

New species of *Gyrodactylus* von Nordmann, 1832 (Monogeneoidea: Gyrodactylidae) from *Gymnodiptychus dybowskii* (Kessler, 1874) (Schizothoracinae) in the Kunes River (Yili River basin), China

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ABSTRACT

Yili River system hosts a diverse fauna of fishes and parasites. *Gymnodiptychus dybowskii* is a rare and endangered aboriginal cold-water fish inhabit in the Yili river system. Our research identified a new species *Gyrodactylus gymnodiptychi* n. sp. isolated from *G. dybowskii* in the Kunes River (Yili River, China). Morphological comparison revealed identifiable differences between the new species and other parasites, including *Gyrodactylus aksuensis*, and *Gyrodactylus tokobaevi*, which are two known parasites living in *G. dybowskii* inhabit in the Aksu River west of Frunze (Kyrgyzstan), as well as *Gyrodactylus montanus* living in *Shizothorax intermedius* inhabited in the Tadjikistan or Uzbekistan. Especially, the dorsal bar of *G. gymnodiptychi* n. sp. was raised at both ends with a hollow, and its hamulus roots were curved inward. The BLASTN search of GenBank did not detect any other *ITS1-5.8S-ITS2* rDNA sequences same as *G. gymnodiptychi*'s. Using the Bayesian Information and Maximum Likelihood methods to analyze the *ITS1-5.8S-ITS2* rDNA gene sequences, we constructed phylogenetic trees for *G. gymnodiptychi* n. sp. Accordingly, our morphological and molecular research indicated that *G. gymnodiptychi* n. sp. was not only a new species of parasites but also the first *Gyrodactylus* member identified in the Yili River in China.

1. Introduction

Yili River system is an international river flowing from the northeast Borohoro Mountains and southeast Halik Mountains to westward through the Yili Basin basin into Kazakhstan. The Borohoro and Halik Mountains are two branches of the Kazakhstan Tian Shan Mountains. Yili River flows 430 km long in China, extending to 3 major tributaries Tekes River, Kunes River, and Kashi River (Ren et al., 1998). Due to the growing freshwater fish farming industry, the cultivation and development of high-quality indigenous fish in the Yili River have become increasingly important for the economic development of the Yili River basin. As the industry shifts from natural ecological environments to intensive and large-scale aquaculture, the living conditions for fish have undergone significant changes. Yili River system hosts a diverse fauna with ten endemic species, including four Schizothoracinae fishes *Gymnodiptychus dybowskii* Kessler, 1987; *Diptychus maculatus* Steindachner, 1866; *Schizothorax argentatus pseudaksaiensis* Herzenstein, 1889; and

Schizothorax argentatus Kessler, 1874; as well as six other fishes (Ren et al., 1998; Guo, 2012; Meng et al., 2018).

Monogeneans of the genus *Gyrodactylus* have been known for almost 190 years for their retention of fully grown daughters in utero until they themselves contain developing embryos (Bakke et al., 2007). *Gyrodactylus* was first described from bream (*Abramis brama*) by von Nordmann (1832). The growing invasion of *Gyrodactylus* into fish farms and wild fields has become a serious endemic disease leading to economic losses (Atkins, 1901; Embury, 1924; Guberlet et al., 1927; Williams, 1964; Johnsen and Jensen, 1991). It has the conservative morphology of particular structures and huge species diversity (Bakke et al., 2007). Soviet scholars have conducted extensive research on this subject including studies on fish parasites in Central Asian countries (Yamaguti, 1965; Gusev, 1985). Previous studies of parasites and their hosts inhabited in the upstream Yili River revealed that two fish subfamilies Schizothoracinae and Botiinae host nine species (eight genera) of parasites, including *Dactylogyrus drjagini* Bychowsky, 1936; *Paradiplozoon*

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schizothorazi Iksanov, 1965; *Rhabdochona opienensis* Hsü, 1933; *Schyzocotyle acheilognathi* Yamaguti, 1934; *Allocreadium schizothoracis* Pande, 1938; *Acanthocephala* sp.; *Chilodonella cyprinid* Moroff, 1902; *Trichodina nobillis* Chen, 1963; and *Trichodina orientalis* Chen et Hsieh in Anon., 1973. (Yao et al., 2013; Niu et al., 2017). Numerous parasites have been observed in fishes inhabited in the upper Yili River system (in China); however, they have not been scientifically studied.

Gymnodiptychus dybowskii belongs to the subfamily Schizothoracinae (Cyprinidae) and is a cold-water fish dwelling in alpine waters. *Gymnodiptychus dybowskii* inhabit in the Syrdarya River, the Balkhash Lake, and the Issyk-Kul Lake in Central Asia. In China, *G. dybowskii* inhabit in the Yili River system, the Junggar basin water system, and the Kaidu River of Xinjiang. *Gymnodiptychus dybowskii* is a rare and endangered aboriginal fish, and it represents an indigenous and ecologically essential species in Xinjiang aquatic ecosystems (Meng et al., 2018). In 2022, *G. dybowskii* was listed as a Class I key-protected aquatic wild animal by the People's Government of Xinjiang Uygur Autonomous Region. Up to now, studies have focused on the physiology and genetics of *G. dybowskii* inhabit in the Yili River system (Niu et al., 2015; Guo et al., 2016); however, its monogenean parasites still remain to be studied. Only *Gyrodactylus aksuensis* and *Gyrodactylus tokobaevi*, the two known parasites infecting *G. dybowskii*, inhabit in the Aksu River west of Frunze (Kirghiz. S.S.R.) (Ergens and Karabekova, 1980).

Currently, identification and nomenclature of *Gyrodactylus* species are still based on morphological characteristics and host specificity. The morphological characterization is primarily based on the measurement of attachment structures (Bakke et al., 2002). Changes of morphological evolution of *Gyrodactylus* have been postulated to be associated with host shifting (Lindenstrøm et al., 2003; Shinn et al., 2004; Bakke et al., 2007). Changes of hamulus and marginal hook size may be associated with environmental changes, such as temperature (Kulemina and Skarlato, 1987; Dávidová et al., 2005). However, these environmental and host factors in association with changes of morphological characteristics still need to be clarified. Recent advancement of molecular technologies and combined uses of molecular and morphological data provide us a platform to further study *Gyrodactylus* taxa (Bueno-Silva and Boeger, 2014; Zahradníčková et al., 2016; García Vázquez et al., 2018).

In this study, we performed morphological characteristics of a novel *Gyrodactylus* organism isolated from the gill and fin of *G. dybowskii* inhabit in the Kunes River, upstream of Yili River system. We used molecular methods and GenBank to study the *ITS1-5.8S-ITS2* rDNA gene sequences and construct phylogenetic trees for the organism to determine the new species *G. gymnodiptychi* n. sp.

