

Article

Unexpected Discovery of an Ectoparasitic Invasion First Detected in the Baikal Coregonid Fish Population

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Abstract: The omul, *Coregonus migratorius* (Georgi, 1775), an endemic fish of Lake Baikal, is the main commercial species of essential economic importance for the extensive region of Eastern Siberia. Despite the continuous 250-year study of Baikal omul and intensive commercial fishing, neither fishers nor biologists have ever mentioned or found external annelid parasites in numerous omul catches. Targeted parasitological studies have also never detected these ectoparasites on the body of this fish. In this study, we present the first reliable information on the existence of specific annelid parasites infecting the pelagic population of Baikal omul and report a 4% infestation of spawners in the Selenga River, the largest tributary of Lake Baikal. We also obtained the main morphometric parameters of the infected fishes, the morphology of the unknown parasite and its feeding strategy. DNA-based analysis was applied to ascertain the taxonomy of the leeches and revealed no closely related taxa existing in international genetic databases (e.g., GenBank) to date. A maximum genetic identity of 89–91% was detected with European *Caspiobdella fadejewi*. The genetic distances in 9–11% of the substitutions from the closest relatives were very high for a reliable molecular identification of a taxon. The Selenga omul leech may represent a potentially new species and genus.

Keywords: Lake Baikal; omul *Coregonus migratorius*; unknown leech parasitemia; DNA-based identification



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1. Introduction

Coregonid fishes (Coregoninae, Salmonidae) are the core elements of the estuarine and freshwater ecosystems of the Arctic Sea basin [1,2], especially in Siberia. The omul, *Coregonus migratorius* (Georgi, 1775), is the main species sought by the commercial fishery in Lake Baikal (Eastern Siberia). The number of omul in Baikal is currently declining, causing concern at the state level, so, starting from 2017, there has been a ban on commercial catches and amateur fishing for this species [3].

Baikal omul belongs to the group of pelagic multirakered (44–54 gill rakers) whitefish with a terminal mouth [4]. Consuming zooplankton, pelagic and benthic amphipods (and other benthic organisms), and larval and juvenile sculpins (Cottoidei), omul, along with the Baikal seal (*Phoca sibirica* Gmelin, 1788), is one of the end links in the food web of the lake's ecosystem. At the same time, omul can also refer to the lake–river migratory (semi-anadromous) whitefish of Siberia. The omul spends most of its life feeding in Lake Baikal and enters its tributaries only for spawning. The largest tributaries, the Upper Angara and Selenga Rivers, which flow into Lake Baikal in its northern and its central parts, respectively, are the spawning grounds for the most numerous schools of omul [5]. In other spawning rivers, there is a low abundance of omul, and hence, their role in natural omul reproduction is negligible.

Morphologically, ecologically and biologically different omul races, forms or populations (in the terminology of different years) inhabit Lake Baikal. According to their morphological traits, three groups were initially identified: the Selenga, the North Baikal and the Chivyrkuy omul races, which were named for the spawning rivers where they

breed [6]. Later, because of some well-marked features, the Posolsk omul population was allocated from the Selenga race as a separate (fourth) population [7]. The term “race” used in the 1930–1950s corresponds to the concept of a “population” in modern terminology (e.g., [8,9]). The population structure of Baikal omul is clearly correlated with its breeding areas [5]. Additionally, according to parameters such as reproductive disunity, and the morphological and biological heterogeneity of populations, three morphoecological groups of Baikal omul have been distinguished: (1) pelagic, (2) coastal pelagic and (3) near-bottom deep-water ones [5]. The reproductive isolation of these groups was confirmed using different methods [10–12]. A compound intraspecific ecological structure of the omul was formed because of the various breeding conditions in the rivers and its existence within the upper layer of the water masses (down to a 400 m depth) in the littoral zone, as well as in the epipelagic and mesopelagic zones of the deep-water lake. Because of the size of the area, the Selenga omul population is the most abundant (about 50–60% of the total abundance) [13]. Breeding in the largest tributary of Lake Baikal, the Selenga omul is adapted to use the food resources across the water area in the lake (epipelagic zone).

Lake Baikal, as the largest ancient reservoir (about 20% of the world’s reserves of unfrozen fresh water and 25–30 million years of geological history) that is rich in biodiversity (more than 3500 species of flora and fauna), has attracted people’s attention since time immemorial [14–16]. Faunal studies of Baikal began in the second half of the 18th century. During the same period, in 1775, Baikal omul was described as a species [17]. The parasitic fauna of the Baikal coregonids unites 44 species [18]. There is information on five leech species that parasitize Baikal fishes: *Baicalobdella cottidarum* Dogiel, 1957, *Codonobdella truncata* (Grube, 1873) and *Codonobdella* sp. feed on different sculpins; *Acipenserobdella volgensis* (Zykoff, 1903) feed exclusively on sturgeon; and *Piscicola* sp. is found on pike (*Esox lucius* Linnaeus 1758) and different types of cyprinids [19–22]. However, these annelid parasites have never been recorded in omul.

