

## Article

# Diversity of the *Piscicola* Species (Hirudinea, Piscicolidae) in the Eastern Palaearctic with a Description of Three New Species and Notes on Their Biogeography <sup>†</sup>

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**Abstract:** Biodiversity is considered one of the most significant parameters for characterizing aquatic environments. The value of species diversity is taken into account when assessing the ecological state and quality of the ecosystem. However, erroneous taxonomic identification distorts biodiversity signification and, consequently, prevents a clear understanding of ecological signals and traits. In this study, we presented the first data on *Piscicola* leech species diversity in the vast area of the Eastern Palaearctic. To avoid misidentifications, an integrated approach was applied, combining modern DNA-based and classical morphological methods. Previously, only one species of the genus, *Piscicola geometra*, was recorded in the whole of Siberia. We discovered three new species: *Piscicola sibirica* sp. nov., *Piscicola khubsugulensis* sp. nov. (Eastern Siberia) and *Piscicola nordica* sp. nov. (European Northeast). The species *Piscicola pojmanskae* was reduced to synonymy with *Piscicola geometra*, whose description was updated. Taxonomic adjustments allowed us to ascertain the eastern edge of the *Piscicola geometra* distribution and to understand the biogeography of the group as a whole. The *Piscicola geometra* range is limited to Western Siberia, while the widespread *Piscicola sibirica* sp. nov. and the purely Khovsgolian *Piscicola khubsugulensis* sp. nov. inhabit Eastern Siberia.

**Keywords:** Palaearctic; Northeast Europe; Eastern Siberia; biodiversity; biogeography; species delimitation; leeches; *Piscicola*; new species



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## 1. Introduction

*Piscicola* de Blainville, 1818 is a genus of annelid worms belonging to the family Piscicolidae Johnston, 1865. The species of this genus are found in the inland waters of Eurasia (22 species), North America (2 species) and South America (1 species) [1–9]. All *Piscicola* representatives are ectoparasites of various fish, and this fact determines their role in the ecosystem. Like any other blood-sucking parasites, piscine leeches eliminate nonviable hosts, thereby regulating host population abundance and maintaining it in a healthy state. By themselves, the bites of piscine leeches are not dangerous and are comparable to mosquito bites on vertebrates. However, the wounds associated with the leech attachment sites may certainly predispose fish hosts to bacterial and fungal infections [10]. There is evidence that piscine leeches serve as the mechanical vectors of viruses [11] and numerous hematopoietic parasites, including trematodes [12,13] and parasitic flagellates [14,15], which are considered pathogenic organisms and which cause many diseases in both humans and aquatic animals.

The first piscine leech species was described by Carl Linnaeus in 1761 [16] but was assigned to the genus *Hirudo* at that time. In 1818, the French zoologist Henri-Marie Ducrotay de Blainville singled out the separate genus *Piscicola* of the Linnaean species, which was still the only known piscine leech. At present, 19 species of *Piscicola* inhabiting the Palaearctic region are known [2–8], which is about half of the species diversity of the Palaearctic Piscicolidae fauna [17]. The trans-Palaearctic *Piscicola geometra* (Linnaeus, 1761)

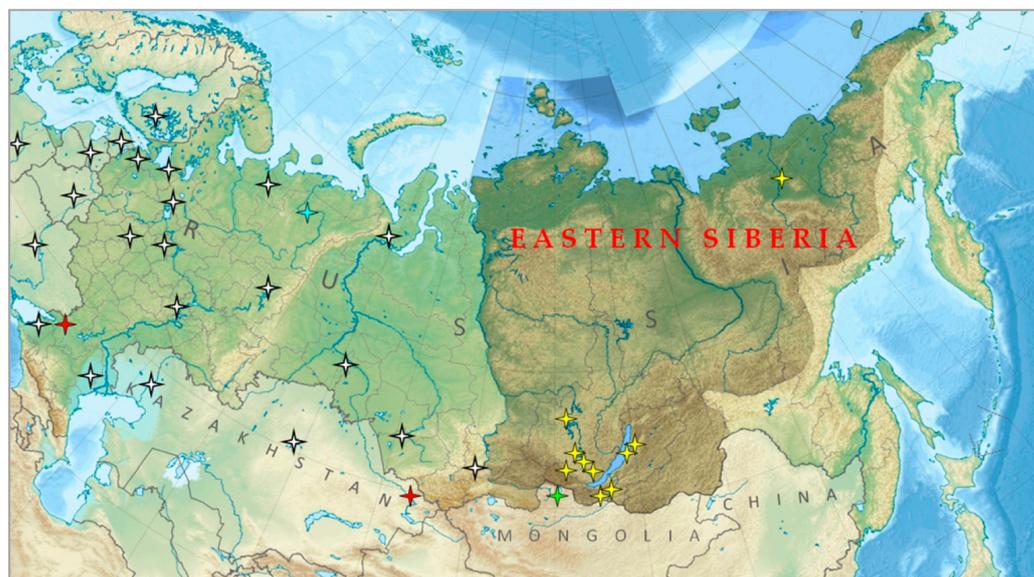
was recognized as the type species for the genus. The rest of the species are found only in European habitats, and 14 of them were discovered in the period of 1997–2013 due to the efforts of Polish researchers [5–8]. At the same time, the leech fauna of the Eastern Palaearctic, and especially Siberia, remains poorly studied. The scarce data obtained earlier suggested that *P. geometra* is distributed in the fresh waters of Western and Eastern Siberia with the exception of Lake Baikal [3,18]. However, recent targeted research has reliably confirmed the presence of *P. geometra* in the Irtysh River basin (Western Siberia) [19–22] but did not find this species in the rivers of Eastern Siberia [23] and certainly not in Lake Baikal [24–27].

Siberia is well known for being rich in natural resources, including fresh waters abounding in a variety of fishes, which, hypothetically, can serve as a food source not only for humans and wild animals but also for parasitic leeches. Therefore, the purpose of this study was to fill the gap in knowledge on the species diversity of the *Piscicola* fauna and its distribution, mainly in the freshwater bodies of Eastern Siberia.

## 2. Materials and Methods

### 2.1. Study Area

Eastern Siberia is a natural region located predominantly in Russia that cover an area of 7,200,000 km<sup>2</sup> [28]. The vast territory of this region stretches from west to east between the valley of the Yenisei River and the watershed ridges along the Pacific coast, stretching south of the Chukotka Peninsula. In the north, Eastern Siberia extends along a wide front to the Arctic Ocean; in the south, it includes the northern parts of Mongolia and China. The length of the territory from north to south is more than 3000 km (Figure 1).



**Figure 1.** Location map of the study region. The territory of Eastern Siberia is highlighted by the shaded background. Asterisks indicate the places where different piscine leeches of the genus *Piscicola* were found: data of this study (red, yellow, green and blue marks, depending on species) and literature data on *Piscicola geometra* (L., 1758) distribution (colorless marks).

The relief is dominated by low and middle mountains. The western part of Eastern Siberia is occupied by the taiga of the Central Siberian Plateau, the largest forest massif in Russia, which is replaced in the north by tundra lowlands, and is preplaced in the south and east by the high mountain ranges of the Eastern Sayan Mountains, the mountains of Transbaikalia and the Kolyma Territory. In some high mountain ranges, alpine landforms have developed in combination with modern glaciations (Orulgan, Momsky, Sunta-Khayata, etc.).

Eastern Siberia is one of the richest regions on the planet with respect to water resources. Due to abundant rainfall and the presence of high mountains and glaciers, there are many large rivers (Yenisei, Angara, Selenga, Vitim, Kolyma, Indigirka, Yana, etc.) and lakes, including Lake Baikal, which is known by the world as the pearl of Siberia. The total length of all rivers is about 700,000 km. The internal waters of Eastern Siberia belong to the basin of the Arctic Ocean (the Kara, the Laptev and the East Siberian Seas).

## 2.2. Sample Collection

For this study, we used biological material collected during 2012–2021 in various inland waters of Northern Eurasia, specifically in Eastern Europe and Siberia; however, most of the samples were collected in the rivers and lakes of Eastern Siberia (Table 1).

Piscine leeches were detected and manually collected directly from infected host specimens or during visual inspection of aquatic vegetation or stone fouling then were immediately fixed in an 80% ethanol solution since alcoholized leech tissues are suitable for both morphological studies and further molecular analyses.

Additionally, samples from the Chivyrkuisky Bay of Lake Baikal (7 indiv.), Yana River (4 indiv.) and Lake Kapylyushi (5 indiv.), which were fixated with 4–7% formalin solution, were used only for morphological analyses and descriptions of species.

Specimens of leeches are kept in the collection of Limnological Institute under the voucher numbers mentioned in Table 1.

## 2.3. Morphological Analysis

Morphological analysis of the piscine leeches was conducted using an MSP-2 var. 2 (LOMO, St. Petersburg, Russia) stereomicroscope. Currently existing taxonomic keys [1,3,4,18] and original descriptions of some species [5–8,29] were used in attempts to determine specimens up to the species level. Novel leech species were additionally examined through scanning electronic microscopy (FEI Company, Hillsboro, OR, USA). Photos of the whole individuals were taken with a NIKON D7000 (Tokyo, Japan) camera.