2. Materials and methods

2.1. Fish and parasite sampling

Samples of *G. dybowskii* were captured using fyke nets from the Kunes River (82°34'49.61" N; 84°44'30.41" E) in the months of July 2018 and December 2019. After sampling, living fish were euthanized by severing the spinal cord posterior to the skull with a single cut. Gills and fins were then surgically isolated for gross examination and isolation of parasites within 24 h. Isolated parasites were preserved in 70% and 95% ethanol for morphometric and molecular analyses, respectively. The definitions of prevalence and mean intensity of infection were used following Bush et al. (1997). All animal procedures were approved by the Xinjiang Agricultural University Animal Care and Use Committee (No. 2019021).

2.2. Morphometric analysis

Twenty-eight intact individuals of *G. gymnodiptychi* n. sp. were isolated from fish bodies, and selected for morphological characterization. Nine individuals were fixed and stained in GAP to reveal features of hamulus, marginal hook, dorsal bar, and ventral bar. Nineteen

individuals were fixed and stained with 4% PL to reveal features of hamulus, marginal hook, and male copulatory organ. Morphological features were microscopically analyzed using a Nikon ECLIPSE E200 imaging optical microscope. The length and width of the body, hamulus, ventral bar, dorsal bar and marginal hook were measured using an EZ-MET software (x86, 6.0.7543) (Shinn et al., 2004; Christison et al., 2005; García-Vázquez et al., 2007). Drawings of parasite morphological features were performed using a camera lucida.

2.3. Molecular analysis

Genomic DNA was extracted from parasites using the EasyPure® Genomix DNA kit (TransGen Biotech, Beijing, China). Fragments of the *ITS1-5.8S-ITS2* ribosomal DNA (rDNA) amplified region were isolated by the PCR technique using the forward primer 5'-TTTCCGTAGGT-GAACCT-3' and the reverse primer 5'-TCCTCCGCTTAGTGATA-3' (Cunningham, 1997). The amplification reaction was performed in a final volume of 50 µL, containing of 1 µL forward primer/reverse primer, 2 µL of DNA, and 25 µL of 2 × Super Master mix (Bio-Rad, CA, USA) and 21 µL of double distilled water. The samples were incubated in the following cycles: 1 cycle at 94 °C for 5 min, 30 cycles at 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 75 s, and the final extension of 1 cycle at 72 °C for 10 min. PCR products were electrophoresed on agarose gels (1%), and DNA fragments were visualized by staining with GelStain (TransGen Biotech). DNA fragments were isolated and purified from agarose slices using an EasyPure® PCR purification kit (TransGen Biotech). Purified DNA fragments were constructed into a plasmid vector, using the pEASY®-T1 Cloning Kit (TransGen Biotech). Then, plasmid DNA was purified with a HiPure Plasmid MiniPrep Kit (TransGen Biotech), followed by sequencing the isolated *ITS1-5.8S-ITS2* rDNA regions using the ABI Cycle Sequencer 3700 (Foster City, CA, USA).

2.4. Phylogenetic analysis

Phylogenetic status of *Gyrodactylus gymnodiptychi* n. sp. collected from the Kunes River was determined by comparing the *ITS1-5.8S-ITS2* rDNA sequences of 46 *Gyrodactylus* species in the GenBank (Table 1). The sequences of *Diplopyrodactylus martini* Prikrylova, Matejusova, Musilova, Gelnar & Harris, 2009 and *Gyrodactylus bychowskii* Albova, 1948 (family Gyrodactylidae) 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 imported into Gblocks 0.91b (Gerard and Jose, 2007) then the conserved sites were extracted using Gblocks 0.91b with the following parameter settings: minimum number of sequences for a conserved/flank position (25/25), maximum number of contiguous non-conserved positions (8), minimum length of a block (10), allowed gap positions (all). The model of molecular evolution were determined according to the corrected Bayesian Information (BI) and Maximum Likelihood (ML) using ModelFinder v1.6.8 (Kalyaanamoorthy et al., 2017). On the basis of the selected model, phylogenetic analyses were performed using two different algorithms: Bayesian Inference (BI) and Maximum Likelihood (ML). Both BI and ML analytical methods were used to determine phylogenetic relationship between parasites, followed by using the Bayesian information criterion (BIC) in ModelFinder v1.6.8 to identify optimal evolutionary model (Posada and Crandall, 1998). The BI analysis was performed by using the MrBayes 3.2 software with the parameter settings nst = 6 and rates = Invgamma (GTR+F+I+G4 model) (Ronquist and Huelsenbeck, 2003). Posterior probability of model parameters was evaluated with the Markov Chain Monte Carlo method (MCMC) running four chains, sampling every 100 generations, for 5,000,000 generations. After checking for convergence, the Burnin sample trees were discarded with the parameter setting at 0.25%. The remaining trees were calculated with the MrBayes 3.2.1. program to determine a 50% majority-rule consensus tree. On the other hand, ML

