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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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Abstract: We investigated the *Gyrodactylus* ectoparasites on relict fishes in the isolated endorheic Central Asian Internal drainage basin in Mongolia (The Hollow) and placed them into the global phylogenetic framework based on internal transcribed spacer regions of the nuclear ribosomal DNA (ITS). Much of the rich Pliocene lacustrine ichthyofauna is extinct. We sampled five riverine survivors: Altai osmans *Oreoleuciscus humilis* and *O. potanini* (Leuciscidae), Mongolian grayling *Thymallus brevirostris* (Salmonidae), and stone loaches *Barbatula conilobus* and *B. cobdonensis* (Nemacheilidae). We found eight species of the subgenus *Gyrodactylus* (*Limnonephrotus*) and four of *G.* (*Gyrodactylus*). Nine species were identified as taxa described earlier, and three were described as new. The endemic Mongolian grayling carried four species, only one of wageneri group typical to salmonids (*Gyrodactylus radimi* sp. nov.), two of nemachili group (*G. zavkhanensis* sp. nov., *G. pseudonemachili* Ergens and Bychowsky, 1967), and *G. amurensis* Akhmerov, 1952 of subgenus *G.* (*Gyrodactylus*). *G. pseudonemachili* was also found on osman and loach. A parasite clade typical for Nemacheilidae was overrepresented by five species (*G. tayshirensis* sp. nov. on *Barbatula conilobus, G. mongolicus* Ergens and Dulmaa, 1970, *G. nemachili* Bychowsky, 1936). Relaxed host specificity mentioned already by Ergens and Dulmaa was evident. In the updated global ITS phylogenies of the two freshwater-restricted subgenera, the parasites from the Mongolian relict populations assumed positions concordant with a hypothesis of multiple ancient introductions from the Euro-Siberian fauna, strong rarefaction and three cases of endemic divergence.

Keywords: tertiary relicts; Central Asian Internal drainage basin; host switching; global phylogeography

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1. Introduction

We investigated the monogenean fish parasites of genus *Gyrodactylus* in the Central Asian Internal drainage basin in Mongolia (abbreviated in this paper as The Hollow). Dulmaa [1] has outlined the extant Mongolian fish fauna and the spatial distribution in an FAO fisheries rapport. The Hollow covers 65% of Mongolian territory and contains 32% of its water resources. The largest Mongolian lakes are situated in the Gobi Valley, where the annual rainfall is only about 100 mm, and the evaporation rate is 900–1000 mm [1]. The water maintaining the lakes flows from the surrounding mountains which in the western and SW range are glaciated.

The Hollow is endorheic, i.e., it has only internal drainages and is not connected via extant waterways to the Arctic or the Pacific Oceans, nor to the Caspian or the Black Seas.

The area has been isolated from Siberia and the Arctic Ocean and Amur watersheds for tens of millions of years since the orogenic changes in the Tertiary Era. Paleontological studies on fish fauna were conducted by Sytchevskaya [2–4] in the western and deepest part of The Hollow, in Lake Khirgis-Nur (49 08′ N, 93 25′ E, 1028.5 m asl), along the banks of the Chono Kharaikh channel, and in the Dzagso-Khaikhan area (right bank of the Zavkhan River). In the Early Pliocene (5.3 to 2.6 Mya) the lake water level was at least 200 m higher than present, and the lake was inhabited by representatives of the Euro-Siberian freshwater ichthyofauna (*Esox*, *Rutilus*, *Leuciscus*, *Abramis*, *Blicca*, *Carassius*, *Tinca*, *Sander* (*Stizostedion*), *Perca*). The lacustrine fauna disappeared by the Middle Pleistocene [2] due to a drying climate and consequential changes in water chemistry and limnology.

Dgebuadze et al. [5] listed twelve endemic and three stocked fish species in the closed basin of The Hollow. Locally harvested species *Coregonus peled* (Gmelin, 1788), *C. migratorius* (Georgi, 1755), and *Esox lucius* (Linnaeus, 1758) have been trafficked into the lakes in the isolated area. Two species of Leuciscidae are endemic: *Oreoleuciscus potanini* (Kessler, 1879), and *O. humilis* Warpachowski, 1889 [6,7], as well as the only salmonid, Mongolian grayling *Thymallus brevirostris* (Kessler, 1879) [8,9]. Even if the taxonomy of loaches is not in the final edition [10–12], the most diverse family in The Hollow was Nemacheilidae with about nine species. The ~12 endemic fish species are significantly less than the 31 species in the nearby Arctic Ocean basin (Yenisey River) and the 45 species in the Pacific Ocean basin (Amur River) in the territory of Mongolia [5].

The Mongolian monogeneans of *Gyrodactylus* von Nordmann, 1832 were described with the highest possible accuracy and coverage by Dr. Radim Ergens and collaborators before the molecular era, both in The Hollow and in the surrounding outflows, the upwaters of Black Irtysh, Yenisey and Amur [13–18]. Most of the information was derived from the material of the Czechoslovak–Mongolian ichthyoparasitological expedition in 1966, which lasted six months and recorded 41 parasite species. The expedition produced the foundation of monogenean knowledge of Central Asia and numerous relevant publications, e.g., Ergens and Dulmaa [17,19,20] and Ergens [13–16,21]. The latest compilation of the morphological data and an identification key are available in the book of Gusev and Bauer [22], in Russian and in Pugachev et al. [23], updated and translated in English.

In this study, we re-visit the *Gyrodactylus* fauna of The Hollow. Through the utilization of molecular "barcoding" by the internal transcribed spacer region of the nuclear ribosomal DNA (from this on, ITS), we attempt to evaluate the consequences of historic catastrophic climate change on the fauna, and also to refresh the taxonomy. The Monogenean parasites are intimately dependent on their hosts, i.e., not able to escape or recolonize by intermediate hosts. The intriguing question is—What happens among the parasites when the host community dramatically deteriorates? A null hypothesis might be that when a host is going to extinction, the parasites disappear even before the host. Among the riverine survivors sampled herein, we confirmed the relaxation of the host specificity reported by Ergens and Dulmaa [18].

2. Material and Methods

2.1. Fish and Parasite Sampling

The fish were collected during the Joint Russian–Mongolian Biological Expedition in August 2012, in four localities in the Central Asian Internal Drainage basin (The Hollow) and one in the Arctic Ocean basin (Figure 1 and Table 1).

The sampling localities and dates, and the fish species collected were as follows.

Tuin River in the Valley of the Lakes, running down to Lake Orog Nuur. Coordinates 45°11′32.0″ N, 100°46′42.8″ E, 1270 m asl. Date—7 August 2012. Only one fish species was caught, the Little Altai Osman *Oreoleuciscus humilis* (10 specimens).

Zavkhan river, the Great Lakes Depression, upstream of the Tayshir Reservoir dam. 46° 39′01″ N, 96° 52′47″ E, 1700 m asl. Date—8 August 2012. A total of 3 fish species were caught: Altai Osman *Oreoleuciscus potanini* (3 specimens), Mongolian grayling *Thy-*

mallus brevirostris (25 specimens), and Stone loach *Barbatula conilobus* Prokofiev, 2016 [12] (11 specimens).

Zavkhan River, downstream of the Tayshir Reservoir dam. 46°41′29″ N, 96°38′46″ E, 1660 m asl. Date—12 August 2012. Only 1 fish species, *Barbatula conilobus* Prokofiev, 2016 (15 specimens).

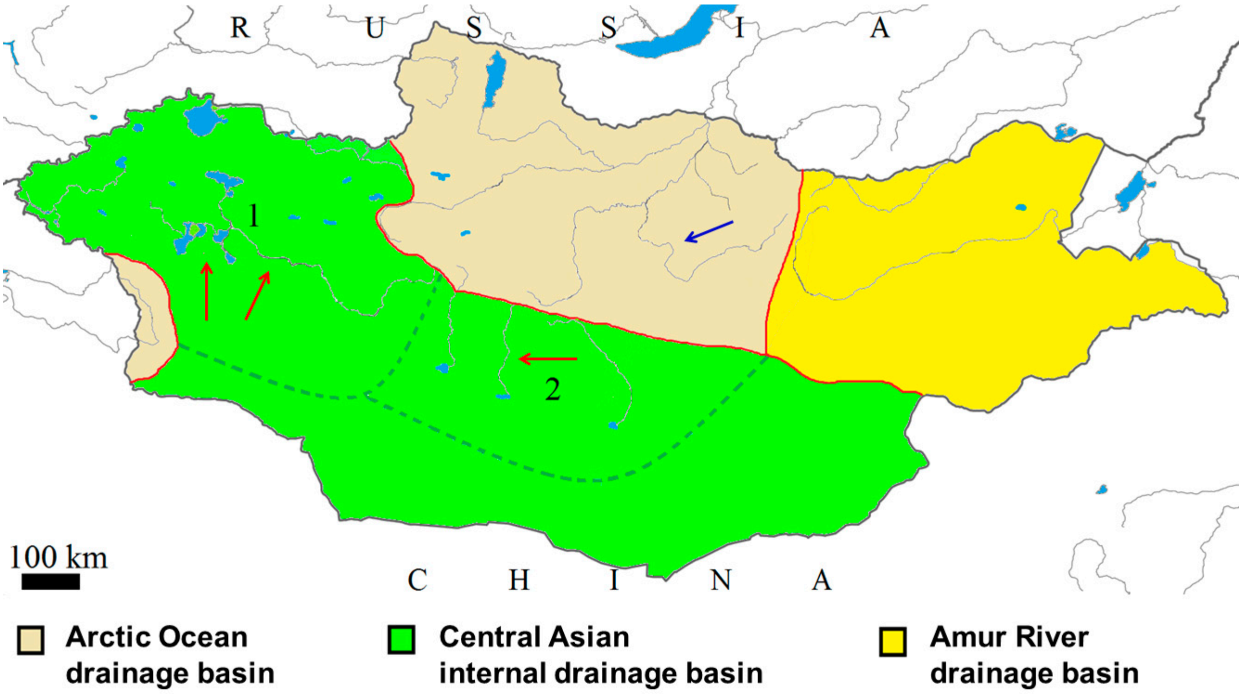

Figure 1. Sampling sites in Mongolia. Red arrows: sampling sites in The Hollow, blue arrow: Tuul River (Tributary of Selenga, draining to Lake Baikal). The red lines demarcate the different drainage areas; green dashed lines demarcate the Great Lakes Depression (1) and the Valley of Lakes (2). More details and names are in the map in Slynko et al. [6] and Kottelat [10].