Regardless of the continuous 250-year history of studying Baikal omul and intensive commercial and recreational fishing, neither fishers nor biologists have ever mentioned or found leeches in numerous omul catches. Special parasitological surveys have also never detected any leech ectoparasites on the body of this fish [19,23–26]. The exception was the report of N. Pronin [27] on once finding a single specimen of *Piscicola geometra* (Linnaeus, 1761) in immature omul from Chivyrkuy Bay. Later, Dugarov and Pronin [26] indicated 34 parasite species infecting Baikal omul, striking off Pronin’s previous finding as an artifact.

In this study, we present the first reliable information on the existence of specific annelid parasites infecting Baikal omul in the Selenga River, as well as a preliminary analysis of the morphological and genetic attributes of the parasite species.

2. Materials and Methods

2.1. Study Area and Habitats

The Selenga River, the largest tributary of Lake Baikal, originates in the territory of Mongolia at the confluence of the Ider and Delgermörön Rivers, and has a predominantly plain appearance (Figure 1). On average, the Selenga River brings about 30 km³ of water into the lake, which is about half of the total inflow into the lake. The watershed covers 447,060 km², almost 82% of the entire area of the lake’s basin [28,29]. The length of the river is 1024 km, including 409 km within Russia. Near the river’s confluence into Lake Baikal, the Selenga forms an extensive delta area of 680 km² (Figure 1). The Selenga delta represents a multi-kilometer alluvion of unconsolidated sediments (about 5.0–5.5 km) onto a steep slope of the Baikal rift. The underwater part of the Selenga delta reaches the west coast of Lake Baikal. The Selenga delta is included on the list of Unique Natural Phenomena of Planetary Significance. It is a part of the Central Protection Zone of Lake Baikal, the UNESCO World Heritage Site.

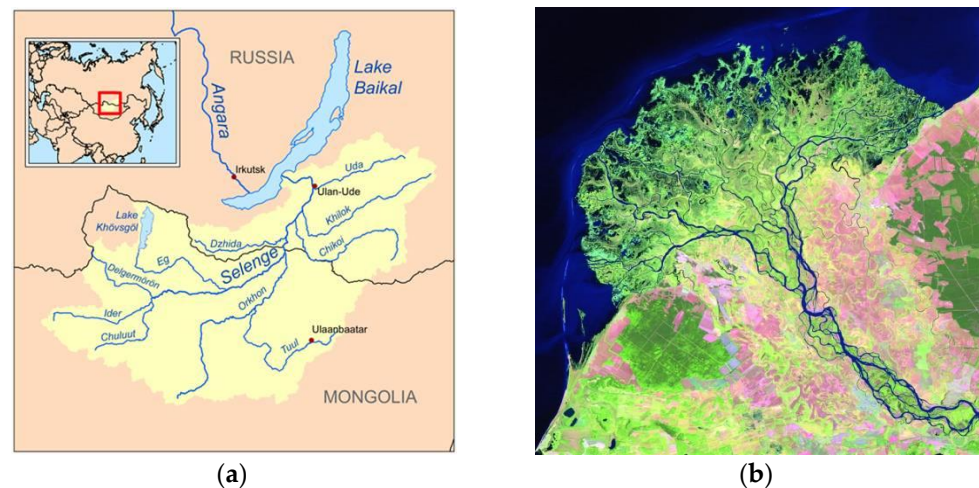


Figure 1. Location map of the study region: (a) The Selenga River and its basin (highlighted in yellow). The Selenga flows northeastwards through Mongolia and Russia, and forms a large delta on the southeast coast of Lake Baikal, the world's largest and deepest freshwater lake. (b) Satellite image of the Selenga River delta from space (Landsat GeoCover image from the USGS).

The waters of the Selenga are rich in fish. According to recent reports, the river is inhabited by 27 species [30,31] that form two unequal environmental groups: migratory and nonmigratory fishes. Cyprinids such as roach (*Rutilus rutilus* (Linnaeus, 1758)), Siberian dace (*Leuciscus baicalensis* (Dybowski, 1758)) and Eurasian minnow (*Phoxinus phoxinus* (Linnaeus, 1758)), as well as predatory fishes such as pike (*Esox lucius*), European perch (*Perca fluviatilis* Linnaeus, 1758) and burbot (*Lota lota* (Linnaeus, 1758)), are the basis of the Selenga ichthyocenosis [32]. Among the nonmigratory fish of the river, there are both valuable commercial and noncommercial fish species. Two rare and endangered species represent valuable commercial fishes: taimen (*Hucho taimen* (Pallas, 1773)) and lenok (*Brachymystax lenok* (Pallas, 1773)).

The group of anadromous (migratory) fish from the Selenga River includes the most valuable commercial species of Baikal fish, namely Baikal sturgeon (*Acipenser baerii baicalensis* Nikolski, 1896), Siberian whitefish (*Coregonus pidschian* (Gmelin, 1788)), Baikal grayling (*Thymallus brevipinnis* Svetovidov, 1931) and Baikal omul (*Coregonus migratorius* (Georgi, 1775)).