Table 1Sequence 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
<i>G. ajime</i> Nitta, 2021	<i>Niwaella delicata</i>	Japan: Kyoto	LC545570	Nitta (2021)
<i>G. anguillae</i> Ergens, 1960	<i>Anguilla australis</i>	Australia: Victoria, Skipton	AB063294	Hayward et al., 2012, unpublished
<i>G. aphyae</i> Malmberg, 1957	<i>Phoxinus phoxinus</i>	Finland: River Merenoja, River Kovda system, White Sea basin	AF484528	Ziętara et al. (2002)
<i>G. arcuatus</i> Bychowsky, 1933	<i>Gasterosteus aculeatus</i>	Finland: Gulf of Bothnia, Baltic Sea	AF328865	Ziętara et al. (2002)
<i>G. brachymystacis</i> Ergens, 1978	<i>Brachymystax lenok</i>	China	GQ368237	Gilmore et al. (2010)
<i>G. branchialis</i> Huyse, Malmberg & Volckaert, 2004	<i>Pomatoschistus marmoratus</i>	France: Vaccares lagoon	DQ821770	Huyse et al. (2006)
<i>G. branchicus</i> Malmberg, 1964	<i>Gasterosteus aculeatus</i>	Russia: Kola Peninsula, White Sea	FJ435199	Rokicka et al. (2009)
<i>G. bubyri</i> Osmanov, 1965	<i>Knipowitschia caucasica</i>	Bulgaria: Atanasovsko Lake	KU355879	Stoyanov et al. (2016)
<i>G. bullatarudis</i> Turnbull, 1956	<i>Poecilia reticulata</i>	Trinidad and Tobago: Lopinot (Arouca) River	AY692024	Cable et al. (2005)
<i>G. cernuae</i> Malmberg, 1957	<i>Gymnocephalus cernuus</i>	Finland: River Oulujoki, Baltic Sea basin	AF484529	Ziętara et al. (2002)
<i>G. derjavini</i> Mikailov, 1975	<i>Oncorhynchus mykiss</i>	Iran: Caspian Sea basin	DQ323402	Rokicka et al. (2007)
<i>G. derjavinoides</i> Malmberg, Collins, Cunningham & Jalali, 2007	<i>Salmo letnica</i>	Macedonia: River Vardar system, Aegean Sea basin	EU304810	Ziętara et al. (2010)
<i>G. gymnodiptychi</i> n. sp.	<i>Gymnodiptychus dybowskii</i>	China: Yili River	MH445968	This study
<i>G. gymnodiptychi</i> n. sp.	<i>Gymnodiptychus dybowskii</i>	China: Yili River	MH445967	This study
<i>G. ginestvae</i> Kvach, Ondračková, Seifertová and Hulak, 2019	<i>Atherina boyeri</i>	Ukraine: Black Sea, Gulf of Odessa	MK550602.2	Kvach et al. (2019)
<i>G. gracilihamatus</i> Malmberg, 1964	<i>Gasterosteus aculeatus</i>	Finland: Gulf of Bothnia, Baltic Sea	AF484532	Ziętara et al. (2002)
<i>G. gurleyi</i> Price, 1937	<i>Goldfish Carassius auratus</i>	China	KC922453	Li et al. (2014)
<i>G. harengi</i> Malmberg, 1957	<i>Clupea harengus</i>	France: Ambleteuse	AJ309295	Matejusová et al. (2003)
<i>G. jiroveci</i> Ergens and Bychowsky, 1967	<i>Barbatula barbatula</i>	Czech Republic	AM502860	Přikrylová et al. (2008)
<i>G. jussii</i> Ziętara & Lumme, 2003	<i>Phoxinus phoxinus</i>	Finland: River Merenoja, River Kovda system, White Sea basin	AY061982	Ziętara and Lumme (2003)
<i>G. leptorhynchi</i> Cone et al., 2013	<i>Syngnathus leptorhynchus</i>	Pacific coast of North America	JX110633	Cone et al. (2013)
<i>G. leucisci</i> Zitnan, 1964	<i>Leuciscus leuciscus</i>	Finland: River Oulujoki, Baltic Sea basin	AF484537	Ziętara et al. (2002)
<i>G. luciopercae</i> Gussev, 1962	<i>Perca fluviatilis</i>	Finland: Gulf of Bothnia, Baltic Sea	AF484541	Ziętara et al. (2002)
<i>G. macronychus</i> Malmberg, 1957	<i>Phoxinus phoxinus</i>	Finland: River Merenoja, River Kovda system, White Sea basin	AY061981	Ziętara and Lumme (2003)
<i>G. medaka</i> Nitta and Nagasawa, 2018	<i>Oryzias latipes</i>	Japan: Tokushima	LC368477	Nitta and Nagasawa (2018)
<i>G. mongolicus</i> Ergens and Dulmaa, 1970	<i>Oreoleuciscus potanini</i>	Mongolia: Chono Kharai river	OQ913868	Lebedeva et al. (2023)
<i>G. nemachili</i> Bikhovski, 1936	<i>Oreoleuciscus potanini</i>	Mongolia: Chono Kharai river	OQ641772	Lebedeva et al. (2023)
<i>G. nipponensis</i> Ogawa and Egusa, 1978	<i>Anguilla japonica</i>	Japan: Shizuoka, Lake Hamana	AB063295	Hayward et al. (2001)
<i>G. notatae</i> King et al., 2009	<i>Menidia menidia</i>	Nova Scotia, Canada	FJ840489	King et al. (2009)
<i>G. orecchia</i> Paladini et al., 2009	<i>Sparus aurata</i>	Adriatic Sea	FJ013097	Paladini et al. (2009)
<i>G. ostendicus</i> Huyse and Malmberg, 2004	<i>Pomatoschistus marmoratus</i>	France: Vaccares lagoon	DQ821768	Huyse et al. (2006)
<i>G. papernai</i> Ergens and Bychowsky, 1967	<i>Salmo salar</i>	Russia: River Vidlitsa, Lake Ladoga system, Baltic Sea Basin	EF446729	Matejusová et al. (2001)
<i>G. poeciliae</i> Harris and Cable (2000)	<i>Poecilia caucana</i>	Venezuela	AJ001844.2	Harris and Cable (2000)
<i>G. proterorhini</i> Ergens, 1967	<i>Proterorhinus semilunaris</i>	Bulgaria: Vidin, Danube	MK584285.2	Kvach et al. (2019)
<i>G. pseudonemacheili</i> Ergens and Bychowsky, 1967	<i>Barbatula conilobus</i>	Mongolia: Zavkhan river	OQ641764	Lebedeva et al. (2023)
<i>G. pterygialis</i> Bychowsky and Polyansky, 1953	<i>Pollachius virens</i>	Norway: Fjord near Bergen	AJ581657	Matejusová et al. (2003)
<i>G. pungitii</i> Malmberg, 1964	<i>Pungitius pungitius</i>	Finland: Lake Ryttilampi, White Sea basin	AF484543	Ziętara et al. (2002)
<i>G. rarus</i> Wegener, 1910	<i>Gasterosteus aculeatus</i>	Finland: Gulf of Bothnia, Baltic Sea	FJ435196	Rokicka et al. (2009)
<i>G. roгатensis</i> Harris, 1985	<i>Cottus gobio</i>	Rogate (West Sussex, England)	AJ011411	Cable et al. (1999)
<i>G. rugiensis</i> Glaser, 1974	<i>Pomatoschistus minutus</i>	France: Vaccares lagoon	DQ821761	Huyse et al. (2006)
<i>G. salaris</i> Malmberg, 1957	<i>Thymallus thymallus</i>	Finland: River Oulankajoki, River Kovda system, White Sea basin	AF484544	Ziętara et al. (2002)
<i>G. salinae</i> Paladini et al., 2011	<i>Aphanius fasciatus</i>	hypersaline environment in Italy	JF950559	Paladini et al. (2011)
<i>G. tayshirensis</i> Lebedeva et al., 2023	<i>Barbatula conilobus</i>	Mongolia: Zavkhan river	OQ641774	Lebedeva et al. (2023)
<i>G. truttae</i> Gloser, 1974	<i>Salmo trutta</i>	Poland: River Wisla system, Baltic Sea basin	EF464681	Rokicka et al. (2007)
<i>G. turnbulli</i> Harris, 1986	<i>Poecilia reticulata</i>	Poland: Gdansk aquarium	EF445942	Lumme and Ziętara (2018)
<i>G. zavkhanensis</i> Lebedeva et al., 2023	<i>Thymallus brevirostris</i>	Mongolia: Zavkhan river	OQ641773	Lebedeva et al. (2023)
<i>Diplogyrodactylus martini</i> Přikrylová, Matejusová, Musilová, Gelnar & Harris, 2009	<i>Polypterus senegalus</i>	Senegal	AM943008	Přikrylová et al. (2009)
<i>Gyrodactylus bychowskii</i> Albova, 1948	salmon	United Kingdom: Scotland	AJ249348	Bruno et al. (2001)

analysis was reconstructed using IQ-TREE (Trifinopoulos et al., 2016) with 10,000 ultrafast bootstraps (Minh et al., 2013). The iTOL (<https://itol.embl.de/>) (Letunic and Bork, 2007) was used to visualise the phylogeny and architecture using files generated from PhyloSuite 1.2.3.

