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Identification of a new species of *Gyrodactylus* von Nordmann, 1832 (Monogenoidea Gyrodactylidae) isolated from *Diptychus maculatus* in Yarkand River, Xinjiang, China

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ABSTRACT

To investigate *Gyrodactylus* infection of fish in the river system of Xinjiang (China), *Gyrodactylus* individuals were isolated from specimens of *Diptychus maculatus*. Morphological characterization and phylogenetic analysis based on *ITS1-5.8S-ITS2* rDNA locus revealed that the gyrodactylids belong to new species. *Gyrodactylus diptychi* n. sp. differs significantly in the morphology of the haptoral structures from 12 known species of *Gyrodactylus* found in fishes of the subfamily Schizothoracinae. In particular, *G. diptychi* n. sp. has a relatively short dorsal bar with thick and large ends, flat and straight hamuli roots, and small ventral bar processes. Furthermore, *G. diptychi* n. sp. is the only representative of *Gyrodactylus* found on *D. maculatus*. Using the BLASTn search of *ITS1-5.8S-ITS2* rDNA sequences in GenBank and the Bayesian Information and Maximum Likelihood methods, we constructed phylogenetic trees for *G. diptychi* n. sp. As a result, our studies clearly identified that *G. diptychi* n. sp. was the first *Gyrodactylus* monogenean isolated from *D. maculatus* and a new species belonged to the subgenus *Limnonephrotus*.

1. Introduction

The monogenean genus *Gyrodactylus* of Nordmann (1832) (Platyhelminthes, Gyrodactylidae) is able to parasitize on the gills and fins of 19 orders of teleost fishes (Wu et al., 2000; Bakke et al., 2002, 2007; Harris et al., 2004). Although most *Gyrodactylus* species are found on single hosts, some exhibit a wider spectrum of hosts (Harris et al., 2004). *Gyrodactylus* infection may cause wounds on gills, secretion of excessive mucus, and breathing difficulty, leading to negative effects on fish growth and natural watershed system. More than 470 nominal specific and subspecific names for *Gyrodactylus* have found in the world literature (Harris et al., 2004). The Gyrodactylidae was limited to species having a viviparous mode of reproduction (Boeger et al., 2020), identification and classification of the diverse *Gyrodactylus* species still rely on morphological characterization of individual features (e.g. opisthaptor) (Bakke et al., 2002). It has been noted that environmental factors may affect their characteristics; thus, additional methods need to be considered to characterize *Gyrodactylus* species (Buenosilva and Boeger, 2014; Zahradníčková et al., 2016).

Diptychus maculates Steindachner, 1866, a member of subfamily Schizothoracinae (family Cyprinidae), is an endangered aboriginal cold-water fish and an indigenous representative of ecologically essential fish species in Xinjiang aquatic ecosystems (Guo, 2012). In 2022, the People's Government of Xinjiang Uygur Autonomous Region categorized *D. maculates* as a Class I key-protected aquatic wild animal. In Xinjiang, *D. maculates* is found in Yarkand River, Yili River, and Tarim River. *Diptychus maculates* is also found in other rivers, including Syr Darya River, Talas River, and the upper stream of River Indus in the Jammu and Kashmir. The Yarkand River hosts a diverse fish fauna with 12 endemic species, consisting of 8 Schizothoracinae fishes species and four other family Cobitidae fishes (Guo, 2012). *Diptychus maculates* are distributed in scattered tributaries and mainstream of Yarkand River at a high elevation of >3300 m and low temperature <12 °C. Although the biological characteristics and genetic diversity of *D. maculates* have been internationally studied (Guo et al., 2009; Yang et al., 2014; Niu et al., 2016; Zhao et al., 2019), our understanding of its parasites in Yarkand River is still largely unclear. Three species of monogenean (*Paradiplozoon yarkandense*, Kadirden et al., 2022; *Dactylogyrus simplex*

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Mizelle, 1937; and *Dactylogyrus linstowi* Bychowsky, 1936) have been reported to parasitize *D. maculates* in Yarkand River (Rong et al., 2020; Rong, 2021; Arken et al., 2022). Whether *D. maculates* may also host the monogenean *Gyrodactylus* remains to be clarified.

In this study, we collected *D. maculates* from the upstream Taxkorgan River of Yarkand River. Morphological characterisation of *Gyrodactylus* specimens found on *Diptychus maculates* was carried out to identify them, and phylogenetic analysis based on *ITS1-5.8S-ITS2* rDNA was used to determine their relationships with congeneric species. Our studies identified a new species *Gyrodactylus diptychi*.

2. Materials and methods

2.1. Fish and parasite sampling

Samples of *D. maculatus* (Scaly osman) were collected from the Yarkand River (75°30′24.47″N; 37°69′41.25″E) in August 2019 and June 2020 using fyke nets. After being captured, the alive fish were transported to the temporary lab, and euthanized by severing the spinal cord posterior to the skull with a single cut. Gills and fins of *D. maculatus* were surgically isolated within 24 h for examination and parasite isolation. The isolated parasites were preserved in 75% and 95% ethanol for further analysis. The definitions of prevalence and mean intensity of infection were used according to Bush et al. (1997). The animal procedures conducted in this study were approved by the Xinjiang Agricultural University Animal Care and Use Committee (No. 2019021).

Table 1
Sequence information of selected *ITS1-5.8S-ITS2* rDNA of *Gyrodactylus* species used for phylogenetic analysis (* species sequenced in this study).

