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New Records of Monogeneans, *Gyrodactylus cyprini* (Gyrodactylidae) and *Dactylogyrus extensus* (Dactylogyridae), Parasitic on Reared Common Carp *Cyprinus carpio* (Cypriniformes: Cyprinidae) in Mie Prefecture, Japan

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Specimens of *Gyrodactylus cyprini* Diarova, 1964 (Monogenea: Gyrodactylidae) and *Dactylogyrus extensus* Mueller and Van Cleave, 1932 (Monogenea: Dactylogyridae) were obtained from the common carp *Cyprinus carpio* Linnaeus, 1758, which was reared in an outdoor pond at the Tamaki Field Station of the Fisheries Technology Institute, Mie Prefecture, Japan. Although *G. cyprini* is a well-known parasite of *C. carpio* in the Palearctic and USA and its existence in Japan was implied by the dermatitis examination of exported koi carp from Japan to Thailand, any specimen of *G. cyprini* has not been recorded in Japan. In addition, I determined some Japanese specimens previously identified as *G. kherulensis* Ergens, 1974 were also *G. cyprini*. This study obtained novel geographical records of these monogeneans, along with redescriptions of three monogenean species and molecular data of *G. cyprini* and *D. extensus*.

Key Words: new to Japan, DNA barcode, freshwater, parasite, Platyhelminthes, gill parasites.

Introduction

The common carp or koi carp, *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae), is a freshwater fish that can be found worldwide (Froese and Pauly 2023). It holds significant importance as both an ornamental fish and a food source, and is extensively aquacultured in numerous countries, including Japan (Hosoya 2001). Wild *C. carpio* populations can be found throughout Japan, but DNA analyses have revealed the coexistence of both native and non-native strains (Mabuchi et al. 2005, 2008). Currently, the pure native strain is exclusively present in Lake Biwa (Mabuchi et al. 2008; Atsumi et al. 2017).

In Japan, 13 nominal species of *Gyrodactylus* von Nordmann, 1832, *Dactylogyrus* Diesing, 1850, and *Eudiplozoon* Khotenovsky, 1984 monogeneans have been reported to parasitize *C. carpio* mainly in fish farms (Table 1). With the recent increase in export activity of koi carp from Japan (Sato 2019), a gyrodactylid monogenean previously unreported in Japan, *G. cyprini* Diarova, 1964, was identified on koi carp imported from Japan to Thailand (Wangsan et al. 2021). This gyrodactylid was recorded in the following countries: USA (Rogers 1968), Uzbekistan (Ergens 1971), Bosnia and Herzegovina (Čanković et al. 1976), Poland (Prost 1980), Spain (Lacasa-Millán and Gutiérrez-Galindo 1995), Egypt (Aly et al. 1995), China (Wang 2000), Iran (Jalali et al. 2005), German (Dzika et al. 2009), Ukraine (Kurovskaya 2004; Davydov et al. 2011), Bulgaria (Margaritov and Kiritsis 2011), Iraq (Mama and Abdullah 2012), Viet Nam (Lysenko 2013), Turkey (Öztürk and Özer 2014), Indonesia (Abadi et al. 2021), and Azerbaijan (see Seyidli et al. 2022).

In this study, laboratory-reared *C. carpio* specimens were examined, and the presence of two monogenean species, *G. cyprini* and *Dactylogyrus extensus* Mueller and Van Cleave, 1932, were identified. This study obtained novel geographical records of these monogeneans, as well as related molecular data. Specimens of *G. kherulensis* Ergens, 1974, previously found in Japanese *C. carpio* were reevaluated based on their morphology, which exhibited characteristics resembling those of *G. cyprini*, such as the bent root of the hamulus and the widening of the side edges of the membrane toward posteriorly (Ogawa and Egusa 1978).

Materials and Methods

Individuals of *C. carpio* were reared in an outdoor pond at the Tamaki Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, adhering to the institute's ethical protocol. The pond was exposed to natural light and was supplied with sand-filtered groundwater (approximately 15°C). On 6 February 2023, three freshly deceased specimens of *C. carpio* (3 years old, standard length: 131–147 mm, mean: 139 mm) were found and transferred on ice to the laboratory. The parasites were examined using a Leica S9 E dissecting microscope (Leica, Wetzlar, Germa-

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ny), and their infection prevalence (P) and intensity of infection (IF) were evaluated according to Bush et al. (1997). The monogeneans from the gills were collected using needles. For molecular analysis, the body of a *Gyrodactylus* specimen was separated from the haptor, whereas the posterior body of a *Dactylogyrus* specimen was separated from the anterior body and haptor using needles. The separated bodies were individually preserved in 99% ethanol in 1.5 mL tubes, while the remaining parts and the other two whole specimens of gyrodactylid were fixed under coverslip pressure using modified picrate glycerin (Nitta and Nagasawa 2018b).

The specimens were captured using an Olympus DP28 digital camera (Olympus, Tokyo, Japan) connected to an Olympus BX51 light microscope (Olympus) or a CANON EOS 70D digital camera (Canon, Tokyo, Japan) fitted onto a Nikon Optiphot-2 compound microscope equipped with phase contrast (Nikon, Tokyo, Japan) for morphological analysis. Measurements were obtained from these images using ImageJ software (version 1.53t, http://rsb.info.nih.gov/ij/), following the methods described by Christison et al. (2005) for gyrodactylids and Nitta and Nagasawa (2023) for the dactylogyrid. The numbering of marginal hook pairs of the dactylogyrid follows Mizelle (1936). The measurement results were presented as a range in micrometers, along with the mean and the number (n) of specimens examined. The newly collected specimens were dehydrated in an ethanol series, clarified in xylene, and mounted in Canada balsam. The specimens were then deposited in the Meguro Parasitological Museum (MPM), Tokyo, Japan. In addition, specimens of *G. kherulensis* (MPM Coll.-Nos 19251, 19606) housed in the collection of MPM were examined.