Chono Kharaikh, the Great Lakes Depression, a river between Khar and Nogoon lakes.47°58′53.4″ N, 93°15′19.0″ E, 1135 m asl. Date—19 August 2012. Two fish species, Altai Osman *Oreoleuciscus potanini* (14 specimens) and Stone loach *Barbatula cobdonensis* (Gundrizer, 1973) (9 specimens).

Tuul River, tributary of Selenga–Yenisey in the Arctic Ocean basin, near Ulaanbaatar. 47°53′10.7″ N, 106°56′01.0″ E, 1290 m asl. Date—28 August 2012. A total of 1 fish species was caught: *Phoxinus* cf. *phoxinus* (Linnaeus 1758) (15 specimens). Kottelat [10] suspected the species identity of the minnow in Tuul.

Skin and nose cavities of each fish specimen were examined under a stereomicroscope. Gills were not inspected. The infected fins were cut and stored in 96% ethanol. For molecular and morphological analysis, the procedure was as follows. The parasites stored in ethanol were cut into two parts, the main body and the opisthaptor. The ITS rDNA sequencing was conducted from the digested main body, so that the specimens were first classified into fifteen species and one hybrid, without names, and the phylogenetic hypothesis was constructed for both subgenera. The parasites on *Phoxinus* in Tuul were identified by the ITS, as the three species were barcoded in Europe [24,25].

The opisthaptors were placed on microscopy slides, treated slightly with proteinase K to make them transparent, and fixed in ammonium picrate glycerin. The morphological evaluation was guided by the species identified through ITS genetic data, and the specimens were measured, drawn, and photographed by microscopes. Comparison with the species described earlier in The Hollow, or more broadly in Mongolia, and the attempt to name the taxa was the final step of the process.

Every parasite specimen was given a short lab code (Table 1) when picked from ethanol and cut into two pieces. The tiny 0.2 mL Eppendorf vials and the microscopy slides were marked with this code, which then followed the sample through the sequencing protocol to different data sets and data processing phases, until the GenBank entry. The code was included in the drawings, photographs, sequencing files, the physical and electronic museum deposits, and finally, in this report.

Table 1. The parasites found on the six host species from the five Mongolian sampling sites.

Locality	Fish Species	Lab Code of Parasite	*Gyrodactylus* Species	Subgenus *
Tuin River	Little Altai Osman (N = 10) *Oreoleuciscus humilis*	Ot2 Ot1, 3, 4, 5, 6	*G. pseudonemachili* Ergens and Bychowsky, 1967 *G. nordmanni* Ergens and Dulmaa, 1970	**L n** **L m**
Zavkhan River above dam	Altai Osman (N = 3) *Oreoleuciscus potanini*	Oz6 Oz2,5 Oz1, 3, 4	*G. radimi* sp. nov. *G. mongolicus* Ergens and Dulmaa, 1970 *G. oreoleucisci* Ergens and Dulmaa, 1970	**L w** **L n** **L m**
	Mongolian grayling (N = 15) *Thymallus brevirostris*	Mg2 Mg1, 6 Mg5, 7, 8 Mg4	*G. zavkhanensis* sp. nov. *G. radimi* sp. nov. *G. pseudonemachili* Ergens and Bychowsky, 1967 *G. amurensis* Akhmerov, 1952	**L n** **L w** **L n** **G**
	Stone loach (N = 11) *Barbatula conilobus*	Lz12	*G. oreoleucisci*	**L m**
Zavkhan River below dam	Stone loach (N = 15) *Barbatula conilobus*	Lz2 Lz4, 5, 6, 8, 9, 10 Lz11, 13, 15, 16, 17, 18 Lz7	*G. sedelnikowi* Gvozdev, 1950 *G. barbatuli* Akhmerov, 1952 *G. pseudonemachili* Ergens and Bychowsky, 1967 *G. tayshirensis* sp. nov.	**G** **G** **L n** **L n**
Chono Kharaikh river	Altai Osman (N = 14) *Oreoleuciscus potanini*	Op4, 5, 6, 8, 9 Op1 Op2, 3, 7	*G. oreoleucisci* *G. dulmaae* Ergens, 1970 *G. mongolicus*	**L m** **G** **L n**
	Stone loach (N = 9) *Barbatula cobdonensis*	Lp1, 2, 4, 5, 6 Lp3	*G. nemachili* Bychowsky, 1936 hybrid *G. nemachili* × *G. mongolicus*	**L n** **L n**
Tuul River, Yenisei basin	Minnow (N = 15) *Phoxinus* cf. *phoxinus*	Ph4, 5, 7, 9, 10 Ph, Ph2 Ph1, 6	*G. aphyae* Malmberg, 1957 *G. albolacustris* Lebedeva, Ziętara and Lumme, 2017 *G. phoxini* Malmberg, 1957	**L w** **L w** **G**

* **L** subgenus *G.* (*Limnonephrotus*), **w** wageneri group, **n** nemachili group, **m** macronychus group. **G** subgenus *G.* (*Gyrodactylus*).

2.2. Morphological Methods

Only parasites with confirmed ITS sequence information were inspected morphologically and identified to species. The comparison with the parasite fauna studied earlier was conducted by utilizing the same methods and recording details. The dimensions recorded and compiled in Pugachev et al. [23] and the description style of Dr. Radim Ergens given their work in this territory. Bridging the old nomenclature with the barcode-supported taxonomy leaves much guesswork for a modern taxonomist, but the molecular produced herein will be widely available for further faunistic work with at least the twelve taxa described here being genetically identifiable.

Measurements of opisthaptor hard parts were performed with a microscope (Nikon) and digital camera (Nikon Optiphot–2) by interactive measuring system IMT iSolution Lite (Ver.7.4, IMT iSolution Inc., NY, USA).

The microscopy slides of the Mongolian specimens are deposited in the Finnish national collection, Central Museum of Natural History (Luomus) with the accession codes KN.372751–KN.372801 (http://id.luomus.fi/KN.372751--KN.372801; accessed on 15 July 2023). A complete list of Museum and GenBank accession codes is in Supplementary Table S1.

2.3. Molecular Analysis

The complete amplification and sequencing of ITS1-5.8SrDNA-ITS2 was made following earlier studies by Ziętara et al. [26,27], Matějusová et al. [28,29], and Přikrylová et al. [30,31]. The ITS sequences were produced from 34 parasite specimens from The Hollow. The mitochondrial *cytochrome* 1 gene (*cox*1) or a fragment of it was sequenced from *G. albolacustris*, *G. aphyae*, *G. mongolicus*, *G. nordmanni*, *G. oreoleucisci*, *G. pseudonemachili*, and *G. radimi* sp. nov. by the methods of Ziętara et al. [32].

The GenBank accessions of the Mongolian specimens are given within the species descriptions and in Supplementary Table S1. New GenBank accessions were also loaded from authors' collections for representatives of subgenus *G.* (*Gyrodactylus*) as OQ672267–OQ672278, and for Russian Far East parasites of *G.* (*Limnonephrotus*) as OQ672243–OQ672253 (Supplementary Materials Tables S2 and S3).

For phylogenetic comparisons, ITS sequences of species from subgenera *Gyrodactylus* (*Limnonephrotus*) and *G.* (*Gyrodactylus*) were either retrieved from the GenBank, or from the authors' collections. The most convenient way to access the data is through utilizing the specified name search and BLAST on the homepage of NCBI, National Center for Biotechnology Information. Altogether, 194 ITS sequences of *G.* (*Limnonephrotu*s), and 64 ITS of *G.* (*Gyrodactylu*s) were used for reconstruction of the phylogenetic hypotheses.

The sequence alignments were made in MEGA7 by Clustal [33]. The phylogenetic hypotheses were derived by NJ method based on K2P distances and tested by bootstrapping 500 rounds. Much of the data from unalignable hypervariable regions were cut off from the genetic distance calculations by using the pairwise deletion option. Despite the specific constraints caused by the functional secondary structure of the ITS [34–36], the ITS offers good resolution for separating evolutionary units (=species. Furthermore, the phylogenetic hypotheses constructed using the ITS complement and support taxonomic classifications [36].

3. Results

Five fish species were found in The Hollow and the sixth in the river Tuul, Yenisey tributary (Table 1). Two species of the Altai osman and the Mongolian grayling were collected. The taxonomy of *Barbatula* is in a dynamic phase. The samples were taken to the laboratory as *Orthrias barbatulus toni*, and the names we use here are deduced from the publications of the locally active authors: *Barbatula conilobus* Prokofiev, 2016 [12], and *Barbatula cobdonensis* (Gundrizer, 1973) [37]. These names were not recorded in the FishBase as of April 2023. Kottelat accepted *B.* (*Nemachilus*) *cobdonensis* as a possible synonym of *B. compressirostris* (Warpachowski, 1897) [10].

The 34 Mongolian parasite specimens from The Hollow barcoded by ITS were classified into twelve species and one hybrid. Eight species (and one hybrid) were assigned to the subgenus *G.* (*Limnonephrotus*), and four to the subgenus *G.* (*Gyrodactylus*). The Mongolian species were then placed within a global phylogenetic framework for the respective subgenus and will be described or redescribed in this context.

We identified the three parasite species on *Phoxinus* from river Tuul through the ITS data, because they were previously studied and barcoded in Europe [24,25].

Subgenus *Gyrodactylus* (*Limnonephrotus*) Malmberg 1970

The subgenus *G.* (*Limnonephrotus*) has been studied intensively in Europe and Asia, mainly because all known economically interesting parasites on salmonids belong to this subgenus. The phylogenetic hypothesis of the subgenus in Figure 2 was based on 194 sequences of ITS. It revealed three major monophyletic multispecies clades, twenty-six species in the wageneri group, nine species in the nemachili group, and eight species in the macronyhus group, all clearly distinct with 100% bootstrap support. These species groups are Palearctic, extending from Europe to the Russian Far East and China.

Figure 2. Phylogenetic hypothesis of the subgenus *G.* (*Limnonephrotus*) based on the Intergenic Transcribed Spacers and 5.8 S rDNA. The red branches represent species from The Hollow.

Only two species of the subgenus are known in North America: *G. crysoleucas* Mizelle and Kritsky, 1967 [38] and the circumboreal *G. salmonis* (Yin and Sproston, 1948) [39], both of the wageneri group. An interesting recent addition to the subgenus is *G. ajime* Nitta, 2021 from Japan [40]. The American *G. crysoleucas* was not included in the tree because of an incomplete ITS.