Parasite	Host species	Locality	GenBank ID	Reference
G. ajime Nitta (2021)	*Niwaella delicata*	Japan: Kyoto	LC545570	Nitta (2021)
G. albolacustris Lumme, ZiÄ;TMtara & Lebedeva (2017)	*Phoxinus phoxinus*	Russia: Ladoga, Baltic Sea basin	EU554412.1	Lumme et al. (2017)
G. anguillae Ergens, 1960	*Anguilla australis*	Australia: Victoria, Skipton	AB063294	Hayward et al., 2012, unpublished
G. aphyae Malmberg, 1957	*Phoxinus phoxinus*	Finland: River Merenoja, River Kovda system, White Sea basin	AF484528	Zięara et al. (2002)
G. arcuatus Bychowsky, 1933	*Gasterosteus aculeatus*	Finland: Gulf of Bothnia, Baltic Sea	AF328865	Zięara et al. (2002)
G. banmae Jin et al., 2022	*Zebrafish*	China	MW353803.1	Jin et al. (2022)
G. brachymystacis Ergens, 1978	*Brachymystax lenok*	China	GQ368237	Gilmore et al. (2010)
G. branchialis Huyse, Malmberg & Volckaert, 2004	*Pomatoschistus marmoratus*	France: Vaccares lagoon	DQ821770	Huyse et al. (2006)
G.branchicus Malmberg, 1964	*Gasterosteus aculeatus*	Russia: Kola Peninsula, White Sea	FJ435199	Rokicka et al. (2009)
G. cernuae Malmberg, 1957	*Gymnocephalus cernuus*	Finland: River Oulujoki, Baltic Sea basin	AF484529	Zięara et al. (2002)
G. derjavini Mikailov, 1975	*Oncorhynchus mykiss*	Iran: Caspian Sea basin	DQ323402	Rokicka et al. (2007)
G. derjavinoides Malmberg, Collins, Cunningham & Jalali, 2007	*Salmo letnica*	Macedonia: River Vardar system, Aegean Sea basin	EU304810	Zięara et al. (2010)
G. diptychi n. sp.*	*Diptychus maculatus*	China	OR762714	This study
G. diptychi n. sp.*	*Diptychus maculatus*	China	OR762715	This study
G. ginestrae Kvach, Ondrač;ková, Seifertová and Hulak (2019)	*Atherina boyeri*	Ukraine: Black Sea, Gulf of Odessa	MK550602.2	Kvach et al. (2019)
G. gurleyi Price, 1937	*Goldfish Carassius auratus*	China	KC922453	Li et al. (2014)
G. gymnodiptychi Zhang et al., 2023	*Gymnodiptychus dybowskii*	China: Yili River	MH4459687	Zhang et al., 2023
G. harengi Malmberg, 1957	*Clupea harengus*	France: Ambleteuse	AJ309295	Matejusová et al. (2003)
G. jussii ZiÄ;TMtara & Lumme (2003)	*Phoxinus phoxinus*	Finland: River Merenoja, River Kovda system, White Sea basin	AY061982	Zięara and Lumme (2003)
G. leptorhynchi Cone et al. (2013)	*Syngnathus leptorhynchus*	Pacific coast of North America	JX110633	Cone et al. (2013)
G. leucisci Zitnan, 1964	*Leuciscus leuciscus*	Finland: River Oulujoki, Baltic Sea basin	AF484537	Zięara et al. (2002)
G. longoacuminatus Zitnan, 1964	*Goldfish*	China	KC922451.1	Li et al. (2014)
G. luciopercae Gussev, 1962	*Perca fluviatilis*	Finland: Gulf of Bothnia, Baltic Sea	AF484541	Zięara et al. (2002)
G. medaka Nitta and Nagasawa (2018)	*Oryzias latipes*	Japan: Tokushima	LC368477	Nitta and Nagasawa (2018)
G. mongolicus Ergens and Dulmaa, 1970	*Oreoleuciscus potanini*	Mongolia: Chono Kharaik river	OQ913868	Lebedeva et al. (2023)
G. nemachili Bikhovski, 1936	*Oreoleuciscus potanini*	Mongolia: Chono Kharaik river	OQ641772	Lebedeva et al. (2023)
G. nipponensis Ogawa and Egusa, 1978	*Anguilla japonica*	Japan: Shizuoka, Lake Hamana	AB063295	Hayward et al. (2001)
G. ostendicus Huyse and Malmberg, 2004	*Pomatoschistus marmoratus*	France: Vaccares lagoon	DQ821768	Huyse et al. (2006)
G. papernai Ergens and Bychowsky, 1967	*Salmo salar*	Russia: River Vidlitsa, Lake Ladoga system, Baltic Sea Basin	EF446729	Matejusová et al. (2001)
G. parvae You et al., 2008	*Pseudorasbora parva*	China: Qinling Mountains	EF450249.1	You et al. (2008)
G. pseudonemacheili Ergens and Bychowsky, 1967	*Barbatula conilobus*	Mongolia: Zavkhan river	OQ641764	Lebedeva et al. (2023)
G. pungitii Malmberg, 1964	*Pungitius pungitius*	Finland: Lake Rytilampi, White Sea basin	AF484543	Zięara et al. (2002)
G. rarus Wegener, 1910	*Gasterosteus aculeatus*	Finland: Gulf of Bothnia, Baltic Sea	FJ435196	Rokicka et al. (2009)
G. rogatensis Harris, 1985	*Cottus gobio*	Rogate (West Sussex, England)	AJ011411	Cable et al. (1999)
G. rugiensis Glaser, 1974	*Pomatoschistus minutus*	France: Vaccares lagoon	DQ821761	Huyse et al. (2006)
G. salaris Malmberg, 1957	*Thymallus thymallus*	Finland: River Oulankajoki, River Kovda system, White Sea basin	AF484544	Zięara et al. (2002)
G. salinae Paladini et al., 2011	*Aphanius fasciatus*	hypersaline environment in Italy	JF950559	Paladini et al. (2011)
G. tayshirensis Lebedeva et al. (2023)	*Barbatula conilobus*	Mongolia: Zavkhan river	OQ641774	Lebedeva et al. (2023)
Diplogyrodactylus martini Prikrylova, Matejusova, Musilova, Gelnar & Harris, 2009	*Polypterus senegalus*	Senegal	AM943008	Přikrylová et al. (2009)
Gyrodactyloides bychowskii Albova, 1948	*salmon*	United Kingdom:Scotland	AJ249348	Bruno et al. (2001)

2.2. Morphometric analysis

A total of 28 individuals of *Gyrodactylus* were collected from host *D. maculatus* and subjected to morphological characterization. *Gyrodactylus* spp. were fixed and stained according to Zhang et al., 2023. The microscopic analysis of morphological features was then conducted using a Nikon ECLIPSE E200 imaging optical microscope. *Gyrodactylus* spp. was palced under a stereomicroscope at × 400–1000 magnification. The various parameters of parasite's body were measured using an EZ-MET software (×86, 6.0.7543) in micrometers according to Shinn et al. (2004). Drawings the morphological features of the parasites were generated utilizing a camera lucida.

2.3. Molecular analysis

The genomic DNA was extracted from parasites using the Genomix DNA kit (EasyPure®) provided by TransGen Biotech, Beijing, China. Fragments of the *ITS1-5.8S-ITS2* ribosomal DNA (rDNA) amplified region were isolated by the PCR technique. The forward primer with the sequence 5′-TTTCCGTAGGTGAACCT-3′ and the reverse primer with the sequence 5′-TCCTCCGCTTAGTGATA-3′ (Cunningham, 1997). The amplification reaction (mixture of 1 μL forward primer, 1 μL reverse primer, 2 μL of DNA template, 25 μL of 2 × Super Master mix [Bio-Rad, CA, USA], and 21 μL of H_2O) was performed as follows: 1 cycle at 95 °C for 5 min and 30 cycles of reactions at 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 75 s, followed by the final extension 1 cycle at 72 °C for 10 min. The PCR products were visualized by electrophoresis on 1% agarose gels and staining with GelStain (TransGen Biotech). The DNA fragments were then isolated and purified from the agarose slices utilizing the PCR purification kit (EasyPure®). Then, purified DNA fragments were sequenced using the ABI Cycle Sequencer 3700 (Foster City, CA, USA) at the Sangon Biotech Co., Ltd. (Shanghai, China).

2.4. Phylogenetic analysis

Sequences were deposited in GenBank and collected *ITS1-5.8S-ITS2* rDNA sequences of 36 *Gyrodactylus* species in the GenBank (Table 1), their accession numbers are cited in the description of each species (Table 1). The sequences of *Diplogyrodactylus martini* Paikrylova, MatÄ;'jusova, Musilova Gelnar and Harris (2009) and *Gyrodactyloides bychowskii* Albova, 1948 were used as two control outgroups. The sequences of the *ITS1-5.8S-ITS2* rDNA genes in the GenBank were identified using the PhyloSuite 1.2.3 (Zhang et al., 2020), and DNA sequences were aligned using MAFFT v7 (Katoh and Standley, 2013). The aligned results were then imported into Globcks 0.91b where the conserved sites were extracted based on specific parameter settings (Gerard and Jose, 2007). The molecular evolution model was determined using the Bayesian Information (BI) and Maximum Likelihood (ML) methods with the assistance of ModelFinder v1.6.8 (Kalyaanamoorthy et al., 2017). The optimal evolutionary model was identified using the Bayesian information criterion (BIC) in ModelFinder v1.6.8 (Posada and Crandall, 1998). Based on the Akaike's information criterion and Bayes information criterion, as implemented in ModelFinder v1.6.8, GTR+F+I+G4 and GTR+F+R4 were chosen as the best-fit model for nucleotide evolution for BI and ML analyses, respectively. For BI analysis, the Markov Chain Monte Carlo method (MCMC) was used to evaluate the posterior probability of model parameters. Four chains were run for 2,000,000 generations, with samples taken every 100 generations. After confirming for convergence, the Burnin sample trees were discarded (parameter set at 0.25 %). The remaining trees were used to calculate a 50% majority-rule consensus tree using the MrBayes 3.2.1. program (Ronquist and Huelsenbeck, 2003). ML analysis was performed using IQ-TREE (Trifinopoulos et al., 2016) with 10,000 ultrafast bootstraps (Minh et al., 2013). The generated files from PhyloSuite 1.2.3 were visualied and the phylogeny and architecture were presented using iTOL (https://itol.embl.de/) (Letunic and Bork, 2007).

