Scale-bar*: 500 μm. *Dendromonocotyle tsutsumii* n. sp. 5, Male copulatory organ of holotype, ventral view; 6, Paratype, vaginal opening, ventral view; 7, Holotype, seminal receptacle and sclerotised distal end of vagina, ventral view; 8, Paratype, egg. *Scale-bars*: 5, 6, 20 μm; 7, 50 μm; 8, 100 μm

opening funnel-shaped, 20–49 (29) wide, followed by muscular bulb, 41–77 (56) long, 27–40 (34) wide (Fig. 6), and narrow duct, 164–491 (310) long, 8–19 (14, n = 9) wide, leading to a gourd-shaped portion, 331–963 (541) long, 114–232 (165, n = 7) wide proximally and 161–338 (238, n = 7) wide distally; its distal portion tapered, leading to narrow, winding sclerotised duct, 47–78 (63) long in straight line, 2–3 (2.4) wide, surrounded by thick muscle fibers, funnel-shaped at both ends, finally connected with a short projection of seminal receptacle (Figs. 7, 9A). Seminal receptacle spherical, 88–192 (134) in diameter. Oötype 324–499 (428) long, 131–199 (164) wide. Uterus muscular, 59–88 (70) wide. Egg tetrahedral, 80–102 (91) long, 79–117 (99) wide with a filament, 94–179 (129, n = 7) long (Fig. 8). Common genital pore at level of ejaculatory bulb (Fig. 4).

Remarks

Dendromonocotyle tsutsumii n. sp. can be distinguished from the other congeners by the combination of the following morphological features: all haptoral septa joining inner ring septum, presence of outer ring septal sclerites, haptor with 55–60 marginal papillae, absence of hamuli, short male copulatory organ, and presence of a narrow sclerotised duct before connection with the seminal receptacle. This new species is most similar to *De. pipinna*, in which the duct between the vagina and seminal receptacle is not sclerotised. This sclerotised structure has never been described before in the known species of *Dendromonocotyle.*

***Dendromonocotyle fukushimaensis* n. sp.**

Type-host: *Dasyatis ushiei* (Jordan & Hubbs), (Myliobatiformes: Dasyatidae), cow stingray; Japanese name: ushi-ei.
Type-locality: Environmental Aquarium Aquamarine Fukushima (36°56′N, 140°54′E), Onahama, Iwaki-shi, Fukushima, Japan (26.viii.2006). The host had been caught off Fukushima in the Pacific (accurate locality not specified), brought into the aquarium.
Site on host: Skin.
Type-material: The holotype and paratypes are deposited in the Meguro Parasitological Museum (MPM coll. nos 21033–21034).
ZooBank registration: The Life Science Identifier (LSID) for *Dendromonocotyle fukushimaensis* n. sp. is urn:lsid:zoobank.org:act:B47BFDAF-056F-4816-A480-B14F034104DF.
Etymology: The new species refers to the locality of the aquarium where the host had been reared.

Description (Figs. 9B, 10–18)

[Based on 5 specimens.] Body, excluding haptor 13,300 (9,100–15,700) long, with maximum width 5,100–7,100 (6,300) at level of testis (Fig. 10). Haptor