The Baikal omul population that is ready to spawn approaches the Selenga at the onset of autumn. The spawning migration of omul to the Selenga begins in mid-August to early September at a water temperature of 10–19 °C and continues for 50–60 days or more until the temperature of the water in the river drops to 2–3 °C, which is significantly lower than in the lake in this period [4,7].

2.2. Sample Collection

For this study, we used biological material obtained in the course of an ichthyological forensic examination concerning an illegal catch of Baikal omul during the spawning period (October 2013). Seized from poachers, a frozen fish was turned over to the Laboratory of Ichthyology at the Limnological Institute (Irkutsk) for investigation, where it was found that the omul was caught with a 38 mm mesh gillnets. In total, 663 fish specimens were studied.

External parasites (ectoparasites) were detected and manually collected directly from infected specimens during visual inspection of the frozen fishes, then immediately fixed in an 80% ethanol solution. Alcoholized leech tissues retained flexibility, facilitating the investigation of annulation and allocation of the genital openings. Moreover, such material is suitable for further molecular analysis. Specimens of leeches are kept in the collection of Limnological Institute under the voucher numbers C0, S65, S71–87, S132–133, S163–164, S166 and K 71–72.

2.3. Taxonomy and Population Affiliation of Fishes

The general biological analysis of the fish material was carried out by a conventional methodology [33], with taxonomic naming according to modern classifications [34]. The number of gill rakers was counted to determine whether the individuals belonged to a specific morphoecological group [5].

2.4. Morphology and DNA-Based Identification of Parasites

Morphological analysis of the ectoparasites was conducted using an MSP-2 var. 2 (LOMO, St. Petersburg, Russia) stereomicroscope. Currently existing taxonomic keys [19,35] were used in attempts to determine the leech species. Photos of the parasite were taken with a NIKON D7000 (Tokyo, Japan) camera.

To avoid the difficulties associated with assessing the species, classic analysis of the morphological characteristics was amplified by DNA-based methods. Molecular analysis was performed on the genome fragment recommended as a standardized marker for DNA barcoding [36–38] to verify the parasite's taxonomy.

DNA was extracted from a small portion (about 5 mg) of the posterior sucker of 10 alcohol-fixed leech specimens with the QIAamp DNA Mini Kit (Cat. No. 51304; Venlo, Netherlands) according to the manufacturer's protocol. This tissue was chosen to prevent contamination by the host's blood. Double-stranded templates of the cytochrome C oxidase subunit I (*cox1*) gene fragment suitable for Sanger sequencing were prepared by PCR amplification with the primers universal to most invertebrates, namely LCO1490 and HCO2198 [39], and Phusion High-Fidelity DNA Polymerase (Thermo Fisher Scientific, Carlsbad, CA, USA). Amplicons were sequenced at CJSC "Syntol" (Moscow, Russia). Chromatograms obtained from the automated sequencers were edited and consensus sequences were created from the complementary strands using MEGA version 11 [40]. The final dataset matrix included 86 terminals and 658 aligned nucleotide characters. Newly sequenced data were deposited in GenBank under the accession numbers KR608789, KR608790 and OP100315–OP100324. Sequences uploaded from GenBank and used as a comparison group or outgroup are described in the Supplementary Materials (Table S1).

A comparison of the nucleotide sequences with the sequence databases, an estimation of the statistical significance of the matches and a search for the regions of local similarity among the homologous DNA fragments were performed using BLAST+ 2.13.0 [41]. The phylogenetic analysis was performed using the maximum likelihood (ML) method implemented in IQ-TREE version 2 [42].

2.5. Parasitological Analysis

The analysis was performed on the basis of a parasitological examination of the fish. To assess the incidence of piscicollis in fish, generally accepted parasitological indicators were used, such as the prevalence (P) and intensity (I) of invasion [43].

3. Results

3.1. Characterization of Fishes

An ichthyological analysis of illegally caught Baikal omul from the lower course of the Selenga River (Figure 2) was conducted in 2013. Six hundred and sixty-three individual coregonid fishes were examined. During the examination, we determined that this catch had been taken with gillnets with a mesh size of 38 mm. There are two omul flows into the Selenga every year: in September and October. A September subpopulation enters the river in the last decade of August or early September and can abide there for up to 60 days, whereas the period of stay of the October subpopulation in the river is more than twice as short. Typical qualities in the spawning shoals of omul are the size, color and number of eggs in mature females. The size and weight of omul vary considerably depending on the habitat and the age of the fish. The maximum age of Baikal omul is 14+ years [4]. According to 50 years of observations, the sexually mature omul of the Selenga population has a size of 312–352 mm and a weight of 0.35–0.52 kg [44]. The three population groups of Baikal

omul (coastal, benthic deep-water and pelagic) are characterized by the different number of frequent gill rakers in a short gill arch. The highest number (44–55) of frequent gill rakers characterizes omul from the Selenga populations as a migrant planktonophagist [5].



Figure 2. The unfrozen Selenga omul with ectoparasite leeches attached to the anal fin.