DNA was extracted from the body using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). DNA of the gyrodactylid was amplified using the ITS1-fm (5′-TAG AGG AAG TAC AAG TCG-3′) and ITS2-rm (5′-CGC TYG AAT CGA GGT CAG GAC-3′) primer pair (García-Vásquez et al. 2015), which amplified the region spanning the 3′-end of the 18S rDNA, the internal transcribed spacer 1, the 5.8S rDNA, the internal transcribed spacer 2, and the 5′-end of the 28S rDNA (ITS1-5.8S-ITS2), and the Trp1F (5′-ATA TAG ACG ATT TGT TTT CA-3′) and 16SR (5′-CAT TTA ATC ATG ATG CAA AAG G-3′) primer pair (Ziętara et al. 2006; Kuusela et al. 2008) to amplify the mitochondrial cytochrome *c* oxidase subunit I gene (*cox1*) sequence. The DNA of the dactylogyrid was amplified using the LSU5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC-3′: Littlewood 1994) and LSU1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′: Tkach et al. 1999; Snyder and Tkach 2001) primer pair to amplify the partial 28S rDNA, and PD-ITS-450F (5′-AGG TGA ACC TGC AGA AGG ATC-3′) and PD-ITS-R (5′-TAA TGC TTA AAT TCA GCG GGT-3′) primer pair to amplify the region spanning ITS1-5.8S-ITS2 (Yamamoto et al. 2011). The PCR reaction was carried out in a 15 µL volume containing 7.5 µL of KOD One PCR Master Mix (Toyobo, Osaka, Japan), 0.45 µL of each 10 μM primer, 0.6 µL of extracted DNA, and 6.0 µL of distilled water. The PCR cycles consisted of an initial denaturation at 98°C for 10 s, followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 56°C for 5 s, extension at 68°C for 10 s, and a final extension at 68°C for 60 s. The PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, USA), and the sequencing was performed commercially (Fasmac Co., Ltd., Kanagawa, Japan). Sequence data and electropherograms were inspected and edited manually using MEGA11 (Tamura et al. 2021). The sequences obtained were submitted to the DNA Data Bank of Japan and compared to other sequences deposited in the International Nucleotide Sequence Databases (INSD) through a BLASTn (http://www.ncbi.nlm.nih.gov/) search conducted on 22 September 2023.

Taxonomic Accounts

Order **Gyrodactylidea** Bychowsky, 1937
[Japanese name: Sandai-chū-moku]
Family **Gyrodactylidae** van Beneden and Hesse, 1863
[Japanese name: Sandai-chū-ka]
Genus ***Gyrodactylus*** von Nordmann, 1832
[Japanese name: Sandai-chū-zoku]
Gyrodactylus cyprini Diarova, 1964
[New Japanese name: Ō-kagi-sandai-chū]
(Fig. 1)

Gyrodactylus kherulensis not of Ergens, 1974: Ogawa and Egusa 1978: 617, fig. 2f-1 (in part).

Description. Body elongate, 407.5–735.8 (580.7, n = 11) long including haptor, 98.3–199.3 (131.9, n = 11) wide at mid-body. Haptor 92.1–201.7 (134.1, n = 12) long, 98.4–178.8 (144.2, n = 11) wide. Pharynx consisting of anterior and posterior pharyngeal bulb, 16.9–51.1 (30.8, n = 12) long, 21.9–41.2 (30.7, n = 11) wide. Male copulatory organ posterior to pharyngeal bulb, slightly sinistral from midline, 22.1–28.0 (25.2, n = 3) in diameter, armed with one large central spine and four or five small spines.

Hamulus total length 69.6–93.6 (84.1, n = 18); shaft straight, 41.9–58.8 (52.6, n = 18) long; point tapered, 32.3–43.9 (39.5, n = 18) long; root 31.4–42.7 (35.9, n = 18) long with anterior tip bent towards the point; ratio of root/total hamulus length 0.83–1.00 (0.91, n = 18); ratio of root/point length 0.40–0.48 (0.43, n = 18); aperture length 22.0–34.3 (27.8, n = 18). Dorsal bar bended, with deep notch in the central lower edge, 1.9–3.8 (2.8, n = 16) long, 9.9–26.2 (15.3, n = 16) wide. Ventral bar slightly straight with a dent in the central portion of the ventral side, 31.3–46.3 (39.2, n = 17) long; 18.6–32.5 (26.5, n = 17) wide; process-to-mid length 1.6–4.7 (3.1, n = 17); length of the median portion 6.6–11.1 (8.4, n = 17); membrane 21.9–34.1 (27.8, n = 17) long, with side edges extending downward vertically or flared, leading to a triangular-shaped lower edge; process absent. Marginal hook, 25.6–31.6 (29.1, n = 17) long; shaft 20.4–25.9 (23.7, n = 17) long, attaching at the midpoint of sickle base. Sickle filament loop 13.4–18.6 (15.5, n = 17) long. Sickles 4.9–6.1

Table 1. Records of nominal species of monogeneans parasitic on *Cyprinus carpio* in Japan.