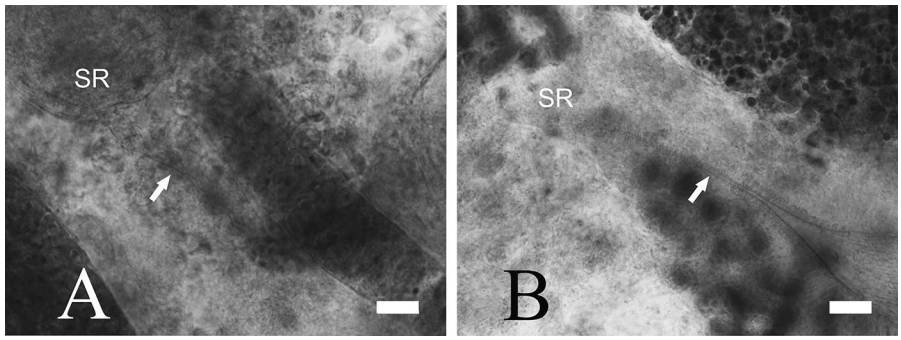
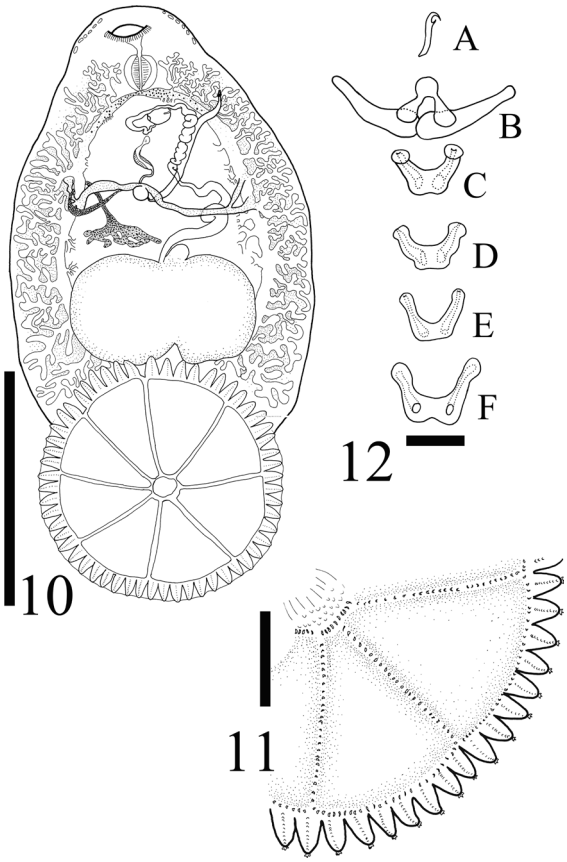


Fig. 9 Photomicrograph of sclerotised part of vagina of *Dendromonocotyle* spp. A, *Dendromonocotyle tsutsumii* n. sp., paratype; B, *Dendromonocotyle fukushimaensis* n. sp., paratype. Arrows indicate sclerotised part. *Abbreviation*: SR, seminal receptacle. *Scale-bars*: 20 μm



Figs. 10–12 *Dendromonocotyle fukushimaensis* n. sp. 10, Holotype, ventral view; 11, Holotype, haptor (part), ventral view; 12, Holotype, haptoral sclerites. *Abbreviations*: A, marginal hooklet; B, terminal papillary sclerite; C, papillary sclerite; D, outer ring sclerite; E, inner ring sclerite; F, septal sclerite. *Scale-bars*: 10, 5 mm; 11, 1 mm; 12, 20 μm

diameter 4,200–6,500 (5,400) (Fig. 10). Haptoral rim with 54–56 (n = 3) marginal haptoral papillae, 176–469 (374) long, 177–328 (239) wide, each armed with 6–13 sclerites (Figs. 10, 11). Hamuli absent. Anterior loculus pair with 6 marginal haptoral papillae each; anterolateral and posterolateral loculus pairs with 7 associated marginal haptoral papillae each; posterior loculus pair with 8 marginal haptoral papillae each, categorised as type A (Vaughan et al., 2008). Marginal hooklets 10–16 (14) long distributed in marginal valve symmetrically between every 4 papillae (Fig. 12A). Five types of sclerites present (Fig. 12): terminal papillary sclerite 20–28 (24) long, 50–67 (60) wide (Fig. 12B); papillary sclerite 13–16 (15) long, 23–29 (26) wide (Fig. 12C); outer ring sclerite 12–19 (14) long, 22–29 (24) wide (Fig. 12D); inner ring sclerite 12–15 (14, n = 4) long, 18–25 (20, n = 4) wide (Fig. 12E); septal sclerite, 17–24 (20) long, 29–39 (34) wide (Fig. 12F). Number of sclerites in outer ring 192–236, in inner ring 44–54 (n = 4).

Oral sucker ventral, 202–491 (315) long, 569–885 (768) wide. Six pairs of anterolateral gland duct openings present (Fig. 10). Pharynx 510–726 (659) long, 587–813 (710) wide. Intestinal caeca bifurcate just posterior to pharynx, extending to posterior end of body proper. Pigment present in caeca.