3. Results

3.1. Taxonomic summary

Gyrodactylus was collected from 69 *G. dybowskii* (fork length:

6.4–18.5 cm), with a prevalence of 91.3% (63 infected fish), and mean intensity was 7.29 (range, 1 to 22). All specimens represented a morphologically similar species but did not correspond to any other *Gyrodactylus* species already identified in the Kunes River.

Class Monogeneoidea Bychowsky, 1937

Subclass Polyonchoinea Bychowsky, 1937

Order Gyrodactylidea Bychowsky, 1937

Family Gyrodactylidae Van Beneden and Hesse, 1863

Gyrodactylus gymnodiptychi n. sp.

Type host: *Gymnodiptychus dybowskii* Kessler, 1874.

Type locality: Kunes River, Xinjiang Uygur Autonomous Region, China (82°34'49.61" N; 84°44'30.41" E).

Site of infection: gills and fins.

Types material: The Holotypes XJLCC20191101 and the Paratypes XJLCC20191102-05 are deposited in the museum of Parasitology at the Xinjiang Agricultural University.

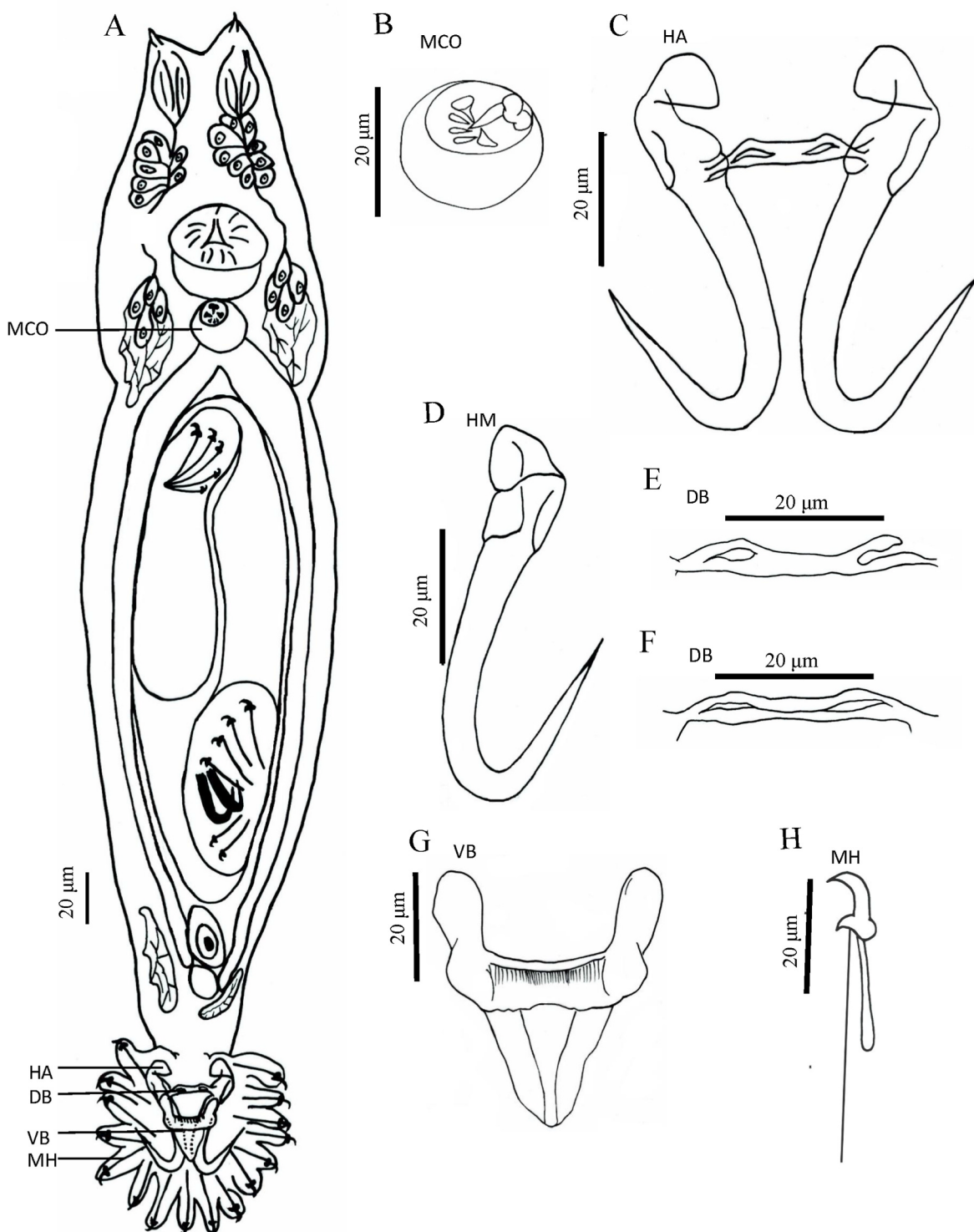


Fig. 1. Holotype of *Gyrodactylus gymnodiptychi* from the gills of *Gymnodiptychus dybowskii*. (A) Whole specimen (composite, ventral view), (B) Male copulatory organ (MCO), (C) Hamuli (HA), (D) Hamulus (HM), (E & F) Dorsal bar (DB), (G) Ventral bar (VB), and (H) Marginal hook (MH).

Genetic material: The *ITS1-5.8S-ITS2* rDNA sequence was deposited in the GenBank (Accession numbers MH445967 and MH445968).

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

Zoo bank: LSID urn:lsid:zoobank.org:pub:34E792F3-9ED8-45C3-A673-FC5C44577BAC.

3.2. Morphology

Based on 28 specimens. Body "gourd-like" shape, fusiform, a depression in the middle of body, total body length 368.0 (223.3–608.0) long, 80 (62.0–136.5) wide. Pharynx bulb 21.1 (13.8–29.7) long, 19.7 (13.6–28.2) wide (Fig. 1A and Table 2). The cecum was posterior to the anterior edge of the testes (Fig. 1A). MCO 12.3 (8.0–19.9) long, 8.5 (6.9–10.2) wide, armed with one central spine, two large spines and three small spines, posterior to pharyngeal bulb (Fig. 1B & 2A). Hamuli 61.6 (57.5–73.7) long, shafts 52.3 (44.9–55.9) long, points 28.2 (18.8–39.0) long, slim; proximal shaft 8.4 (7.9–11.2) wide, curved. Aperture distance 19.1 (18.8–23.8) long, hamulus aperture angle 31.7° (26.9°–33.5°), hamulus root 22.4 (18.8–25.8) long, inward and curved (Fig. 1C & 2B). Dorsal bar 29.2 (21.7–38.6) long, 2.2 (1.6–3.3) wide, the middle flat, straight, with a hollow at each end (Fig. 1E and F and 2C & D). Ventral bar 40.7 (35.8–53.1) long, 8.0 (6.5–10.9) wide, ventral bar processes 12.1 (7.8–14.3) long, ovoid; ventral bar membrane 19.9 (15.9–22.3) long (Fig. 1G & 2E). Marginal hook 38 (30.4–45.6) long, hook shaft 32.6 (24.1–37.9) long, rounded bottom; marginal hook sickle

8.7 (6.4–10.6) long, curved, tilted forward; sickle point 5.2 (3.6–5.7) wide, sickle distal 3.7 (2.7–5.0) wide. Marginal hook toe 2.23 (2.1–2.8) long, marginal hook aperture 7.1 (7.0–8.5) long, hook instep 1.0 (0.9–1.3) high, and filament loop 12.2 (12.0–16.1) long (Fig. 1H & 2F).

