

Survivors from a Pliocene Climatic Catastrophe: *Gyrodactylus* (Platyhelminthes, Monogenea) Parasites of the Relict Fishes in the Central Asian Internal Drainage Basin of Mongolia

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Supplementary material

Table S1. List of *Gyrodactylus* species from Mongolia. GenBank and Museum accessions.

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Figure S1. The nemachili species group of subgenus *G.* (*Limnonephrotus*). Phylogenetically informative indel segment of ITS1.

Figure S2. Sequencing phenograms (ABI files) of the hybrid *G. nemachili* x *G. mongolicus* (Lp3) from the Forward and Reverse primers.

Figure S3. The hypervariable segment of the ITS1 of subgenus *G.* (*Gyrodactylus*).

Figures S4–S16. Photos of the species described or redescribed in this paper.

Table S1. List of *Gyrodactylus* species from Mongolia. Museum accession: <http://id.luomus.fi/> followed by the specimen's code.

Museum code	Box #	Parasite species	Host	ID	GenBank ITS rDNA	Genbank <i>cox1</i>	Locality
KN.372751	1	<i>Gyrodactylus radimi</i> sp. nov.	<i>T. brevirostris</i>	Mg1	OQ641783	OQ661864	Zavkhan river, above dam
KN.372752	2			Mg6	OQ641784	-	
KN.372753	3		<i>O. potanini</i>	Oz6	OQ641785	-	
KN.372754	4	<i>Gyrodactylus oreoleucisci</i>	<i>O. potanini</i>	Oz3	OQ641777	OQ661868	Zavkhan river, above dam
KN.372755	5	Ergens & Dulmaa, 1970		Oz4	OQ641776		
KN.372756	6		<i>B. conilobus</i>	Lz12	OQ641778		Zavkhan river, below dam
KN.372757	7		<i>O. potanini</i>	Op4	OQ916397		Chono Kharaikh
KN.372758	8			Op5	OQ916395		
KN.372759	9			Op6	OQ916396		
KN.372760	10			Op8	OQ916394		
KN.372761	11			Op9	OQ916398		
KN.372762	12	<i>Gyrodactylus nordmanni</i>	<i>O. humilis</i>	Ot1	OQ641779	OQ661869	Tuin river
KN.372763	13	Ergens & Dulmaa, 1970		Ot3	OQ641780	-	
KN.372764	14			Ot4	OQ641781	-	
KN.372765	15			Ot5	OQ641782	-	
KN.372766	16			Ot6	-	-	
KN.372767	17	<i>Gyrodactylus nemachili</i>	<i>B. cobdonensis</i>	Lp1	-	-	Chono Kharaikh
KN.372768	18	Bychowsky, 1936		Lp2	OQ641770	-	
KN.372769	19			Lp4	OQ641771	-	

KN.372770	20			Lp5	-	-	
KN.372771	21			Lp6	OQ641772	-	
KN.372772	22	<i>Gyrodactylus pseudonemachili</i>	<i>B. conilobus</i>	Lz1	OQ641760	-	Zavkhan river below dam
KN.372773	23	Ergens & Bychowsky, 1967		Lz11	OQ641761	-	
KN.372774	24			Lz13	OQ641762	-	
KN.372775	25			Lz14	OQ641763	-	
KN.372776	26			Lz15	OQ641764	-	
KN.372777	27			Lz16	OQ641765	-	
KN.372778	28			Lz17	OQ641766	-	
KN.372779	29			Lz18	OQ641767	-	
KN.372780	30		<i>T. brevirostris</i>	Mg3	OQ641755	-	Zavkhan above dam
KN.372781	31			Mg5	OQ641757	-	
KN.372782	32			Mg7	OQ641756	-	
KN.372783	33			Mg8	OQ641758	-	
KN.372784	34		<i>O. humilis</i>	Ot2	OQ641759	OQ661866	Tuin river
KN.372785	35	<i>Gyrodactylus mongolicus</i>	<i>O. potanini</i>	Oz2	OQ641769	OQ661870	Zavkhan river, above
KN.372786	36	Ergens & Dulmaa, 1970		Oz5	OQ641768	-	
KN.372787	37			Op2	OQ913866	-	Chomo Kharaiikh
KN.372788	38			Op3	OQ913867	-	
KN.372789	39			Op7	OQ913868	-	
KN.372790	40	<i>Gyrodactylus tayshirensis</i> sp. nov.	<i>B. conilobus</i>	Lz7	OQ641774	-	Zavkhan river, below dam