Testis single, 2,300–3,700 (2,800) long, 3,100–4,500 (4,000) wide. Vas deferens originating from anterior end of testis, tightly coiled, running antero-sinistrally, passing dorsal to vagina, tapering and curving dextrally in front of ejaculatory bulb, delated to form seminal vesicle, 126–217 (172) wide, then entering into ejaculatory bulb posteriorly (Fig. 13). Ejaculatory bulb 372–552 (484) long, 340–621 (456) wide. Male copulatory organ (MCO), sclerotised, gently curved, 159–177 (171) long in a

straight line, 19–34 (26) wide at base, 5–10 (7) at distal end (Fig. 14); encircled by collar near distal end, thickened centrally, 14–19 (16) long, 14–15 (15) wide, 1.5–2.4 (2.1) thick in middle; donut-shaped ring at base of collar 1.2–4.1 (3.7) long, 17–20 (19) wide, 3.6–4.6 (4.0) thick; 12–22 (16) from distal end of collar to distal end of MCO, 130–143 (138) from base of MCO to ring (Fig. 15A, B).

Ovary branched, 1,239–1,713 (1,550) wide, looping right intestinal ceacum dorso-ventrally, leading to seminal receptacle (Fig. 13). Vaginal opening 6–9 (8, n = 2) wide in slightly sinistral field of body at level of posterior part of pharynx (Fig. 10). Vaginal duct narrow initially, 63–77 (70, n = 2) long, 2.5–3.0 (2.8, n = 2) wide, followed by glandular part, gradually widening distally, 294–399 (347) long, 41–62 (52, n = 2) wide, directing centrally at level of ejaculatory bulb, dorsal to vas deferens, leading to narrow sclerotised duct, 82–127 (114, n = 4) long, 0.9–1.4 (1.1) wide, surrounded by muscle fibers, funnel-shaped at both ends, finally connected with seminal receptacle (Figs. 9B, 16), spherical, 197–369 (298) in diameter. Oötype muscular, 441–540 (469) long, 172–186 (177) wide (Fig. 17). Common genital pore at level of ejaculatory bulb (Fig. 10). Egg tetrahedral, 64–94 (84) wide, with a filament 225–414 (319) long (Fig. 18).

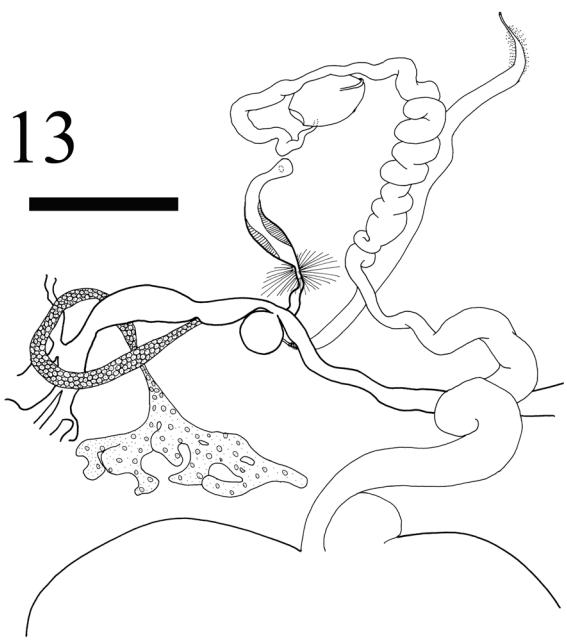
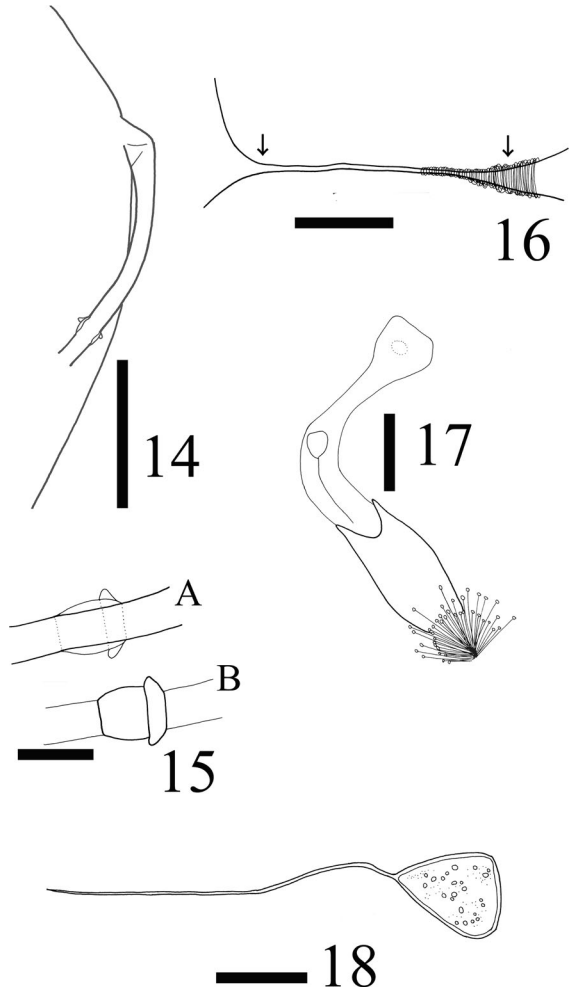