3. Results

3.1. Infection indices

After capturing *D. maculatus* from Yarkand River, we selected a total of 107 individuals (tail fork length: 6.4–18.5 cm) from our collection. Studying these individuals, we detected the prevalence of *Gyrodactylus* infection at 82% and the mean intensity of infection at 7.

Order Gyrodactylidea Bychowsky, 1937.

Family Gyrodactylidae Van Beneden and Hesse, 1863.

Genus *Gyrodactylus* Nordmann, 1832.

***Gyrodactylus diptychi* n. sp.** (Figs. 1 and 2)

Type-host: *Diptychus maculates* Steindachner, 1866.

Site of infection: fins.

Type-locality: Yarkand River, Xinjiang Uygur Autonomous Region, China (75°30′24.47″N; 37°69′41.25″E).

Type-material: The Holotypes: XJBCC20201201, paratypes: XJBCC20201202-06.

GenBank accession number: *ITS1-5.8S-ITS2* rDNA: OR762714 and OR762715.

Etymology: The species was named by referring to the genus of host *D. maculatus* from which it parasitized.

Zoo bank: LSID urn:lsid:zoobank.org:pub:0FD61239-608F-4E82-8323-5CE2CEB20573

3.2. Morphological characterization of Gyrodactylus diptychi n sp.

In order to clarify the morphological characteristics of *Gyrodactylus* from *Diptychus maculates*. Descirption based on 28 flattned specimens (Figs. 1 and 2, Table 2). Body "gourd-like" shape, fusiform, total body length 436.4 (369.4–493.6) long, 108.8 (101.1–115.2) wide. Pharynx bulb 27.5 (16.8–29.7) long, 22.3 (14.6–27.2) wide. The cecum not spread to anterior edge of the testes. MCO 16.6 (8.03–19.8) long, 15.6 (7.6–17.7) wide, armed with one central spine, two large spines and four small spines, posterior to pharyngeal bulb (Figs. 1C & 2D). Hamuli 83.7 (78.5–86.9) long, shafts 60.2 (56.7–63.0) long, tapered end; points 35.6 (32.3–38.2) long; proximal shaft 9.5 (9.0–10.2) wide, curved (Figs. 1A, 2A and 2B); Aperture distance 27.4 (25.7–30.6) long, hamulus aperture angle 38.5° (32.6°–48.7°), hamulus root 28.6 (21.9–32.7) long, inward and curved (Figs. 1B & 2C). Dorsal bar 20.1 (18.9–25.3) long, 2.5 (1.8–3.4) wide, the middle flat straight, short, and both ends were thick and big (Figs. 1D and 2E). Ventral bar 38.0 (30.7–41.5) long, 13.8 (11.5–15.2) wide, with short processes and medium triangular membrane, ventral bar processes 3.1 (2.8–3.4) long, ventral bar membrane 24.1 (19.2–26.3) long, "U" shape with a weak central ridge (Figs. 1E & 2F). Marginal hook 35.0 (32.5–37.9) long, hook shaft 29.1 (27.1–31.9) long, rounded bottom; marginal hook sickle 6.3 (5.3–7.2) long, sickle shaft approximately perpendicular to the base, curved, tilted forward and aligned the toe; sickle point 2.9 (2.4–3.7) wide, sickle distal 2.7 (2.1–3.7) wide. Marginal hook toe 1.7 (1.1–2.5) long, marginal hook aperture 4.7 (2.0–5.9) long, hook instep 1.0 (0.8–1.4) high, and filament loop 1.0 (0.8–1.4) long (Fig. 1F and G & 2G & 2H).

3.3. Remarks

To clarify any features of the newly identified *G. diptychi* n. sp. distinctive from already known members of *Gyrodactylus*, we compared the morphological features of *G. diptychi* n. sp. with *G. hemivicinus* Ergens et Daniyarov, 1976; *G. kafirniganensis* Ergens and Karabekova (1980) and *G. montanus* Gusev (1985). We also compared the marginal hooks of *G. diptychi* n. sp. with 12 *Gyrodactylus*, which are parasitic to Schizothoracinae. As depicted in Fig. 3, *G. diptychi* n. sp. exhibited similar marginal hook morphology to the *G. gymnodiptychi* Zhang et al., 2023 isolated from *Gymnodiptychus dybowskii*, Kessler, 1874; However, *Gyrodactylus diptychi* n. sp. had a slender sickle, a longer marginal hook toe and a longer marginal hook proximal (Fig. 3). As depicted in Fig. 4,

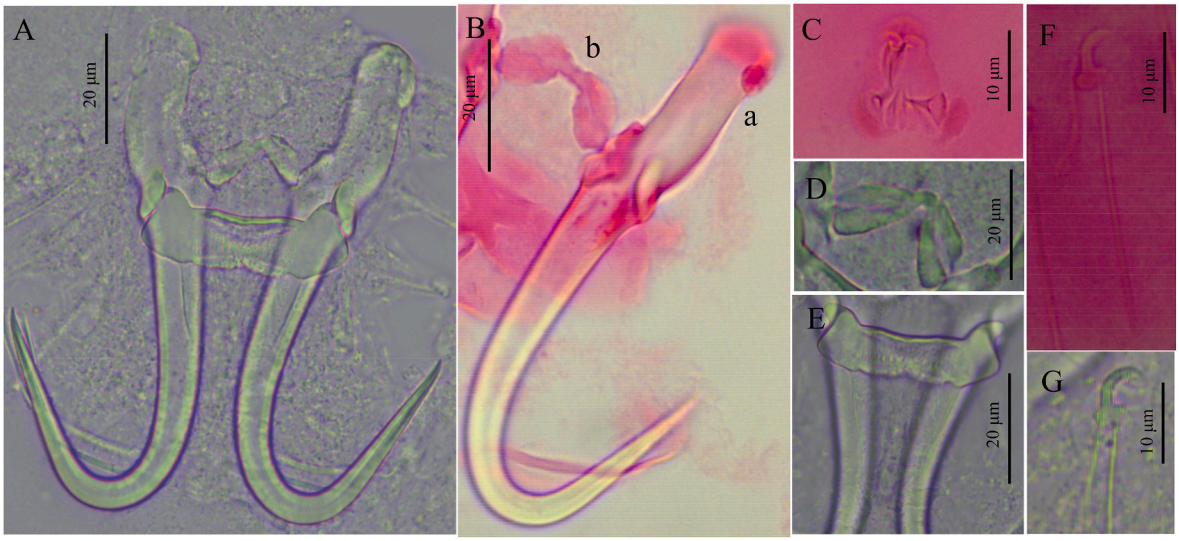

Fig. 1. Light micrographs of *Gyrodactylus diptychi* n. sp. (A) Hamulus-bar complex, (B) Hamuli (HA), (C) Male copulatory organ (MCO), (D) Dorsal bar (DB), (E) Ventral bar (VB), (F) Marginal hook (MH), (G) Marginal hook sickle(MHS).