## 2.4. DNA-Based Species Identification

To verify the leeches' taxonomy and exclude possible errors in species identification, the classical morphological approach was supplemented with DNA methods. Molecular analysis was performed using the genome fragment recommended as a standardized marker for DNA barcoding [30–32].

DNA was extracted from a small portion (about 5 mg) of the posterior sucker of 28 alcohol-fixed leech specimens with the QIAamp DNA Mini Kit (QIAGEN, Venlo, The Netherlands) according to the manufacturer's protocol. This tissue was chosen to prevent contamination with the host's blood. Double-stranded templates of the cytochrome C oxidase subunit I (*cox1*) gene fragment suitable for sequencing were prepared through PCR amplification with the primers universal to most invertebrates, namely LCO1490 and HCO2198 [33] and Taq DNA polymerase ("Lytech" Co. Ltd., Moscow, Russia). Amplicons were sequenced at CJSC "Syntol" (Moscow, Russia). Sequence chromatograms were checked manually, and consensus sequences were created from the complementary strands using MEGA11 [34]. Newly sequenced data were deposited in GenBank under the accession numbers KM095103, KM095104 and OQ152290–OQ152315 (Table 1).

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+ ver. 2.13.0 [35]. Sequences downloaded from GenBank and used as a comparison group or outgroup are described in the Supplementary Materials (Table S1).

DNA-based species delimitation was performed through GMYC [36] based on ultrametric phylogenetic tree inferred using Bayesian method implemented in BEAST ver. 2.6.6 [37]. The Bayesian phylogeny reconstruction was carried out using evolutionary model recommended by jModelTest [38] with a total of 200 million MCMC generations, where every

10,000th generation was sampled and where the first 10% of generations were discarded as burn-ins. A relaxed lognormal molecular clock parameter was chosen based on the recommendations due to the fact that the 95% highest posterior density of the substitution coefficient rate variation (CV) did not include zero value [39]. The convergence of ESS statistics was checked using Tracer ver. 1.7.1 [40]. Visualization of phylogeny was conducted in FigTree ver. 1.4.4 [41]. Genetic distances (p) were calculated in MEGA11 [34].

**Table 1.** List of the *Piscicola* spp. specimens used in this study with indication of voucher, sampling location and GenBank accession number of newly sequenced data.

Species	Voucher	Locality <sup>1</sup>	GenBank Accession	
<i>Piscicola geometra</i> (L., 1761)	C218-220 KM3-11	EU: Temernik River, RR, Russia; 47.276107, 39.718591	OQ152291-3 8	
	C221	EU: Temernik River, RR, Russia; 47.286388, 39.723242	OQ152294	
	F88 L1-L5	WS: Bukhtarma reservoir, Kazakhstan; 49.616111, 83.525833	OQ152290 5	
	L6-L7	WS: Bukhtarma reservoir, Kazakhstan; 49.616944, 83.571667	2	
	L8-L11	WS: Bukhtarma reservoir, Kazakhstan; 49.627500, 83.455833	4	
<i>Piscicola nordica</i> sp. nov.	C169	EU: Lake Golovka, RK, Russia; 67.493504, 64.050113	OQ152314	
<i>Piscicola sibirica</i> sp. nov.	C2 B39-40 B38, A1-22	ES: Angara River, IR, Russia; 52.432133, 104.109404 ES: Angara River, IR, Russia; 52.535392, 103.964607	OQ152295 OQ152304-5 23	
	BS76-77 BS31-33	ES: Bratsk reservoir, IR, Russia; 53.095889, 103.356420	OQ152296-7 3	
	M27-29	ES: Irkutsk reservoir, IR, Russia; 51.998870, 104.664175	OQ152301-3	
	BR1-8	ES: Irkutsk reservoir, IR, Russia; 51.962708, 104.713775	8	
	BR10-20	ES: Irkutsk reservoir, IR, Russia; 52.118985, 104.481527	11	
	C175	Olkha River, IR, Russia; 52.138078, 104.073423	1	
	M1 C222	Irkut River, IR, Russia; 51.919409, 103.697399 Irkut River, IR, Russia; 52.280077, 104.209310	1 1	
	C140	Belaya River, IR, Russia; 52.866246, 103.310471	1	
	G80 KM77-78 C137	ES: Lake Gusinoe, RB, Russia; 51.116221, 106.363094	KM095104 3	
	G81	ES: Lake Tsaidam, RB, Russia; 50.564094, 105.788088	KM095103	
	K65-67	ES: Ina River, RB, Russia; 53.654650, 109.840142	OQ152298-300	
	K79-80 K81-K82 P1-P7	ES: Lake Baikal, RB, Russia; 51.953118, 106.127163 ES: Lake Baikal, RB, Russia; 53.745792, 109.110156	OQ152306-7 2 7	
	S1-S4	ES: Yana River, RS, Russia; 67.553945, 133.367242	4	
	<i>Piscicola khubsugulensis</i> sp. nov.	K86-87 C183-184	ES: Lake Khovsgol, Mongolia; 51.080021, 100.477723	OQ152308-9 OQ152310-1
		K18-19 X494-496	ES: Lake Khovsgol, Mongolia; 50.441656, 100.181546	OQ152312-3 4
<i>Cystobranchnus mammilatus</i> (Malm, 1863) <sup>2</sup>	B44 KM1-2	EU: Pechora River, RK, Russia; 65.427151, 52.134787	OQ152315 2	

<sup>1</sup> EU—Europe, including RK—Republic of Komi and RR—Rostov Region; WS—Western Siberia, ES—Eastern Siberia, including IR—Irkutsk Region, RB—Republic of Buryatia and RS—Republic of Sakha (Yakutia). <sup>2</sup> denotes the only non-*Piscicola* species which was sequenced in this study.

### 3. Results

The definition of the species boundaries in many groups of animals, especially invertebrates, is often ambiguous due to the insufficient number of visually distinguishable morphological features and the lack of a unified approach to assessing their taxonomic and phylogenetic significance. This hampers species determination and often leads to the formation of polytypic species that represent a flawed taxonomic system. A prime example of this is Hirudinea, including the family Piscicolidae. The taxonomy of this group has been revised many times [1,3,4,18,42], and this process is currently ongoing [5]. To eliminate the so-called “human factor”, to resolve the difficulties associated with assessing the estimation of species boundaries and to, therefore, attain a more accurate determination of species diversity, genetic approaches based on statistical bioinformatics were integrated with the classical analysis of morphological characters.

The modern approach to the taxonomy of organisms includes the use of molecular phylogeny and the statistical methods for processing its results. Data on the genetic structure of a taxon allows not only the determination of its relationship with the rest but also the provision of a technique for reliably determining the place of an unknown specimen in the existing classification. To date, the DNA barcoding technique has been successfully introduced for these purposes [30–32]. DNA barcoding as a formalized method for including genetic attributes in a taxonomic analysis elucidates the intellectual content of taxonomy from a molecular perspective. The method hypothesizes the existence of a barcode gap, visualizing a significant difference in the frequency distribution of the intra- and interspecific variations of the nucleotide composition in DNA marker sequences.

#### 3.1. DNA Identification of Leeches

The performed molecular genetic analysis made it possible to decipher the nucleotide sequences of the *cox1* gene fragment of 639–657 bp in length for 28 specimens, including 23 Siberian and 5 European piscine leeches (Table 1). This part of the genome was selected since it was recognized as a versatile tool for the DNA barcoding of Metazoa [30–32] and especially useful for the molecular identification of leeches (e.g., [43–48]).