Fig. 13 Reproductive system of *Dendromonocotyle fukushimaensis* n. sp., holotype, ventral view. *Scale-bar*: 500 µm



Figs. 14–18 *Dendromonocotyle fukushimaensis* n. sp. 14, Paratype, male copulatory organ, ventral view; 15, Holotype, donut-shaped structure near distal end of male copulatory organ; 16, Paratype, seminal receptacle and sclerotised distal end of vagina, ventral view; 17, Paratype, distal end of vagina, ventral view; 18, Paratype, egg. *Scale-bars*: 14, 18, 100 µm; 15, 17, 20 µm; 16, 50 µm

Remarks

Dendromonocotyle fukushimaensis n. sp. can be distinguished from the other congeners by the combination of the following morphological features: all haptoral septa joining inner ring septum, presence of outer ring septal sclerites, haptor with 54–56 marginal papillae, absence of hamuli, short male copulatory organ, and presence of a narrow sclerotised duct before connection with the seminal receptacle.

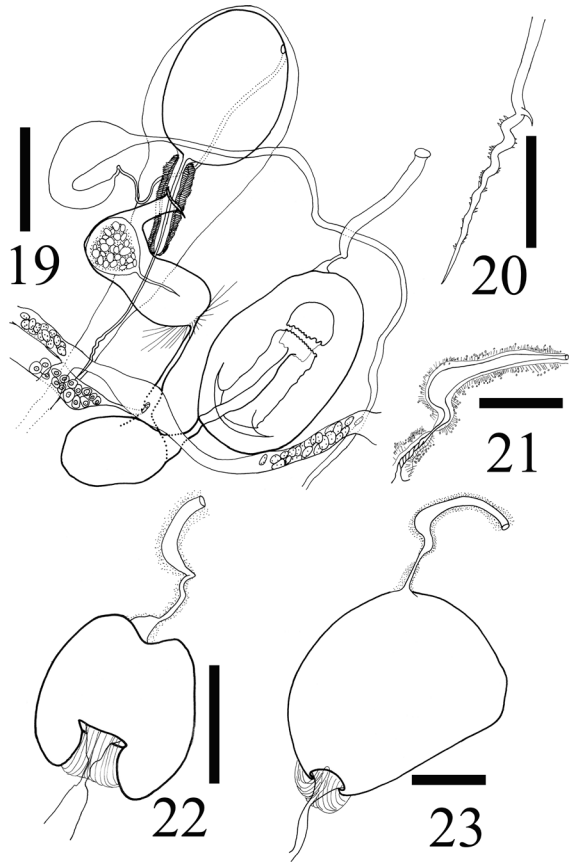
This new species is most similar to *Dendromonocotyle centrourae* Cheng & Whitaker, 1993, from which it differs in the body size (9.1–15.7 mm long in *De. fukushimaensis* n. sp. *vs* 6.32–7.22 mm long in *De. centrourae*), the number of sclerites in the outer ring of the haptor (192–236 *vs* 150–170), the terminal papillary sclerites (two outer prongs slightly curved inward, about 60 µm wide *vs* two outer prongs strongly bent inward, about 30 µm wide; Chisholm & Whittington, 1995), male copulatory organ (encircled by a collar with a donut-shaped ring *vs* encircled by a donut-shaped ring only) and the duct between the vagina and seminal receptacle (sclerotised *vs* non-sclerotised). Geographical distribution of the hosts is also different, coastal area of Japan for *De. fukushimaensis vs* Atlantic and the Mediterranean Sea for *De. centrourae* (see Cheng & Whitaker, 1993).