New species from China, Xinjiang Province, Uyghur Autonomous Region, deposited in GenBank (MH445967 and MH445968) by Xie and Yue in 2018, and hosted by Naked osman *Gymnodiptychus dybowskii* (Kessler, 1874) were included in the phylogenetic hypothesis. It has not been named but made an important extension to the phylogeography of the nemachili group.

Redescriptions of old and descriptions of new species from The Hollow, Mongolia

Family Gyrodactylidae Beneden and Hesse, 1864

Genus *Gyrodactylus* von Nordmann, 1832

Subgenus *G.* (*Limnonephrotus*) Malmberg, 1970.

Gyrodactylus radimi sp. nov.

Type host and locality: *Thymallus brevirostris* (Mg1, Mg6), Zavkhan River, The Hollow, Mongolia.

Other hosts: *Oreoleuciscus potanini* (Oz6), Zavkhan River, Mongolia.

Prevalence and intensity of infection: for *Thymallus brevirostris* 8%; 1 and 1 worm per host specimen; *O. potanini*–one of three fish, one worm per host specimen.

Specimens deposited in the museum collection: holotype—KN.372751, paratypes—KN.372752 and KN.372753.

ZooBank registration: the Life Science Identifer (LSID) for *Gyrodactylus radimi* sp. nov. is urn:lsid:zoobank.org:act:3AF532A6-82F2-4920-A416-FE32E042A036.

Description (Figure 3, Supplementary S4): Anchor 58–60 µm, anchor root 17–19 µm, anchor shaft 46–49 µm and point 28–32 µm. Ventral bar 6–8 × 23–25 µm, with short processes and medium triangular membrane, 16–17 µm long. Dorsal bar delicate, 1–2 × 29–31 µm. The total length of marginal hooks is 38–40 µm, sickle 8–9 µm long with bent point not extending toe. The sickle filament loop is short, not reaching half of the handle.

Molecular sequence data: ITS length 1231 bp, GenBank accessions OQ641783–OQ641785. A fragment (1244 bp) of *cox*1 (Mg1) was deposited (OQ661864). Both ITS and *cox*1 show that *G. radimi* sp. nov. belongs to the wageneri species group of the subgenus *G.* (*Limnonephrotus*).

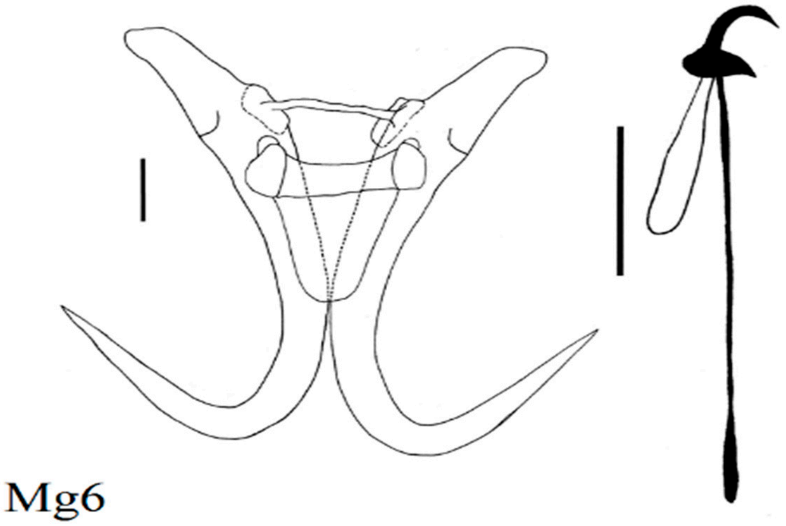

Figure 3. *Gyrodactylus radimi* sp. nov. on *Thymallus brevirostris*. Scale bars 10 µm.

Taxonomic remarks: This was the only wageneri group species in our sample from the Great Lakes Depression, and this conclusion was derived from morphological, ITS, and *cox*1 data. Numerous wageneri group monogeneans were previously known on salmonids, and some were already from the territory of Mongolia, while not in The Hollow. The molecular data immediately excluded numerous parasites recorded on salmonids and thymallids. The three specimens were not a genetix match with *G. salmonis* Yin and Sproston, 1948, *G. salaris* Malmberg, 1957 (or syn. *G. thymalli* Žitňan, 1960), *G. magnus* Konovalov, 1967 (or syn. *G. brachymystacis* Ergens, 1978), *G. taimeni* Ergens, 1971, *G. truttae* Gläser, 1974, *G. derjavini* Mikailov, 1975, *G. teuchis* Lautraite, Blanc, Thiery, Daniel and Vigneulle, 1999, or *G. derjavinoides* Malmberg, Collins, Cunningham and Jalali, 2007. The nearest (but not very close) phylogenetic relatives of *G. radimi* sp. nov. were *G. lucii* Kulakowskaja, 1951, and *G. derjavinoides* (Figure 2), both barcoded only in Europe.

When the above salmonid parasites were excluded, we searched for candidates from the described species lacking a DNA barcode. One candidate species was *Gyrodactylus lenoki* Gusev 1953, a parasite of *Brachymystax lenok* (Pallas, 1773) from the Amur River. The host *B. lenok* has been recorded in Mongolia, in Lake Terkhiin Tsagaan, and River Tuul, but not in The Hollow [41]. Interestingly, the *G. lenoki* original forms "typical, forma A and forma B" differed clearly in the shape of marginal hooks (Figures 10 and 11 in Ergens [41]), and Ergens himself was casting doubt over the species delineation. The marginal hooks of specimens from Zavkhan resembled the forma A, but all measurements were clearly smaller than the measurements of either typical, or A or B. The length of anchors of Zavkhan specimens was 58–60 µm, while the published range among *G. lenoki* was 89–102 µm.

We suggest that we have here an interesting case of "taxonomic mimicry". Ergens [16] mentions *G. thymalli* on European grayling in Hron and Hnilec (Danube basin, type locality of *G. thymalli* Žitňan 1960) but also in Nikolka and Penzhina rivers in Russian Far East, Kamtchatka. Pugachev et al. [23] repeated: "[*G. thymalli*] found on fins of *Thymallus thymallus* Linnaeus, 1758, *T. arcticus* Pallas, 1776, and *T. brevirostris*; species widespread in the area of its hosts". Perhaps the researchers overlooked the grayling parasites, and recorded them as *G. thymalli*, as was the habit in Europe at the time [42].

Etymology. The species was named after our great predecessor Dr. Radim Ergens (1933–2007).

Subgenus: *G.* (*Limnonephrotus*), macronychus group.

***Gyrodactylus oreoleucisci* Ergens and Dulmaa, 1970.**

Type host and locality: Dwarf Altai osman *Oreoleuciscus humilis,* Lake Telmen, North-western Khangay, Arctic Ocean basin, Mongolia.

Present hosts and localities: *Oreoleuciscus potanini* (Oz1, 3, 4) and *Barbatula conilobus* (Lz12) in Zavkhan River, *Oreoleuciscus potanini* in Chono Kharaikh River (Op4, 5, 6, 8, 9).

Prevalence and intensity of infection: *O. potanini* in Zavkhan River–3 of 3 fish, 1 worm per fish; *O. potanini* in Chono Kharaikh River 3 of 14 fish, 1–3 worms per fish; *Barbatula conilobus* in Zavkhan River–1 of 9 fish with one worm.

Specimens deposited in museum collection: KN.372754–KN.372761.

Description of specimens linked with ITS DNA (Figure 4, Supplementary S5): Anchor 67–71 µm; anchor root 22–25 µm, anchor shaft pronounced, 48–51 µm and point 30–32 µm. Ventral bar 4–6 × 26–29 µm, with short processes and membrane, 14–16 µm long. Dorsal bar thin, 2–2 × 25–34 µm. Marginal hook 27–31 µm, with sickle 7–9 µm.

Molecular sequence data: ITS length 1141 bp, GenBank accessions OQ641775–OQ641778 and OQ916395–OQ916398. Fragments of the mitochondrial *cox*1 (940 bp) from Zavkhan (Oz1, 3) were deposited as OQ661867 and OQ661868. They differed by 12 transitions and 4 transversions (d_{MCL} = 0.021), suggesting a permanently large population of the parasite.

Remarks: This species corresponds well with the description of the parasite on the nominal host in Khar Lake of the Zavkhan River system [18].

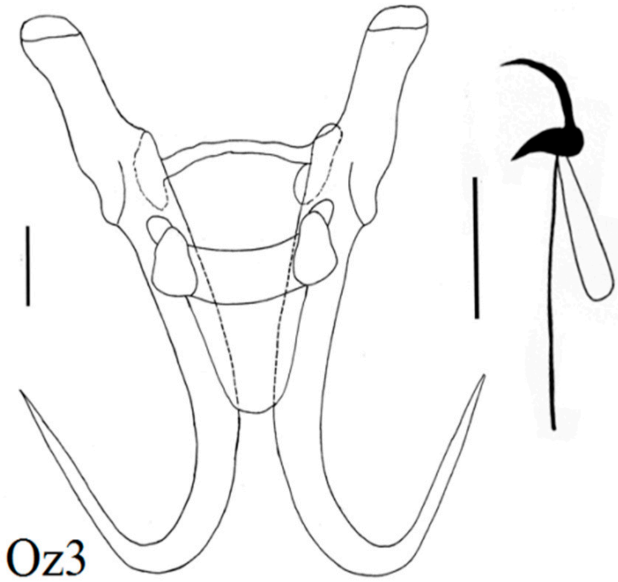

Figure 4. *Gyrodactylus oreoleucisci* Ergens and Dulmaa, 1970 on *Oreoleuciscus potanini*. Scale bars 10 µm.

***Gyrodactylus nordmanni* Ergens and Dulmaa, 1970**

Type host: Altai osman *Oreoleucisus pewzowi* Herzenstein, 1883 (Valid name is *Oreoleuciscus potanini* (Kessler, 1879)) in lake Sangiyn Dalay, Arctic Ocean basin, Mongolia.

Present host: Dwarf Altai osman *Oreoleuciscus humilis* in Tuin River, Valley of Lakes (Ot1, 3, 4, 5, 6).

Prevalence and intensity of infection: 4 of 10 fish, 1–2 worms per host.

Specimens deposited in museum collection: KN.372762–KN.372766.