3.3. Remarks

To understand the association of *G. gymnodiptychi* n. sp. with known members of *Gyrodactylus*, we compared the morphological features of *G. gymnodiptychi* n. sp. with *Gyrodactylus aksuensis* Ergens and Karabekova (1980); *Gyrodactylus tokobaevi* Ergens and Karabekova (1980); and *Gyrodactylus montanus* Bychowsky, 1957; Ergens and Karabekova (1980); Gusev, 1985). As depicted in Fig. 3, compared with *G. aksuensis*, the dorsal bar of *G. gymnodiptychi* n. sp. was raised at both ends with a hollow, but *G. aksuensis* was lanker and narrower than *G. gymnodiptychi* n. sp. (Fig. 3A and B). *Gyrodactylus gymnodiptychi* n. sp. exhibited similar ventral bar morphology to the *G. tokobaevi* (Fig. 3A and C). In both species, their ventral bar processes were prominent, but hamulus roots of *G. gymnodiptychi* n. sp. were curved inward. In addition, the dorsal bar of *G. gymnodiptychi* n. sp. had a straight center and a projection with a hollow at both ends, but the *G. tokobaevi* only had prominent ends without hollow. Additionally, the MCO of *G. gymnodiptychi* n. sp. had three spines fewer than *G. tokobaevi* (Fig. 3A and C). *Gyrodactylus gymnodiptychi* n. sp. exhibits similar dorsal bar morphology to the *G. montanus*. In both species, their dorsal bars had a hollow at both ends of the projection, but the hamulus root of *G. gymnodiptychi* n. sp. was

Table 2

The comparison of *Gyrodactylus gymnodiptychi* with other morphologically similar species.

Measurement	N	<i>G. gymnodiptychi</i> (n = 28) Present study	<i>G. tokobaevi</i> (Ergens, 1980)	<i>G. montanus</i> (Gusev, 1985)	<i>G. aksuensis</i> (Ergens, 1980)
(length and width, µm)		Average (range)	Average (range)	Range	Average (range)
Total body , length	25	368.0 (223.3–608.0)			
Total body , width	28	80.0 (62.0–136.5)			
Pharynx , length × width	28	21.1 (13.8–29.7) × 19.7 (13.6–28.2)			
Opisthaptor , length × width	28	87.9 (62.0–110.7) × 87.3 (66.9–145.0)			
Male copulatory organ , length × width	13	12.3 (8.0–19.9) × 8.5 (6.9–10.2)			
MCO spines	6	1L, 5S	1L, 8S	1L, 6S	
Hamulus					
Total length	28	61.6 (57.5–73.7)	62–65 (65)		
Aperture distance	28	19.1 (18.8–23.8)			
Point shaft width	28	8.4 (7.9–11.2)			
Point length	28	28.2 (18.8–39.0)	28–29 (29)	21–33	13 (13–14)
Distal shaft width	28	5.4 (4.2–6.9)			
Shaft length HSL	28	52.3 (44.9–55.9)	42–44 (44)	63–78	22 (22–23)
Inner curve length	28	5.1 (3.63–6.61)			
Aperture angle	28	31.7° (26.9°–33.5°)			
Point curve angle	28	12.6° (8.2°–19.2°)			
Inner aperture angle	28	36.0° (33.0°–39.5°)			
Root length	28	22.4 (18.8–25.8)	19–21 (21)		
Ventral bar					
Length	18	40.7 (35.8–53.05)	29 (27–30)	33–45	15 (15–16)
Width	12	8.0 (6.5–10.9)	7 (6–7)	9–15	3 (3–4)
Process to mid-length	1	4.6 (n = 1)			
Mid-length	1	8.5 (n = 1)			
Process length	9	12.1 (7.8–14.3)			
Membrane length	9	19.9 (15.9–22.3)	17–20 (18)		
Dorsal bar					
Length	13	29.2 (21.7–38.6)	22 (20–22)	22–45	15 (15–16)
Width	13	2.2 (1.6–3.3)	3 (3–4)	3–6	1
Marginal hook					
Total length	28	38 (30.4–45.6)	29–31	40–53	21–22
Shaft length	28	32.6 (24.1–37.9)			
Sickle length	28	8.7 (6.5–10.6)	6–7	7–8	5–5.5
Sickle point width	21	5.2 (3.6–5.7)			
Toe length	8	2.2 (2.1–2.8)			
Sickle distal width	24	3.7 (2.7–5.0)			
Aperture	7	7.1 (7.0–8.5)			
Instep/arch height	7	1.0 (0.9–1.3)			
Filament loop	20	12.2 (12.0–16.1)			