Amongst the 663 omul fishes available for morphological measurements, the sex ratio of females to males was 1:2. The results of detailed measurement are given in Table 1. Thus, in the materials from the Selenga, there were mainly sexually mature individuals with an age of 7 to 12+ years. Physical parameters such as their body length and weight ranged from 302 to 376 mm and from 0.29 to 0.56 kg, respectively (Table 1). The number of gill rakers in the individuals studied ranged from 47 to 52, which proves that the illegally caught fishes belonged to the Selenga population according to the common classification [5].

Table 1. Main characteristics of Baikal omul from the Selenga River (data from October 2013).

Gender and Life Stage	Number of Specimens	SL *, mm	Weight, g
Spawning females	3	321–353	444–520
Spawned females	217	315–376	288–540
Spawned males	443	302–366	289–557

* SL, standard length.

3.2. Parameters of Leech Parasitemia in the Selenga Omul Population

The parasitological inspection of studied fishes revealed 27 piscine leeches. This was the first case of finding leeches parasitizing Baikal omul, despite long-term research into this fish in the lake. Leeches were detected only on post-spawning individual fish. Taking the number of fish investigated and the amount of leech material gathered into account, the prevalence of leech parasitemia in the Selenga spawning omul was at least 4.07% in 2013. The infection intensity was one parasite specimen per fish. Parasites were situated at the fins, mostly the anal, abdominal and thoracic fins (Figures 2 and 3). No leech samples were found at the dorsal fin. There is a high probability of an underestimation of the intensity of parasitemia due to possible loss of parasites during the process of catching the fish because some leeches could have detached from the host before fixing and thus been excluded from the analysis.



Figure 3. Location of leech ectoparasites on Baikal omul caught in the Selenga River: (a) at the abdominal fin and (b) at thoracic fin.

3.3. Parasite Morphology

Overall, 27 leech specimens that had been frozen and refixed in an 80% ethanol solution were available for morphological analysis. This method of sample preparation was not conducive to the safety of the morphological characteristics and considerably hindered the analysis. Nevertheless, the analysis succeeded in defining the main parameters of the external morphology.

The specimens of piscine leeches taken from Baikal omul had similar body proportions. The length of the parasites ranged from 6 to 22 mm. The maximal body width was in the back part of the urosome and varied within 1.0–2.8 mm for different individuals. The largest specimen was 22 mm in length and had a width of 1.2 mm. The maximum width of 2.8 mm was recorded in a specimen measuring 12.8 mm long. The average body length in these piscicolids was 10.4 mm, with a width of 1.9 mm. The values of the mode for length and width were approximately the same: 9.6 mm and 2.0 mm, respectively.

The color of living parasites is unknown. After freezing to -20°C , the leeches had a uniform brownish-green tint (Figure 4). The coloration was caused by brown pigment cells that were evenly distributed all over the body's surface. Some specimens had two well distinguished pairs of eyes, similar to most representatives of the family Piscicolidae (Johnston, 1865): one pair of rod-like eyes and another pair of punctiform eyes (Figure 4). The suckers were well developed. The anterior and posterior suckers had an almost equal size and a rounded shape. The posterior sucker was attached to the urosome medially; its diameter was slightly larger than the greatest width of the adjacent part of the body.



Figure 4. Morphology of the leech, an ectoparasite of the Selenga omul population. The largest specimen (15 mm in length). Scale = 5 mm. A close-up of the anterior sucker with well-preserved eyes is shown in the bottom left corner. The arrows indicate the eyes.

3.4. Identification of the Parasites

Despite the adequate preservation of the frozen samples and their availability for morphological analysis, the cumulative set of morphological traits obtained did not allow us to attribute the omul leech to any currently known species. Morphologically, the closest species belong to the genera *Caspiobdella* Epstein, 1966, *Acipenserobdella* Epstein, 1969 and *Cystobranchnus* Diesing, 1859.

Acipenserobdella volgensis (Zykoff, 1903) and *Cystobranchnus mammilatus* (Malm, 1863) were previously recorded as a part of the Selenga River's parasitic fauna [19,35], whereas *Caspiobdella* have never been found there. All known *Caspiobdella* mainly inhabit the Caspian Sea, as well as the northwestern tributaries of the Black Sea, including the entire Danube basin [19]. However, recently, they have been increasingly found in European water bodies, being assigned as invasive species (e.g., [45,46]).

Cystobranchnus mammilatus is a specific parasite of the burbot (*Lota lota*). Despite the similar size, unlike the unclassified omul leech from the Selenga, *C. mammilatus* has a large posterior sucker with a diameter of up to three times wider than its body. Moreover, the eyes of *C. mammilatus* are poorly developed or absent.

Another relative that is morphologically close to the Selenga leeches, *Cystobranchnus salmositicus* (Meyer, 1946), inhabits the fresh waters of North America [47]. In contrast to the Selenga leech found in Baikal omul (Figure 4), *C. salmositicus* is approximately 50% larger and, which is especially important, has 11 pairs of large pulsating vesicles on the lateral edges of the urosome.