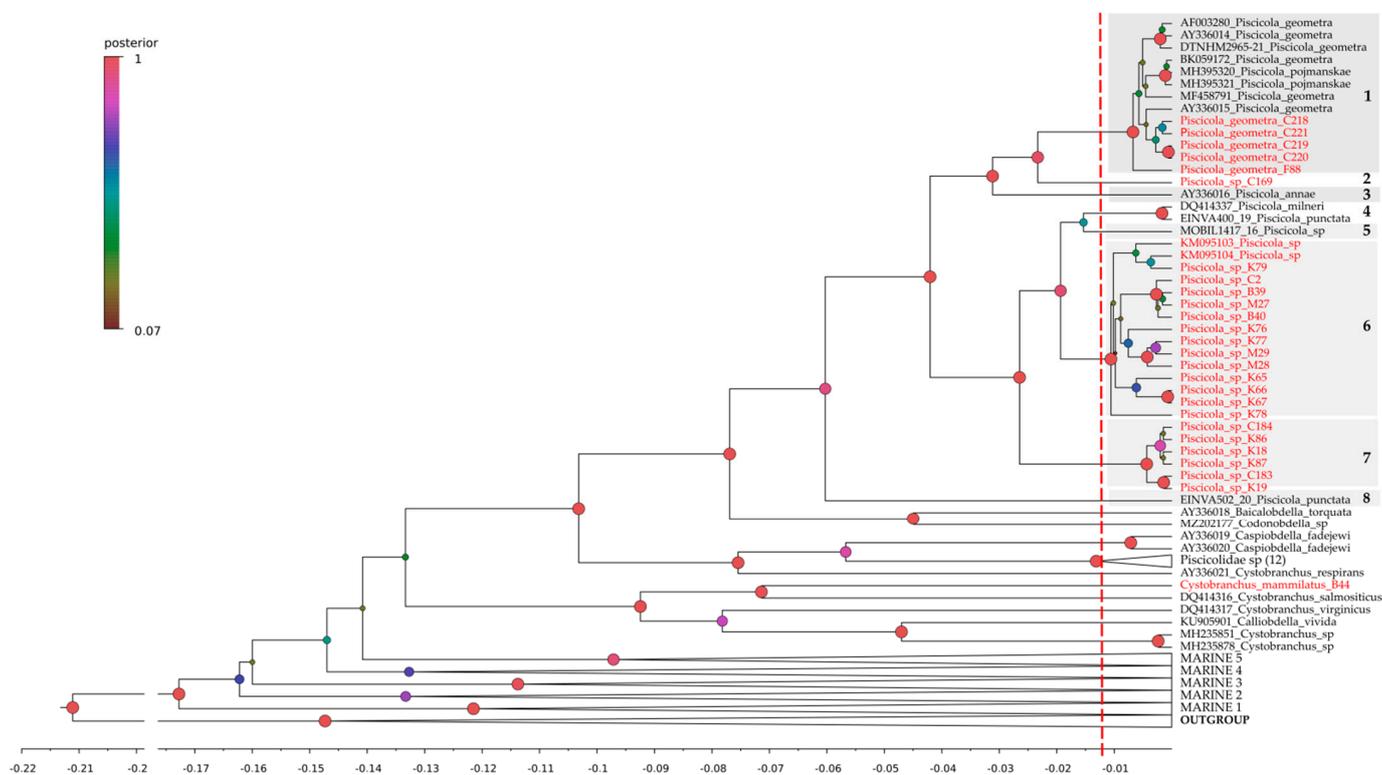
The BLAST analysis revealed the nucleotide sequences most similar to the Siberian samples throughout the worldwide genetic data available at the moment. The resulting set of *cox1* sequences consisted of 100 sequences belonging to different organisms (with an identity of 83% and higher), including 85 representatives of Piscicolidae, whose genetic identity ranged from 83.82 to 97.84%. The *cox1* sequences obtained from the BLAST list belonged to 25 genera of piscine leeches. At the top of the list, there were 10 sequences of the genus *Piscicola* representatives with an identity ratio of 93.35 to 97.84% (Table 2).

**Table 2.** Top 10 Piscicolidae homologous sequences selected using BLAST with the statistical significance of the matches.

Description	Max Score	Query Cover	Identity	Length, bp	Accession
<i>Piscicola milneri</i>	1120	100%	97.84%	665	DQ414337
<i>Piscicola geometra</i>	976	100%	93.83%	15135	OX030972
<i>Piscicola geometra</i>	976	100%	93.83%	14788	BK059172
<i>Piscicola geometra</i>	976	100%	93.83%	651	AF003280
<i>Piscicola pojmanskae</i>	924	95%	93.68%	617	MH395321
<i>Piscicola pojmanskae</i>	924	95%	93.68%	617	MH395320
<i>Piscicola geometra</i>	939	96%	93.63%	634	MF458791
<i>Piscicola annae</i>	922	95%	93.53%	629	AY336016
<i>Piscicola geometra</i>	920	95%	93.53%	631	AY336014
<i>Piscicola geometra</i>	913	95%	93.35%	628	AY336015

The comparison group used for the analysis of the Siberian piscine leeches was formed mainly on the basis of the BLAST data and was supplemented with single findings obtained directly from the GenBank and BOLD databases. The *cox1* sequences of freshwater

fish-parasitic leeches were selected and uploaded with the utmost care since there are few of them in the international genetic databases. On the contrary, redundant marine species data were not included in order not to overload the analysis. Seven homologous sequences of flat leeches (Glossiphoniidae), the closest family to fish leeches, were selected as the outgroup: AY047325 *Haementeria molesta*, MF150168 *Helobdella stagnalis*, MH643798 *Hemiclepsis marginata*, MH670853 *Glossiphonia verrucata*, OK350346 *Placobdella costata*, AY047318 *Theromyzon tessulatum* and LC413906 *Torix tukubana*. The final dataset matrix consisted of 116 terminals (62 freshwater and 47 marine and brackish water fish leeches) and 657 aligned nucleotide characters. Based on the resulting alignment, an ultrametric Bayesian tree was reconstructed (Figure 2); then, on this tree, using the GMYC algorithm [36], a threshold between the intraspecific and interspecific variability equal to 1.19 was statistically calculated (red dotted line in Figure 2).



**Figure 2.** Bayesian tree based on the barcoding genome fragment alignment of 116 Hirudinea specimens, including data on 81 freshwater and marine piscine leeches and 7 glossiphoniids, which were used as comparison group and outgroup, respectively. Tip labels of the 28 *cox1* sequences assembled in this work are colored in red. The red dotted line indicates the threshold separating intra- and interspecies variability.

The phylogenetic tree showed that the group of the Siberian leeches' sequences formed two distinct lineages within the branch of the *Piscicola* representatives, with the highest values of the Bayesian posterior probability (nos. 6 and 7 in Figure 2). One lineage united 6 *cox1* sequences of the piscine leeches from Lake Khovsgol, and the other one united 15 sequences from the different rivers and lakes of Eastern Siberia (Table 1). Both Siberian lineages appeared to be closely related to the North American *Piscicola milneri* (Verrill, 1871) (Figure 2); nevertheless, the genetic distances (p) between these three groups ranged from 2.53% to 3.92% (Table 3). According to the DNA barcoding theory, such a number of nucleotide substitutions corresponds to the species level of genetic variability [30–32] and confirmed the species status of these three leech groups (the two Siberian lineages and *P. milneri*).

**Table 3.** Estimates of average evolutionary divergence over sequence pairs within and between the *Piscicola* species groups.

No.	Species Group	P	SE	1	2	3	4	5	6	7	8	9
1	<i>P. annae</i>	n/c	n/c		0.0083	0.0092	0.0085	0.0105	0.0098	0.0094	0.0070	0.0081
2	<i>P. geometra</i>	0.0043	0.0014	0.0491		0.0090	0.0022	0.0104	0.0094	0.0090	0.0089	0.0072
3	<i>P. milneri</i>	0.0015	0.0015	0.0598	0.0577		0.0093	0.0104	0.0048	0.0054	0.0068	0.0086
4	<i>P. pojmanskae</i>	0	0	0.0487	0.0047	0.0559		0.0104	0.0096	0.0046	0.0093	0.0078
5	<i>P. punctata</i>	n/c	n/c	0.0734	0.0754	0.0677	0.0697		0.0112	0.0104	0.0107	0.0107
6	<i>Piscicola</i> sp.	n/c	n/c	0.0637	0.0594	0.0152	0.0570	0.0734		0.0058	0.0067	0.0093
7	<i>P. sibirica</i> *	0.0102	0.0020	0.0653	0.0640	0.0253	0.0628	0.0749	0.0259		0.0074	0.0090
8	<i>P. khubsugulensis</i> *	0.0022	0.0013	0.0615	0.0565	0.0308	0.0556	0.0726	0.0265	0.0392		0.0090
9	<i>P. nordica</i> *	n/c	n/c	0.0447	0.0386	0.0546	0.0389	0.0738	0.0581	0.0607	0.0565	

\* New species discovered during this study: *P. sibirica* sp. nov. (the different Siberian waters), *P. khubsugulensis* sp. nov. (Lake Khovsgol) and *P. nordica* sp. nov. (Lake Golovka).

In addition, the sequence of a single leech caught in Lake Golovka (Northeastern Europe, Table 1) formed a separate branch that was phylogenetically closest to the *Piscicola geometra* cluster (no. 2 in Figure 2). The genetic distance (p) separating these species was  $3.86 \pm 0.70\%$  (Table 3).

Noticeably, the *Piscicola geometra* cluster itself (no. 1 in Figure 2) included newly sequenced specimens from southeastern Europe and southern part of Western Siberia as well as both specimens of *Piscicola pojmanskae* from Poland (MN395320 and MN395321). The intraspecific polymorphism of *P. geometra*, with and without the *P. pojmanskae* sequences, was 0.44 and 0.43% (Table 3), respectively. Such a low level of genetic differences undoubtedly indicated their species identity.