KN.372791	41	<i>Gyrodactylus zavkhanensis</i> sp. nov.	<i>T. brevirostris</i>	Mg2	OQ641773	-	Zavkhan river, above dam
KN.372792	42	<i>G. nemachili</i> x <i>G. mongolicus</i>	<i>B. cobdonensis</i>	Lp3		-	Chono Kharaikh river
KN.372793	43	<i>Gyrodactylus dulmaae</i> Ergens, 1970	<i>O. potanini</i>	Op1	OQ641791		Chono Kharaikh river
KN.372794	44	<i>Gyrodactylus sedelnikowi</i> Gvozdev, 1950	<i>B. conilobus</i>	Lz2	OQ641800	-	Zavkhan river, below dam
KN.372795	45	<i>Gyrodactylus amurensis</i> Akhmerov, 1952	<i>T. brevirostris</i>	Mg4	OQ641801	-	Zavkhan river, above dam
KN.372796	46	<i>Gyrodactylus barbatuli</i> Akhmerov, 1952	<i>B. conilobus</i>	Lz4	OQ641794		Zavkhan river, below dam
KN.372797	47			Lz5	OQ641795		
KN.372798	48			Lz6	OQ641796		
KN.372799	49			Lz8	OQ641797		
KN.372800	50			Lz9	OQ641798		
KN.372801	51			Lz10	OQ641799		
KN.372802	52	<i>Gyrodactylus aphyae</i> Malmberg, 1957	<i>P. cf. phoxinus</i>	Ph4	OQ641787	OQ661865	Tuul river, Ulaanbaatar
KN.372803	53			Ph5	-		
KN.372804	54			Ph7	OQ641788	-	
KN.372805	55			Ph8	-	-	
KN.372806	56			Ph9	OQ641789	-	
KN.372807	57			Ph10	OQ641790	-	
KN.372808	58	<i>Gyrodactylus albolacustris</i>	<i>P. cf. phoxinus</i>	Ph	KU365755	KU365756	Tuul river, Ulaanbaatar
KN.372809	59	Lumme, Ziętara & Lebedeva, 2017		Ph2	OQ641786	-	
KN.372810	60	<i>Gyrodactylus phoxini</i> Malmberg, 1957	<i>P. cf. phoxinus</i>	Ph1	OQ641792		Tuul river, Ulaanbaatar

Table S2. Sequences obtained in present study for macronychus group *Gyrodactylus* from Bolshaya Ussurka, Vladivostok Region, Russian Far East.

Species	Host	ID	ITS rDNA
<i>Gyrodactylus</i> cf. <i>oxycephali</i>	<i>Rhynchocypris lagowskii</i>	1Pla1	OQ672243
<i>Gyrodactylus</i> cf. <i>oxycephali</i>	<i>Rhynchocypris lagowskii</i>	1Pla2	OQ672244
<i>Gyrodactylus</i> cf. <i>oxycephali</i>	<i>Rhynchocypris lagowskii</i>	1Pla3	OQ672245
<i>Gyrodactylus</i> cf. <i>oxycephali</i>	<i>Rhynchocypris lagowskii</i>	2Pla6	OQ672246
<i>Gyrodactylus</i> cf. <i>oxycephali</i>	<i>Rhynchocypris lagowskii</i>	2Pla1	OQ672247
<i>Gyrodactylus</i> cf. <i>mantshuricus</i>	<i>Rhynchocypris percnura</i>	Ppe2	OQ672248
<i>Gyrodactylus</i> cf. <i>mantshuricus</i>	<i>Rhynchocypris percnura</i>	Ppe1	OQ672249
<i>Gyrodactylus</i> cf. <i>konovalovi</i>	<i>Rhynchocypris lagowskii</i>	2Pla4	OQ672250
<i>Gyrodactylus</i> cf. <i>konovalovi</i>	<i>Rhynchocypris lagowskii</i>	2Pla3	OQ672251
<i>Gyrodactylus</i> cf. <i>konovalovi</i>	<i>Rhynchocypris lagowskii</i>	2Pla2	OQ672252
<i>Gyrodactylus</i> cf. <i>lagowskii</i>	<i>Rhynchocypris lagowskii</i>	2Pla5	OQ672253

Table S3. Subgenus *G. (Gyrodactylus)* sequences obtained in present study.