Dendromonocotyle akajeii Ho & Perkins, 1980

Host: *Hemitrygon akajei* (Muller & Henle) (Myliobatiformes: Dasyatidae).
Locality: Lake Hamana (34°25′N, 136°22′E), Shizuoka Prefecture, Japan (29.vii.2003).
Site on host: Dorsal skin.
Voucher material: 20 specimens deposited in the Meguro Parasitological Museum (MPM coll. no. 21007).

Redescription (Figs. 19–23)

[Based on 10 specimens.] Body, excluding haptor 2,264–3,806 (3,203) long, with maximum width 1,183–2,075 (1,670) at level of testis. Haptor diameter 905–1,238 (1,088). Haptoral rim with 55–57 marginal haptoral papillae, 74–105 (86) long, 23–49 (38) wide, each armed with 6–8 sclerites. Hamuli absent. Anterior loculus pair with 6 marginal haptoral papillae each, anterolateral and posterolateral loculus pairs with 7 associated marginal haptoral papillae each, posterior loculus pair with 8 marginal haptoral papillae each, categorised as type A (Vaughan et al., 2008). Marginal hooklets 7–12 (10) long distributed in marginal valve symmetrically between every 4 papillae. Four types of sclerites present; terminal papillary sclerite 7–14 (9) long, 16–39 (27) wide; papillary sclerite 3–7 (5) long, 8–15 (11) wide; outer ring sclerite 4–9 (7, n = 9) long, 11–17 (13, n = 9) wide; inner ring sclerite 5–8 (7, n = 9) long, 10–18 (13, n = 9) wide; septal sclerite, outer side 7–14 (9, n = 9) long, 14–26 (19, n = 9) wide, inner side 6–11 (8, n = 9) long, 13–22 (17, n = 9) wide . Number of sclerites in outer ring 119–120 (n = 2), in inner ring 33–34 (n = 2).



Figs. 19–23 *Dendromonocotyle akajeii*. 19, Reproductive system, ventral view; 20, Distal end of the male copulatory organ, ventral view; 21, Distal end of the vagina, ventral view; 22, 23 Vagina showing different degrees of invagination, ventral view. *Scale-bars*: 19, 200 µm; 20, 50 µm; 21–23 100 µm

Oral sucker ventral, 18–87 (55) long, 100–181 (143) wide. Eye-spots antero-dorsal to pharynx. Pharynx 168–254 (216) long, 181–299 (261) wide. Intestinal caeca bifurcate just posterior to pharynx, extending to posterior end of body proper. Pigment present in caeca.

Testis single, 289–639 (416) long, 572–1,204 (750) wide. Vas deferens originating from anterior part of testis, running anteriorly, passing right vitelline duct and vaginal duct dorsally, and at level of intestinal bifurcation, turning dextrad to form seminal vesicle in slightly right field of body. Seminal vesicle sausage-shaped, deeply bent in middle, 53–77 (65) wide

(Fig. 19); narrow duct emerging at its base, directing antero-sinistrally and soon folding back postero-sinistally, finally leading to anterior part of ejaculatory bulb (Fig. 19). Ejaculatory bulb ellipsoidal, 187–310 (262) long, 138–267 (208) wide (Fig. 19). Sclerotised male copulatory organ narrow, straight and long, 431–490 (470) long, 4–7 (5) wide (Fig. 20), with hook-like projection at beginning of a spiral, armed with spike-like projections in distal portion, 75–113 (101) long (Figs. 19, 20). Proximal portion of male copulatory organ covered with muscular sheath, 202–320 (258) long, 35–58 (46) wide (Fig. 19). Entire male copulatory organ covered with thin sheath (Fig. 19).