Species	Site of infection	Locality		Reference
		Prefecture	Site	
Gyrodactylus cyprini Diarova, 1964	Gill arch	Mie	Fish pond	This study
	Fin, gill	Niigata	Fish farm	This study; Ogawa and Egusa (1978)[a]
	Fin, gill	Nagano	Fish farm	This study; Ogawa and Egusa (1978)[a]
Gyrodactylus kherulensis Ergens, 1974	Fin, gill	Niigata	Fish farm	Ogawa and Egusa (1978)
	Fin	Hokkaido	Lake Tōro	Ogawa (1994)
	—	Shiga	Lake Biwa	Timoshkin et al. (2011)
Gyrodactylus sprostonae Ling, 1962	Gill arch	Nagano	Fish farm	Ogawa and Egusa (1978)
Gyrodactylus stankovici Ergens, 1970	—	Shiga	Lake Biwa	Timoshkin et al. (2011)
Dactylogyrus anchoratus (Dujardin, 1845)	Gill	Ibaraki	Fish farm	Ogawa and Egusa (1979a)
	Gill	Tokyo	Fish farm	Ogawa and Egusa (1982)
Dactylogyrus biwaensis Ogawa and Egusa, 1982	Gill	Shiga	Lake Biwa	Ogawa and Egusa (1982)
Dactylogyrus extensus Mueller and Van Cleave, 1932	Gill	Hiroshima	Fish farm	Imada et al. (1975); Nakatusgawa and Muroga (1977)
	Gill	Niigata	Fish farm	Ogawa and Egusa (1979b); Oe and Hoshino (2002)
	Gill	Nagano	Fish farm	Ogawa and Egusa (1979b)
	Gill	Gunma	Fish farm	Ogawa and Egusa (1979b)
	Gill	Ibaraki	Fish farm	Ogawa and Egusa (1979b)
	Gill	Chiba	Fish farm	Ogawa and Egusa (1979b)
	Gill	Shizuoka	Fish farm	Ogawa and Egusa (1979b)
	Gill	Tokyo	Fish farm	Ogawa and Egusa (1979b)
	Gill	Hokkaido	Fish farm	Nagasawa et al. (1989); Ogawa (1994)
	Gill	Shiga	Fish farm	Ogawa and Egusa (1982)
	Gill	Okinawa	Hija River; Asatomata River	Nitta and Nagasawa (2015, 2018b)
	Gill	Mie	Fish pond	This study
Dactylogyrus falciformis Achmerow, 1952	Gill	Hokkaido	Lake Toro	Nagasawa et al. (1989); Ogawa (1994)
Dactylogyrus minutus Kulwieć, 1927	Gill	Nagano	Fish farm	Ogawa and Egusa (1977)
	Gill	Hiroshima	Fish farm	Nakatsugawa and Muroga (1977)
	Gill	Shiga	Lake Biwa	Ogawa and Egusa (1982)
	Gill	Okinawa	Asatomata River	Nitta and Nagasawa (2018a)
Dactylogyrus mrazeki Ergens and Dulmaa, 1968	Gill	Shiga	Lake Biwa	Ogawa and Egusa (1982)
Dactylogyrus sahuensis Ling, 1973	Gill	Shiga	Lake Biwa	Ogawa and Egusa (1982)
Dactylogyrus takahashii Ogawa and Egusa, 1982	Gill	Shiga	Lake Biwa	Ogawa and Egusa (1982)
Eudiplozoon kamegaii Nishihira and Urabe, 2020	Gill	Gunma	Tone River	Kamegai et al. (1965, 1966a, b)[b]; Kamegai (1968a)[b]
	Gill	Ibaraki	Lake Kasumigaura	Kamegai et al. (1966a, b)[b]; Kamegai (1968a, 1970a)[b]
	Gill	Tokyo	Yabuki-cho	Kamegai et al. (1966a)[b]; Kamegai (1968a)[b]
	Gill	Shiga	Lake Biwa	Kamegai (1968a)[b]; Timoshkin et al. (2011)[c]; Nishihira and Urabe (2020)
	Gill	Nagano	Lake Suwa	Kamegai (1974)[b]; Kimura (1977)[b]
	Gill	Saitama	Ogawa-cho	Kamegai (1968b)[b]
	Gill	Hokkaido	Lake Onuma; Ishikari River; Lake Akan; Lake Tōro[d]	Kamegai (1976)[b]; Nagasawa et al. (1989)[c]; Ogawa (1994)[c]
	Gill	—	—	Kamegai et al. (1967)[b]; Kamegai (1968a, c, 1969, 1970b, 1973)[a]
Eudiplozoon nipponicum (Goto, 1891)	Gill	—	—	Hirose et al. (1987)[b, e]

[a] Reported as *Gyrodactylus kherulensis* (see the results).
[b] Reported as *Diplozoon nipponicum* Goto, 1891 (see Nishihira and Urabe 2020).
[c] Reported as *Eudiplozoon nipponicum* (Goto, 1891) (see Nishihira and Urabe 2020).
[d] The collection sites are including *E. nipponicum* obtained from *Carassius* spp.
[e] Hirose et al. (1987) experimentally infected *C. carpio* with oncomiracidia from diplozoids (reported as *D. nipponicum*) that parasitize on *Carassius langsdorfii* Temminck and Schlegel, 1846 from the Tama River in Tokyo. Nishihira and Urabe (2020: 11) treated this record as *E. kamegaii*; however, in this paper, this record is reposited as *E. nipponicum* because *Eudiplozoon* species that parasitize on wild *Carassius* species are treated as *E. nipponicum* (Nishihira and Urabe 2020).