Species	Host	ID	ITS rDNA	Locality
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	7Vk4	OQ672267	Lumijoki, Bothnian Bay, Finland
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	1Su1	OQ672268	Siuruanjoki, Bothnian Bay, Finland
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	Pho7_15_25_26_29	OQ672269	Siurua, Finland
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	Bol6	OQ672270	River Bolshaya Uya, Onega Lake, Russia
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	1Kn6	OQ672271	Kamennaya River, White Sea basin, Russia
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	2St2	OQ672272	Strelna River, Kola Peninsula, Russia
<i>Gyrodactylus phoxini</i>	<i>Phoxinus phoxinus</i>	6Slk3	OQ672273	Smolniczek, SE Poland
<i>Gyrodactylus magnificus</i>	<i>Phoxinus phoxinus</i>	Pho19_16_23	OQ672274	Siurua, Finland
<i>Gyrodactylus decorus</i>	<i>Alburnoides bipunctatus</i>	Al1	OQ672275	Vidlitsa River, Ladoga Lake basin, Russia
<i>Gyrodactylus</i> sp.	<i>Rhynchocypris percunura</i>	Ph4_5_6	OQ672276	Vladivostok region, Russia
<i>Gyrodactylus sedelnikowi</i>	<i>Barbatula barbatula</i>	Gs_BK	OQ672277	Bolshie Kozli, Archangelsk region, Russia
<i>Gyrodactylus perccotti</i>	<i>Perccottus glenii</i>	Per1_2_3_4	OQ672278	Invasive, Poland

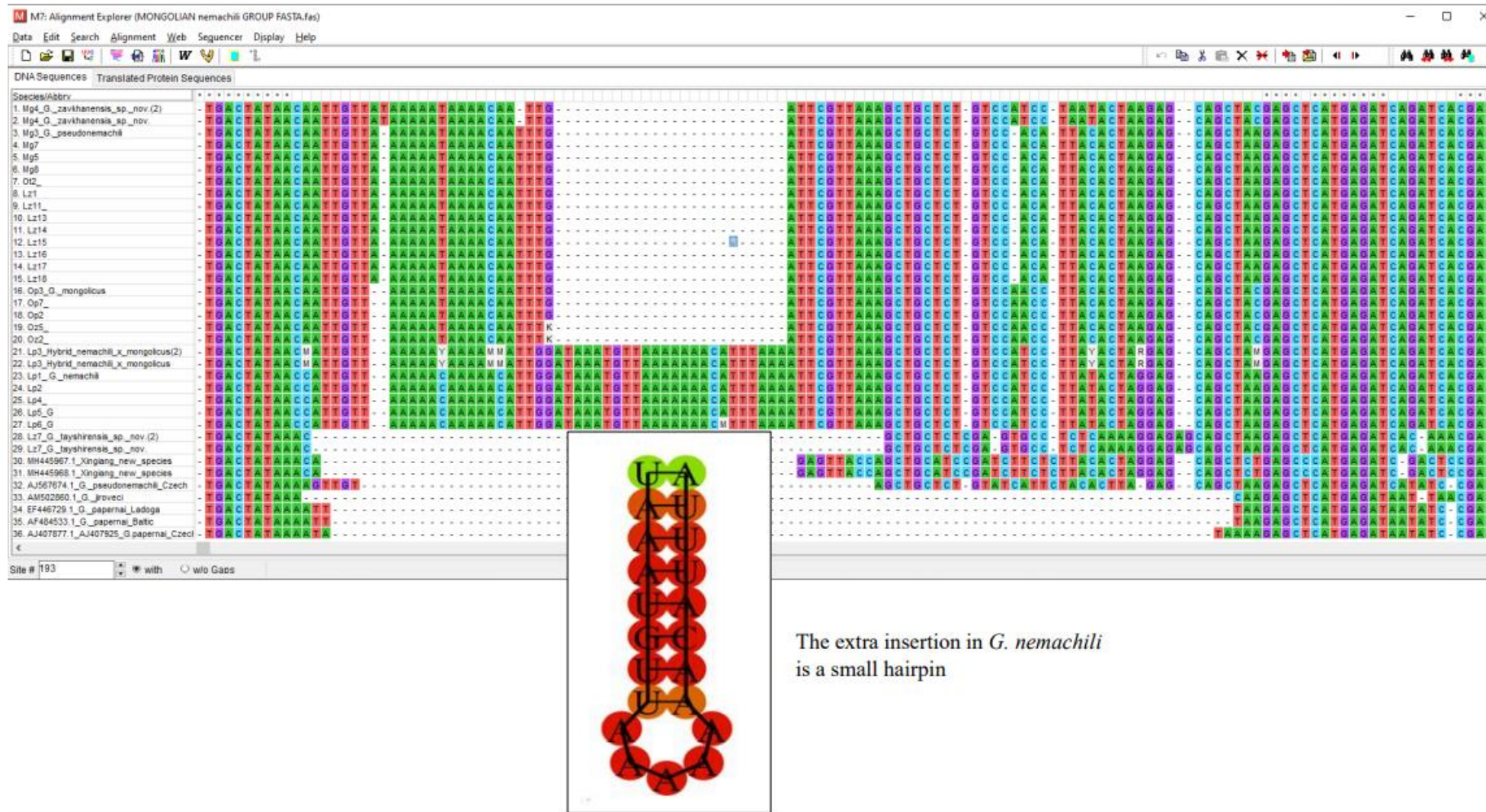
Table S4. List of *Gyrodactylus* species from the Hollow found previously and now.