Acipenserobdella volgensis exclusively parasitizes sturgeons (Acipenseridae Bonaparte, 1831), including Baikal sturgeon living in the Selenga River. Unlike the leeches found in omul, *A. volgensis* has a body twice as large, anterior and posterior suckers of different sizes, and less pigmentation on the ventral side.

Regarding *Caspiobdella*, there are three presently known species of this genus: *C. caspica* (Selensky, 1915), *C. fadejewi* Epstein, 1961 and *C. tuberculata* Epstein, 1966. None of them is similar to the Selenga omul piscicolids. Unlike the Selenga leeches, *C. tuberculata* is a small-sized leech (up to 6 mm) with a well-defined trachelosome and large posterior suckers (1.5 times wider than the urosome). Both *C. caspica* and *C. fadejewi* have a specific coloration that is typical only for these species.

The comparison with related species indicated that the morphoecological status of the omul leech does not correspond to any of them. Thus, despite some similarity to certain Palaearctic and Nearctic taxa, the leeches parasitizing Baikal omul apparently have a unique set of morphological characteristics. This may indicate either the discovery of a potentially new species of fish leeches or the need for additional material to study their vital morphology (size, color, pigmentation, eyes, ocelli, vesicles, etc.). However, live omul leeches, whose biology is associated with the reproduction of its fish host, are difficult to obtain due to strict government regulations aimed at protecting the spawning population of omul. Therefore, DNA identification was undertaken to confirm the findings based on the morphology.

Twelve nucleotide sequences of the leeches were generated and deposited into GenBank (KR608789–KR608790 and OP100315–OP100324). The length of the genome fragments encoding part of mitochondrial cytochrome *c* oxidase (*cox1*) was 649–676 bp. This part of genome was selected because it is recognized as a versatile tool for DNA barcoding of Metazoa [36,37] and is especially useful for the molecular identification of leeches [48–50].

The BLAST analysis revealed the most similar nucleotide sequences throughout the world's available genetic databases. The resulting set of *cox1* sequences consisted of 100 sequences belonging to different organisms (with an identity of 81% and higher), including 92 representatives of Piscicolidae, whose genetic identity ranged from 81.43% to 90.54%. The *cox1* sequences of fish leeches found on the BLAST list belonged to 27 genera of fish leeches. At the top of the list, there were 10 sequences of freshwater piscicolids with an identity ratio of 86.87% to 90.54% (Table 2).

Table 2. Top 10 Homologous Piscicolidae sequences selected by BLAST, with the statistical significance of the matches.

Description	Max Score	Query Cover	Identity	Length, bp	Accession
<i>Caspiobdella fadejewi</i>	830	95%	90.54%	628	AY336019
<i>Caspiobdella fadejewi</i>	826	95%	90.38%	626	AY336020
<i>Piscicola</i> sp.	778	98%	88.25%	674	KM095104
<i>Piscicola milneri</i>	774	100%	87.92%	665	DQ414337
<i>Piscicola geometra</i>	769	100%	87.77%	15135	OX030972
<i>Piscicola geometra</i>	765	99%	87.83%	651	AF003280
<i>Piscicola</i> sp.	763	99%	87.81%	676	KM095103
<i>Piscicola geometra</i>	763	100%	87.61%	14788	BK059172
<i>Piscicola geometra</i>	737	96%	87.54%	634	MF458791
<i>Branchellion parkeri</i>	736	100%	86.87%	665	DQ414308

To form a set of molecular data, first of all, it is necessary to determine the composition of the comparison group and the outgroup. The comparison group should consist of homologous sequences used as a reference when determining the target group's similarity, while the outgroup allows for the phylogeny to be rooted, which is essential for understanding the evolution of traits along a phylogeny [50]. The comparison group for the analysis of omul leeches was formed using the BLAST data and directly supplemented with individual findings of piscicolid sequences in GenBank, provided that the sequence length was at least 600 bp. At the same time, the *cox1* sequences of freshwater and brackish-water leeches parasitizing fish are scarce in the international genetic databases, so they were selected and uploaded with special care. On the other hand, redundant data on marine species were eliminated not to overload the analysis. Seven homologous sequences of flat leeches (Glossiphoniidae), the closest family to fish leeches, were selected as the outgroup. The final dataset consisted of 86 *cox1* sequences belonging to 20 freshwater and brackish-water leeches and 47 marine fish leeches, together representing 35 genera of Piscicolidae.

The phylogenetic tree showed that the group of the omul leech sequences formed a single lineage within the freshwater representatives of the Piscicolidae branch, with 99.7% probability according to UFBoot [51] (Figure 5). In the tree, 12 *cox1* sequences of omul leeches formed a separate clade and appeared to be closely related to the European species *Caspiobdella fadejewi* (Figure 5), which corresponds to the morphological data.