Description of the specimens linked with ITS DNA (Figure 5, Supplementary S6): Anchor 65–71 µm; anchor root 20–24 µm, anchor shaft pronounced 49–55 µm and point 31–33 µm. Ventral bar 5–7 × 25–27 µm, with short processes and medium triangular membrane, 14–17 µm long. Dorsal bar delicate, 1–2 × 16–21 µm. Marginal hook 28–28 µm, with sickle 8–9 µm.

Molecular sequence data: ITS length 1191 bp, GenBank Accessions OQ641779–OQ641782. Mitochondrial *cox*1 (full length, based on Ot1, 3, 4, 5, and 6) was deposited as OQ661869. It was noteworthy that all five *cox*1 sequences were identical, as a contrast to the next relative *G. oreoleucisci*, where two sequences differed by 2%.

Remarks: The species *G. nordmanni* was originally described from gills of *Oreoleucisus pewzowi* in lakes Sangiyn Dalay and Khar by Ergens and Dulmaa [18]. Slynko et al. [6] included the fish in these waters to the species *O. humilis*. Our parasite specimens had slightly larger anchors compared to data reported in Ergens and Dulmaa [18]. They were morphologically very close to the *G. oreoleucisci* in Khar Lake of the Zavkhan River system, but genetically distinct in the ITS (Figure 2). The *cox*1 distance between *G. oreoleucisci* and *G. nordmanni* was 19.8% (See Section 4.3).

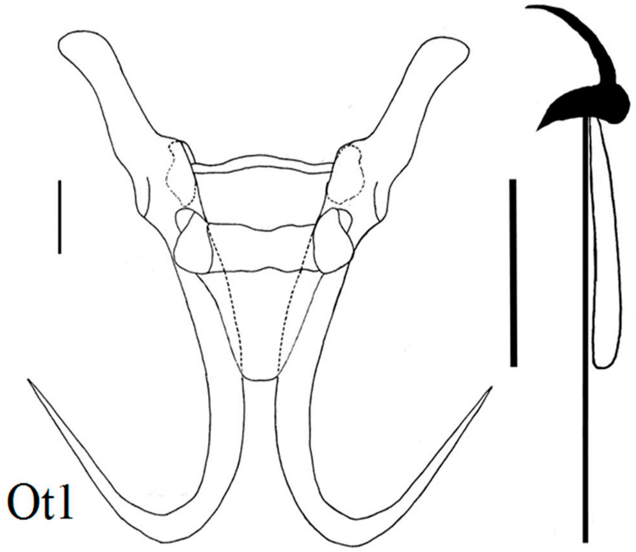

Figure 5. *Gyrodactylus nordmanni* Ergens and Dulmaa, 1970 on *Oreoleuciscus humilis.* Scale bars 10 µm.

Subgenus *G.* (*Limnonephrotus*), nemachili group.

***Gyrodactylus nemachili* Bychowsky, 1936.**

Type host and locality: Gray loach *Nemachilus dorsalis*, (valid name *Triplophysa dorsalis* (Kessler, 1872)), Kyrgyzstan.

Present host and locality: *Barbatula* sp., probably *B. cobdonensis,* Chono Kharaikh River (Lp1, Lp2, Lp4, Lp5, Lp6).

Prevalence and intensity of infection: five of nine fish, one worm per host.

Specimens deposited to museum collection: KN.372767–KN.372771.

Description of the specimens linked with the ITS DNA (Figure 6, Supplementary S7): Anchor 35–39 µm long with folded root; anchor root 6–9 µm; anchor shaft 21–22 µm and point 31–32 µm. Ventral bar 7–10 × 19–22 µm, with small processes and medium rounded membrane, 12–18 µm long. Dorsal bar 2–3 × 20–24 µm. Marginal hook 23–26 µm, with sickle 5–6 µm.

Molecular sequence data: ITS length 1267 bp, GenBank accessions OQ641770–OQ641772.

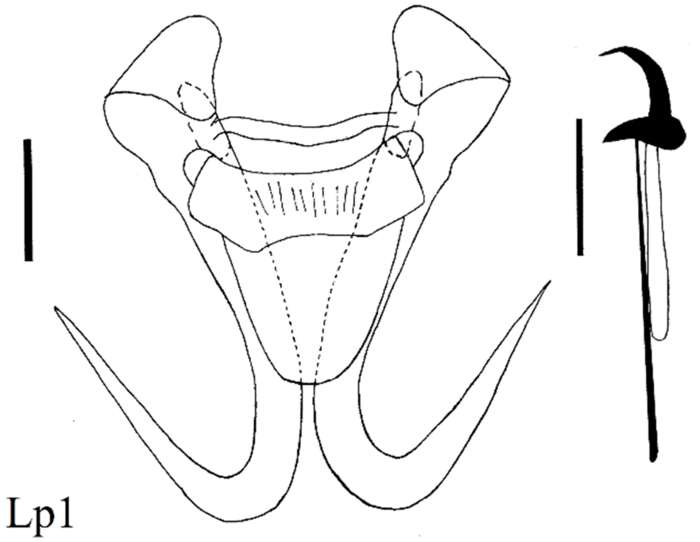

Figure 6. *Gyrodactylus nemachili* Bychowsky, 1936 on *Barbatula cobdonensis*. Scale bars 10 µm.

***Gyrodactylus pseudonemachili* Ergens and Bychowsky, 1967**

Type host: Gray loach *Nemachilus dorsalis* (valid name *Triplophysa dorsalis* (Kessler, 1872)), Kyrgyzstan.

Present hosts and localities: *Barbatula sp.,* probably *B. conilobus,* Zavkhan River, above Tayshir reservoir (Lz1, 11, 13, 15, 16, 17, 18), *Thymallus brevirostris*, Zavkhan river, above Tayshir reservoir (Mg3, 5, 7, 8), *Oreoleuciscus humilis,* Tuin River (Ot2).

Prevalence and intensity of infection: *B.* cf. *conilobus*–40%, 1–2 worms per fish; *T. brevirostris*–16%, 1 worm per fish; *O. humilis,* Tuin River–1 of 10 fish with 1 worm.

Specimens deposited to the museum collection: KN.372772–KN.372784.

Description of the specimens linked with ITS DNA (Figure 7, Supplementary S8): Anchor 58–65 µm; folded anchor root 20–25 µm; robust anchor shaft 40–45 µm and point 27–32 µm. Ventral bar 5–9 × 25–29 µm, with short processes and membrane, 17–22 µm. Dorsal bar 2–3 × 17–29 µm. Marginal hook 26–29 µm, with sickle 6–7 µm. There is a paired snail-like structure near the dorsal bar (similar to *G. zavkhanensis* sp. nov.), which is not mentioned in earlier descriptions.

Molecular sequence data: ITS length 1242 bp, GenBank accession OQ641755–OQ641767. The full 1548 bp mitochondrial *cox*1 of the specimen Ot2 (host *Oreoleuciscus humilis*, Tuin river) was deposited as OQ661866.

Remarks: The species was found in two distant sampling sites and on three hosts. It is closest in dimensions and morphological features to *G. pseudonemachili* which was originally described from the gills of *Nemachilus dorsalis* from Kyrgyzstan by Ergens and Bykhovsky [43]. Later, this name was used for monogeneans found on gills, fins, skin, and in nasal cavities of *Nemachilus strauchi* (Kessler, 1874), *Barbatula toni* Linnaeus, 1758, and *Oreoleuciscus humilis* in different water bodies of Mongolia [44,45]. The name was previously attached to an ITS barcode AJ567674, but far away from the provenience of the type. Species *G. pseudonemachili* was described in Asia, and the name was adopted for a species from the Czechia [30]. Both taxa are included in Supplementary Figure S1 and in the phylogenetic hypothesis in Figure 2. We suggest that the European species should be renamed.

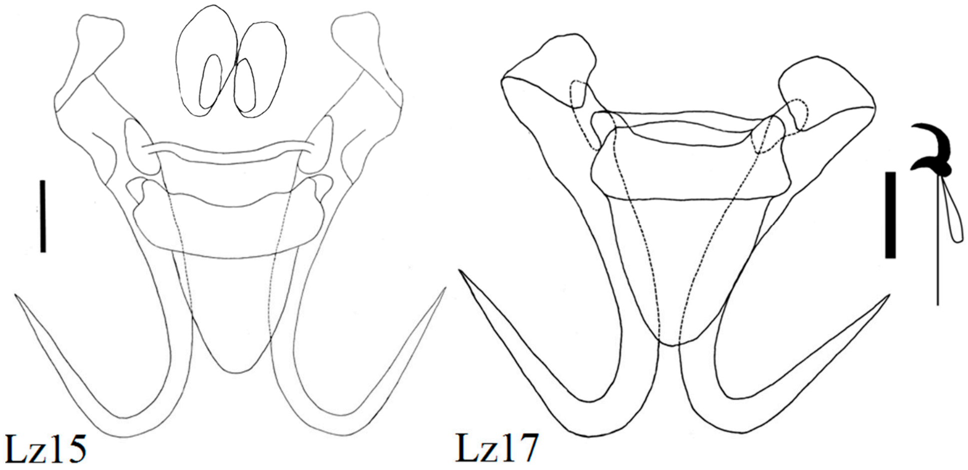

Figure 7. *Gyrodactylus pseudonemachili* Ergens and Bychowsky, 1967 on *Barbatula conilobus*. Specimen Lz17 has deep folds of anchor. Specimen Lz15 has more open folds of anchor. Scale bars 10 µm.

Gyrodactylus mongolicus **Ergens and Dulmaa, 1970**

Type host and locality: Dwarf Altai osman *Oreoleuciscus humilis,* River Teysin (Tess), Ubsunur (Uvs) Hollow, Big Altai range, Mongolia.

Present host and localities: *Oreoleuciscus potanini,* Zavkhan River (Oz2, 5), Chono Kharaikh River (Op3, 7), in the Great Lakes Depression.

Prevalence and intensity of infection: in Zavkhan River–1 of 3 fish with 2 worms; in Chono Kharaikh River–2 of 14 fish; 1 worm per fish.

Specimens deposited: KN.372785–KN.372789.

Description of the specimens linked with ITS DNA (Figure 8, Supplementary S9): Anchor 56–61 µm with folded root; anchor root 13–14 µm, anchor shaft 42–46 µm, point 29–30 µm. Ventral bar 6–9 × 27–28 µm, with short processes and medium membrane 17–19 µm. Dorsal bar 3–4 × 29–31 µm. Marginal hook 31–32 µm, with sickle 7–8 µm.

Molecular sequence data: ITS length 1242 bp, GenBank Accessions OQ641768–OQ641769 and OQ913866–OQ913868. A 1518 bp fragment of *cox*1 was deposited as OQ661879.