Two sequences of the North American leech *Piscicola punctata* (Verrill 1871) found in the BOLD database (EINVA400\_19 and EINVA502\_20), as seen in Figure 2, belonged to different phylogenetic lineages (nos. 4 and 8). Indeed, the genetic distance between them was  $6.85 \pm 1.01\%$ , which did not allow them to be attributed to the same species. At the same time, the sequence of *P. punctata* EINVA400\_19 was clustered together with *P. milneri* with a 100% posterior probability (no. 2 in Figure 2), and the genetic distance (p) was only 0.15% (Table 3). Obviously, the specimen EINVA400\_19 was incorrectly assigned to *P. punctata* and, with a high degree of probability, belongs to *P. milneri*.

As for the *Piscicola* sp., a piscine leech from Alaska (no. 5 in Figure 2), the position of this specimen was not so well-defined. According to the BI-tree, the *cox1* sequence of the *Piscicola* sp. was phylogenetically related to the *Piscicola milneri* lineage (Figure 2) with a genetic difference between them of  $1.52 \pm 0.46\%$  (Table 3), which was still within the acceptable level of intraspecific variability by the DNA barcoding theory [30–32]. However, the GMYC species delimitation analysis suggested the assignment of the *Piscicola* sp. from Alaska to a separate species, leaving room for its attribution to *P. milneri*, since the threshold confidence interval allowed this.

Thus, depending on the taxonomic status of the Alaskan specimen, there were seven or eight genetically recognizable *Piscicola* species within the studied dataset.

### 3.2. Morphological Identification of Piscine Leeches

The biological material of the 117 specimens of piscine leeches collected in the Palaearctic (Table 1) was available for a morphological analysis. Most of the specimens from the western part of Russia and from Eastern Kazakhstan were reliably identified as *Cystobranchus mammilatus* (Malm, 1863) and *Piscicola geometra* (L., 1761) (Table 1), while only one specimen from Lake Golovka was attributed to a potentially new species because its morphology was unmatched to the known species. The results obtained using the classical morphological approaches were consistent with the molecular genetic data on species delimitation (Figure 2).

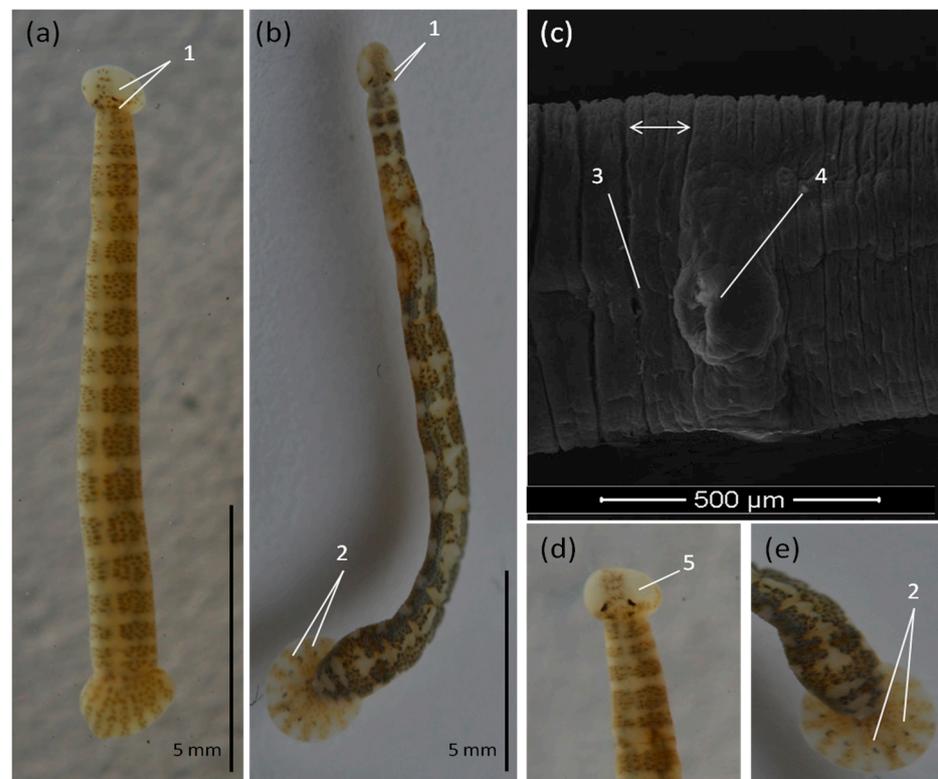
The specimens of piscine leeches from Eastern Siberia were represented by two morphologically different groups that had no analogues among the currently described species.

Therefore, three new species were identified during the morphological investigation. Their taxonomic status was supported by the DNA-based species delimitation analyses (Figure 2, Table 3).

### 3.3. Taxonomy of Studied *Piscicola* and Description of New Species

Phylum:	ANNELIDA Lamarck, 1809
Classis:	CLITELLATA Michaelsen, 1919
Subclassis:	HIRUDINEA Lamarck, 1818
Order:	RHYNCHOBDELLIDA (Blanchard, 1894)
Family:	PISCICOLIDAE Johnston, 1865 (syn. ICTHYOBDELLIDAE Leuckart, 1863)
Genus:	PISCICOLA de Blainville, 1818
Type species:	<i>Piscicola geometra</i> (Linnaeus, 1761)
Species:	<b><i>Piscicola geometra</i> (Linnaeus, 1758)</b> (Figure 3)

**Neotype:** Russia, Temernik River in Rostov Region, a right tributary of the Don, from aquatic vegetation, leg. E. Matveenko (28-X-2015).



**Figure 3.** Morphology of *Piscicola geometra* (L., 1758): (a,b) body proportions and pigmentation of ethanol-fixed specimens from European part of Russia (left) and Western Siberia (right), (c) body fragment with gonopores (SEM), (d) anterior sucker and trachelosome and (e) part of urosome with caudal sucker. Numerical designations: 1—eyes, 2—punctiform eye-like spots, 3—female genital opening, 4—male genital opening and 5—depigmented area.

**Paratypes:** There were 11 specimens from the aquatic vegetation of the Temernik River in the Rostov Region of Russia, leg. E. Matveenko (28-X-2015); 12 specimens were from the benthic samples of the Bukhtarma reservoir in the Irtysh River, Kazakhstan, leg. L. Fedorova (11-IX-2015).

**Specimens deposited:** The neotype (voucher LIN\_C220) and 23 paratypes (vouchers C218, C219, C221, KM3-11, F88 and L1-11) were preserved in 80% ethanol and were deposited in the collection of Irina Kaygorodova, Limnological Institute.

**Revised description:** The body of the specimens had a total length of up to 50 mm [3], including the suckers. The maximum known length of the European specimens is about 33 mm [4]. Leeches from our collection were rather small and had a body length of 10–15 mm, with an urosome width of up to 1.3 mm. The body was smooth, thin-walled, evenly elongated, round in the cross section and indistinctly differentiated into the trachelosome and urosome. Eleven pairs of small pulsatory vesicles were apparent in the lateral margins of the urosome (Figure 3). Pulsatory vesicles were small and obscure, each covering at least two annuli; they were difficult to see after fixing the specimens.

The suckers were relatively large and round; the oral sucker was up to 1.0 mm, and the caudal sucker was up to 2.0 mm in diameter. The caudal sucker was located excentrically and almost twice exceeded the maximum body width.

There were two pairs of cephalic eyes on the oral sucker; the eyes shape was typical for members of the genus *Piscicola*, while the pigmentation of the cephalic end formed a specific pattern (Figure 3d). The upper pair of crescentiform eyes was located at the lower border of the depigmented area of the cephalic end; the lower pair was represented by punctiform eyes located at the base of the sucker.

On the caudal sucker, there were 12–14 eye-like spots separated by dark pigmented rays.

The color of both the live and fixed leeches was brownish-olive. On the dorsal side, there was a geometric pattern characteristic of this species that was formed by one medial bright band and multiple transverse bright stripes and that was evenly distributed from the head to the caudal sucker. The arrangement of the transverse bright stripes clearly correlated with the lateral vesicles in the urosome and extended into the trachelosome; this created a cruciform pattern on the dorsal surface (Figure 3a). Sometimes this pattern was broken into repeating cross-shaped spots (Figure 3b). The number of segmental stripes or spots was 19 or 20, depending on how noticeable the last stripe near the caudal sucker was. The ventral side was always lighter. The segmental pattern here was less pronounced or not visible at all.

The external reproductive system of *P. geometra* consisted of the male and female openings (gonopores), a well-developed copulatory region within which the copulatory gland opened (Figure 3c). The gonopores were separated by three annuli. There were also three annuli between the female pore and the opening of copulatory gland. The sperm ducts were simply looped [1,3]. The tops of the ejaculatory canals did not reach the fourth ganglion or were at its level [3].

**Ecology:** The species lives in both flowing and stagnant water bodies with a favorable oxygen regime. In floodplain water bodies, it dies out in winter due to a decrease in the level of oxygen dissolved in the water. Nevertheless, it is known that *Piscicola geometra* does not fall into an anabiotic state at low temperatures, unlike other leeches [18].

This leech feeds on the blood of various fish without showing any host-specificity. Like other *Piscicola* species, it is not a permanent ectoparasite and, once satiated, leaves the host's body; therefore, *P. geometra* spends most of its life at the bottom among the aquatic vegetation.