The genetic similarity was analyzed in the group of freshwater leeches, which is genetically closest to the studied omul leeches according to the results of BLAST and ML phylogeny (Table 2, Figure 5). Intraspecific genetic polymorphism should not exceed the threshold of 1–2%, according to the DNA barcoding theory [36]. Calculations showed (Table 3) that this parameter in all studied freshwater piscicolid species was within this threshold (*Caspiobdella fadejewi*, $1.14 \pm 0.41\%$; *Piscicola milneri*, $1.63 \pm 0.40\%$; *Piscicola geometra* (including *P. pojmanskae*), $0.4 \pm 0.17\%$), which indicates the genetic homogeneity of these species. The genetic polymorphism in the group of 12 new leech species from the Selenga is $0.88 \pm 0.20\%$ of nucleotide substitutions per site (Table 3), which is also within the acceptable level and is even lower than in *C. fadejewi* and *P. milneri*. Since these values coincide with the intraspecific differences typical of piscicolid leeches [52], we should conclude that all omul leeches most likely belong to the same species.

The genetic distance between the omul leeches' sequences and those of *C. fadejewi* is $8.05 \pm 1.09\%$, which is the minimum compared with the genetic distances with other freshwater Piscicolidae species (Table 3). According to the hypothesis underlying the DNA barcoding approach [36,37], this level of genetic distance is sufficient to conclude that these phylogenetic groups are taxonomically independent and therefore belong to different species. Nevertheless, the genetic distance of 8.05% is still twice as high for the species within the same genus. For example, the genetic distances between different species within the related freshwater genus *Piscicola* range from 4.82 to 5.50%. Similarly, we cannot assign the omul leech from the Selenga River to the genus *Caspiobdella*. Unfortunately, the international base of publicly available DNA sequences contains no nucleotide sequence of

the *cox1* gene belonging to other fish leeches from the Selenga River, *C. mammilatus* and *A. volgensis*.