Mongolian species of <i>Gyrodactylus</i>	Detected in The Hollow 2012	Reported earlier*	ITS barcode
<i>G. radimi</i> sp. nov.	x		x
<i>G. thymalli</i> Zitnan (syn. of <i>G. salaris</i>)		x	In Europe
<i>G. oreoleucisci</i> Ergens & Dulmaa, 1970	x	x	x
<i>G. nordmanni</i> Ergens & Dulmaa, 1970	x	x	x
<i>G. pewzowi</i> Ergens, 1980		x	
<i>G. mongolicus</i> Ergens & Dulmaa, 1970	x	x	x
<i>G. nemachili</i> Bychowsky, 1936	x	x	x
<i>G. pseudonemachili</i> Ergens & Bychowsky, 1967	x	x	x
<i>G. jiroveci</i> Ergens & Bychowsky, 1967		x	in Europe
<i>G. tayshirensis</i> sp. nov.	x		x
<i>G. zavkhanensis</i> sp. nov.	x		x
<i>G. dulmaae</i> Ergens, 1970	x	x	x
<i>G. minimus</i> Malmberg, 1957		x	
<i>G. sedelnikowi</i> Gvosdev, 1950	x	x	x
<i>G. amurensis</i> Akhmerov, 1952	x		x
<i>G. barbatuli</i> Akhmerov, 1952	x	x	x
<i>G. minimus</i> Malmberg, 1957		x	
<i>G. magnificus</i> Malmberg, 1957		x	in Europe
<i>G. limneus</i> Malmberg, 1964 (syn. of <i>G. phoxini</i>)		x	in Europe
<i>G. llewellyni</i> Ergens & Dulmaa, 1967		x	
<i>G. menschikowi</i> Gvosdev, 1950		x	
<i>G. malmbergensis</i> Prost, 1974		x	
Total number of species	12	17	

* data of Paranlejamts (1993), Pugachev (2002).

Eight species were common in old and new collections, and three new species were described. One name was adopted from Amur basin. Ten species which were reported earlier were not found, or identified with another name. Four names used by earlier authors were recorded and barcoded in Europe, two of them synonymized and one used for at least two taxa.

Supplementary Figure S1. The nemachili species group of subgenus *G. (Limnonephrotus)*. Phylogenetically informative indel segment of ITS1 including European and Mongolian species and one new from Xinjiang, Uigur autonomic area, China on *Gymnodiptychus dybowskii*, Naked osman (Kessler, 1874). The European species *G. jiroveci* and *G. papernai* have the shortest sequence.



Supplementary Figure S2. Sequencing phenograms (ABI files) of the hybrid *G. nemachili* x *G. mongolicus* (Lp3) from the Forward and Reverse primers. The sequence after the heterozygotic indel was a mess. In this case, both parents were known.