Remarks: The morphology of the specimens, the host, and the type locality agree with the original description of Ergens and Dulmaa [18], repeated in Figure 474 in Pugachev et al. [23].

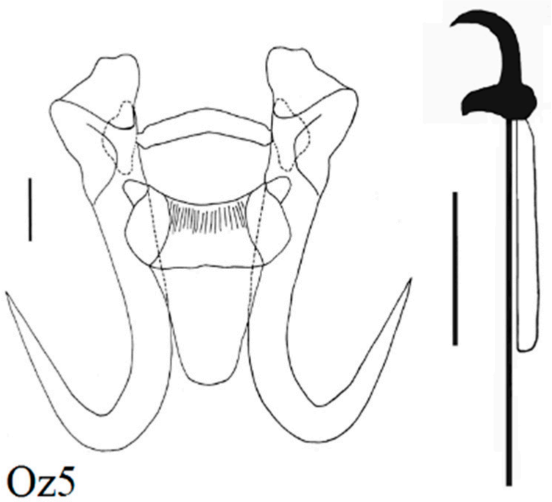

Figure 8. *Gyrodactylus mongolicus* Ergens and Dulmaa 1970 on *Oreoleuciscus potanini*. Scale bars 10 µm.

***Gyrodactylus tayshirensis* sp. nov.**

Type host and locality: Stone loach *Barbatula conilobus* Prokofiev 2016, Zavkhan River (Lz7), Mongolia. One worm was found on eleven fish.

Specimen deposited in museum collection as a holotype–KN.372790.

ZooBank registration: the Life Science Identifer (LSID) for *Gyrodactylus tayshirensis* sp. nov. is urn:lsid:zoobank.org:act:A99706C6-71E6-4492-AAE1-8DB7FFEB835B.

Description (Figure 9, Supplementary S10): Anchor robust 52 µm long with folded root; anchor root 20 µm, anchor shaft 42 µm and point 27 µm. Ventral bar 8 × 27 µm, with short processes and a pronounced membrane 17 µm long. Dorsal bar 3 × 23 µm. Marginal hook 30 µm, with sickle 7 µm.

A genus-specific note. The rules of taxonomy were created long before molecular confirmation was possible. A minimum of three specimens was needed to describe a species. In fact, a *Gyrodactylus* worm represents a mother, a daughter, and a granddaughter, so three specimens. Furthermore, the worm is optionally hermaphroditic, thus representing both sexes. As a logical consequence, we dare to name this species and the next one based on an apparently single specimen.

Molecular sequence data: ITS length 1164 bp, GenBank Accession OQ641774. It was noteworthy that the large ITS1 insertion, which had various but related forms in the four other Mongolian species of nemachili group (Supplementary Figure S1), was completely missing in the specimen Lz7. It was also missing in the Xinjiang parasite (MH445967–8) and in all European species (including the European *G. pseudonemachili*). The insertion was thus a molecular synapomorphy of the four nemachili group species in The Hollow, suggesting their common invading ancestor and subsequent diversification.

Etymology: The species is named after the name of Tayshir reservoir lake in River Zavkhan where the parasite was found.

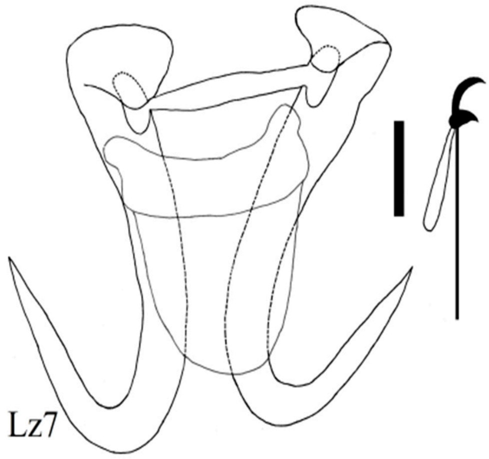

Figure 9. *Gyrodactylus tayshirensis* sp. nov. on *Barbatula conilobus*. Scale bar 10 µm.

***Gyrodactylus zavkhanensis* sp. nov**

Type host and locality: Mongolian grayling *Thymallus brevirostris*, Zavkhan River, Mongolia (Mg2). One parasite was found in twenty-five fish.

Specimen deposited in the museum collection as a holotype–KN.372791.

ZooBank registration: the Life Science Identifer (LSID) for *Gyrodactylus zavkhanensis* sp. nov is urn:lsid:zoobank.org:act:2217E71A-76AF-4FA6-885D-F2934420FD96.

Description (Figure 10, Supplementary S11): Anchor 52 µm; anchor root 24 µm, anchor shaft 43 µm and point 28 µm. Ventral bar 5 × 28 µm, with short processes and membrane 18 µm long. Dorsal bar delicate, 2 × 27 µm. Marginal hook 27 µm, with sickle 6 µm. There is a paired snail-like structure near to the dorsal bar, which is not mentioned in earlier descriptions.

Molecular sequence data: ITS length 1194, GenBank accession OQ641773.

Etymology: The species was named after the river–Zavkhan (also Dzavhan or Dzabkhan)–where the parasite was found.

Remarks: The parasite is morphologically and metrically very close to the Mongolian species *G. pseudonemachili* defined here, but the ITS is clearly distinct (Figure 2, Supplementary Figure S1).

Ecological remark: The nemachili group species are characteristically parasites of the stone loach family (Nemacheilidae/Barbatulidae), while in the present Mongolian collection we had several cases where they were collected from other hosts. This was already observed by Ergens and Dulmaa [18]. This may indicate the relaxation of host specificity (possibly during the environmental catastrophe), and deserves further investigation.

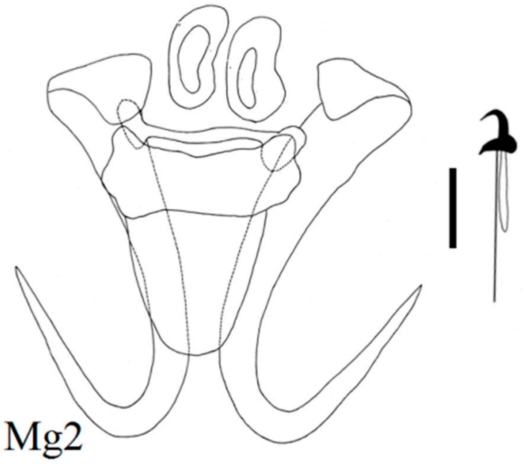

Figure 10. *Gyrodactylus zavkhanensis* sp. nov. on *Thymallus brevirostris*. Scale bar 10 µm.

Hybrid *G. nemachili x G. mongolicus*

Host and locality: One hybrid parasite was found among nine specimens of *Barbatula cobdonensis*, Chono Kharaikh River (Lp3).

Specimen deposited in the museum collection: KN.372792.

Description of the specimen confirmed by ITS (Figure 11, Supplementary S12): Anchor 58 µm long with fold; anchor root 23 µm, anchor shaft pronounced, 28 µm and point 37 µm. Ventral bar 5 µm × 27 µm, with short processes and membrane 17 µm long. Dorsal bar 3 × 20 µm. Marginal hook 33 µm, with sickle 10 µm.

Molecular sequence data: Molecular sequence of the specimen Lp3 was a combination of two different sequences and blurred after the heterozygous indel both with forward and reverse primers, illustrated in Supplementary Figure S2. The sequence in Supplementary Figure S1 is a reconstruction of the sequence from forward and reverse readings. In the alignable segments there were 21 heterozygotic sites (1.7%), fitting with the differences between *G. nemachili* and *G. mongolicus*. The parental lineages were present in the same population, while observed on different hosts. Considering the viviparity, a hybrid represents from the beginning a clone: a daughter born from the fertilized egg, and the apomictic granddaughter. When the clone continues the parthenogenetic propagation, the proportions of the maternal and paternal genomes remain equal, as demonstrated in the upper panel in Supplementary Figure S2. Thus, the hybridization was not necessarily recent, and the hybrid clone was sympatric with the parental lineages. There was no hint of free or general sexual reproduction among the parasites in the Chono Kharaik River.

Remark: The haptor dimensions of the hybrid were all as large or larger than in the larger parent, *G. mongolicus*. Anchor root folding was more open than in both parents.

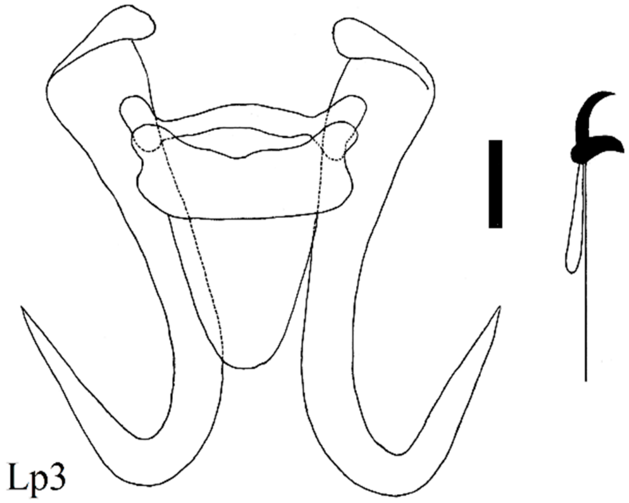

Figure 11. *Gyrodactylus* hybrid *G. nemachili* × *G. mongolicus* on *Barbatula cobdonensis*. Scale bar 10 µm.

Taxonomic remark: After adding five Mongolian and one Chinese species of *nemachili* group to the global phylogenetic hypothesis of now nine species (Figure 2 and Supplementary Figure S1), some classical taxonomic remarks were necessary. The group indeed needs molecular clarification, due to wide geographical distribution and a highly variable host spectrum of stone loaches (FishBase, https://www.fishbase.se/, accessed on 1 June 2023).

The name *G. papernai* was finally (sic) associated with a parasite on *Barbatula barbatula* in the type locality in Czechia (AJ407877 + AJ407925). These ITS1 and ITS2 sequences were first published as *G. jiroveci* Ergens and Bychowsky, 1967 by Matějusová et al. [28] and corrected to *G. papernai* Ergens and Bychowsky, 1967 by Přikrylová et al. [30]. Consequently, the same two names were used for a parasite on *Barbatula barbatula* in Finland and in Vidlitsa, Ladoga, where it was observed as an accidental visitor on *Salmo salar* Linnaeus, in 1758. It was first named *G. jiroveci* in Ziętara and Lumme [24]; but corrected to *G. papernai* in Ziętara et al. [27], following the Czech authorities. This example shows the power (and pain) of DNA barcoding: the taxonomy has developed into a self-correcting science, and the authority of the "world specialists" is given to GenBank. *G. papernai* is a synonym of *G. jiroveci* still in Pugachev et al. [23], but two separate taxa with these names are in the phylogenetic hypotheses since Přikrylová et al. [30].