**Geographic distribution:** According to Nesemann and Neubert [4], the distribution of *Piscicola geometra* as a whole remains unknown because it is often confused with other piscicolid species. Being a typical representative of the Palaearctic fauna, this species was thought to be found throughout Northern Eurasia with the exception of the Kamchatka Peninsula and Lake Baikal [3,18]. However, our study showed that the Yenisei River serves as the eastern boundary of its distribution.

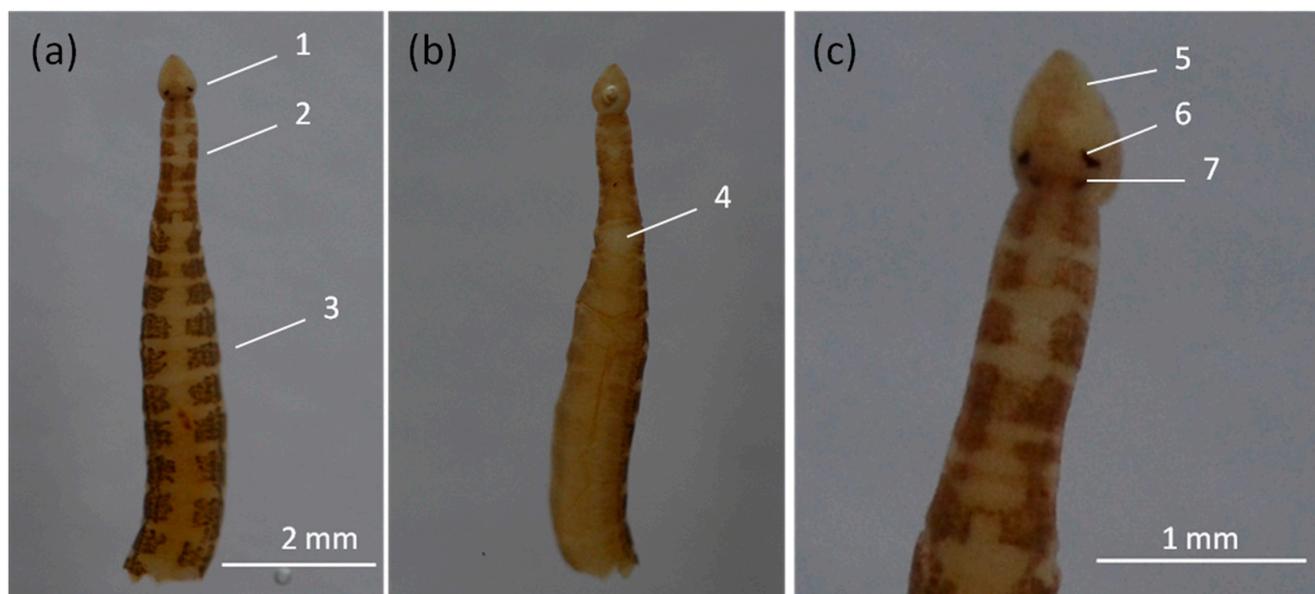
Species: *Piscicola nordica* sp. nov. Kaygorodova (Figure 4)

**Holotype:** Russia, Lake Golovka, a member of Kharbey lake system, from the whitefish *Coregonus lavaretus* (L., 1758), leg. M. Baturina (01-VIII-2012).

**Specimens deposited:** One specimen from the dorsal fin of a whitefish was fixed in 80% ethanol and was deposited in the collection of Irina Kaygorodova, Limnological Institute.

**Etymology:** The species name was chosen to emphasize the geographic confinement of this miniature leech to the harsh conditions of the Russian North.

**Description:** There was only one immature specimen at our disposal, and the DNA-based identification of which clearly indicated its species status within the genus (Figure 2, Table 3). The body length of the junior individual was 6 mm, excluding the suckers. The trachelosome was 2 mm long and 0.2–0.4 mm wide; its smallest diameter was at the cephalic end at the base of the oral sucker. The maximal diameter of the urosome was 1.0 mm. The body was elongated, rounded in the cross section in vivo and indistinctly differentiated into the trachelosome and urosome; the surface was smooth and thin-walled. The body shape of the fixed specimens changed to a flattened one. The pulsatory vesicles were very small, each covering two annuli. The anterior sucker was pointed, spear-shaped and up to 0.6 mm long. The caudal sucker was accidentally lost, so its size was unknown (Figure 4).



**Figure 4.** Morphology of *Piscicola nordica* sp. nov.: (a) dorsal view, (b) ventral view and (c) anterior part of body. Numerical designations: 1—oral sucker, 2—trachelosome, 3—urosome, 4—copulatory area, 5—depigmented area, 6—crescentiform eye and 7—baculiform eye.

At the cephalic end, there were two pairs of eyes and a specific fungaceous pigmentation pattern (Figure 4c). The upper pair of the typical crescentiform eyes was located at the lower border of the depigmented area of the cephalic end, while the lower pair was represented by baculiform eyes located at the base of the sucker.

The color of the living leech was greenish-olive, and, after fixation, the coloration changed to a brownish color. On the dorsal body side, there was a geometric pattern formed by a medial light band and transverse stripes crossing it (Figure 4a). The transverse light stripes corresponded with the pulsating vesicles located laterally on the urosome and continued along the trachelosome to the head sucker. The ventral side of the body was equally less pigmented and, therefore, lighter than the dorsal side.

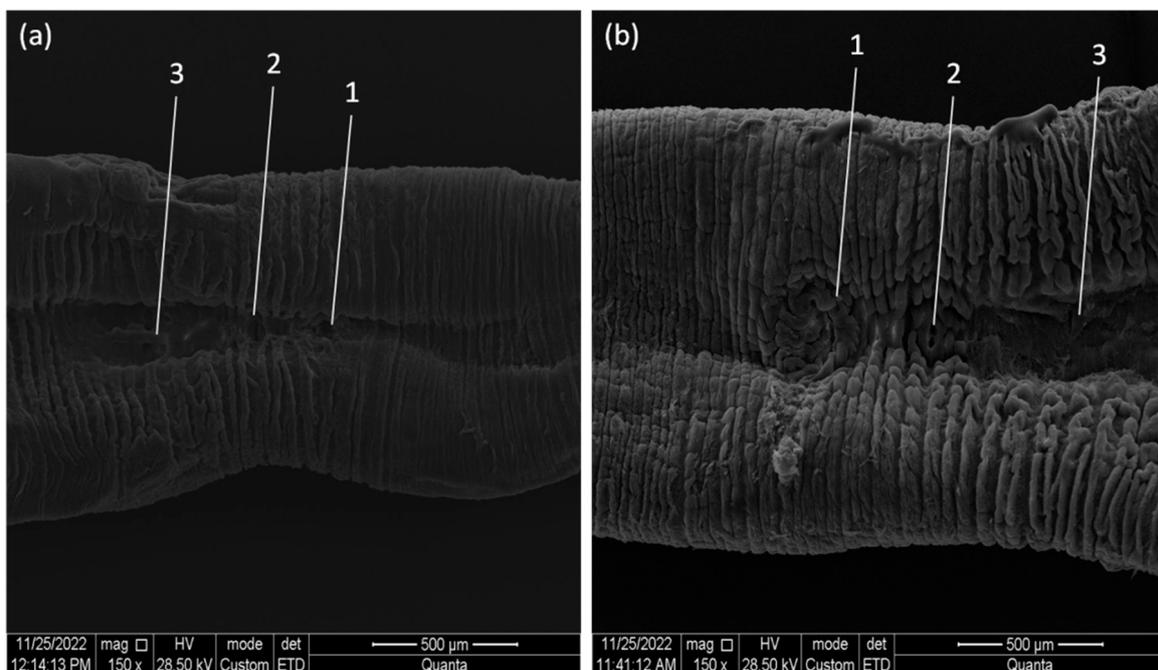
The reproductive system was not fully developed but had a typical arrangement of the genital pores (male and female) and copulatory area (Figure 4b). The gonopores were separated by four annuli.

**Ecology:** The leech is an ectoparasite of fish, apparently preferring the salmonids of the genus *Coregonus*.

**Geographic distribution:** Kharbey lake system, Republic of Komi, Russia.

Species: *Piscicola sibirica* sp. nov. Kaygorodova (Figures 5 and 6)

**Figure 5.** Morphology of *Piscicola sibirica* sp. nov.: (a) exterior of living junior leech from Lake Baikal (voucher K79), (b) region of cephalic end with trachelosome and sucker, (c) fragment of urosome with caudal sucker, (d) exterior of fixed adult leech from Angara River (voucher KM15), (e) anterior end of fixed specimen from the Irkutsk reservoir (M51) and (f) external morphology of fixed specimen (M51). Numerical designations: 1—cephalic end, 2—eyes, 3—caudal sucker, 4—eye-like spots, 5—depigmented area, 6—median band and 7—pulsatory vesicles.