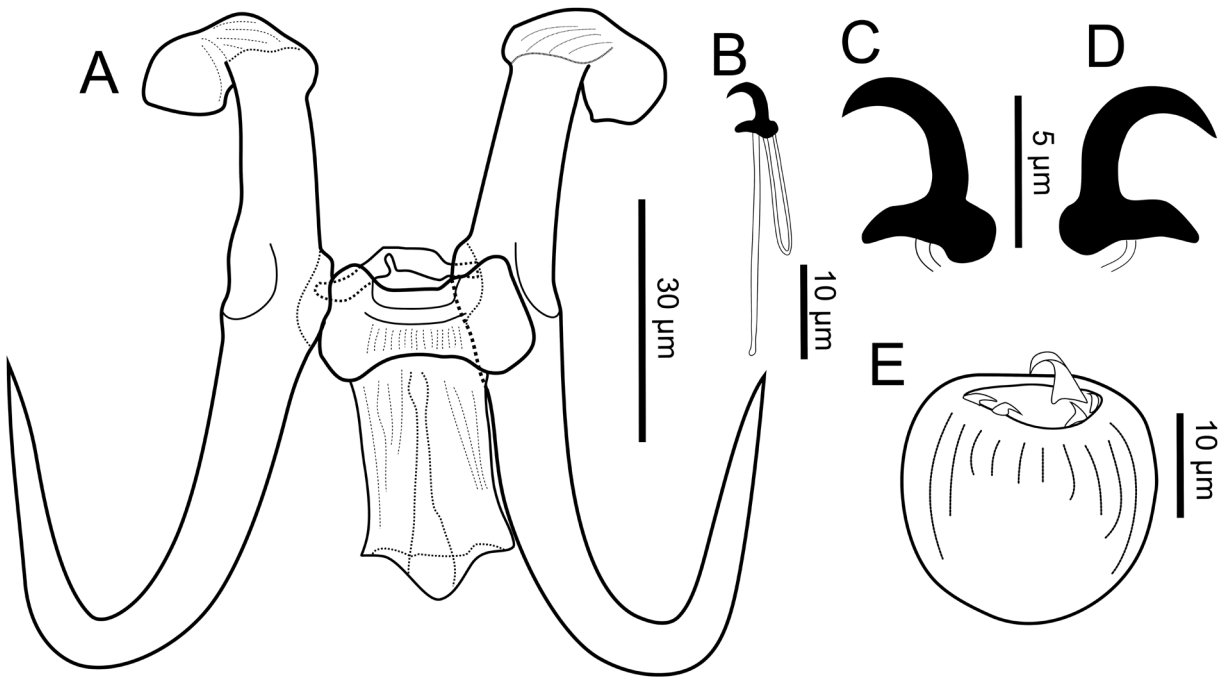


Fig. 1. *Gyrodactylus cyprini* Diarova, 1964 (A–C, E: MPM Coll.-No. 21980; D: MPM Coll.-No. 19251). A, Central hook complex (ventral view); B, marginal hook; C, D, marginal hook sickle; E, male copulatory organ.

(5.4, n = 17) long; sickle proper extending from the base, curving inward at mid-length to sickle point; point slightly exceeding the toe or not exceeding; distal width 3.1–4.7 (4.0, n = 17); proximal width 3.7–4.8 (4.2, n = 17); toe triangular, its point facing downward; knob slightly angular, heel rounded; aperture distance 4.1–5.4 (4.9, n = 17).

Material examined. Newly obtained specimens: three specimens (MPM Coll.-No. 21980) from Mie Prefecture; specimens previously identified as *G. kherulensis*: 13 specimens from Niigata Prefecture [MPM Coll.-No. 19251a (Slide Nos A0244–0255)]; two specimens from Nagano Prefecture [MPM Coll.-No. 19251b (Slide No. A0229)].

Localities. Outdoor pond (34°28′25.7″N, 136°39′42.3″E) at the Tamaki Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency in Mie Prefecture; Niigata Prefectural Inland Water Fisheries Experiment Station, Nagaoka City in Niigata Prefecture; Saku City in Nagano Prefecture, Japan.

Host. *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae).

Site on host. Gill arch, gill filament, fin.

Prevalence and intensity of infection. P = 33.3%, IF = 3 in Mie Prefecture.

Etymology. The newly proposed Japanese name, "Ō-kagi" refers to the large hamulus of *G. cyprini*, which is one of the largest among all *Gyrodactylus* species. Additionally, the name "sandai-chū" refers to the genus *Gyrodactylus*.

Molecular analysis. The ITS1-5.8S-ITS2 (1174 bp; INSD accession number LC764379) and *cox1* (1648 bp; LC764379) sequences obtained from a *G. cyprini* specimen (MPM Coll.-No. 21980) were determined. A BLASTn analysis of the ITS1-5.8S-ITS2 fragment returned no identical much, and the closest hit was *Gyrodactylus longoacuminatus* Žitňan, 1964 [ON117571, unpublished data from China; AY278043, Ziętara and Lumme (2004) from Poland], which had the highest similarity with 88.47–88.68% and a coverage of 76–82%. The closest hit to the *cox1* sequence was *Gyrodactylus gurleyi* Price, 1937 [KU659806, Zou et al. (2016) from China] at 85.01% similarity with 100% coverage.

Remarks. *Gyrodactylus cyprini* was described based on six specimens that were parasitic on the gill arches of common carp in Almaty, Kazakhstan (Diarova 1964). This species is characterized by the large hamulus, the shape of the tip of the hamulus root, which Diarova (1964) described as "resemble the shape of the feet, shod in children's shoes", the absence of the ventral bar process, and the side edges of the membrane that widen toward posteriorly (Diarova 1964; Ergens 1985; Ergens et al. 2010). In this study, the measurements and morphology of the sclerotized parts of the newly collected specimens were consistent with previous descriptions of *G. cyprini* (Table 2).

Haptoral sclerotized structures of *G. kherulensis* described by Ogawa and Egusa (1978: fig 2f) showed large hamuli, the bent tip of the hamulus root, and the widened membrane which are considered characteristics of *G. cyprini*. These are features not found in other descriptions of *G. kherulensis*, including the original description (Ergens 1974, 1985; Ali et al. 1988; Ergens et al. 2010; Maseng 2010; Abdullah and Mama 2013; Muhammad et al. 2013). After reviewing the specimens in the MPM collection, two species, *G. cyprini* and *G. kherulensis* were detected in the materials used in Ogawa and Egusa (1978) (MPM Coll.-No. 19251). Those specimens and newly collected materials represent the first record of *G. cyprini* in Japan.