Supplementary Figure S3 The hypervariable segment of the ITS1 of subgenus *G.* (*Gyrodactylus*) to demonstrate the DNA barcoding for readers less accustomed with it. Some sequences are duplicated for visibility. The “barcoding” function is immediately clear. Genetic distances may be quantitative and gradual, but ITS separates species qualitatively. The sequences of *G. sedelnikowi* from Mongolia (OQ641800) and from the White Sea basin (OQ672277) are identical in this segment, and the Czech sequence (AJ407891) differs by two nucleotides, but no indels.





Supplementary Figure S4. *Gyrodactylus radimi* sp. nov. on *Thymallus brevirostris*. Scale bars 10 μ m.



Supplementary Figure S5. *Gyrodactylus oreoleucisci* Ergens & Dulmaa 1970 on *Oreoleuciscus potanini*. Scale bars 10 μ m.



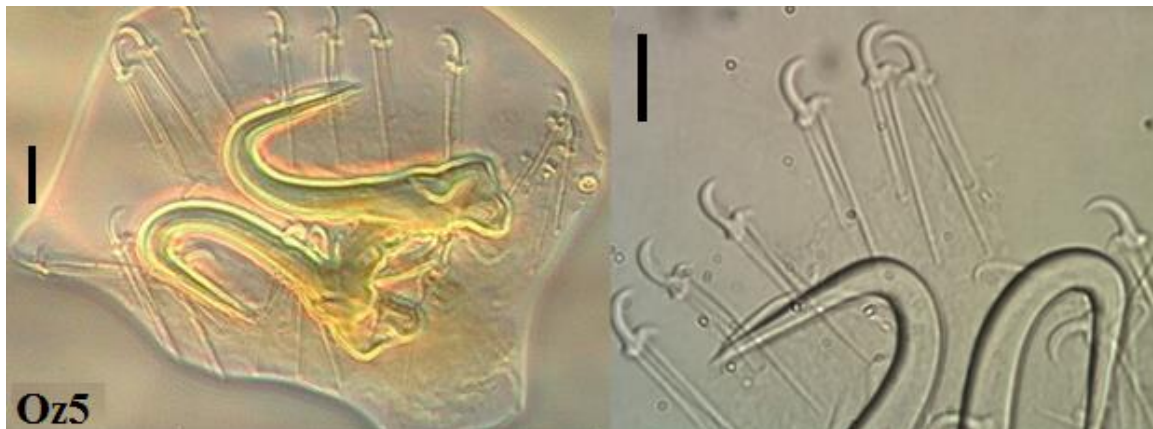
Supplementary Figure S6. *Gyrodactylus nordmanni* Ergens & Dulmaa, 1970 on *Oreoleuciscus humilis*, Tuin river. Scale bars 10 μ m.



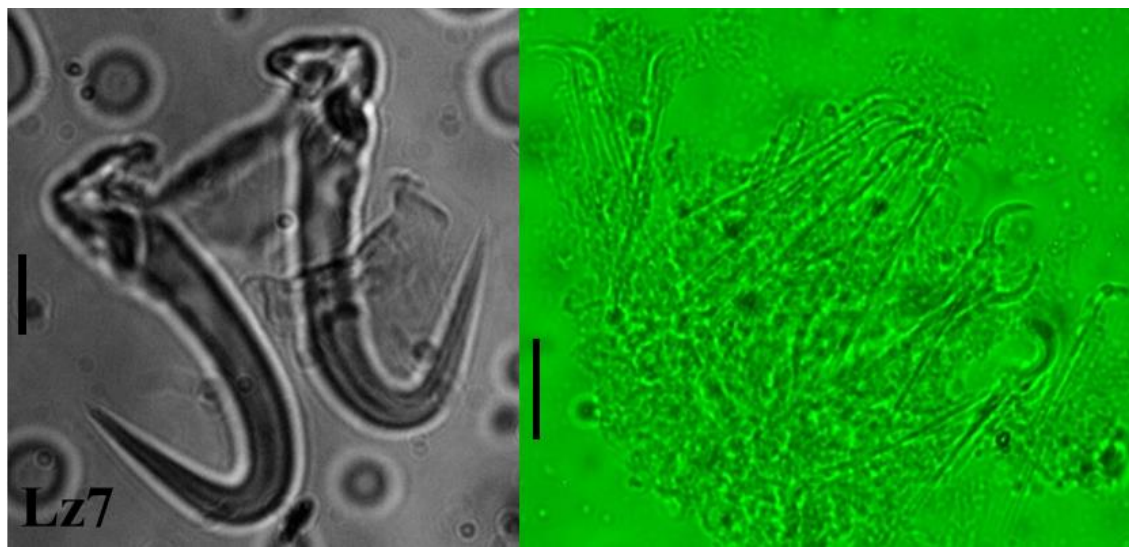
Supplementary Figure S7. *Gyrodactylus nemachili* Bychowsky 1936, on *Barbatula cobdonensis*. Scale bars 10 μ m.