Sixty million year old subgenus *Gyrodactylus* (*Gyrodactylus*) Malmberg 1970

The phylogenetic hypothesis of the subgenus *G.* (*Gyrodactylus*) in Figure 12 includes the African clade (*G. nigritae* Přikrylová, Blazek and Vanhove, 2011, *G. rysavyi* Přikrylová, Blazek and Vanhove, 2011, *G. alekosi* Přikrylová, Blazek and Vanhove, 2011, *G. synodonti* Přikrylová, Blazek and Vanhove, 2011) [31], because they are among the bridge species connecting our phylogenetic hypothesis with that of Boeger et al. [46]. Into the phylogenetic tree displayed in Figure 12, we also included two Far East Asian clades, macracanthus and granoei groups presented by Reyda et al. [47]. One unnamed Indian species (KM434259) was also added to show the range of the geographical distribution.

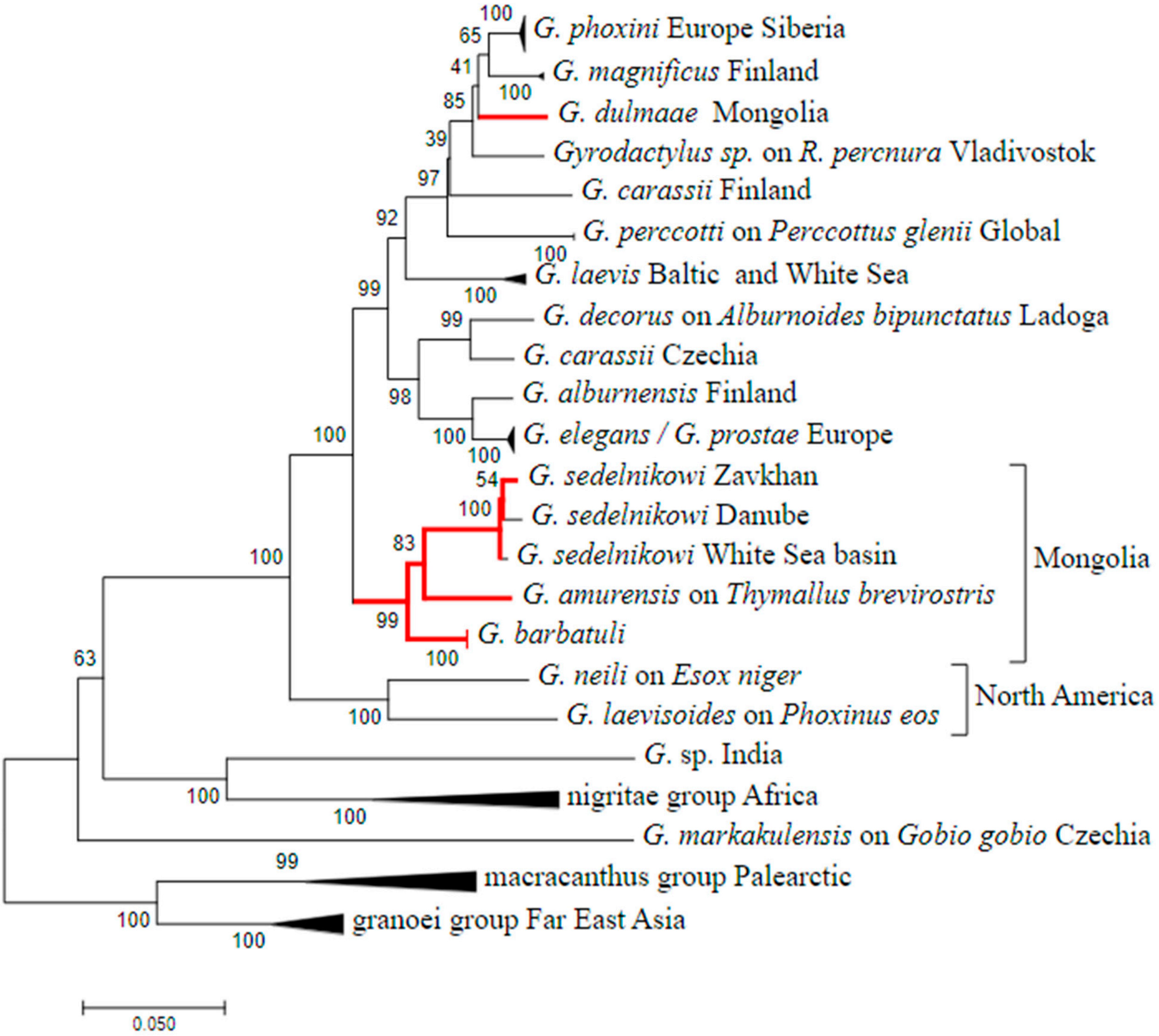

Figure 12. The phylogenetic hypothesis of subgenus *G.* (*Gyrodactylus*) based on the ITS1 -5.8S -ITS2. A total of 65 nucleotide sequences formed the 1138 sites long alignment. The compressed clades were described earlier [31,47].

Gyrodactylus dulmaae **Ergens, 1970**

Type host and locality: *Barbatula toni*(*Nemachilus barbatula toni*) in different places of Mongolia (not strictly defined).

Present host and locality: Skin of O*reoleuciscus potanini,* Chono Kharaikh River (Op1). One parasite was found on fourteen fish.

Specimen deposited in museum collection: KN.372793.

Description of the single specimen linked with ITS DNA (Figure 13, Supplementary S13). Anchor 34 µm; anchor root 12 µm, bends ventrally; anchor shaft pronounced, 28 µm and point 16 µm. Ventral bar 6 × 11 µm, with short processes and narrow triangular membrane, 11 µm. Dorsal bar delicate, 1 × 7 µm. Marginal hook 21 µm, with sickle, 5 µm.

Molecular sequence data: ITS length 912 bp, GenBank Accession OQ641791.

Remarks: This specimen corresponded well with the type described by Ergens [48]. Ermolenko [49] reported *G. dulmaae* on gills of *B. toni* in waterbodies of Southern Maritime Territory, Russia. Pugachev [44] identified it in the nasal cavity of *Phoxinus phoxinus* from the Selenga River.

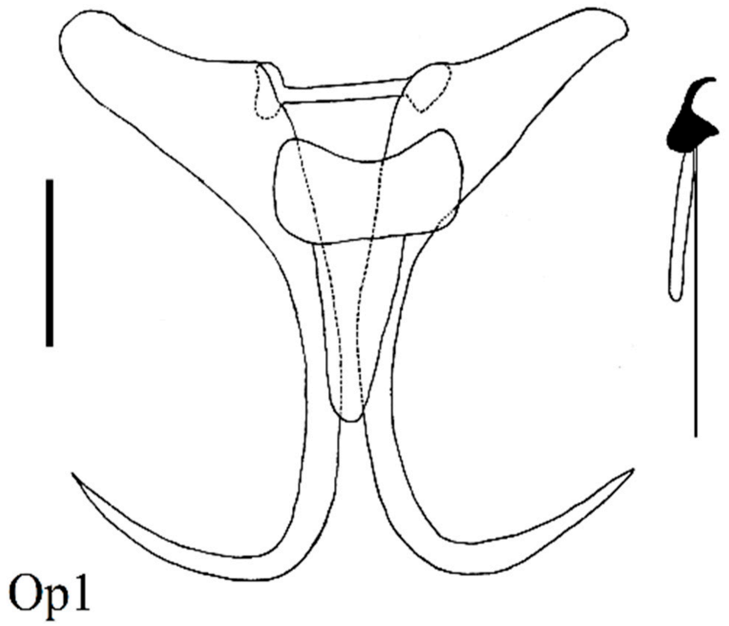

Figure 13. *Gyrodactylus dulmaae* Ergens, 1970 on *Oreoleuciscus potanini*. Scale bar 10 µm.

Gyrodactylus sedelnikowi **Gvozdev, 1950**

Type host and locality: *Barbatula toni markakulensis* (nomen dubium), Lake Markakul, Altai Mountains, Kazakhstan (former Soviet Union).

Present host and locality: Stone loach *Barbatula conilobus* in Zavkhan River, below the Tayshir Reservoir (Lz2). One worm was found on eleven fish.

Specimen deposited in museum collection: KN.372794.

Description (Figure 14, Supplementary S14): Anchor 38 µm; broad anchor root 11 µm; anchor shaft 30 µm, point 19 µm. Ventral bar 7 × 15 µm, without processes, and medium narrow membrane, 14 µm. Dorsal bar 2 × 6 µm. Marginal hook 20 µm with sickle 7 µm.

Molecular sequence data: ITS length 904 bp, GenBank Accession OQ641800.

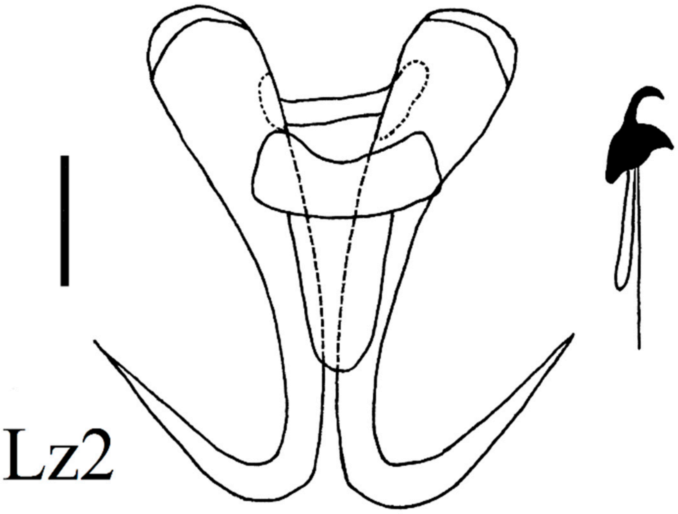

Figure 14. *Gyrodactylus sedelnikowi* Gvozdev, 1950 on *Barbatula conilobus*. Scale bar 10 µm.