Table 2. Morphological measurements (in micrometers) and armature of male copulatory organ of *Gyrodactylus cyprini* from *Cyprinus carpio*.

Source of data		This study	This study	Diarova (1964); Agapova (1966)	Rogers (1968)	Čanković et al. (1976)	Prost (1980)	Ergens (1985); Ergens et al. (2010)	Abdullah and Mama (2013)
Number of specimens examined		3	15	6	8	—	1	—	—
Locality		Japan: Mie	Japan: Niigata, Nagano	Kazakhstan: Almaty	USA: Alabama	Bosnia and Herzegovina	Poland	—	Iraq: Kurdistan
Site of infection		Gill arch	Fin, gill filament	Gill arch	—	Fin, gill	Skin	—	Skin
Body	length	632.2–657.7	407.5–735.8	421–451	370–540	—	470–780	500–900	550–800
	width	104.2–139.3	98.3–199.3	64–73	75–110	—	55–80	—	—
Pharynx	length	39.7–51.1	16.9–41.1	21.5–30	—	—	43	—	—
	width	29.6–36.3	21.9–41.2	25–34	—	—	38	—	—
male copulatory organ	diameter	25.5 × 22.0	22.1–28.0	19	17–21	—	—	—	—
	spines	1 large (L), 4 small (S)	1L, 5S	1L, 3S	1L, 5S	—	—	1L, 2 medium, 3S	—
Haptor	length	140.3–201.7	92.1–157.7	—	—	—	168	—	—
	width	148.8–178.8	98.4–168.4	—	—	—	153	—	—
Hamulus	total length	83.5–85.8	69.6–93.6	103–120	89–97	100–110	115	88–140	85–140
	shaft length	56.8–58.8	41.9–57.9			72–74	65–67	68–84	70–82
	point length	37.7–40.5	32.3–43.9	34–51	37–45	49–52	46	34–53	36–51
	root length	35.2–36.9	31.4–42.7	—	—	—	50	56–60	56–60
	aperture length	27.5–30.5	22.0–34.3	—	—	—	—	—	—
	ratio of root/point length	0.88–0.97	0.83–1.00	0.92*	0.93*	1.12*	1.39*	1.12–1.23*	1.20*
	ratio of root/total length	0.42–0.43	0.40–0.48	0.42*	0.40*	0.50*	0.44*	0.45–0.46*	0.49*
Dorsal bar	total length	3.1–3.8	1.9–3.7	—	4	5–6	5	4–7	4–6
	total width	12.9–18.0	9.9–26.2	—	19	15–17	27	13–21	12–20
Ventral bar	total length	36.9–41.7	31.3–46.3	—	—	—	53	—	—
	total width	26.5–28.9	18.6–32.5	34–38	29–32	35–37	32	29–38	30–40
	process–to–mid length	3.1–4.0	1.6–4.7	—	—	—	—	—	—
	median length	8.8–11.1	6.6–9.6	8–10	8–11	13–15	12	8–17	8–18
	membrane length	23.7–29.4	21.9–34.1	34–43	28–35	40–42	37	28–44	28–41
Marginal hook	total length	27–29.5	25.6–31.6	25–30		31–34	31	25–37	27–38
	shaft length	21.8–23.8	20.4–25.9	—	24–25		26	—	—
	sickle length	5.3–6.1	4.9–6.0	—	6–7	6–7	7	5–7	—
	distal width	4.0–4.5	3.1–4.7	—	—	—	6	—	—
	proximal width	3.9–4.2	3.7–4.8	—	—	—	5	—	—
	aperture distance	4.7–5.1	4.1–5.4	—	—	—	—	—	—
	filament length	15.2–16.8	13.4–18.6	—	8–12	—	14	—	—

* Ratios were estimated from figures.

Gyrodactylus kherulensis Ergens, 1974
[Japanese name: Koi-sandai-chū]
(Fig. 2)

Description. Body elongate, 462.1–697.5 (617.7, n = 6) long including haptor, 80.5–147.3 (112.6, n = 6) wide at mid-body. Haptor 91.0–122.5 (105.4, n = 6) long, 108.3–142.4 (115.7, n = 6) wide. Pharynx consisting of anterior and posterior pharyngeal bulb, 23.3–33.9 (33.1, n = 6) long, 23.3–39.3 (33.1, n = 6) wide. Male copulatory organ posterior to pharyngeal bulb, slightly sinistral from midline, 27.3–28.1 (27.7, n = 2) in diameter, armed with one large central spine and four small spines.

Hamulus total length 60.1–79.6 (68.4, n = 6); shaft straight, 40.1–50.8 (44.2, n = 6) long; point tapered, 30.7–41.4 (33.8, n = 6) long; root 18.4–30.3 (23.8, n = 6) long with anterior tip straight or slightly bent; ratio of root/total hamulus length 0.60–0.76 (0.70, n = 6); ratio of root/point length 0.31–0.38 (0.35, n = 6); aperture length 20.3–25.9 (22.4, n = 6). Dorsal bar bended, with deep notch in the central lower edge, 2.2–2.8 (2.4, n = 6) long, 11.7–16.9 (14.1, n = 6) wide. Ventral bar slightly straight with a dent in the central portion of the ventral side, 28.1–39.6 (33.4, n = 6) long; 19.4–25.4 (23.2, n = 6) wide; process-to-mid length 1.9–3.8 (2.6, n = 6); length of the median portion 6.6–9.3 (7.6, n = 6); membrane 19.9–27.4 (23.5, n = 6) long, with side edges vertical or slightly tapered downward and leading to a triangular-shaped lower edge; process 1.0–1.6 (1.3, n = 6) long. Marginal hook, 24.8–31.2 (28.7, n = 6) long; shaft 19.5–24.8 (28.7, n = 6) long, attaching at the midpoint of sickle base. Sickle filament loop 14.6–16.7 (15.5, n = 6) long. Sickles 5.0–5.5 (5.3, n = 6) long; sickle proper extend-