Ovary looping right vitelline duct, receiving common vitelline duct and short duct from seminal receptacle before leading to oötype (Fig. 19). Oötype, 212–316 (270) long, 104–146 (121) wide, directing antero-dextrally, ventral to male copulatory organ, followed by short uterus curving antero-sinistrally, leading to genital pore (Fig. 19). Seminal receptacle 97–151 (118) long, 145–251 (184) wide just behind joining of right and left vitelline ducts (Fig. 19). Vagina opening ventrally, slightly sinistral to left intestinal caecum at level of posterior end of ejaculatory bulb (Fig. 19). Vagina consisting of distal duct, 214–314 (257) long, central spheroid body, 261–403 (342) long, 182–307 (226) wide and proximal duct, 231–399 (339) long (Fig. 19). Main part of distal duct glandular, followed by short, non-glandular connecting part, leading to narrow, twisted, glandular portion to connect with spheroid body (Fig. 21). Tip of proximal duct funnel-shaped, sclerotised, 20–53 (34) long, 35–93 (54) wide, leading to seminal receptacle (Fig. 19). Proximal duct showing different degrees of invagination into spheroid body (Figs. 19, 22, 23). Egg tetrahedral, 33–110 (82) long, 61–114 (87) wide with a filament, 39–127 (95) long (Fig. 19).

Remarks

Hemitrygon akajei is distributed widely in Japanese waters. *Dendromonocotyle akajeii* was recorded on *H. akajei* (as *Dasyatis akajei*) from the Sea of Japan (Ho & Perkins, 1980). In the present study, the monogenean was collected from the same host species on the Pacific side. It remains to be studied whether *De. akajeii* is also widely distributed as the fish host.

The female reproductive system of *De. akajeii* is redescribed in this paper. The spheroid body of the vagina, interpreted as a seminal receptacle by Ho & Perkins (1980) in their new species description, shows different shapes depending on the degree of invagination of the proximal part. No such different states of the vagina were reported in the original description. Olson & Jeffries (1983) reexamined the type-specimens of *De. akajeii* and suggested that what Ho & Perkins (1980) described as a seminal receptacle was a spermatophore. Later, Chisholm & Whittington (1995) noted that it was not a spermatophore but the seminal receptacle as in Ho & Perkins (1980). However, it was revealed that the vagina is much longer, followed by a spherical seminal receptacle.

Discussion

To date, there are 20 species of *Dendromonocotyle* including *De. tsutsumii* n. sp. and *De. fukushimaensis* n. sp., which are the second and third species of the genus reported from Japanese waters. Four host species have been recorded as hosts of *De. akajeii*, *De. tsutsumii* n. sp. and *De. fukushimaensis* n. sp. in Japan: *H. akajei*, *Da. matsubarai*, *Da. ushiei* and *M. tobijei*. Thirty-two species of Myliobatiformes are present in Japanese waters (Nakabo, 2013). Many more *Dendromonocotyle* species will be found from the remaining 28 species of Japanese rays.

Species of *Dendromonocotyle* are generally host-specific. Only four out of 18 species described so far infect more than one species of host: *Dendromonocotyle octodiscus* Hargis, 1955 from *Dasyatis say* (Lesueur), *Dasyatis marmorata* (Steindachner) and *Urolophus jamaicensis* (Cuvier); *De. citrosa* Vaughan, Chisholm & Christison, 2008 from *Dasyatis chrysonota* (Smith) and *Maculabatis gerrardi* (Gray); *De. ukuthena* Vaughan, Chisholm & Christison, 2008 from *M. gerrardi* and *Himantura uarnak* (Forskål); and *De. colorni* Chisholm, Whittington & Kearn, 2001 from *H. uarnak* and *M. gerrardi*. *Dendromonocotyle tsutsumii* n. sp. is another species of the genus collected from two species of rays, *M. tobijei* and *Da. matsubarai.* These rays belong to different families, as in the case of *De. octodiscus*, but are common species in the Pacific coastal areas of Japan with overlapping habitats (Nakabo, 2013). *Dendromonocotyle tsutsumii* may have a wider host range,

as many more Myliobatiformes rays inhabit Japanese waters, which remains to be studied.

Of the 20 species, only *De. tsutsumii* n. sp. and *De. fukushimaensis* n. sp. have a sclerotised part at the distal end of the vaginal duct before connection with the seminal receptacle. Muscle bundles at the base of the sclerotised part may control the flow of sperm from the vagina into the seminal receptacle.