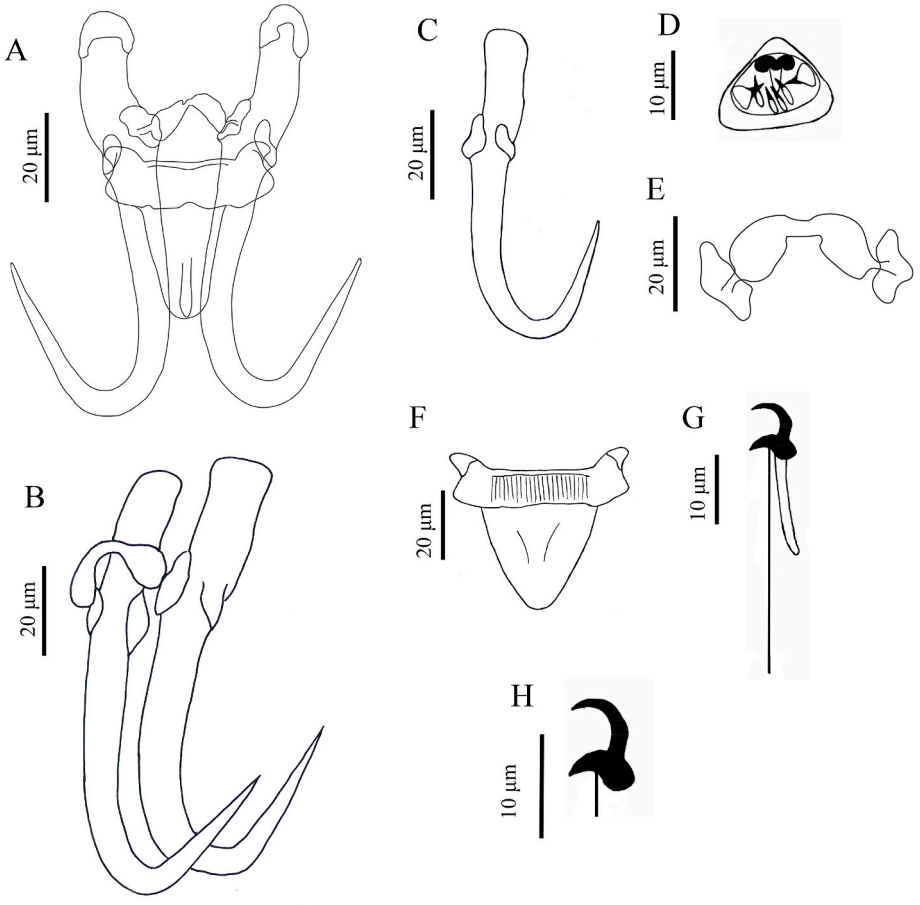

Fig. 2. Line drawings of the haptoral structures of *Gyrodactylus diptychi* n. sp. (A & B) Hamulus-bar complex, (C) Hamuli (HA), (D) Male copulatory organ (MCO),(E) Dorsal bar (DB), (F) Ventral bar (VB), (G) Marginal hook (MH), (H) Marginal hook sickle(MHS).

G. diptychi n. sp. exhibited similar ventral bar morphology to the *G. hemivicinus*; on both species, their dorsal bars were short, but the dorsal bar of *G. diptychi* n. sp. was flat straight in middle and thick and big at both ends. The MCO of *G. diptychi* n. sp. had only one spine that was fewer than *G. hemivicinus* (Fig. 4A and B). *Gyrodactylus diptychi* n. sp. exhibited same number of MCO' spines and exhibited similar ventral bar morphology to the *G. hemivicinus* and *G. kafirniganensis* (Fig. 4A and C); however, the dorsal bar of *G. diptychi* n. sp. was short and flat straight in middle, and thick and big at both ends. *Gyrodactylus diptychi* n. sp. exhibited similar hamuli morphology to the *G. montanus*; on both

Table 2
The comparison of *Gyrodactylus diptychi* with other morphologically similar species.

Measurement	*Gyrodactylus diptychi* n. sp. (n = 28) Present study	*G. hemivicinus* (Ergens, 1976)	*G. kafirniganensis* (Ergens, 1976)	*G. montanus* (Gusev, 1985)
(length and width, μm)	Average (range)	Average (range)	Range	Range
Total body, length	436.4 ± 39.8 (369.4–493.6)	–	–	–
Total body, width	108.8 ± 23.7 (101.1–115.2)	–	–	–
Pharynx, length × width	27.5 ± 2.1 (16.8–29.7) × 22.3 ± 1.6 (14.6–27.2)	–	–	–
Opisthaptor, length × width	83.9 ± 2.9 (71.9–104.3) × 84.9 ± 2.7 (74.6–113.0)	–	–	–
Male copulatory organ, length × width	16.6 ± 3.9 (8.03–19.8) × 15.6 ± 2.7 (7.6–17.7)	–	–	–
MCO spines	1L, 6S	1L, 5S	1L, 6S	1L, 6S
Hamulus				
Total length	83.7 ± 3.2 (78.5–86.9)	62–71	–	–
Aperture distance	27.4 ± 1.8 (25.7–30.6)	–	–	–
Point shaft width	9.5 ± 0.5 (9.0–10.2)	–	–	–
Point length	35.6 ± 2.1 (32.3–38.2)	25–30	21–33	21–33
Distal shaft width	5.5 ± 0.4 (4.9–6.1)	–	–	–
Shaft length	60.2 ± 2.3 (56.7–63.0)	47–50	63–78	63–78
Inner curve length	6.5 ± 1.9 (4.8–9.7)	–	–	–
Aperture angle	38.5° ± 5.6° (32.6°–48.7°)	–	–	–
Point curve angle	11.7° ± 1.8° (9.5°–14.2°)	–	–	–
Inner aperture angle	43.0° ± 6.4° (35.9°–53.5°)	–	–	–
Root length	28.6 ± 3.8 (21.9–32.7)	15–25	–	–
Ventral bar				
Length	38.0 ± 4.4 (30.7–41.5)	30 (31–35)	33–45	33–45
Width	13.8 ± 1.8 (11.5–15.2)	6 (5–9)	9–15	9–15
Process to mid–length	5.2 ± 1.3 (3.1–6.8)	–	–	–
Mid–length	7.6 ± 0.6 (6.8–8.4)	–	–	–
Process length	3.1 ± 0.2 (2.8–3.4)	–	–	–
Membrane length	24.1 ± 2.9 (19.2–26.3)	15–22	–	–
Dorsal bar				
Length	20.1 ± 2.7 (18.9–25.3)	25 (21–27)	22–45	22–45
Width	2.5 ± 0.7 (1.8–3.4)	2	3–6	3–6
Marginal hook				
Total length	35.0 ± 1.9 (32.5–37.9)	30–33	40–53	40–53
Shaft length	29.1 ± 1.6 (27.1–31.9)	–	–	–
Sickle length	6.3 ± 0.5 (5.3–7.2)	7-9	7–8	7–8
Sickle point width	2.9 ± 0.4 (2.4–3.7)	–	–	–
Toe length	1.7 ± 0.6 (1.1–2.5)	–	–	–
Sickle distal width	2.7 ± 0.5 (2.1–3.7)	–	–	–
Aperture	4.7 ± 1.4 (2.0–5.9)	–	–	–
Instep/arch height	1.0 ± 0.2 (0.8–1.4)	–	–	–
Filament loop	11.8 ± 2.1 (8.0–14.1)	–	–	–

Note: "–" means no date.