Supplementary Figure S8. *Gyrodactylus pseudonemachili* Ergens & Bychowsky, 1967, on *Barbatula conilobus*. Scale bars 10 μ m.



Supplementary Figure S9. *Gyrodactylus mongolicus* Ergens & Dulmaa 1970, on *Oreoleuciscus potanini*. Scale bars 10 μ m.



Supplementary Figure S10. *Gyrodactylus tayshirensis* sp. nov. on *Barbatula conilobus*. Scale bars 10 μ m.



Supplementary Figure S11. *Gyrodactylus zavkhanensis* sp. nov. on *Thymallus brevirostris* (Mg2). Scale bar 10 µm.



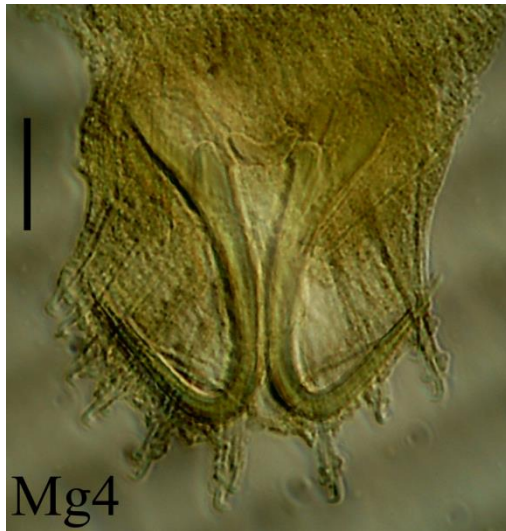
Supplementary Figure S12. *Gyrodactylus* hybrid *G. nemachili* x *G. mongolicus* on *Barbatula cobdonensis*. Scale bar 10 µm.



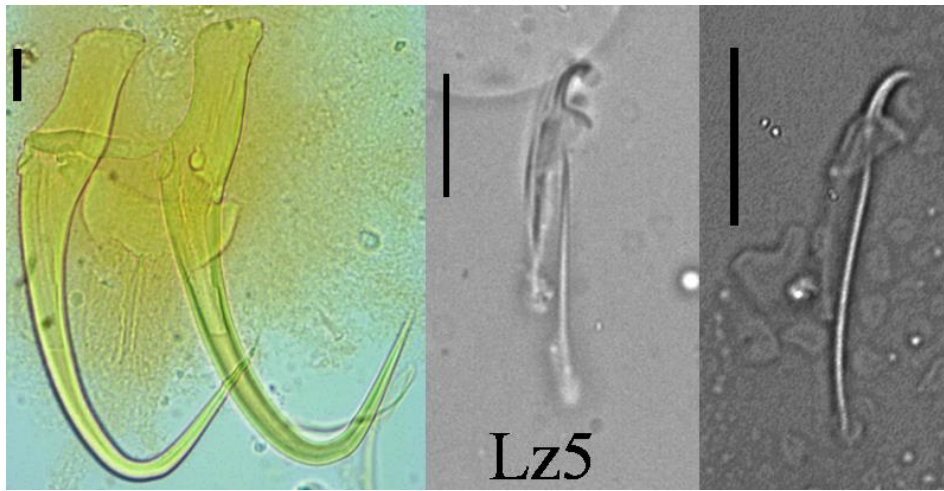
Supplementary Figure S13. *Gyrodactylus dulmae* Ergens, 1970, on *Oreoleuciscus potanini*. Scale bar 10 µm.



Supplementary Figure S14. *Gyrodactylus sedelnikowi* Gvozdev, 1950, on *Barbatula conilobus*. Scale bar 10 μm .



Supplementary Figure S15. *Gyrodactylus amurensis* Akhmerov, 1952, on *Thymallus brevirostris*. Scale bar 10 μm .



Supplementary Figure S16. *Gyrodactylus barbatuli* Akhmerov, 1952, on *Barbatula conilobus*. Scale bars 10 μ m.