Remarks: The morphology and dimensions of the parasite corresponded well with the type *G. sedelnikowi* in Lake Markakol in the Irtysh watershed, Kazakhstan [48]. It has been reported on the gills of *B. barbatula*, *B. toni*, and *B. toni markakulensis,* as widespread. Two described species have been suggested to be synonyms: *G. amurensis*, Akhmerov, 1952, and *G. dubious*, Roman, 1956 [23]. Below, we tentatively suggest a valid species status for *G. amurensis*.

Through comparison of the ITS sequence, the Mongolian parasite is close enough to be judged as conspecific with *G. sedelnikowi* on *B. barbatula* from Vlára River, (AJ407891 + AJ407935, Danubian Basin) [28] and on *B. barbatula* from River Bolshie Kozli (OQ672277, Arkhangelsk Region, White Sea Basin, Russia). The parasite from the type locality Lake Markakul is not barcoded, and the host species spectrum needs updating [12,50,51].

***Gyrodactylus amurensis* Akhmerov, 1952**

Type host and locality: Stone loach *Nemachilus barbatulus*, Lake Bolon', Amur basin, Habarovsk region, Russia

Present host and locality: *Thymallus brevirostris,* Zavkhan River, Mongolia. One parasite was found on twenty-five fish examined.

Specimen deposited: KN.372795.

Description of the specimen linked with ITS DNA (Figure 15, Supplementary S15) based on one specimen Mg4: Anchor 34 µm; wide anchor root 12 µm; anchor shaft 28 µm and point 16 µm. Ventral bar 6 × 16 µm, with a membrane 15 µm long. Dorsal bar delicate 2 × 10 µm. Marginal hook 21 µm, with sickle 6 µm.

Molecular sequence data: ITS length 911 bp, GenBank Accession OQ641801.

Remarks: *G. amurensis* is the first species of subgenus *G.* (*Gyrodactylus*) ever reported on a salmonid, and the third non-wageneri (*G. papernai* and *G. arcuatus* were reported as accidentals [27]).

The specimen Mg4 corresponded well with *Gyrodactylus amurensis* which was described by Akhmerov [52] from the gills of *Nemachilus barbatulus* from Bolon' Lake. Morphologically, *G. amurensis* was most close to *G. sedelnikowi* and has been considered as its junior synonym [23]. The ITS made a clear distinction (Figure 12; Supplementary Figure S3). This allows us to resurrect the species *Gyrodactylus amurensis* Akhmerov, 1952 and confirm the first case of its presence in The Hollow. Some uncertainty remains, and barcoding of the parasites from the type of area should be performed.

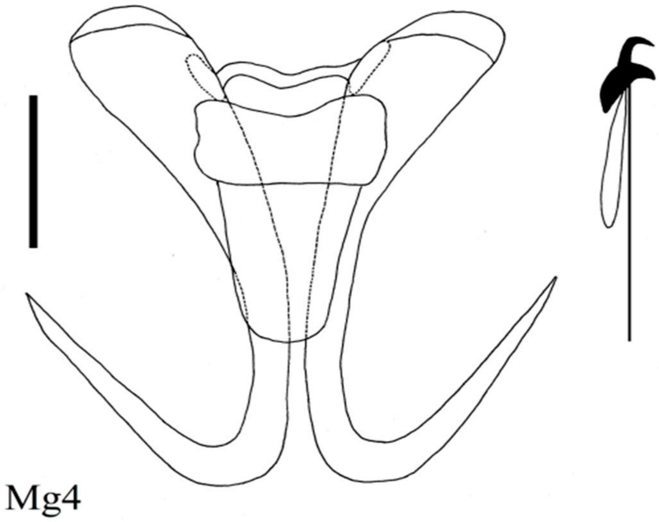

Figure 15. *Gyrodactylus amurensis* Akhmerov, 1952 on *Thymallus brevirostris*. Scale bar 10 µm.

Gyrodactylus barbatuli **Akhmerov, 1952**

Type host and locality: Stone loach *Nemachilus barbatulus* Lake Bolon', Amur basin, Habarovsk region, Russia.

Present host and locality: Skin of *Barbatula conilobus* Prokofiev, 2016 [12]. Zavkhan River below the Tayshir Reservoir, Mongolia. In this site, all 15 fish were infected carrying 1–7 worms per host specimen.

Curiously, the present specimens from one locality were easily classified into the two "forms" (size classes) suggested in the previous treatments [23].

Specimens deposited: KN.372796–KN.3727801.

Descriptions (Figure 16, Supplementary S16):

Specimens fitting with Forma typica (Lz 6, 8, 9, 10): Anchors 72–73 µm; anchor root 22–25 µm, anchor shaft pronounced, 54–55 µm, point 30–32 µm. Ventral bar 10–11 × 24–28 µm, without processes; membrane 25 µm. Dorsal bar 2–3 × 20–22 µm. Marginal hook 25–28 µm, with sickle 7–8 µm.

Specimens fitting with Forma B (Lz4, 5): Anchors 88–89 µm; anchor root 26–29 µm, anchor shaft pronounced, 69–71 µm, point 38–41 µm. Ventral bar 14–15 × 30–33 µm, without processes; membrane 28–30 µm. Dorsal bar 3 × 26–27 µm. Marginal hook 28–29 µm, with sickle 8–9 µm.

Molecular sequence data: ITS length 910 bp, GenBank Accession ITS (OQ641794–OQ641799) based on Lz4, 5, 6, 8, 9, 10, representing both size classes.

Remarks: The specimens corresponded well with the separate types described on the nominal hosts [23]. There was no variation in the ITS sequence. The species has been recorded in River Selenga, lake Terkhiin Tsagaan and other water bodies in Mongolia [45,48]. It was also reported from Lake Onega in Russian Karelia [53] and on an atypical host *Leuciscus idus* in the Yenisey River [37]. The morphospecies seems to be widespread in Eurasian water bodies on *Nemachilus* or *Barbatula*.

There was an interesting phylogenetic observation concerning the opisthaptor dimensions in the subgenus *G.* (*Gyrodactylus*). The three species *G. sedelnikowi*, *G. amurensis,* and *G. barbatuli* were very closely related in the ITS phylogeny, but morphologically problematic. The former two (as well as *G. dulmaae*) were small, with anchor lengths of 34–38 µm, but the anchors of *G. barbatuli* were not only variable but also twice as long, 72–89 µm. Correspondingly, the marginal hooks of the small species were 20–21 µm, but 25–29 µm in *G. barbatuli.* The habitus of the haptor of *G. barbatuli* is not truncated as typical for the subgenus, but the marginal hook is diagnostic to the subgenus.

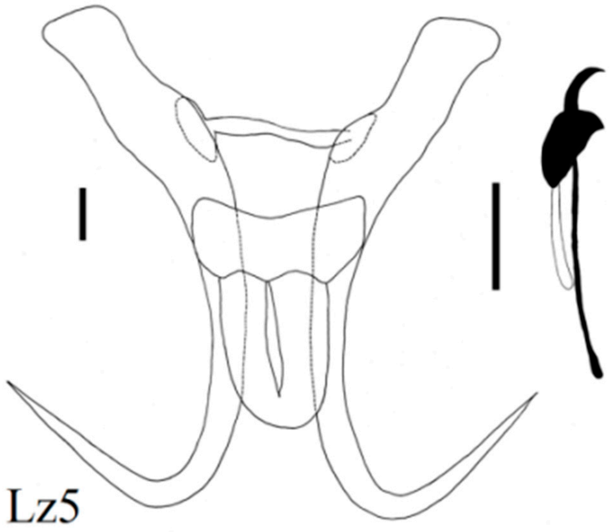

Figure 16. *Gyrodactylus barbatuli* Akhmerov, 1952 on *Barbatula conilobus*. Scale bars 10 µm.

4. Discussion

4.1. The Two Freshwater Subgenera of Gyrodactylus Were Defined by Malmberg 1970

A molecular synapomorphy of the subgenus *G.* (*Limnonephrotus*) (Figure 2) is the extremely conservative structure of “four fingers” in the ITS2. The first three fingers (hairpins) are defined by a forward motif GTATTACACG, and the fourth by a modified CATACACG. According to Boeger et al. [46], the age of the subgenus *G.* (*Limnonephrotus*) is less than 30 Million years. The bridge species connecting their phylogeny with ours are *G. rutilensis* Gläser, 1974 (possible syn. of *G. gracilihamatus* Malmberg, 1964), *G. rhodei* Žitňan, 1964, *G. salaris* Malmberg, 1957, *G. salmonis* and G. *gobiensis* Gläser, 1974. The present extensions of the subgenus to the Russian Far East, China, and Japan define the distribution as mostly Palearctic, with only a few species in North America.

In the subgenus *G.* (*Gyrodactylus*), the ITS1–5.8S–ITS2 segment is clearly shorter than in other subgenera, with less than 912 bp among the four Mongolian species. Another molecular synapomorphy is a motif AAACACAACACA in the ITS2 causing two small extra hairpins in the final loop of the secondary structure. The African clade (*G. nigritae*, *G. rysavyi*, *G. alekosi,* and *G. synodonti*), *G. carassii* Malmberg, 1957 from Europe and *G. sedelnikowi* Akhmerov, 1952 from Europe and Mongolia were the bridge species connecting our phylogenetic hypothesis with that of Boeger et al. [46], who estimated the age to be about 60 Million years. Only two species are known from North America.

In the phylogenetic tree displayed in Figure 12, we also included two Far East Asian clades, macracanthus and granoei groups presented by Reyda et al. [47]. As a curiosity, the macracanthus clade contains the frog parasite *G. jennyae* Paetow, Cone, Huyse, McLaughlin and Marcogliese, 2009, which was imported to North America together with two other Far East Asian parasite species by the globally invasive Asian fish *Misgurnus anguillicaudatus* (Cantor, 1842) [47].

4.2. The Faunal Poverty of The Hollow

The orogenic changes isolated the Central Asian lowland from all in- and outflows and main watery connections by the end of the Middle Pleistocene [2]. Prior to this, the paleontological data show that the ichthyofauna was typical Euro-Siberian, and even the present observations on the Monogeneans support these findings. The parasite fauna contains representatives from diverse segments of the phylogenetic trees, in *G.* (*Limnonephrotus*) from three species groups, and in *G.* (*Gyrodactylus*), from two. We may conclude that at least five independent introductions of parasites had occurred prior to the final isolation, but most probably, many more.

In the comparison of the earlier faunistic information (Supplementary Table S4), eight parasite species were common in the old collection of seventeen species, and in the new collection of twelve. Three new species were described. One name was adopted from the Amur basin. Ten species that were reported earlier were not found or we identified them with another name. Four names used by earlier authors in Mongolia were recorded and barcoded in Europe, two of them synonymized, and one used for at least two taxa.