Species of *Dendromonocotyle* are divided into two main groups by the type of the male copulatory organ; species with a short, arched tube and species with a long, straight tube. The latest key to *Dendromonocotyle* species proposed by Vaughan & Chisholm (2009) was based primarily on the types of the male copulatory organ. Three new species have been added to *Dendoromonocotyle* since Vaughan & Chisholm (2009): *Dendromonocotyle rajidicola* Irigoitia, Chisholm & Timi, 2016; *De. tsutsumii*; and *De. fukushimaensis.* Therefore, including the three species, the key to species of *Dendromonocotyle* has been revised in this paper.

Key to the species of *Dendromonocotyle*

1a Male copulatory organ not extending beyond level of posterior portion of ejaculatory bulb ……..… 2
1b Male copulatory organ extending beyond level of posterior portion of ejaculatory bulb ..…… 5
2a Distal end of vaginal duct sclerotised ……. 3
2b Distal portion of vaginal duct not sclerotised …. 4
3a Male copulatory organ with donut-shaped structure at mid-length ..…. *De. fukushimaensis* n. sp.
3b Male copulatory organ lacking donut-shaped structure at mid-length … …*De. tsutsumii* n. sp.
4a Male copulatory organ with donut-shaped structure at mid-length .... ……..… *De. centrourae*
4b Male copulatory organ lacking donut-shaped structure at mid-length ..…… *De. pipinna*
5a Haptoral septal pairs 2 and 3 not joining inner ring septum; outer ring of septal sclerites absent; hamuli present .. ……. *De. californica*
5b All haptoral septa joining inner ring septum; outer ring of septal sclerites present; hamuli present or absent .....…… 6
6a Haptor with 38 or 42 marginal papillae 7
6b Haptor with 56 marginal papillae 9
7a Haptor with 38 marginal papillae; spines present at distal end of muscular sheath surrounding male copulatory organ .....……. *De. lasti*
7b Haptor with 42 marginal papillae 8
8a Distal end of male copulatory organ looping, accessory filaments present ……. *De. ardea*
8b Distal end of male copulatory organ not looping, accessory filaments absent; vaginal pore armed with spines ..... *De. taeniurae*
9a Hamuli present 10
9b Hamuli absent 13
10a Inner wall of vagina armed with spines ..... *De. ukuthena*
10b Inner wall of vagina unarmed 11
11a Proximal end of vagina a sclerotised tightly coiled duct; haptoral papillae armed with 3–4 sclerites ..... *De. colorni*
11b Proximal end of vagina not a sclerotised tightly coiled duct 12
12a Haptoral papillae armed with 7–8 sclerites including terminal papillary sclerite; male copulatory organ not extending to level of ovary ….…….. *De. akajeii*
12b Haptoral papillae armed with 4–5 sclerites including terminal papillary sclerite; male copulatory organ extends to level of ovary …… *De. lotteri*
13a Distal end of male copulatory organ lacking accessory filament …….. 14
13b Distal end of male copulatory organ with accessory filament(s) .....…….…….. 16
14a Male copulatory organ with distinct spherical inflation at mid-length ..... *De. urogymni*
14b Male copulatory organ without distinct spherical inflation at mid-length 15
15a Male copulatory organ extending beyond level of ovary; distal end simple tube coming to a point; terminal papillary sclerite keyhole-shaped ……..…… *De. kuhlii*
15b Male copulatory organ extending to level just beyond posterior portion of ejaculatory bulb; distal end widens; ovary anchor-shaped … .....…….. *De. torosa*
16a Distal end of male copulatory organ with 2 crisscrossed sperm ducts ..... *De. bradsmithi*
16b Distal end of male copulatory with single sperm duct 17
17a Distal end of sperm duct within male copulatory organ ending subterminally ……... *De. octodiscus*

17b Distal end of sperm duct within male copulatory organ ending terminally 18
18a Distal end of sperm duct within male copulatory organ looping once ..... *De. cortesi*
18b Distal end of sperm duct within male copulatory organ not looping 19
19a Male copulatory organ short, not extending past level of testis ..... *De. citrosa*
19b Male copulatory organ extending past level of testis ..... *De. rajidicola*

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Compliance with ethical standards

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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