**Figure 6.** External organs of the *Piscicola sibirica* sp. nov. reproductive system: (a) specimen from the Bratsk reservoir (voucher BS31) and (b) specimen from the Irkutsk reservoir (voucher M28). Numerical designations: 1—male gonopore, 2—female gonopore and 3—external copulatory area.

**Holotype:** Russia, Irkutsk reservoir in the Angara River near Taltsy, from pike *Esox lucius* (L., 1758), leg. P. Anoshko (15-V-2020).

**Paratypes:** 97 specimens in total were from the different waters of Eastern Siberia (Table 1), and, of them, 1 specimen was from a grayling *Thymallus baicalensis* Dybowski, 1874 of the Angara River, leg. M. Ageev (11-IX-2016); 25 specimens were from the pike of the Angara River near Angarsk, leg. E. Dzuba (01-V-2018); 5 specimens were from the blanket algae of the Bratsk reservoir in the Angara River near Svirsk, leg. A. Kupchinski (03-VIII-2012); 2 specimens were from the pike of the Irkutsk reservoir in the Angara River near Taltsy, leg. P. Anoshko (15-V-2020); 8 specimens were from the elodea of the Irkutsk reservoir in the Angara River near Bolshaya Rechka, leg. P. Anoshko (10-IV-2019); 11 specimens were from the pike of the Irkutsk reservoir in the Angara River, leg. E. Dzuba (02-V-2018); 1 specimen was from a benthic sample of the Olkha River, a right tributary of the Irkut, leg. E. Matveenkov (09-I-2016); 2 specimens were from the Irkut River, leg. I. Knizhin (20-VI-2014); 1 specimen was from a benthic sample of the Belaya River, a left tributary of the Angara, leg. T. Sitnikova (26-VIII-2011); 1 specimen was from the perch of Lake Gusinoe and 3 specimens were from an aquatic vegetation of Lake Gusinoe, leg. I. Kaygorodova (28-VII-2013); 1 specimen was from the pike of Lake Tsaidam, leg. I. Kaygorodova (27-VII-2013); 3 specimens were from the macrophytes of the Ina River, a left tributary of the Barguzin, leg. L. Sukhanova (03-X-2012); 4 specimens were from an aquatic vegetation of the Posolsky Sor of Lake Baikal, leg. I. Kaygorodova (01.08.2012); 7 specimens were from the perch of the Chivyrkuy Bay of Lake Baikal, leg. N. Pronin (data unknown); 4 specimens were from the Yana River, leg. V. Samusenok (17-VIII-2002).

**Specimens deposited:** Type material was fixed and preserved in 80% ethanol, except for the specimens from the Chivyrkuy Bay of Lake Baikal (seven specimens) and the Yana River (four specimens), which were initially fixed in 4% formalin. The holotype (voucher LIN\_M28) and paratypes (vouchers BS31-33, BS77, BS78; M27-29, KM12-31, BR2-8, BR10-20, B38-40, C2, A1-22, C175; M11, C222, C140, K79-80, K81-K82, P1-7, G80, KM77, KM78, C137; G81, K65-67 and S1-S4) were placed in the collection of Irina Kaygorodova, Limnological Institute.

**Etymology:** The name was chosen to reflect the geographic range of the new species and to emphasize its wide distribution in Siberia.

**Description:** The body was up to 6–31 mm of the total length, excluding the suckers. The biggest specimens were found in the Irkutsk reservoir; their length was 22.8 mm on average, with an urosome width of up to 4.0 mm. The body was evenly elongated, round in the cross section of the live leeches (Figure 5a) and indistinctly differentiated into the trachelosome and urosome; the surface was smooth and thin-walled. The body of fixed specimens often changed to a flattened shape (Figure 5d). The pulsatory vesicles were small, each covering at least two annuli; they were difficult to see in the living leeches but were clearly visible in the fixed individuals (Figure 5f).

The suckers were relatively large and round; the oral sucker was up to 2.0 mm and the caudal sucker was up to 4.0 mm in diameter. The caudal sucker was located eccentrically; the ratio of its diameter to the maximal body width was 1.4–1.5 on average. At the cephalic end, there were two pairs of eyes of a typical shape, while the pigmentation of the oral sucker formed a species-specific pattern (Figure 5a,b,e). The upper pair of crescentiform eyes was located at the lower border of the depigmented area of the cephalic end; the lower pair was represented by punctiform eyes located at the base of the sucker. On the caudal sucker, there were 8–10 eye-like spots separated by dark pigmented rays (Figure 5c,d,f).

The color of the living leeches was greenish-olive, and, after fixation, it changed to light or dark brown. Three bright light bands ran along the body: a median band on the dorsal body surface and one on each of the lateral sides (Figure 5a,d,f). The lateral light stripes connected the pulsatory vesicles located on the urosome and continued along the trachelosome up to the head sucker. The ventral side was always lighter due to a less-pigmented broad medial band, while the trachelosome and both margins of the urosome remained darkly pigmented.

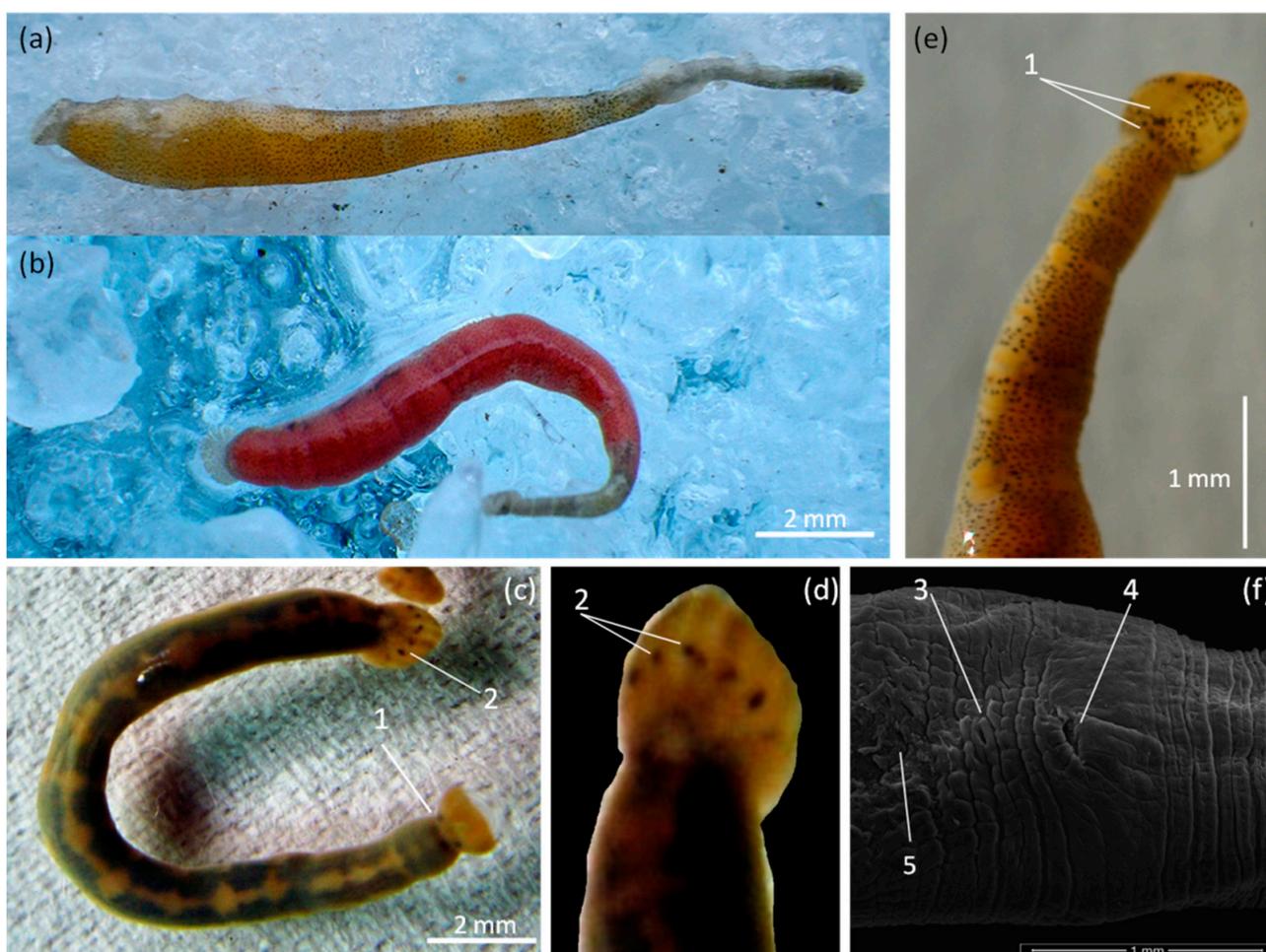
The external reproductive system of the *Piscicola sibirica* sp. nov. was typical for the genus and consisted of the genital pores (male and female openings) and the copulatory region within which the copulatory gland opened (Figure 6). The copulatory zone was well developed. The gonopores were separated by three annuli; however, there was only one annulus between the female pore and the copulatory gland opening.