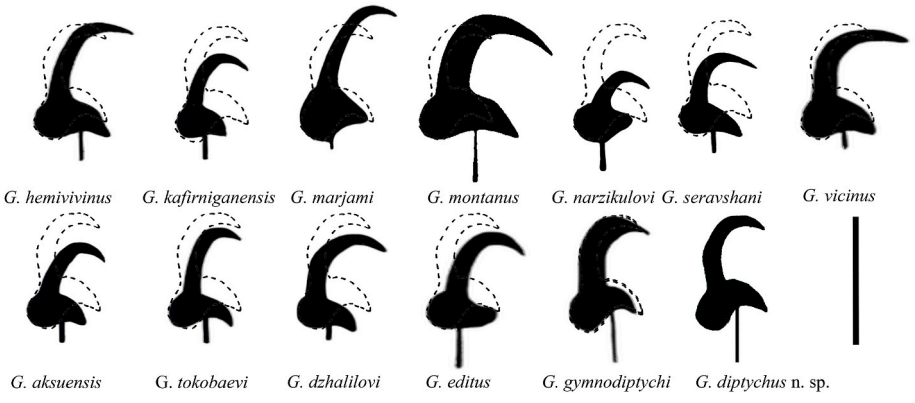

Fig. 3. Marginal hook sickles of *Gyrodactylus diptychi* n. sp.; *G. hemivicinus* from Gusev (1985); *G. kafirniganensis* from Gusev (1985); *G. marjami* from Gusev (1985); *G. montanus* from Gusev (1985); *G. narzikulovi* from Gusev (1985); *G. seravshani* from Gusev (1985); *G. vicinus* from Gusev (1985); *G. aksuensis* from Gusev (1985); *G. tokobaevi* from Gusev (1985); *G. dzhalilovi* from Gusev (1985); *G. editus* from Gusev (1985); *G. gymnodiptychi* from Zhang et al., 2023; Scale bars: 20 μm.

species, their MCO armed with one central spine, two large spines, and four small spines; however, the dorsal bars of *G. montanus* had a hollow at both ends of the projection (Fig. 4A and D). The results indicated *G. diptychi* n. sp. having a short dorsal bar with thick and big ends that was distinctive from 12 species of gyrodactylid isolated from the fish subfamily Schizothoracinae.

3.4. Molecular characterization

To determine the *ITS1-5.8S-ITS2* rDNA sequence of *G. diptychi* n. sp.,

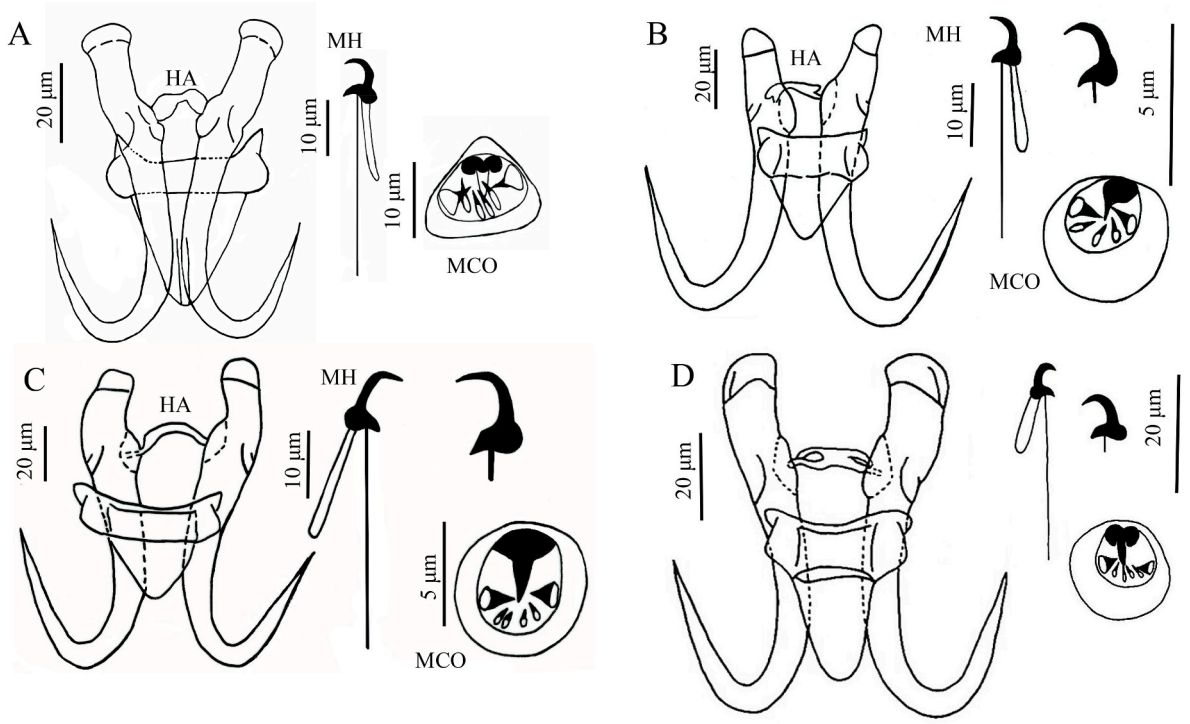

Fig. 4. Morphological comparison of **(A) *Gyrodactylus diptychi*** n. sp., (B) *Gyrodactylus hemivicinus*, (C) *Gyrodactylus kafirniganensis*, and (D) *Gyrodactylus montanus*.

we selected two specimens for gene analysis; the results revealed that the length of these gene fragments from both specimens was 1191 bp (GenBank: OR762714 and OR762715) sharing >99% identity. BLASTn inquiry study of the *ITS1-5.8S-ITS2* gene fragment did not reveal any genes matched in GenBank (Altschul et al., 1997; Benson et al., 2007). The results revealed sequence homology of *G. diptychi* n. sp. (GenBank:

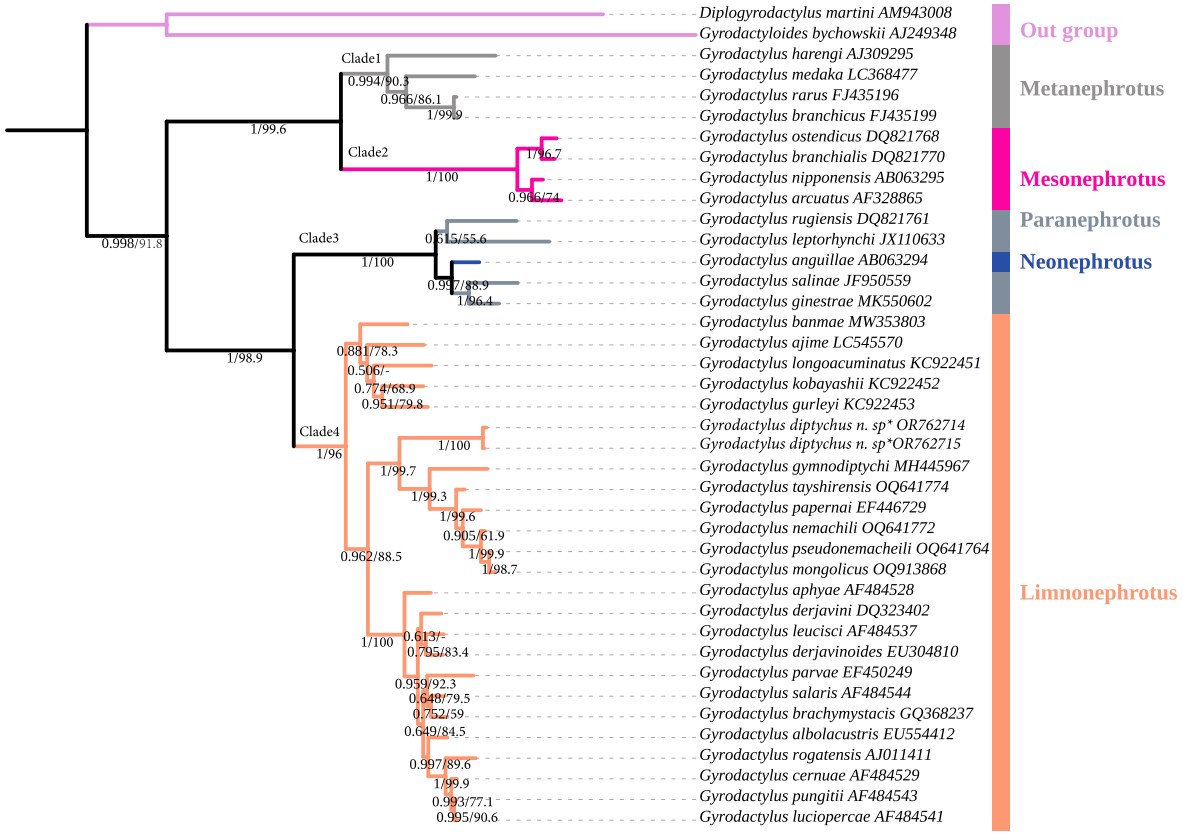

Fig. 5. The phylogenetic tree constructed by the BI and ML methods based on the *ITS1-5.8S-ITS2* rDNA sequences from selected *Gyrodactylus* species. Numbers along branches represent Bayesian posterior probability/ML bootstrap support.

MH445967) to *Gyrodactylus* sp. MB-2017 (749/833, 89.92%); *G. tayshirensis* Lebedeva et al. (2023) (844/943, 89.50%); *G. pseudonemacheili* Ergens and Bychowsky, 1967 (820/925, 88.65%); *G. gymnodiptychi* (827/923, 89.60%); *G. papernai* Ergens and Bychowsky, 1967 (818/915, 89.40%); *G. nemachili* Bikhovski, 1936 (823/926, 89.88%); and *G. mongolicus* (824/924, 89.18%). The BLASTn query of the *5.8S* rDNA fragment detected 6 identical matches of species, which belong to the subgenus *Limnonephrotus* Malmberg, 1964.

3.5. Phylogenetic positioning of G. diptychi n sp.

To investigate the relationship of *G. diptychi* n. sp. to other *Gyrodactylus* members, we used BI and ML methods to construct the phylogenetic trees, using *ITS1-5.8S-ITS2* gene sequences, of *G. diptychi* n. sp. and *Gyrodactylus* members. As shown in Fig. 5, BI and ML analytic methods yielded two similar phylogenetic trees with minor variations in statistical values. *Gyrodactylus diptychi* n. sp. formed a clade with *G. gymnodiptychi*, *G. tayshirensis*, *G. papernai*, *G. nemachili*, *G. pseudonemacheili*, and *G. mongolicus*. Both phylogenetic trees indicated that *G. diptychi* n. sp. was closely related to *Gyrodactylus* members and belonged to the subgenus *Limnonephrotus* Malmberg, 1964.

4. Discussion

The studies identified a new species of the monogenean *G. diptychi* n. sp. having a relatively short dorsal bar with thick and big ends, flat and straight hamuli roots, and small ventral bar processes; these features were distinctive from 12 already known species of the genus *Gyrodactylus* isolated from the fish subfamily members of Schizothoracinae but not *D. maculatus*. The newly identified *G. diptychi* n. sp. was the first *Gyrodactylus* isolated from *D. maculatus*.

Monogeneans (*Dactylogyrus* genus and *Paradiplozoon* genus) were reportedly isolated from *D. maculatus* in China and Russia (Gusev, 1985; Arken et al., 2020; Rong et al., 2020; Rong, 2021; Arken et al., 2022). Twelve species of *Gyrodactylus* were reportedly isolated from the subfamily Schizothoracinae; among these species, *G. gymnodiptychi* was found in China (Zhang et al., 2023), *G. tokobaevi* and *G. aksuensis* were found in Aksu River (west of Bishkek) (Ergens and Karabekova, 1980), and other species were found in Czech Republic (Gusev, 1985). The morphological studies revealed that *G. diptychi* n. sp. shared similar feature of Marginal hook with *G. gymnodiptychi* but not with other 11 species. *G. diptychi* n. sp. shared a similar feature of relatively flat and straight hamuli roots with 9 species except *G. gymnodiptychi*, *G. narzikulovi*, and *G. aksuensis* (Ergens and Karabekova, 1980; Gusev, 1985; Zhang et al., 2023). *G. diptychi* n. sp. shared a similar feature of small ventral bar processes with 10 species except *G. gymnodiptychi* and *G. tokobaevi* (Ergens and Karabekova, 1980; Gusev, 1985). Interestingly, *G. diptychi* n. sp. had unique features of a short dorsal bar with non-hollow thick and big ends in contrast to other *Gyrodactylus* species carrying long and slender dorsal bar with a hollow at ends (Gusev, 1985).

Blast studies indicated that the *ITS1-5.8S-ITS2* genes of *G. diptychi* n. sp. were distinguishable from all other *Gyrodactylus* species listed in the GenBank. The constructed phylogenetic trees indicated that *G. diptychi* n. sp. was closely related to members of the subgenus *Limnonephrotus*, in which *Gyrodactylus* sp. MB-2017 (KY848504) and *G. gymnodiptychi* (MH445967, MH445968) shared ≈ 90% homology of the *ITS1-5.8S-ITS2* genes with *G. diptychi* n. sp. However, *Gyrodactylus* sp. MB-2017 is a parasite of a *D. maculatus*-unrelated fish host *Aulopyge huegelii* Heckel, 1843; and *G. gymnodiptychi* is a parasite of host *G. dybowskii*. The phylogenetic tree also revealed that *G. diptychi* n. sp. was closely related to the members of *G. nemachili*-group, including *G. nemachili*, *G. pseudonemacheili*, *G. tayshirensis*, *G. papernai*, *G. mongolicus*, and *G. gymnodiptychi* (Lebedeva et al., 2023). In general, *G. nemachili*-group shows a common characteristic of the anchor with folded inner root (Lebedeva et al., 2023); in contrast, *G. diptychi* n. sp. showed a straight inner root of the anchor. In addition, *G. nemachili*-group members are reportedly isolated from the host families of Cyprinidae, Cobitidae, and Bagridae (Arken et al., 2022; Lebedeva et al., 2023); however, *G. diptychi* n. sp. was isolated only from the host subfamily Schizothoracinae belonged to the family Cyprinidae.