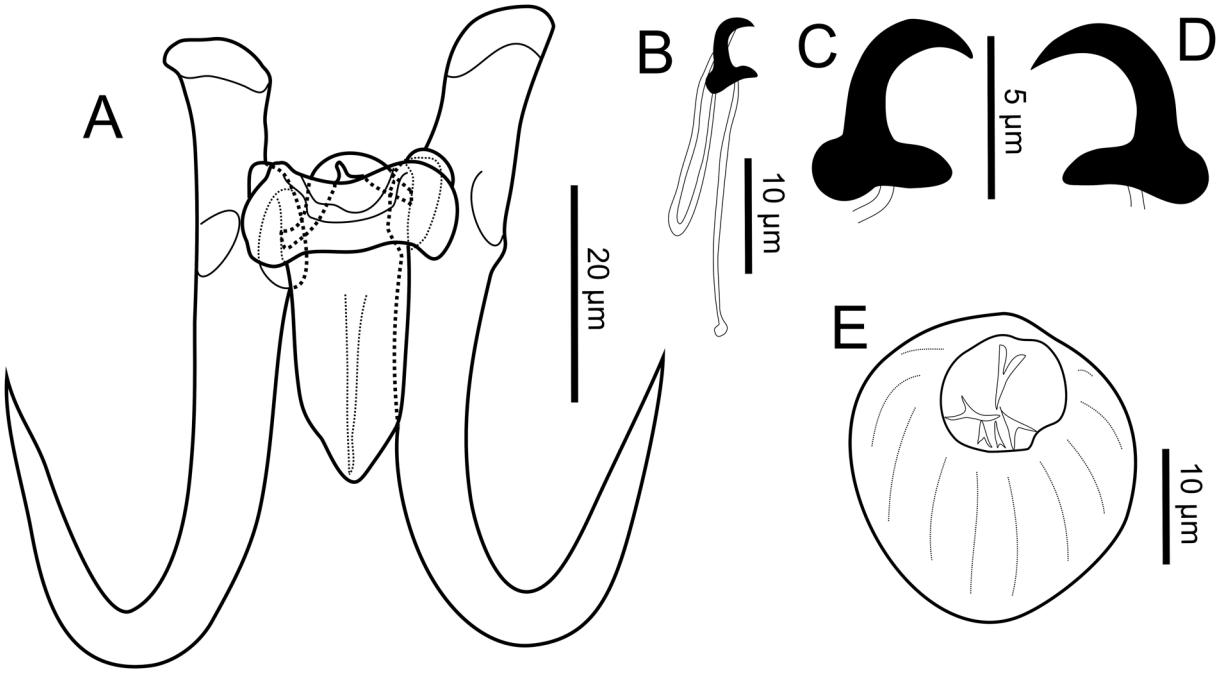


Fig. 2. *Gyrodactylus kherulensis* Ergens, 1974 (A–C, E: MPM Coll.-No. 19606; D: MPM Coll.-No. 19251). A, Central hook complex (ventral view); B, marginal hook; C, D, marginal hook sickle; E, male copulatory organ.

ing from the base, curving inward at mid-length to sickle point; point slightly exceeding the toe; distal width 2.9–4.3 (3.7, n = 6); proximal width 3.7–4.7 (4.2, n = 6); toe rounded triangular; knob not pronounced, heel rounded; aperture distance 4.4–5.2 (4.9, n = 6).

Material examined. Two specimens from Niigata [MPM Coll.-No. 19251a (Slide No. A0244)]; four specimens from Hokkaido [MPM Coll.-No. 19606 (Slide Nos A5179–A5181)].

Localities. Niigata Prefectural Inland Water Fisheries Experiment Station, Nagaoka City in Niigata Prefecture; Lake Tōro, Shibecha Town in Hokkaido.

Host. *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae).

Site on host. Fin.

Remarks. *Gyrodactylus kherulensis* was originally described from *C. carpio* in Mongolia, Russia, and Estonia (Ergens 1974). This species is distinguished from congeners by morphological characteristics, including a deep notch at the posterior edge of the dorsal bar, small processes on the ventral bar, and a *G. cotti*-type of the marginal hook sickle (Ergens 1985; Ergens et al. 2010). In this study, six specimens from the MPM collection were subjected to reexamination, leading to their identification as *G. kherulensis* based on consistent measurements and morphology of the sclerotized parts, aligning with previous descriptions (Table 3). While *G. kherulensis* exhibits similarities with *G. cyprini* concerning the dorsal bar's deep notch, the shape of the marginal hook, and the curved root tip, certain morphological features set them apart. Specifically, *G. kherulensis* can be distinguished by the absence of outward extension of the lateral edges of the membrane, the presence of a ventral bar process, and a shorter root in relation to the hamulus and point lengths (root/hamulus length: 28–37% in *G. kherulensis* vs. 40–50% in *G. cyprini*; root/point length: 59–80% in *G. kherulensis* vs. 83–123% in *G. cyprini*) (see Tables 2, 3).

Family **Dactylogyridae** Bychowsky, 1933
[Japanese name: Shi-sen-chū-ka]
Subfamily **Dactylogyrinae** Bychowsky, 1937
[Japanese name: Shi-sen-chū-aka]
Genus ***Dactylogyrus*** Diesing, 1850
[Japanese name: Yubigata-mushi-zoku]
Dactylogyrus extensus Mueller and Van Cleave, 1932
[Japanese name: Koi-yubigata-mushi]
(Fig. 3)

Description. Three pairs of head organs. Two pairs of eye-spots with some dissociated eyes. Pharynx 118.9 long, 117.2 wide. Male copulatory organ consisting of tubular penis and accessory piece, 77.1 long. Penis slender, bending strongly at base, length 85.5. Sclerotized accessory piece rod-shaped with brash-shaped tip, length 60.0. Pair of dorsal hamulus, 74.9 long; length to notch 65.7 long; outer root 25.5 long; inner root 14.3 long; point 17.9 long. Dorsal bar total length 20.3, median length 18.7, total width 48.7. Ventral bar absent. Marginal hooks 7 pairs; hook lengths: pair I 32.3; pair II 34.5; pair III 36.2; pair IV 33.2; pair V 31.1, pair VI 33.8; pair VII 34.1 µm. Pair of needles located near tip of fifth marginal hooks, 8.6 long.