The scarcity of the wageneri group species was obvious. While this group dominates in the faunistic collections in Europe [24,28–30], partly guided by the interest in salmonid parasites, it was represented by one species only in The Hollow, *G. radimi* sp. nov. We conclude that the decline and destruction of the lacustrine fish fauna severely affected the wageneri group.

The prevalence and intensity of *Gyrodactylus* infections among the Mongolian fish samples were in general very low. Even the multispecies infections were apparently well below any pathogenic threshold. This corresponds with the authors' observations in Northern Europe. The status quo exists among undisturbed host–parasite communities.

4.3. Endemic Diversification

The indications of endemic diversification in The Hollow are clear. Among the nemachili group, the five Mongolian species were a monophyletic clade, including no insertion in *G. tayshirensis* sp. nov., a shared short insertion in *G. zavkhanensis* sp. nov., *G. pseudonemachili* and *G. mongolicus,* and the longest insertion in *G. nemachili* (Supplementary Figure S1).

The two species of the macronychus group, *G. oreoleucisci* and *G. nordmanni* were not very close (*cox*1 d_{MCL} = 0.198), but they were more related to each other than to other members of the minnow (*s. lato*) specific macronychus group, which has species both east and west of Mongolia.

In the subgenus *G.* (*Gyrodactylus*), the Mongolian species *G. sedelnikowi, G. amurensis*, and *G. barbatuli* formed a trio, suggesting endemic diversification in The Hollow. These three species' names were adopted from descriptions outside of The Hollow, and the species deserve more detailed investigations. As upstream-dwelling fauna, the stone loaches and their parasites appear to be isolated and differentiating populations. At least *G. sedelnikowi* crossed the Altai mountain range to reach Lake Markakol and Europe (or vice versa). The fourth Mongolian species *G. dulmaae* was quite close to *G. phoxini*, which was found on *Phoxinus* in Europe and in Tuul River in Mongolia.

When The Hollow was drying historically, the water chemistry also changed [2]. The deteriorating limnological conditions destroyed the lake fauna, but the fish species which had been living in the rivers, running partially from glaciers, survived. A direct quote from Dr. Eugenya Sytchevskaya: "Thus inhabitants of fast-flowing, mostly cold, well-aerated streams with gravel bottoms, *Thymallus brevirostris* and *Nemachilus barbatulus* (sic), as well as representatives of the eurybiont genus *Oreoleuciscus* became common" [2]. The taxonomy of the Nemacheilidae is in continuous turbulence. Our samples were tentatively named *Barbatula conilobus* (Zavkhan River) [12] and *Barbatula cobdonensis* (Chono Kharaikh) [54], increasing our sample to five fish species, but the loaches came from the field by the name *Orthrias barbatulus toni* [55].

The present ichthyofauna of The Hollow, as well as the fossil list of Sytchevskaya [2–4] is missing representatives of Cottidae, Cobitidae and the riverine cyprinoids *Phoxinus*, *Eupallasella* and *Rhynchocypris*.

After the isolation, the drying climate led to the decrease in the lake water level by more than 200 m. The Great Lakes Depression and the Valley of Lakes were connected 5.5 million years ago as a single large lake [6]. In the eastern part, the river Tuin now runs in sand in the lake Orog Nuur and is completely isolated from our other sampling sites. Only one fish species, but two parasite species were found in Tuin, and the ITS of *G. pseudonemachili* was identical in Tuin and in the other sampling sites and hosts.

On the other side of the mountains, the main watersheds of rivers Irtysh, Ob, and Yenisei join and drain at present east of Ural, but they were draining to a huge proglacial Komi Ice Lake in the West Siberian plain only 90–80 kyr ago. The large lake directed the Siberian waters west to Ural and drained southwards, to the Aral Lake and Caspian Sea [56–58]. The Komi Lake also connected the Baltic and White Sea refugial fauna with the Siberian Fauna, explaining the distribution of *G. aphyae*, *G. albolacustris,* and *G. phoxini* in Europe and Tuul River near Ulaanbaatar on *Phoxinus phoxinus* (dubious name) [10]. The genetic divergence of the mitochondrial *cox*1 gene of the parasites between Tuul in Mongolia and Ladoga was 8.4–11.8% (Table 2), of the same magnitude as the divergence of 9% between the *Phoxinus* populations [59,60]. The GenBank accessions of *cox*1 for Mongolian *G. albolacustris* and *G. aphyae* were KU365756 and OQ661865, respectively.

The waters in rivers Tuin and Zavkhan are assumed to be separated by 5.5 million years [6]. The parasites *G. nordmanni* and *G. oreoleucisci* in Tuin and Zavkhan show a 22.5% divergence in the mitochondrial *cox*1, but the hosts *Oreoleuciscus humilis* and *O. potanini* differ by only 4.2% in the *cytochrome B*. This may suggest that the parasites originate from the mountain upwaters and have been separated much longer than the hosts connected in the great lake.

Table 2. Some genetic *p*-distance estimates between parasites and hosts between Europe and Asia, and within The Hollow, based on mitochondrial genes.

Species	Populations		mtDNA	Distance	Source
G. albolacustris	Tuul	Ladoga	*cox*1	8.4%	[61]
G. aphyae	Tuul	Ladoga	*cox*1	11.8%	Present study
G. nordmanni vs. *G. oreoleucisci*	Tuin	Zavkhan	*cox*1	19.8%	Present study
O. humilis vs. *O. potanini*	Tuin	Zavkhan	*cyt* B	4.2%	[6]
P. phoxinus	Tuul	Ladoga	*cyt* B	8.9–9.1%	[59]

Four parasite species were described earlier in Mongolia from the spined loach *Cobitis taenia* [14,38], which were not found in The Hollow, and the parasites were missing, too. From the sister genera *Phoxinus, Eupallasella,* and *Rhynchocypris* (see [11,62]), twelve *Gyrodactylus* species were determined, but these hosts and parasites too are missing in The Hollow.

Many of the fish species presented as hosts of the species in our global parasite phylogenies are treated in the available comprehensive phylogenetic analyses of the Eurasian fish: Cyprinidae [62–64], Salmonidae (especially *Thymallus*) [8,9,65], Cobitidae [66], Cottidae [67], and Nemacheilidae [68], *Pungitius* [69], *Esox* [70], *Sander* [71], Percidae [72] and *Lota* [73]. As of 14 May 2023, there were >1900 DNA sequence fragments named *Barbatula* in the GenBank, and we predict that a complete phylogeography of stone loaches may be possible in the near future.

4.4. Host Specificity Lost in Hard Times in the Compressed Ecosystem

The loss of host specificity of the nemachili group species was first pointed out by Ergens and Dulmaa [18], p. 10. In the description of *G. mongolicus* (nemachili group) as a parasite of *Oreoleuciscus potanini* in Khar Lake in the Zavkhan River system, they wrote:

> "[I]t is most difficult to explain the occurrence of *G. mongolicus* on the host *Oreoleuciscus humilis*, *O. pewzowi* and *O. potanini*, because this parasite is a typical member of the morphological group of the species *G. nemachili*, comprising parasites which are characteristic of several Cobitidae (sic) (*Nemachilus*, *Lefua*). It may only be suggested that some of these parasites started gradually to exchange their hosts living under the same ecological conditions and that this process

> led finally to a definitive adaptation to the new host both physiologically and morphologically. Never, however, did not find *G. mongolicus* on any species of the genus *Nemachilus* or possibly on any other genus of Cobitidae (sic).”

Confirmation of the observation of Ergens and Dulmaa may be the most significant scientific result of our investigations. Our specimens of *G. mongolicus* were indeed recorded on Altai osman, in two localities. This has been possibly a permanent (evolutionary) host switch. A complementary observation from our collection was that the most numerous nemachili group species *G. pseudonemachili* was found on *Oreoleuciscus*, *Thymallus*, and *Barbatula.* Only the morphologically variable *G. barbatuli* was found in one site and one host only, on *Barbatula conilobus.* However, the lost host specificity as a hypothetical response to the climatic catastrophe is an interesting scenario but needs sample sizes a magnitude larger than the present, and collections in many more rivers and creeks in The Hollow and surrounding mountain ranges. The variability of the ITS offers good opportunities to study large numbers of parasites by utilizing cost-effective PCR-RFLP identification.

4.5. A Conclusive Endnote

> “Clearly, the best guesses are not at all scientific results but can be considered as scientific hypotheses”. Kottelat in “Fishes of Mongolia” [10].

The above confession of the world fish specialist Maurice Kottelat, after spending a weekend in Central Mongolia, but years spent in the European museums which maintain the Mongolian specimens, fits extremely well with our current approach here.

Through our examination of only 49 parasite specimens, we extend the knowledge of the *Gyrodactylus* parasites of fishes in Mongolia very little. Radim Ergens and his collaborators inspected perhaps thousands of worms, and more fish species or isolates, and we feel ourselves painfully incompetent with the task. We missed several species found earlier in The Hollow. We tried to connect our specimens and barcodes the best we could with the names and descriptions provided by the classical authors, but sometimes it felt nearly impossible and resulted in what we consider to be an educated guess. We dared to give three new names when there was no possible correspondence with described species in The Hollow or in the territory of Mongolia. So, instead of an extension of the knowledge, we created some fixed points for future research.

Gyrodactylus is not an easy genus for taxonomists. However, the parasites which now are “tagged” or “barcoded” by molecular markers are linked to the global phylogeographic framework. Supported by the molecular barcoding, the descriptions connect the Central Asian Internal drainage basin of Mongolia with the outside world east and west, south and north, and help to understand the evolution of the two parasite subgenera in the continent. Unavoidably, the results of this study were only a rough outline of the whole phylogeography, but the data helped to scale future work. The resolution achieved through analysis of the *Gyrodactylus* ITS rDNA offers an interesting insight into the phylogeography of the genus. The parasites serve as a kind of epigenetic marker of the host, evolving faster and differentiating sharply via largely clonal propagation in the allopatric populations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15070860/s1, Table S1: List of *Gyrodactylus* species from Mongolia. GenBank and Museum accessions; Table S2: New ITS sequences obtained in present study for macronychus group of *Gyrodactylus* from Russian Far East; Table S3: New ITS sequences of the subgenus *G.* (*Gyrodactylus*); Table S4: List of *Gyrodactylus* species from The Hollow found previously and now; 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.

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