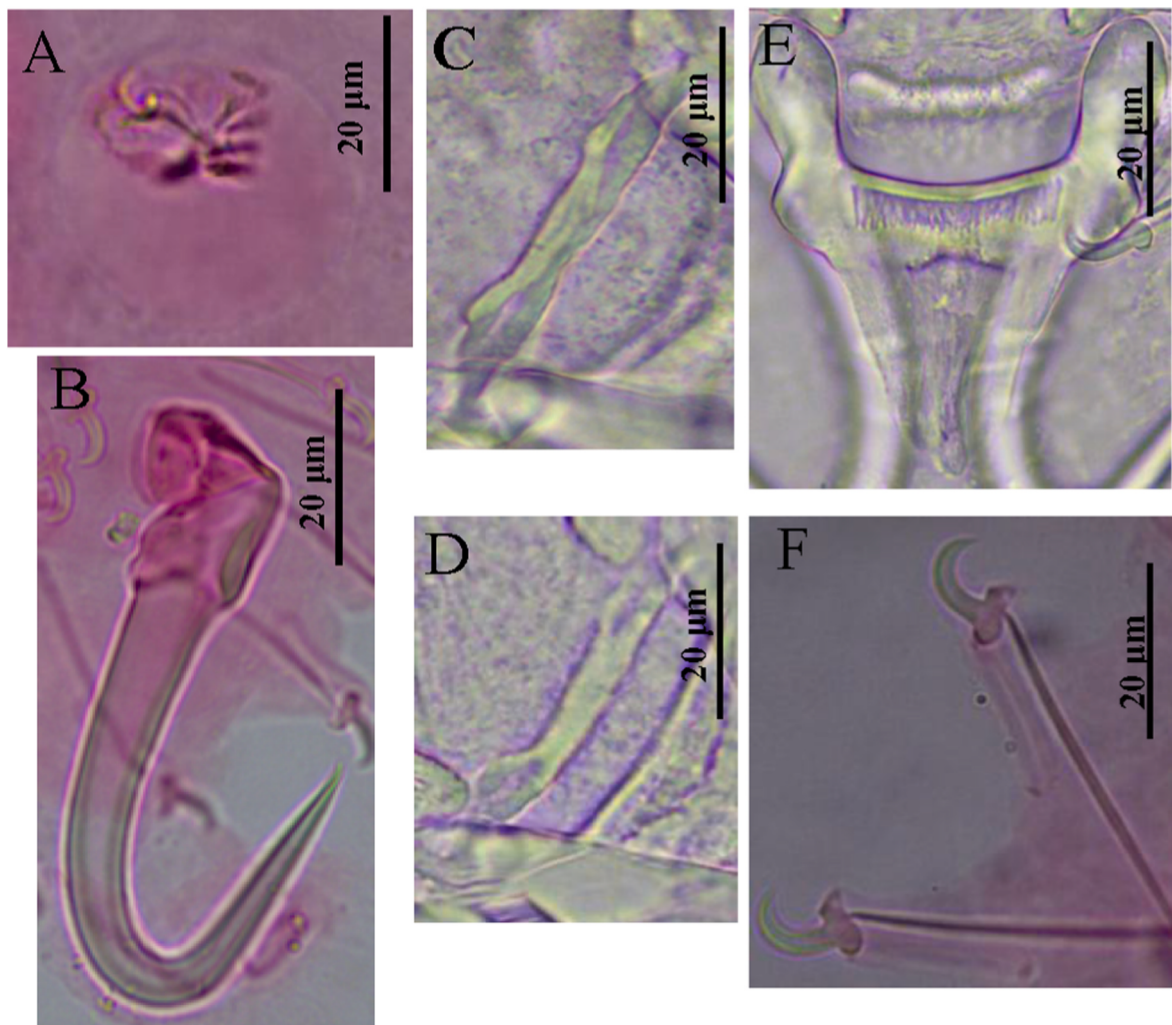


Fig. 2. Light micrographs of the haptoral structures of *Gyrodactylus gymnodiptychi* from the gills of *Gymnodiptychus dybowskii*. (A) Male copulatory organ (MCO), (B) Hamulus (HA), (C & D) Dorsal bar (DB), (E) Ventral bar (VB), and (F) Marginal hook (MH).

curved inward and stouter than *G. montanus*. In addition, the ventral bar processes of *G. gymnodiptychi* n. sp. were more prominent than *G. montanus* (Fig. 3A and D). The results clearly revealed identifiable morphological differences between *G. gymnodiptychi* n. sp. and other *Gyrodactylus* members. In addition, *G. gymnodiptychi* n. sp. was the only one showing a hollow dorsal bar and curved hamulus root that were distinct from the other eleven species, carrying non-hollow dorsal bars and straight hamuli roots, of gyrodactylid isolated from the fish sub-family Schizothoracinae.

3.4. Molecular identification

The results determined that the two DNA sequences of 1202 bp (GenBank: MH445967) and 1199 bp (GenBank: MH445968) shared 99.46% identity, indicating that they were the same species. The results of a BLASTn search (Altschul et al., 1997) of the ITS1-5.8S-ITS2 fragment revealed no identical hits with entries in GenBank (Benson et al., 2007). *Gyrodactylus gymnodiptychi* n. sp. (GenBank: MH445967) appeared most closely related to *Gyrodactylus tayshirensis* (862/935, 92.19%, OQ641774) obtained from the *Barbatula conilobus* (Cypriniformes, Nemacheilidae, *Barbatula*) in Zavkhan river (Mongolia), *Gyrodactylus jiroveci* (834/906, 92.05%, AM502860) (Příkrylová et al., 2008) from *Barbatula barbatula* (Cypriniformes, Nemacheilidae, *Barbatula*) collected in Czech Republic, *Gyrodactylus papernai* (833/905, 92.04%, EF446729) (Ziętara et al., 2008) from *Salmo salar* (Salmoniformes, Salmonidae,

Salmo) collected in Vidlitsa River, Lake Ladoga system (Russia), *Gyrodactylus mongolicus* (942/1045, 90.14%, GenBank: OQ913868) and *Gyrodactylus nemachili* (939/1044, 89.94%, OQ641772) obtained from the *Oreoleuciscus potanini* (Cypriniformes, Leuciscidae, *Oreoleuciscus*) in Chono Kharai river (Mongolia), and *Gyrodactylus zavkhanensis* (933/1038, 89.88%, OQ641773) obtained from the *Thymallus brevirostris* (Salmoniformes, Salmonidae, *Thymallus*) in Zavkhan river (Mongolia). A BLASTn query of the 5.8S rDNA fragment detected 19 identical matches of species including *Gyrodactylus mongolicus* Ergens and Dulmaa, 1970 (OQ913866, OQ913868, OQ641769, OQ641768), *Gyrodactylus* cf. *lagowskii* (OQ672253), *Gyrodactylus* cf. *kononovi* (OQ672250), *Gyrodactylus* cf. *mantshuricus* (OQ672249, OQ672248), *G. tayshirensis* (OQ641774), *Gyrodactylus zavkhanensis* (OQ641773), *Gyrodactylus nemachili* Bikhovski, 1936 (OQ641772, OQ641771, OQ641770), *Gyrodactylus pseudonemacheili* Ergens and Bychowsky, 1967 (OQ641767, OQ641764, OQ641758, OQ641756), *G. papernai* Ergens and Bychowsky, 1967 (EF446729, AF484533).

3.5. Phylogenetic analysis

We used BI and ML methods to topologically construct phylogenetic trees. As shown in Fig. 4, these two methods yielded two similar phylogenetic trees and only minor differences in statistical support values for some nodes. Both trees showed that all *Gyrodactylus* taxa were split into two major evolutionary lineages, and further divided into four