Material examined. One specimen (MPM Coll.-No. 21981).

Locality. Outdoor pond (34°28′25.7″N, 136°39′42.3″E) at the Tamaki Field Station, Fisheries Technology Institute, Japan Fisheries Research and Education Agency, Mie Prefecture, Japan.

Table 3. Morphological measurements (in micrometers) and armature of male copulatory organ of *Gyrodactylus kherulensis* from *Cyprinus carpio*.

Source of data		This study	Ergens (1974)	Ergens (1985); Ergens et al. (2010)	Ali et al. (1988)	Maseng (2010)	Abdullah and Mama (2013)
Number of specimens examined		6	11	—	—	126	—
Locality		Japan: Niigata, Hokkaido	Mongolia, Russia, Estonia	—	Iraq: Babylon	South Africa: Kuilsriver,	Iraq: Kurdistan
Site of infection		Fin	Gill, fin, skin, nasal cavity	—	Gill	Gill, skin, fin	Skin
Body	length	462.1–697.5	—	<400	470	316–401	350–420
	width	80.5–147.3	—	—	58	65–113	80–110
Pharynx	length	23.4–33.9	—	—	—	—	—
	width	23.3–39.3	—	—	—	—	—
male copulatory organ	diameter	27.3–28.1	—	—	—	9–16	—
	spines	1 large (L), 4 small (S)	—	1L, 2 medium, 3S	—	1L, 6S	—
Haptor	length	91.0–122.5	—	—	—	69–103	—
	width	108.3–142.4	—	—	—	63–114	—
Hamulus	total length	60.1–79.6	61–78	61–78	64	67–82	60–76
	shaft length	40.1–50.8	45–57	45–57	41	34–46	47–55
	point length	30.7–41.4	29–33	29–33	27	23–40	29–31
	root length	18.4–30.3	19–30	19–30	23	21–33	20–28.7
	aperture length	20.3–25.9	—	—	—	20–24	—
	ratio of root/ point length	0.60–0.76	0.59–0.77*	0.78–0.80*	0.60*	0.71–0.75*	0.75*
	ratio of root/ total length	0.31–0.38	0.28–0.35*	0.36–0.37*	0.31*	0.36–0.37*	0.36*
Dorsal bar	total length	2.2–2.8	3	3	4	2–3	3
	total width	11.7–16.9	10–17	10–17	19	11–20	11–17
Ventral bar	total length	28.1–39.6	—	—	—	28–40	—
	total width	19.4–25.4	20–26	20–26	19	21–27	20–25
	process–to–mid length	1.9–3.8	—	—	—	2–4	—
	Process length	1.0–1.6	—	—	—	1–3	—
	median length	6.6–9.3	8–10	7–9	9	6–8	6–9
	membrane length	19.9–27.4	19–26	19–26	23	20–28	16–23
Marginal hook	total length	24.8–31.2	26–31	26–31	23	24–31	25–32
	shaft length	19.5–24.8	—	—	—	18–26	—
	sickle length	5.0–5.5	6–7	6–7	4	5.1–6.4	—
	distal width	2.9–4.3	—	—	—	3–4	—
	proximal width	3.7–4.7	—	—	—	4–5	—
	aperture distance	4.4–5.2	—	—	—	4–6	—
	filament length	14.6–16.7	—	—	—	—	—

* Ratios were estimated from figures.

Host. *Cyprinus carpio* Linnaeus, 1758 (Cypriniformes: Cyprinidae).

Site on host. Gill filament.

Prevalence and intensity of infection. P = 33.3%, IF = 1.

Molecular analysis. The 28S rDNA (1172 bp; LC764381) and ITS1-5.8S-ITS2 (984 bp; LC764380) sequences from a *D. extensus* specimen (MPM Coll.-No. 21981) were determibed. A BLASTn analysis of the 28S fragment returned *D. extensus* [MT997188, unpublished from China; AJ969944, Šimková et al. (2006) from Czech Republic; AY553629, Wu et al. (2005) from China; and MZ352176, Attia et al. (2022) from Egypt] as having the greatest similarity at 99.23–100% with 66–69% coverage. The closest hits to the newly generated sequence of ITS1-5.8S-ITS2 were also *D. extensus* sequences [AJ564129, Šimková et al. (2004) from Czech Republic; KM277459, Wen (2015) from China; and MZ358891, unpublished data from India] at 100% similarity with 42–46% coverage.

Remarks. The specimen was identified as *D. extensus* based on the morphology of the male copulatory organ and haptoral hard parts, as described in previous studies by Mueller and Van Cleave (1932), Ogawa and Egusa (1979b), Long (2000), Ergens et al. (2010), and Nitta and Nagasawa (2015). In Japan, *D. extensus* was found in 11 prefectures, including Hokkaido, Niigata, Nagano, Gunma, Ibaraki, Chiba, Shizuoka, Tokyo, Shiga, Hiroshima, and Okinawa prefectures (Table 1), and the current study represents the first record of *D. extensus* in Mie Prefecture.