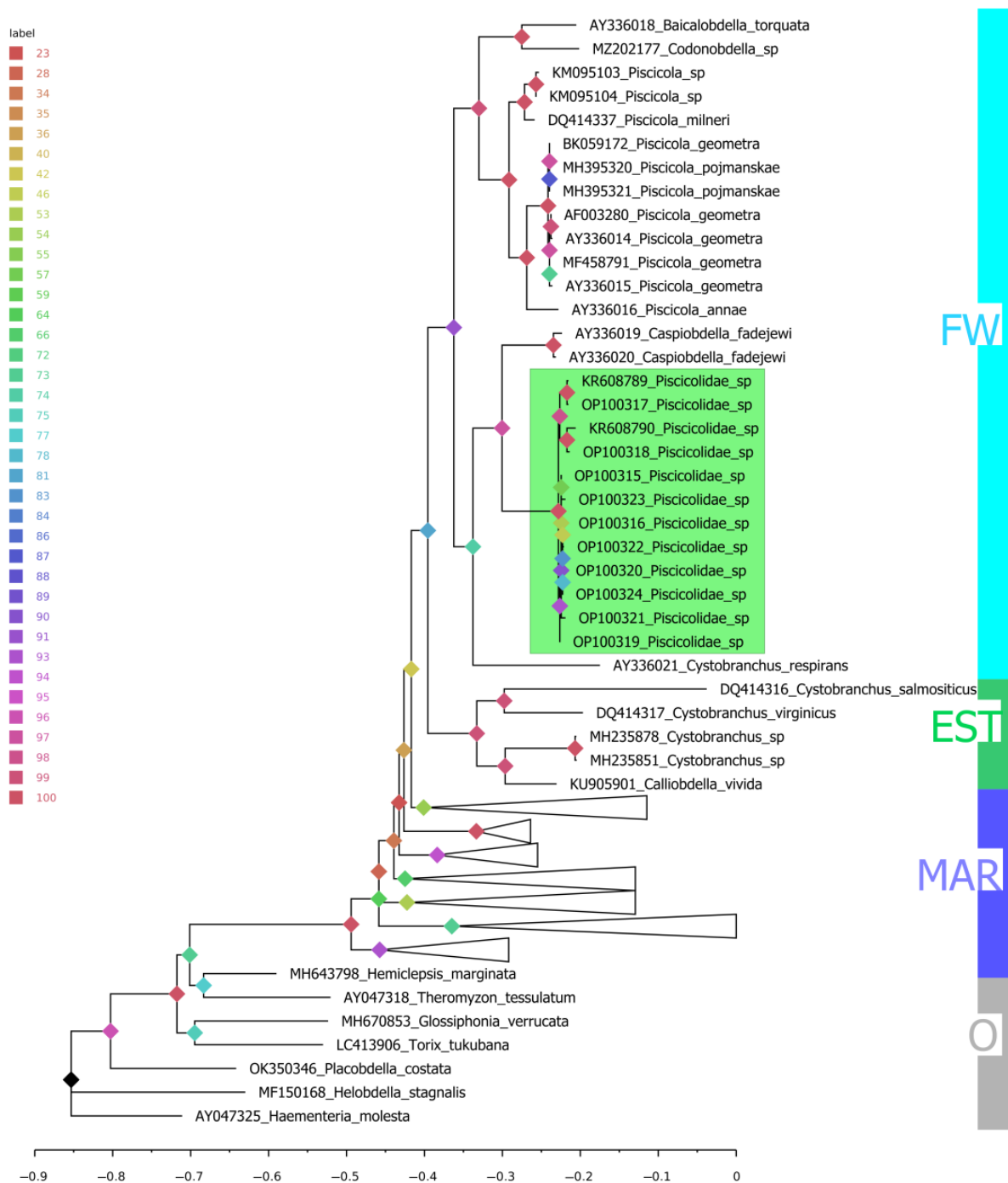


Figure 5. Maximum likelihood tree visualizing the phylogenetic position of the studied omul leeches from the Selenga River, the clade of which is highlighted in green. The colors of the labels in the tree nodes reflect the level of probability according to UFBoot [51], the values of which are deciphered in the heat map shown in the left corner. On the right, freshwater, estuarine and marine leeches, and the outgroup are marked as FW, EST, MAR and O, respectively. The phylogenetic lineages of marine leeches are presented in contracted form. A full version of this tree is shown in the Supplementary Materials (Figure S1).

Table 3. Net evolutionary divergence (p) within and between selected sequences of groups of freshwater leeches.

Species Group	p	SE	1	2	3	4	5	6	7	8
<i>Baicalobdella torquata</i>	n/c	n/c		0.0128	0.0112	0.0142	0.0109	0.0125	0.0120	0.0131
<i>Caspiobdella fadejewi</i>	0.0114	0.0041	0.1234		0.0132	0.0133	0.0114	0.0117	0.0121	0.0109
<i>Codonobdella</i> sp.	n/c	n/c	0.0931	0.1340		0.0136	0.0114	0.0118	0.0122	0.0133
<i>Cystobranchnus respirans</i>	n/c	n/c	0.1552	0.1324	0.1422		0.0128	0.0134	0.0141	0.0132
<i>Piscicola milneri</i>	0.0163	0.0040	0.0959	0.1078	0.1046	0.1275		0.0085	0.0084	0.0114
<i>Piscicola geometra</i>	0.0040	0.0017	0.1173	0.1057	0.1119	0.1450	0.0526		0.0085	0.0120
<i>Piscicola annae</i>	n/c	n/c	0.1127	0.1185	0.1111	0.1471	0.0550	0.0482		0.0122
Omul leech	0.0088	0.0020	0.1322	0.0805	0.1388	0.1322	0.1085	0.1137	0.1237	

Since the morphological and molecular methods were inefficient for defining the species of the omul leech, we can assume that the piscicolids parasitizing Baikal omul may belong to a new taxon for science on both the species and genus level.

4. Discussion

Illegal and unregulated fishing is recognized as a widespread problem for natural resource management. Illegal activity harvests more fishery resources than what is sustainable. The burden of unregulated fish poaching in the Baikal region is an escalating concern, significantly undermining the management of hydroecosystems and fish stocks [3,53]. The greatest fish population losses caused by illegal activity occur during periods of fish spawning. Moreover, there are human health risks from the zoonotic parasites in illegal catches [54], which have been underestimated to date.

Parasitizing fish leeches are not as harmless as might seem at first glance. These ectoparasites negatively affect the health of their host. The ulcerations associated with leech attachment sites certainly debilitate the host fish and may predispose the hosts to bacterial and fungal infections [55]. There is evidence that fish leeches serve as mechanical vectors of viruses [56] and numerous hematopoietic (including trematodes [57,58]) and parasitic flagellates [59,60], which are considered to be pathogenic organisms that cause many diseases in both humans and aquatic animals.

Despite sufficient knowledge of the ecology and parasitic fauna of Baikal omul, there has been no reliable information about the infection of this fish by leeches. This has been primarily caused by underestimation of the importance of ectoparasites and, as a result, the lack of proper attention by researchers to this group. Another more likely reason is the inaccessibility of material due to the appearance of leech invasions in omul exclusively during the spawning period when the omul harvest is limited by law. In this regard, we had the unique opportunity to conduct these studies and detect a specific invasion by leeches without causing additional harm to the spawning omul.

Unfortunately, the illegal catch seized by the police from poachers and handed over for research did not provide any information about the biology and ecology of the leeches. Nevertheless, based on known facts about the behavior of Baikal omul before and during spawning, as well as the ecology of its spawning grounds in the Selenga River, we have assumed hypothetically possible mechanisms of fish infection.

Primarily, the fact that leeches were found on fish in the spawning grounds may suggest that they feed on omul eggs and accidentally fall onto the fish. Moreover, there is much evidence about the feeding of leeches on the eggs of fish and amphibians (e.g., [19,61]), but this feeding strategy is rare and has been often omitted entirely in reviews about leeches. However, the infection of pink salmon eggs (*Oncorhynchus gorbuscha* (Walbaum, 1792)) with freshwater leeches (*Cystobranchnus salmositicus*) has long been reported [62]. Furthermore, recent studies in Lake Baikal have shown that numerous juveniles of the leech *Baicalobdella cottidarum* feed on the eggs of the stone sculpin (*Paracottus knerii* (Dybowski, 1874)) [22].