In Yarkand River, six native members of the fish subfamily Schizothoracinae are regarded as endangered species and Class I key-protected aquatic wild animals (Guo, 2012). Only three species, two species of the genus *Dactylogyrus* and one species of the genus *Paradiplozoon*, of Monogenea have been recently reported in the Yarkand River (Arken et al., 2020; Arken et al., 2022). Accordingly, Schizothoracinae-associated monogeneans are still largely unclear. Thus, it is important to advance our knowledge of monogeneans, which parasitize Schizothoracinae, in order to develop effective methods for protecting Schizothoracinae members from monogenean infection.

5. Conclusion

Our combined morphological, molecular, and genetic approaches identified a new monogenean species, *G. diptychi* n. sp., to the fish host *D. maculatus* of the subfamily Schizothoracinae.

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Availability of data and material

All data produced for this study are provided in the manuscript.

Data availability statement

The sequence data is uploaded to the NCBI GenBank and the raw sequences are available under the accession of OR762714 and OR762715.

CRediT authorship contribution statement

Cui-lan Hao: Writing – review & editing, Writing – original draft, Visualization, Software, Funding acquisition, Data curation, Conceptualization. **Wen-run Zhang:** Resources, Data curation. **Kadirden Arken:** Data curation. **Jin-pu Wang:** Data curation. **Cai-xia Shi:** Data curation. **Li Zhang:** Data curation. **Cheng Yue:** Supervision.

Declaration of competing interest

The authors declare no conflict of interest.

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References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Arken, K., Guo, A.M., Hao, C.L., Zhang, W.R., Rong, M.J., Song, P.H., Yue, C., 2020. A new species of *Dactylogyrus, D. diptychus* n. sp. (Monogenea: Dactylogyridae), from Schizothoracine fishes (Cyprinidae: Schizothoracinae) in the Xinjiang Uygur autonomous region, China. Acta Hydrobiol. Sin. 44, 663–669.

Arken, K., Hao, C.L., Guo, A.M., Zhang, W.R., Rong, M.J., Kamal, W., Tian, S.L., Kadir, M., Yue, C., 2022. A new species of *Paradiplozoon* (Monogenea: Diplozoidae), A gill parasite of the Schizothorax fish (Cyprinidae: Schizothoracinae) from the Yarkand River, Xinjiang, China. Acta Parasitol. 67, 330–339.

Bakke, T.A., Cable, J., Harris, P.D., 2007. The biology of gyrodactylid monogeneans: the "Russian-doll killers". Adv. Parasitol. 64, 161–376.

Bakke, T.A., Harris, P.D., Cable, J., 2002. Host specificity dynamics: observations on gyrodactylid monogeneans. Int. J. Parasitol. 32, 281–308.

Benson, D.A., Mark, B., Lipman, D.J., James, O., Wheeler, D.L., 2007. GenBank. Nucleic Acids Res. 35 (Database issue), D21–D25.

Boeger, W.A., Kritsky, D.C., Patella, L., Bueno-Silva, M., 2020. Phylogenetic status and historical origins of the oviparous and viviparous gyrodactylids (Monogenoidea, Gyrodactylidea). Zool. Scripta 00, 1–13.

Bruno, D.W., Collins, C.M., Cunningham, C.O., Mackenzie, K., 2001. *Gyrodactyloides bychowskii* (Monogenea: Gyrodactylidae) from sea-caged Atlantic salmon *Salmo salar* in Scotland: occurrence and ribosomal RNA sequence analysis. Dis. Aquat. Org. 45, 191–196.

Buenosilva, M., Boeger, W.A., 2014. Neotropical Monogenoidea. 58. Three new species of *Gyrodactylus* (Gyrodactylidae) from Scleromystax spp. (Callichthyidae) and the proposal of COII gene as an additional fragment for barcoding gyrodactylids. Folia Parasitol. 61, 213–222.

Bush, A.O., Lafferty, K.D., Lotz, J.M., Shostak, A.W., 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J. Parasitol. 83, 575–583.

Cable, J., Harris, P.D., Tinsley, R.C., Lazarus, C.M., 1999. Phylogenetic analysis of *Gyrodactylus* spp. (Platyhelminthes: Monogenea) using ribosomal DNA sequences. Can. J. Zool. 77, 1439–1449.

Cone, D.K., Appy, R., Baggett, L., King, S., Gilmore, S., Abbott, C., 2013. A new gyrodactylid (Monogenea) parasitizing bay pipefish (*Syngnathus leptorhynchus*) from the Pacific Coast of North America. J. Parasitol. 99, 183–188.

Cunningham, C.O., 1997. Species variation within the internal transcribed spacer (ITS) region of *Gyrodactylus* (Monogenea:Gyrodactylidae) ribosomal RNA genes. J. Parasitol. 83, 215–219.

Ergens, R., Karabekova, D.U., 1980. Two new species of *gyrodactylus* (Monogenea) from Kirghizian *Diptychus dybowskii* (Cypriniformes). Folia Parasitol. 27, 89–91.

Gerard, T., Jose, C., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. Syst. Biol. 56, 564–577.

Gilmore, S.R., Abbott, C.L., Cone, D.K., 2010. The placement of *Gyrodactylus salmonis* (Yin & Sproston) in the molecular phylogeny of studied members of the *Gyrodactylus wageneri*-group parasitizing salmonids. J. Fish. Dis. 33, 461–467.

Guo, Y., 2012. The Fishes of Xinjiang. Xinjiang Science and Technology Press, Urumqi.

Guo, Y., Meng, W., Liu, J., Zhang, R.M., Cai, L.G., 2009. Comparison of morphological characters of *Diptychus maculates* in different rivers of Xinjiang. Chin. J. Fish. 22, 1–5.

Gusev, A.V., 1985. Key to the Parasites of Freshwater Fish Fauna of the USSR, vol. 2 (in Russian), Leningrad Science Publishing Company.

Harris, P.D., Shinn, A.P., Cable, J., Bakke, T.A., 2004. Nominal species of the genus *Gyrodactylus* von Nordmann 1832 (Monogenea: Gyrodactylidae), with a list of principal host species. Syst. Parasitol. 59, 1–27.

Hayward, C.J., Iwashita, M., Ogawa, K., Ernst, I., 2001. Global spread of the eel parasite *Gyrodactylus anguillae* (Monogenea). Biol. Invasions 3, 417–424.

Huyse, T., Pampoulie, C., Audenaert, V., Volckaert, F.A.M., 2006. First report of *Gyrodactylus* spp. (Platyhelminthes: Monogenea) in the western Mediterranean Sea: molecular and morphological descriptions. J. Parasitol. 92, 682–690.

Jin, X., Li, W., Cheng, Y., Li, M., Wu, S., Zou, H., Wang, G., 2022. Description of *Gyrodactylus banmae* n. sp. (Monogenea, Gyrodactylidae) parasitic on zebrafish, Danio rerio. Parasitol. Int. 87, 102531.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Haeseler, A.v., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods 14, 587–589.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780.

Kvach, Y., Ondračková, M., Seifertová, M., Hulak, B., 2019. *Gyrodactylus ginestrae* n. sp. (Monogenea: Gyrodactylidae), a parasite of the big-scale sand smelt, *Atherina boyeri* risso, 1810 (Actinopterygii: Atherinidae) from the Black sea. Parasitol. Res. 118, 3315–3325.