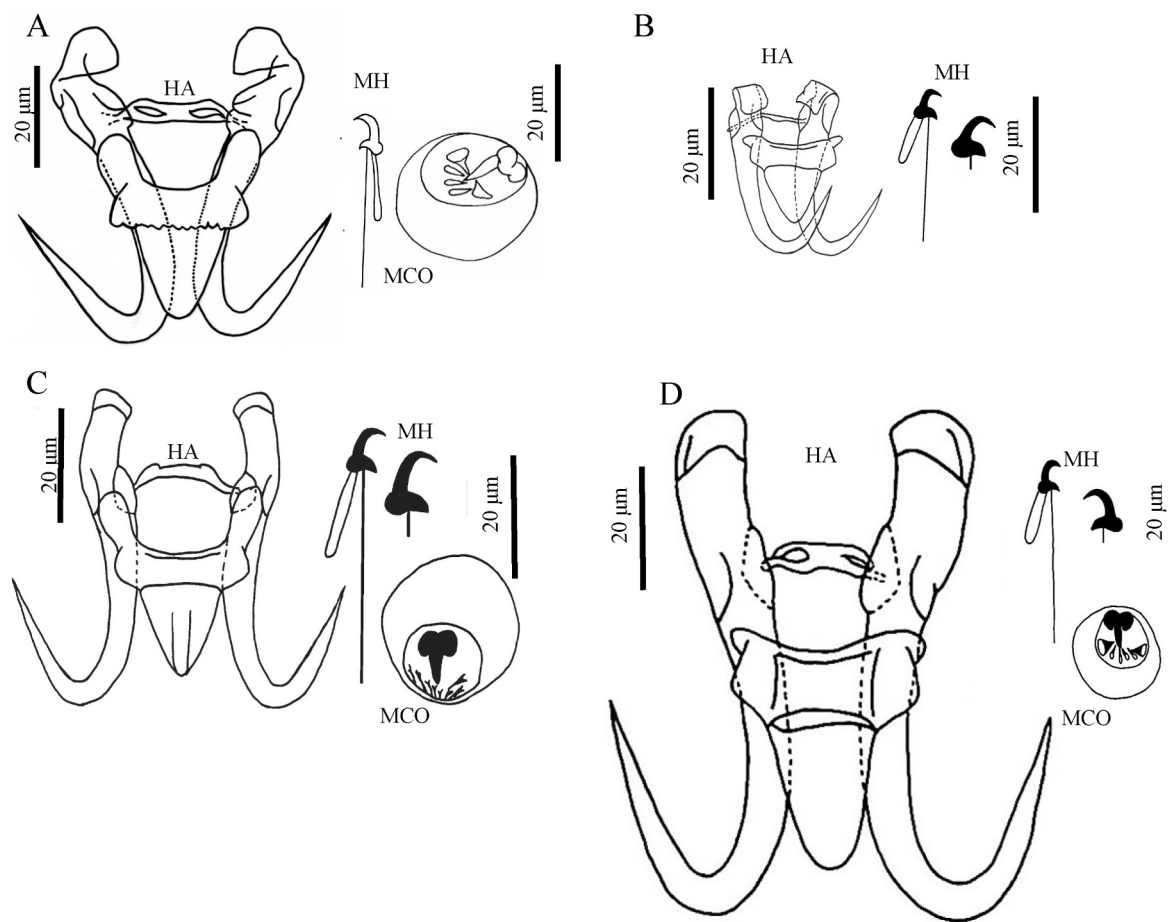


Fig. 3. Morphological comparison of (A) *Gyrodactylus gymnodiptychi*, (B) *Gyrodactylus aksuensis*, (C) *Gyrodactylus tokobaevi*, and (D) *Gyrodactylus montanus*. A - original drawing; B and C - from Ergens and Karabekova (1980); D - from Gusev (1985).

clades. The first lineage consisted of two subgenera: clade 1 and clade 2. Clade 1 was composed of the subgenus *Metanephrotus*, which included six host families (Atherinidae, Adrianichthyidae, Clupeidae, Gasterosteidae, Sparidae, and Gadidae). Clade 2 consisted of the subgenus *Mesonephrotus*, which included four host families (Gadidae, Gobiidae, Sparidae, Gasterosteidae, and Poeciliidae). The second lineage consisted of two subgenera: clade 3 and clade 4. Clade 3 consisted of one species of *Gyrodactylus* in the subgenus *Neonephrotus* and five species of *Gyrodactylus* in the subgenus *Paranephrotus*. These species were found on hosts belonging to the families Anguillidae, Aphaniidae, Atherinidae, Gobiidae, Gadidae, and Syngnathidae. Additionally, *G. gymnodiptychi* n. sp. was discovered along with 23 species of *Gyrodactylus* in the subgenus *Limnephrotus*, forming a sister group in clade 4. The nodes supporting this relationship were well supported in both the BI and ML trees. The hosts of *Limnephrotus* species belonged to the families Nemacheilidae, Cyprinidae, Salmonidae, Cobitidae, Gasterosteidae, Cottidae, and Percidae. These phylogenetic trees commonly indicated that *G. gymnodiptychi* n. sp. was closely associated with several *Gyrodactylus* members isolated from fishes inhabited in the river of Russia, Mongolia and Czech Republic. A clade of *G. gymnodiptychi* n. sp. diverged first, then *G. papernai*, *G. zavkhanensis*, *G. pseudonemacheili*, *G. mongolicus*, *G. nemacheili*, *G. jiroveci* and *G. tayshirensis* formed a clade.

4. Discussion

In this communication, we used morphological and molecular methods to identify a new species of parasite *Gyrodactylus gymnodiptychi* n. sp. for the first time. The *G. gymnodiptychi* n. sp. was the only one showing a hollow dorsal bar and curved hamulus root that were distinct

from the other eleven gyrodactylid members. *Gyrodactylus gymnodiptychi* n. sp. shared the same fish host *G. dybowskii* with two already known monogeneans *G. tokobaevi* and *G. aksuensis*.

The parasite fauna of fishes in the Yili River is largely unknown. Although *G. gymnodiptychi* n. sp. shares the same host *G. dybowskii* with *G. tokobaevi* and *G. aksuensis*, our newly described species was isolated from *G. dybowskii* living in the Yili River, while *G. tokobaevi* and *G. aksuensis* inhabit in the unrelated Aksu River west of Frunze (Ergens and Karabekova, 1980). Eleven species of *Gyrodactylus* have been reported from subfamily Schizothoracinae, including *G. hemivivinus* Ergens and Daniyarov, 1976; *G. kafirniganensis* Ergens and Daniyarov, 1976; *G. marjami* Allamuratov and Gushev, 1969; *G. montanus* Bychowsky, 1957; *G. narzikulovi* Ergens and Dzhalilov, 1979; *G. seravschani* Osmanov, 1965; *G. vicinus* Bychowsky, 1957; *G. aksuensis*, 1980; *G. tokobaevi*; *G. dzhalilovi* Ergens and Ashurova, 1984; and *G. editus* Dzhalilov and Ashurova, 1980; Ergens and Karabekova (1980); Gusev, 1985). The studies revealed that in comparison with *G. gymnodiptychi* n. sp., *G. narzikulovi* and *G. aksuensis*, other 9 species showed relatively flat and straight hamuli roots (Ergens and Karabekova, 1980; Gusev, 1985). These species shared a feature of small ventral bar processes, except for *G. gymnodiptychi* n. sp. and *G. tokobaevi*, which had prominent ventral bar processes (Ergens and Karabekova, 1980; Gusev, 1985). In these species, our identified new *G. gymnodiptychi* n. sp. sharing the feature of a dorsal bar with a hollow at each end of the projection with *G. montanus*. Additionally, *G. gymnodiptychi* n. sp. was distinguishable from *G. tokobaevi* and *G. montanus* by its hollow at each end of dorsal bar and a curved hamulus root (Ergens and Karabekova, 1980; Gusev, 1985). The newly identified *G. gymnodiptychi* n. sp. was the only one carrying both a hollow at each