**Ecology:** The new species is a pronounced oxyphile living in rivers and lakes with a high content of dissolved oxygen in the water; it is sensitive to pollution and eutrophication. Preferring meso- or oligotrophic waters, the *Piscicola sibirica* sp. nov., however, does not enter the open parts of Lake Baikal and occurs mainly in the warmed bays of the lake (Posolsky Sor and Chivyrkuy Bay). The *Piscicola sibirica* sp. nov. does not fall into anabiosis during winter like *P. geometra*.

This leech was found to parasitize various fish (pike, perch, and, occasionally, grayling) without showing any host specificity. The *Piscicola sibirica* sp. nov. belongs to facultative ectoparasites since, after fattening, it leaves the host, spending most of its time as part of a free-living benthos; therefore, it is often found among aquatic vegetation.

**Geographic distribution:** The new species is widely distributed throughout the freshwaters of Eastern Siberia, outside of which it has not been found (Figure 1).

Species: *Piscicola khubsugulensis* sp. nov. Kaygorodova (Figure 7)



**Figure 7.** Morphology of *Piscicola khubsugulensis* sp. nov.: (a,b) body proportions and pigmentation of living individuals (voucher C183-184), (b) the glow of the spermatophore, (c) fixed specimen (voucher X495), (d) posterior sucker, (e) anterior sucker and trachelosome and (f) body fragment with genital openings of the X495 specimen (SEM). Numerical designa: 1—eyes, 2—eye-like spots, 3—female genital opening, 4—male genital opening and 5—copulatory gland.

**Holotype:** Mongolia, Lake Khovsgol, from Kosogol grayling *Thymallus nigrescens* Dorogostaisky, 1923, leg. A. Mamontov (28-VI-2011).

**Paratypes:** Four specimens parasitizing grayling were from the type habitat, leg. A. Mamontov (28-VI-2011); four specimens from another locality of Lake Khovsgol also parasitized grayling, leg. M. Lazarev and O. Kirilchik (24-III-2012).

**Specimens deposited:** The holotype (LIN\_495) and nine paratypes (K86, R87, C183, C184, 494, 496 and 497) were fixed in 80% ethanol and deposited in the collection of Irina Kaygorodova, Limnological Institute.

**Etymology:** The species was named to emphasize its endemism to the unique Mongolian lake; however, in honor of the author, the Russian pronunciation of the lake name was taken as the basis for the Latin name of the species.

**Description:** The body was up to 15–22 mm of the total length, excluding the suckers. On average, the leeches were 18 mm long and 1.9 mm wide at the largest part of the urosome. The body of the leeches was elongated, evenly expanding posteriorly, round in the cross section and indistinctly differentiated into the trachelosome and urosome (Figure 7a,b); the surface was smooth and thin-walled. The pulsatory vesicles were small, each covering at least two annuli; they were clearly visible in both the live and ethanol-fixed leeches (Figure 7b,c).

The suckers were relatively small and round and were about the same size; both were up to 2.5 mm in diameter. The caudal sucker was located excentrically, and the ratio of its diameter to the maximal body width was 0.84 on average. On the caudal sucker, there were 6–8 thick eye-like spots located at the base of the dark pigmented rays (Figure 7d).

At the cephalic end, there were two pairs of eyes of a typical shape; the pigmentation of the oral sucker formed a specific fungaceous pattern (Figure 7e). The upper pair of crescentiform eyes was located at the lower border of the depigmented area of the cephalic end; the lower pair was represented by punctiform eyes located at the base of the oral sucker.

The color of the living leeches varied within yellowish-brown tints; after fixation, the color of the pigmentation changed to light brown or dark green. Three rows of bright light spots ran along the body: a median band on the dorsal surface of the body (Figure 7c) and one row of spots on each of the lateral sides. The lateral bright spots coincided with the pulsating vesicles of the urosome and continued along the trachelosome in the direction to the head sucker. In addition, there was a mid-dorsal depigmented strip on which the same medial bright spots were strung like beads (Figure 7c). The dorsal pattern was not always clearly visible, especially on the live leeches (Figure 7a,b). The width and degree of the depigmentation of the median dorsal light stripe varied in different individuals; in some, it was very thin and barely noticeable (Figure 7a,b), and, in others, on the contrary, it was well expressed (Figure 7c). The pigmentation of the ventral side of the body was similar to that of the dorsal side but was without bright spots.

The external reproductive system of the *Piscicola khubsugulensis* sp. nov. was typical for the genus and consisted of the genital pores (male and female openings) and the copulatory region within which the copulatory gland opened (Figure 7f). The copulatory zone was well developed. The gonopores were separated by two annuli; there were also two annuli between the female pore and the copulatory gland opening.

**Ecology:** This species is endemic to Lake Khovsgol; it feeds by sucking the blood of local grayling exclusively.

**Geographic distribution:** The range of the *Piscicola khubsugulensis* sp. nov. is limited to Lake Khovsgol, Mongolia.

## 4. Discussion

### 4.1. Adjustments in the *Piscicola* Species Diversity

The determination of faunal diversity is a fundamentally important link that lays the foundation for making decisions on conservation activities. However, the accuracy of the results obtained often depends on the chosen methodological approach and the qualifications of the researcher. Morphological identification is known to be the most vulnerable and

dependent on the subjective opinion of the researcher, especially in taxonomically complex groups such as invertebrates. The consequences of an incorrect assessment of biodiversity go far beyond purely taxonomic issues. The most common errors in morphological taxonomy involve both the oversplitting of closely related organisms into distant species and the overlumping of distant organisms into a single highly variable species, which can impede any downstream analysis. The presence of overrepresented and underrepresented species in datasets can lead to distorted conclusions in ecological modeling and, as a result, to incorrect solutions to environmental problems.

To avoid the difficulties associated with morphologically assessing species, DNA-based methods have been developed. The first successful project on the use of genetic information for solving the problems of taxonomic assignment was DNA barcoding, and a threshold of the genetic distance of about 2% was established [30,31], indicating the maximum value of intraspecific divergence for the *cox1* fragment [33]. More recently, new genetic approaches have been developed that use the time of divergence [49], the nature of branching [50] and the frequency of genetic distances [51] to distinguish between species. These methods have been successfully applied to various groups, including leeches [47,48].

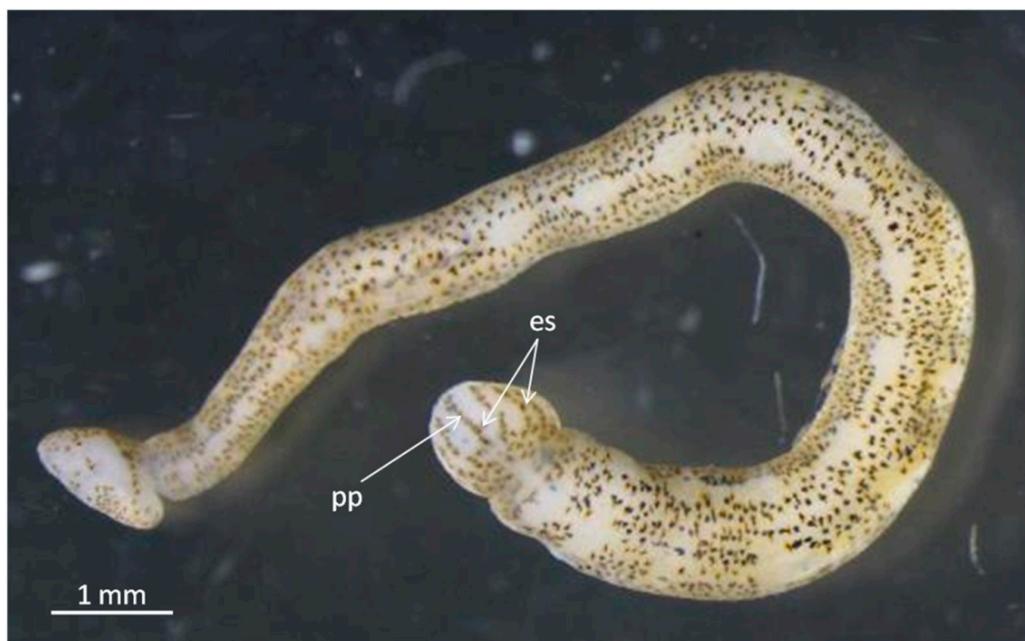
Taking advantage of the DNA-based approach, we were able to confidently identify seven *Piscicola* species within the available genetic dataset: four previously known (*P. geometra*, *P. annae*, *P. milneri* and *P. punctata*) and three new species (*P. nordica* sp. nov., *P. sibirica* sp. nov. and *P. khubsugulensis* sp. nov.). At the same time, some errors were revealed in the morphological determination of the samples whose genetic data were publicly available through international databases (GenBank and BOLD).