Discussion

Fish diseases affecting cultured *C. carpio*, attributed to *Gyrodactylus* species, have been extensively documented

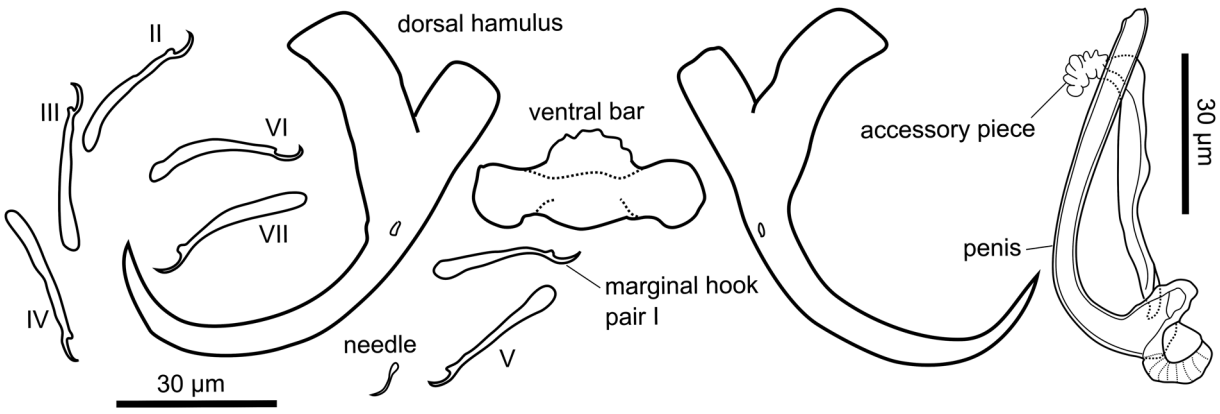


Fig. 3. Sclerotized parts of *Dactylogyrus extensus* Mueller and Van Cleave, 1932 (MPM Coll.-No. 21981). Male copulatory organ, ventral view.

across Japan (Fisheries Agency 1980). Nonetheless, the specific identification of monogeneans responsible for these infections remains largely deficient. While more than ten *Gyrodactylus* species have been documented in *C. carpio* within the Palearctic region (Pugachev et al. 2010), only four of these species have been reported in Japan (see Table 1). Consequently, there exists a high likelihood of the presence of yet unreported species. Therefore, it is imperative to conduct a comprehensive survey of the *Gyrodactylus* fauna in *C. carpio*, accompanied by detailed morphological descriptions and molecular data. Additionally, future studies should investigate the pathogenicity of each species to gain a deeper understanding of their impact on carp health.

Gyrodactylus cyprini and *G. kherulensis* found worldwide, including Japan. However, the origins of these species remain elusive, and it is also unclear whether they have been present in Japan since ancient times. Lake Biwa, an ancient freshwater lake, is home to a native Japanese strain of *C. carpio* (Mabuchi et al. 2005; Atsumi et al. 2017). So far, seven species of monogeneans have been reported from *C. carpio* inhabiting the lake (see Table 1). While, two dactylogyrid monogeneans, *Dactylogyrus biwaensis* Ogawa and Egusa, 1982 and *D. takahashii* Ogawa and Egusa, 1982, have only been reported from *C. carpio* inhabiting the lake (Ogawa and Egusa 1982; Pugachev et al. 2010) and would be endemic in the lake. The specimens of *G. kherulensis* noted in Lake Biwa warrant reexamination (Timoshkin et al. 2011). Further investigation of the *Gyrodactylus* parasites infesting the *C. carpio* in this region will make us able to discuss the original species diversity and establishment of alien species of *Gyrodactylus* in Japan.

The two species of *Gyrodactylus* reported within this investigation, *G. cyprini* and *G. kherulensis*, exhibit a striking resemblance in terms of morphology, thereby posing challenges in differentiation. Nevertheless, disparities in the configuration and dimensions of the root along with the ventral bar serve as diagnostic characters for these two species, thus precluding their synonymization. Contemporary descriptions and identifications of *Gyrodactylus* species underscore the value of genetic information (e.g., Hansen et al. 2007); regrettably, DNA data pertaining to *G. kherulensis* is presently unavailable. Subsequent inquiries of taxonomical assessments will necessitate genetic scrutiny of specimens of both taxa.

Most studies have reported on *G. cyprini* obtained from the gills and body surface of *C. carpio*; however, *Carassius auratus* (Linnaeus, 1758) (Cyprinidae) and *Pseudorasbora parva* (Temminck and Schlegel, 1846) (Gobionidae) have also been recorded as hosts of *G. cyprini* in Bulgaria, Ukraine, and Indonesia (Margaritov and Kiritsis 2011; Yurishinets and Zaichenko 2015; Yevtushenko 2020; Abadi et al. 2021). These records lack morphological description and molecular data. Two of the four reported cases were found in fish ponds where *C. carpio* was infected with *G. cyprini* in Bulgaria and Ukraine (Margaritov and Kiritsis 2011; Yevtushenko 2020). Although *Gyrodactylus* is generally considered host-specific, accidental infection is known to occur among other fish species that share the same habitat or are transported in the same vessel (Ziętara et al. 2008; Grano-Maldonado et al. 2011). Margaritov and Kiritsis (2011) reported a less frequent occurrence of *G. cyprini* on *P. parva*, and suggested that the above records were obtained as a result of temporary parasitism.

DNA data of *D. extensus* has been documented from multiple regions, including the Czech Republic, Egypt, China, India, and Japan, with limited diversity (Šimková et al. 2004, 2006; Wu et al. 2005; Wen 2015; Attia et al. 2022; this study), because *D. extensus* is most likely to have artificially expanded its distribution along with *C. carpio*. It is expected that *G. cyprini* exhibits low genetic diversity, as well as *D. extensus*. Future molecular investigations of monogeneans parasitic on *C. carpio* in various regions are expected to clarify the process and associated risk of host codispersal.

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Declarations

Competing interests. The author declares no conflicts of interest.

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