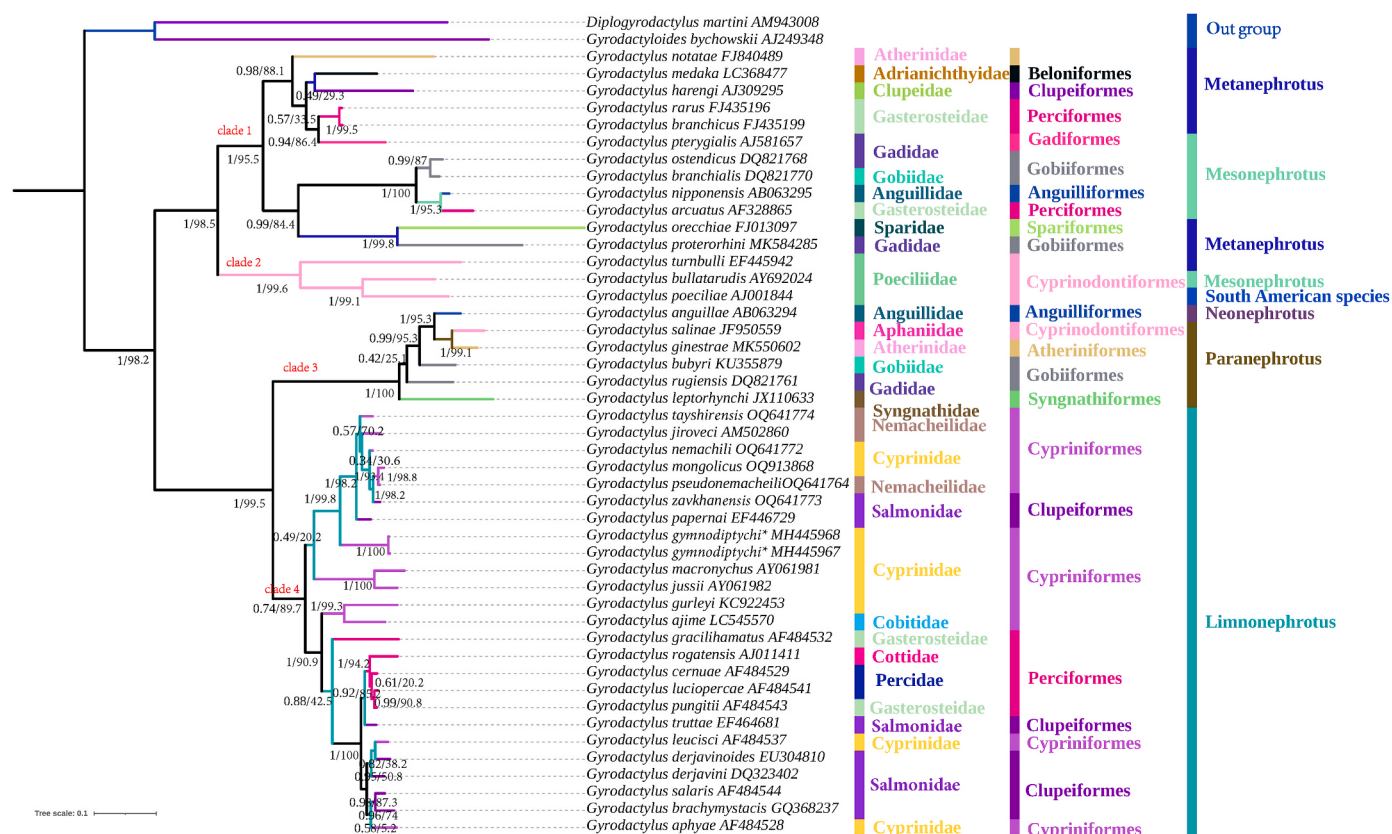


Fig. 4. Phylogenetic tree generated by the Bayesian Inference (BI) and Maximum Likelihood (ML) method based on *ITS1-5.8S-ITS2* rDNA sequences of selected *Gyrodactylus*. *Diplogyrodactylus martini* and *Gyrodactylus bychowskii* were used as the outgroup. The numbers at nodes indicate posterior probabilities and bootstrap branch support (%). Taxonomic identity were shown to the right: Family and Order of host fish, subgenus of *Gyrodactylus*. * *Gyrodactylus gymnodiptychi* in the phylogenetic tree.

end of dorsal bar and a curved hamulus root in contrast to other Schizothoracinae members carrying only one of the two features (Gusev, 1985).

The blast studies indicated that the *ITS1-5.8S-ITS2* rDNAs of *G. gymnodiptychi* n. sp. isolated from the fish *G. dybowskii* were distinguishable from all other *Gyrodactylus* species listed in the GenBank. The constructed phylogenetic trees indicated the association of *G. gymnodiptychi* n. sp. with the subgenus *Limnonephrotus*. Two other subgenus members *G. tayshirensis* (OQ641774) and *G. jiroveci* (AM502860) shared high degrees of 92.19% and 92.05% homology of the *ITS1-5.8S-ITS2* rDNA genes with *G. gymnodiptychi* n. sp., respectively; however, both of them are parasites of a *G. dybowskii*-unrelated genus host *Barbatula* identified in the Mongolia and Czech Republic (Přikrylová et al., 2008). Whether *Barbatula* members may host *G. gymnodiptychi* n. sp. remains to be determined.

In Xinjiang, eleven native members of the fish subfamily Schizothoracinae are Class I key-protected aquatic wild animals (Guo, 2012). Only the two members *G. dybowskii* and *Schizothorax pseudaksaiensis* are known hosts of monogeneans (Yao et al., 2013). The association of Schizothoracinae members and monogeneans is still largely unclear. Thus, it is important to advance our knowledge of monogeneans, such as *Gyrodactylus* members, which parasitize Schizothoracinae, in order to formulate effective methods for protecting Schizothoracinae members from monogenean-related diseases.

5. Conclusion

Newly investigated gyrodactylids from *Gymnodiptychus dybowskii* in Yili River are clearly distinguished from other members of the genus *Gyrodactylus* on the basis of morphological and genetic data.

Gyrodactylus gymnodiptychi is proposed as a new species.

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Availability of data and material

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

Authors contribution

Conception and Design, Sample Collection, Morphological analyses, Drew the morphological figures, Data analysis, and Manuscript Preparation: Wen-Run Zhang; Funding acquisition, Sample collection, Data curation, and Writing: Cui-Lan Hao; Sample collection: Kadirden Arken, Meng-Jie Rong, Sheng-Li Tian, Munira Kadir; Funding acquisition, Supervision: Cheng Yue. All authors read and approved the final version of the manuscript.

Data availability statement

The sequence data is uploaded to the NCBI GenBank and the raw sequences are available under the accession of MH445967 and MH445968.

Declaration of competing interest

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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