Unlike salmon and sculpin, Baikal omul does not lay eggs compactly in clutches and does not bury them in the ground. The spawning of omul in the Selenga River takes place in the riverbed, where the depth usually exceeds 1.5 m, which seems to be an adaptation to the conditions of egg incubation [63]. Boulder and pebble grounds with a small admixture of coarse-grained sand are the most preferred. Thus, the entire channel of the Selenga River with suitable bottom substrates (64 km²), except for the sites without a current, is a potential spawning ground for omul. Special studies on the spawning grounds in the tributaries of Lake Baikal indicated that in the beginning, the eggs are in a suspended state, then the river sand encrusts the eggs' surface, which leads to an increase in their specific gravity [44]. Some part of the omul eggs sticks to the substrate (large stones and boulders), and the other part, having lost its stickiness due to the adhered grains of sand, rolls under the stones, where it stays during the entire incubation period. Consequently, there are at least two circumstances that are not favorable for the long-term presence of leeches on the omul eggs, namely that the eggs are encrusted with sand because of their inaccessibility, and because of the danger of being eaten together with the eggs by other aquatic organisms. Thus, the Baikal omul's eggs are not a reliable food source for piscine leeches; and the assumption that feeding on fish eggs can contribute to the infection of the omul by leeches is clearly untenable.

The most probable scenario is that the life strategy of the Selenga piscine leech may be similar to that of the ancient leech *Acanthtobdella peledina* (Grube 1850), another parasite of Siberian salmonids [64]. Both leeches are not capable of rapid displacement, especially outside the host, to whose body they are securely held by suckers. Therefore, the moment of infection is vital for the successful existence of these parasites. How do the leech infestations of Baikal omul occur? Since the leeches were found on Baikal omul that had been illegally caught in spawning grounds, the answer to this question probably lies in the ecology of fish spawning, especially features such as the fishes' cluster formation, the timing of entry and the duration of stay in the spawning grounds, which are most likely to contribute to infection by leeches. Additionally, it is necessary to consider the main characteristics of the fish spawning grounds as potential habitats for the leeches, namely, the bottom substrate types, current speed and depth. These data can serve as a basis for obtaining information on leech's ecology and the host–parasite relationships.

Reaching puberty, Baikal omul makes spawning migrations, moving from feeding places to spawning places. The physiological state of anadromous spawning migration in Baikal omul occurs after a 6–15-year period of life in the lake [4,44]. Before spawning migration, Baikal omul forms shoals near the estuaries of spawning rivers; after that, it enters the rivers. The migration of the Selenga population begins at the end of August and continues, gradually weakening, until the freeze-up (end of October) [63]. The distance that the spawning shoal travels along the river ranges from 120 to 450 km from the estuary. Therefore, the duration of the spawning migration of omul in the Selenga can be about 60 days. Moreover, the speed of the spawning stock moving upstream the river is uneven and depends on external factors (water temperature and daily cycles) [4]. Heavy autumn rains often raise the water level and sharply reduce its temperature, forcing the omul to settle in pits until the flood subsides. Furthermore, numerous observations have shown that, under favorable weather conditions, the most optimal fish movement was detected in the dark (evening and night), while during the day, the omul remained in the pits and reaches with a minimal current. While in such pits and reaches of the Selenga, Baikal pelagic omul would become easy prey for leeches, which usually prefer such warm places as their habitat. The long-term spawning migration of the host fish provides the leech with a reliable food base for this period and, hence, successful preparation for reproduction.

Leeches, presumably together with the downstream migrant omul, return to the lower reaches of the Selenga River, feed for more time on the omul, and then leave the host fish in pits, places that are convenient for wintering and breeding. Such a scenario is more plausible, since leeches have never been found on the feeding omul in Lake Baikal.

Apparently, the leeches do not leave the river and remain in its lower reaches to repeat the successful scenario next year. Thus, the life cycle of the Selenga leech closes.

The death of up to 90% of omul that spawned in the Selenga River is explained by the extended length and duration of the migration path along the Selenga River (up to 450 km) [4,44]. The mass death of the spawned Baikal omul in the Selenga, in particular, is associated with the influence of parasites on the fishes' organism, already weakened by the grueling migration [65]. Our results confirm this assumption, as leeches also could contribute to weakening of the omul, largely through further infection with more aggressive parasites and pathogens.

5. Conclusions

The most striking result of this study is the first detection of parasitemia caused by an ectoparasitic leech in the commercial Baikal omul, despite the close attention previously given to its parasitic fauna.

The leeches were found on the pelagic omul during its spawning period in the Selenga River when any omul fishing is prohibited. Cooperation with the police allowed us, without causing additional damage to the omul population, to have access to illegally caught fish.

The piscine leech belongs to a new species and probably to a new genus, according to the morphological and molecular data. To describe a new taxon, a deeper morphoanatomical study of the leech is required.

In the Discussion, we present possible scenarios for the leech's infection of omul and an explanation of the absence of such parasitemia in Baikal omul in the lake.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/fishes7050298/s1>. Table S1: Taxon names and GenBank accession numbers for 74 leeches used in phylogenetic analysis. Figure S1: The full version of the ML tree based on the *cox1* sequences most closely related to the Selenga leeches parasitizing Baikal omul.

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