A classic example of species oversplitting was detected within *P. geometra*. The sequences of the *P. pojmanskae* specimens were grouped within a single phylogenetic lineage with those of the geographically heterogeneous *P. geometra* (Figure 2). Turning to the morphological description of *P. pojmanskae* [5,29,52], we found that its morphological differences were taxonomically insignificant and could easily be attributed to the variability of *P. geometra*'s characters. There are 10 morphological characters of *P. pojmanskae* that distinguish this species from *P. geometra* according to the original description [29]; all the diagnostic characters indicated by the author are most likely unreliable since they refer either to individually variable metric features of external and internal morphology (e.g., body length, copulatory area length, conducting tissue length and width of vector tissue), subjective assessments (body shape and shape of its individual parts) or even dubious assessments (coloration pattern and distance between gonopores). In the photos of these species presented by Kendzierska et al. [52], the similarity in the dorsal pigmentation, the shape of suckers and the arrangement of the pigmentation on them is clearly seen. As for the distance between the gonopores, an important trait in the taxonomy of leeches, the number of annuli found in *P. geometra* varies in different works. There are five annuli between the gonopores according to A. Bielecki [5,29]. In a later article, he and his colleagues had already found 4–5 annuli [52]. The results of the morphological analysis obtained in this work convincingly demonstrated the presence of three annuli between the male and female genital openings in geographically distinct *P. geometra* (Figure 3), confirming the opinion of a group of American scientists [1,53]. Thereby, the reduction of *P. pojmanskae* into a synonymy of the species *P. geometra* was supported by both the morphological and genetic data.

Furthermore, there is a hypothesis [54] that the taxonomic status of the recently described piscicolid species from Poland (including [5,29]) is controversial since most of them are the morphological forms of *P. geometra* rather than individual species from an ecological point of view. In addition, when compared with the other species described by A. Bielecki in 1997 [5], their author argued that *P. pojmanskae* is morphologically close not only to *P. geometra* but also to four species (*P. margaritae*, *P. elishebae*, *P. jarai* and *P. pomorskii*), which, under the new conditions, calls into question the species status of more than just *P. pojmanskae*. Indeed, a species richness of about 17–20 species for each biocenotic type is known to be typical of the aquatic environments of Central Europe. [4,54]. However,

15 species of the genus *Piscicola* alone were reported in Poland [5–8,29], while in other parts of the Palaearctic, only 8 species of this genus have been recorded, including the three new species described in this paper. Against this background, the Polish faunistic list of piscicolids looks somewhat redundant and requires revision.

Another misattribution was discovered among Nearctic species: one of the *Piscicola punctata* accessions in the BOLDsystem database (EINVA400\_19) was found to be genetically homogenous with *Piscicola milneri* (Figure 2, Table 3). The external morphological features of the disputed specimen from Lake Superior, USA, confirmed its belonging to *Piscicola milneri* (Figure 8). The specimen EINVA400\_19 had an obvious ray pigmentation and eye-like spots on the caudal sucker, which is inconsistent with the taxonomic diagnosis of *P. punctata*, which is characterized by the absence of any pigmentation on the caudal sucker [1,42,53].



**Figure 8.** Appearance of the *Piscicola* leech from Lake Superior, United States. The image of the specimen EINVA400\_19 was retrieved from <https://www.boldsystems.org> (accessed on 10 January 2023). Arrows indicate pigmented pattern (pp) and eye-like spots (es) on the caudal sucker.

#### 4.2. Biogeography of the *Piscicola* Fauna

The freshwater Piscicolidae fauna is recognized as generally Holarctic and predominantly Western Palaearctic with the exception of the representatives of the genera *Myzobdella* (Neotropical) and *Limnotrachelobdella* (Eastern Palaearctic), which might be secondary invaders from the sea [17]. According to current knowledge, there are 62 fish leech species in the world [7,8,17]; of them, 45 species (including three new species described in this study) are distributed in the Palaearctic, and 7 are distributed in the Nearctic.

The diversity of the genus *Piscicola* now amounts to 28 species, the vast majority of which occur in the Northern Hemisphere, except for *P. platensis* Cordero, 1933 from South America. Only two species of *Piscicola* (*P. milneri* and *P. punctata*) have been recorded in North America so far, and only *P. milneri* was found in Alaska [1].

The two species *P. geometra* and *P. sibirica* sp. nov. are the most common species in the Palaearctic (Figure 9). In the Lake Baikal catchment area along with the Angara, Selenga and their basins, not a single individual of *P. geometra* was found despite their targeted search in this study. The Angara and Yenisei Rivers represent a single hydrological complex; apparently, *P. geometra* is also absent in the Yenisei although the ecological conditions of its existence in both the Yenisei and the Angara differ little from the Ob, for example, where *P. geometra* is undoubtedly present (Figure 1). However, in nature, a free niche is

never empty. Indeed, the fish leech *P. sibirica* sp. nov. was found in the different water bodies of the Angara-Baikal Basin (Table 1), performing the same ecological function as *P. geometra*. The geographic boundary separating the ranges of these fish leeches coincides with the valley of the Yenisei River (Figure 9). Along the Yenisei, there is a visual division into Western and Eastern Siberia, which have different geological structures and times of formation [55]. In this regard, the range of *P. geometra* in the east does not extend beyond the West Siberian Plain, while its geographic vicariate *P. sibirica* sp. nov. is distributed in Eastern Siberia, the most ancient (Precambrian) part of the continent (Figure 9).



**Figure 9.** Biogeography of vicarious species of *Piscicola* in the Northern Hemisphere: Extrapolation of distribution and occurrence data on *Piscicola geometra* (light-blue area), *Piscicola milneri* (light-green area), and *Piscicola sibirica* sp. nov. (light-red area) collected under this study and from publicly available records. The watershed areas of the Baikal–Angara and the Yana River (regions where *Piscicola sibirica* sp. nov. was recorded) are highlighted in red and marked with numbers 1 and 2, respectively.

*Piscicola milneri* is another phylogenetically related species that was often confused with *P. geometra* in the past. This species was reported from the Great Lakes region located in the eastern part of the United States, including Alaska; and was detected throughout Canada [1]. According to our data, the North American *P. milneri* and the East Siberian *P. sibirica* sp. nov. are genetically the closest species among *Piscicola* (a 2.56 % of nucleotide substitution difference, Table 3) despite being from different continents (Figure 9). Recently, researchers studying the Earth’s crust have proven the existence of a single Precambrian continent (from 1.8 billion to 700 million years ago) which united modern North America and Eastern Siberia; thus, the Siberian–American continent existed before the formation of Eurasia [56], which may explain the similarity of the Siberian and American leech fauna. The formation of the Bering Strait separated the continents of Eurasia and North America about 5.5 million years ago [57] and finally interrupted the gene flow, which contributed to the divergence and subsequent speciation within the Nearctic fauna, and, in particular, led to the appearance of *P. milneri*. This young North American species continued the same ecological mission as its predecessor *P. sibirica* sp. nov.

Another new species described from Eastern Siberia, the *Piscicola khubsugulensis* sp. nov., inhabits Lake Khovsgol (a part of the Baikal–Angara Basin) and does not occur outside this lake. The factor limiting the distribution of this leech species is probably the habitat of its host fish—the Kosogol grayling (*Thymallus nigrescens* Dorogostaisky, 1923), which is endemic to Khovsgol.

## 5. Conclusions

1. Three new species of the genus *Piscicola* from the Eastern Palaearctic (*P. nordica* sp. nov., *P. sibirica* sp. nov. and *P. khubsugulensis* sp. nov.) were first discovered using DNA-based identification and were then morphologically differentiated and described.

2. The species *Piscicola pojmanskae* Bielecki 1994 was reduced to a synonym for *Piscicola geometra* (L., 1761) since they are genetically, morphologically and ecologically identical; the species diversity of the Piscicolidae fauna from the Western Palaearctic is most likely redundant and requires revision.
3. Due to these findings, the description of *Piscicola geometra* (L., 1761) required revision and clarification, which eventually led to the establishment of the neotype.
4. The geographic range of *Piscicola geometra* (L., 1761), previously known as a trans-Palaearctic species, turned out to be limited in the east by the Yenisei River.
5. The species *Piscicola geometra* and *Piscicola sibirica* sp. nov., the most widespread species in the Palaearctic, as well as the Nearctic *Piscicola milneri* are geographic vicariates of each other.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15010098/s1>, Table S1: Taxa names and GenBank accession numbers for 88 leeches used in phylogenetic analysis.

**Author Contributions:** Conceptualization and methodology, I.K.; validation, E.M.; formal analysis, E.M.; resources and data curation, I.K.; writing—original draft preparation, I.K.; writing—review and editing, I.K.; visualization, I.K.; supervision, I.K.; funding acquisition, I.K. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** The original genetic data of this study can be openly accessed at <https://www.ncbi.nlm.nih.gov/>, using reference numbers KM095103, KM095104 and OQ152290-OQ152315. New species of fish leeches were registered in ZooBank (lsid:zoobank.org:pub:8489DEB9-E0B4-44B1-99DD-11A2D1CCF5C3).

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