Lebedeva, D., Ziętara, M., Mendsaikhan, B., Ermolenko, A., Lumme, J., 2023. Survivors from a pliocene climatic catastrophe: *gyrodactylus* (Platyhelminthes, Monogenea) parasites of the relict fishes in the central asian internal drainage basin of Mongolia. Diversity 15, 860.

Letunic, I., Bork, P., 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23, 127–128.

Li, R.R., Li, W.X., Wu, X.D., Wang, G.T., 2014. Identification of *gyrodactylus* species in goldfish (carassius auratus) through morphological study and the analysis of the rdna its sequence. Acta Hydrobiol. Sin. 38, 903–909.

Lumme, J., Zitara, M.S., Lebedeva, D.y., 2017. Ancient and modern genome shuffling: Reticulate mito-nuclear phylogeny of four related allopatric species of *Gyrodactylus* von Nordmann, 1832 (Monogenea: Gyrodactylidae), ectoparasites on the Eurasian minnow Phoxinus phoxinus (L.) (Cyprinidae). Syst. Parasitol. 94, 183–200.

Matejusová, I., Gelnar, M., Verneau, O., Cunningham, C.O., Littlewood, D., 2003. Molecular phylogenetic analysis of the genus *Gyrodactylus* (Platyhelminthes: Monogenea) inferred from rDNA ITS region: subgenera versus species groups. Parasitology 127, 603–611.

Matejusová, I., Gelnar, M., McBeath, A.J.A., Collins, C.M., Cunningham, C.O., 2001. Molecular markers for gyrodactylids (Gyrodactylidae: Monogenea) from five fish families (Teleostei). Int. J. Parasitol. 31, 738–745.

Minh, B.Q., Nguyen, M.A.T., Haeseler, A.v., 2013. Ultrafast approximation for phylogenetic bootstrap. Mol. Biol. Evol. 30, 1188–1195.

Nitta, M., 2021. A new monogenean species, *Gyrodactylus ajime* n. sp. (Gyrodactylidae), parasitic on *Niwaella delicata* (Niwa), an endemic loach of Japan. Syst. Parasitol. 98, 307–319.

Nitta, M., Nagasawa, K., 2018. *Gyrodactylus medaka* n. sp. (Monogenea: Gyrodactylidae) parasitic on wild and laboratory-reared medaka Oryzias latipes (Beloniformes: Adrianichthyidae) in Japan. Parasitol. Int. 67, 651–658.

Niu, J.G., Zhang, T., Liu, H., Zhang, F., Cai, L.G., Yu, Y., 2016. Study on Artificial Domestication and Breeding Techniques of *Diptychus maculates* Scientific Fish Culture, vol. 12, pp. 10–12.

Paladini, G., Huyse, T., Shinn, A.P., 2011. *Gyrodactylus salinae* n. sp. (Platyhelminthes: Monogenea) infecting the south European toothcarp *Aphanius fasciatus* (Valenciennes) (Teleostei, Cyprinodontidae) from a hypersaline environment in Italy. Parasites Vectors 4, 100.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Přikrylová, I., Matějusová, I., Musilová, N., Gelnar, M., Harris, P.D., 2009. A new gyrodactylid (Monogenea) genus on gray bichir, *Polypterus senegalus* (Polypteridae) from Senegal (West Africa). J. Parasitol. 95, 555–560.

Rokicka, M., Lumme, J., Ziętara, M.S., 2007. Identification of *Gyrodactylus* ectoparasites in Polish salmonid farms by PCR-RFLP of the nuclear ITS segment of ribosomal DNA (Monogenea, Gyrodactylidae). Acta Parasitol. 52, 185–195.

Rokicka, M., Lumme, J., Ziętara, M.S., 2009. Two new Antarctic *Gyrodactylus* species (Monogenoidea): description and phylogenetic characterization. J. Parasitol. 95, 1112–1119.

Rong, M.J., 2021. Studies on the Taxonomy, Phylogeny and Correlation between Schizothorax and *Dactylogyrus* in Yarkand River Basin. Xinjiang Agricultural University.

Rong, M.J., Arken, K., Zhang, W.R., Hao, C.L., Guo, A.M., Kadir, M., Tian, S.L., Yue, C., 2020. A new record of *Dactylogyrus simplex* in gills of *Diptychus maculates* from Yarkand River basin, China. Freshw. Fish. 50, 46–51.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Shinn, A.P., Hansen, H., Olstad, K., Bachmann, L., Bakke, T.A., 2004. The use of morphometric characters to discriminate specimens of laboratory-reared and wild populations of *Gyrodactylus salaris* and *G. thymalli* (Monogenea). Folia Parasitol. 51, 239–252.

Trifinopoulos, J., Nguyen, L.T., Haeseler, A.V., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44, W232–W235.

Wu, B.H., Lang, S., Wang, W.J., 2000. Fauna in China:Platyhelminthes: Momogenea. Science Press, Beijing.

Yang, T.Y., Meng, W., Ma, Y.W., Cai, L.G., Guo, Y., 2014. Genetic structure analysis of Diptychus maculatus between two water systems in Xinjiang based on mitochondrial COI and Cytb gene sequences. Freshw. Fish. 44, 41–47.

You, P., Easy, R.H., Cone, D.K., 2008. *Gyrodactylus parvae* n. sp. (Monogenea) from the fins and body surface of *Pseudorasbora parva* (Cyprinidae) in Central China. Comp. Parasitol. 75, 28–32.

Zahradníčková, P., Barson, M., Luus-Powell, W.J., Přikrylova, I., 2016. Species of *Gyrodactylus* von Nordmann, 1832 (Platyhelminthes: Monogenea) from cichlids from Zambezi and Limpopo river basins in Zimbabwe and South Africa: evidence for unexplored species richness. Syst. Parasitol. 93, 679–700.

Zhang, D., Gao, F., Jakovli, I., Zou, H., Wang, G.T., 2020. PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies. Mol. Ecol. Resour. 20, 348–355.

Zhang, W.R., Hao, C.L., Arken, K., Rong, M.J., Tian, S.L., Kadir, M., Yue, C., 2023. New species of *Gyrodactylus* von Nordmann, 1832 (Monogenoidea: Gyrodactylidae) from *Gymnodiptychus dybowskii* (Kessler, 1874) (Schizothoracinae) in the Kunes River (Yili River basin), China. Int. J. Parasitol.-Par. 22, 136–145.

Zhao, X.F., Mi, B.H., Liang, L.Q., Ma, Y.Q., Sun, B., Chang, Y.M., 2019. Genetic diversity analysis of *Gymnodiptychus dybowskii* and diptychus maculates. Chin. J. Fish. 32, 10–24.

Ziętara, M.S., Huyse, T., Lumme, J., Volckaert, F.A., 2002. Deep divergence among subgenera of *Gyrodactylus* inferred from rDNA ITS region. Parasitology 124, 39–52.

Ziętara, M.S., Lumme, J., 2003. The crossroads of molecular, typological and biological species concepts: two new species of *Gyrodactylus* Nordmann, 1832 (Monogenea: Gyrodactylidae). Syst. Parasitol. 55, 39–52.

Ziętara, M.S., Rokicka, M., Stojanovski, S., Lumme, J., 2010. Introgression of distant mitochondria into the genome of *Gyrodactylus salaris*: nuclear and mitochondrial markers are necessary to identify parasite strains. Acta Parasitol. 55, 20–28.