

Article

Taxonomy and Melanism Patterns of Freshwater Leeches in the Genus *Glossiphonia* (Hirudinea: Glossiphoniidae) from Northeast Asia [†]

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urn:lsid:zoobank.org:act:C77498B1-390B-42FC-BBA8-4DEAF5332856.



Citation: Bolotov, I.N.; Eliseeva, T.A.; Kondakov, A.V.; Kropotin, A.V.; Aksenova, O.V.; Bespalaya, Y.V.; Travina, O.V.; Gofarov, M.Y.; Kim, S.K.; Lee, J.H.; et al. Taxonomy and Melanism Patterns of Freshwater Leeches in the Genus *Glossiphonia* (Hirudinea: Glossiphoniidae) from Northeast Asia. *Diversity* **2023**, *15*, 756. <https://doi.org/10.3390/d15060756>

Academic Editors: Michael Wink and Simon Blanchet

Received: 29 April 2023

Revised: 30 May 2023

Accepted: 4 June 2023

Published: 8 June 2023



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Abstract: Freshwater leeches belonging to the genus *Glossiphonia* Johnson, 1816 are fairly common benthic annelids in freshwater ecosystems throughout Eurasia, but the taxonomy of this group remains poorly resolved, and the species content of some local faunas is unsatisfactorily known. For example, it was thought that the only widespread species, *Glossiphonia complanata* (Linnaeus, 1758), dwells in Northeast Asia. Here, we revise the Northeast Asian *Glossiphonia* leeches with an integrative taxonomic approach. This revision is based on the largest DNA-sequence and morphological datasets collected to date. Two *Glossiphonia* species are recorded from the region. First, *Glossiphonia koreaensis* **sp. nov.**, a putative regional endemic species, was discovered in South Korea. Second, a valid name and complete description are provided for *Glossiphonia mollissima* Moore, 1898 (non-Grube, 1871). The latter name was wrongly applied through misidentification and, according to ICZN (Article 49), is unavailable. Hence, we propose a new name, *Glossiphonia moorei* **sp. nov.**, in memory of Dr. J. Percy Moore, who discovered this leech. It is a prospective trans-Beringian species, ranging through Eastern Siberia, the Russian Far East, and Alaska (although Alaskan records are based on nonsequenced samples), showing a significant proportion of melanic individuals in samples north of the Arctic Circle. The latter pattern may reflect substrate-induced cryptic coloration (camouflage), but this hypothesis needs to be statistically checked in the future. Finally, a complete checklist of *Glossiphonia* species is presented.

Keywords: South Korea; Far East; Siberia; new species; cryptic coloration; freshwater hydrobionts

1. Introduction

Representatives of the genus *Glossiphonia* Johnson 1816 are among the most common freshwater leeches in various water bodies of Eurasia and North America [1,2]. Traditionally, it was thought that one species in this genus, *G. complanata* (Linnaeus, 1758), has an enormous Holarctic range, expanding from Europe through Northeast Asia to North America [1–3]. However, a growing body of modern DNA-based research indicates that

the distribution of this species is confined to Europe and that its records from Asia and North America should be attributed to other species [4–8].

It was shown that at least two species-level *Glossiphonia* phylogenetic lineages belonging to the so-called *verrucata* group (subgenus *Boreobdella* Johansson, 1929) could be delineated in Northeast Asia [8]. The first species was discovered in South Korea and does not have an available Linnean name, although it was repeatedly recorded under the name *Glossiphonia complanata* [9,10]; this identification was found to be incorrect [8].

The taxonomic history of the second species is much complicated. Moore [11] discovered it in leech samples from the Bering Island (Commander Islands) deposited in the U.S. National Museum (currently the National Museum of Natural History (USNM), Washington, DC, USA) in 1898, but erroneously identified it as a specimen of *Clepsine mollissima* Grube, 1871. The latter nominal taxon was described from Lake Baikal [12]. Based on a thorough morphological examination of the Bering Island sample, Moore [11] transferred this leech to the genus *Glossiphonia* and thus a new combination, *Glossiphonia mollissima* (Grube, 1871), was created. Later, Livanow [13] disputed this opinion and stated that Grube's species is a member of the genus *Theromyzon* Philippi, 1867 and that Bering Island specimens may belong to a new, still undescribed species of *Glossiphonia*. Half a century later, Moore and Meyer [14] referred the Bering Island leech to as *Glossiphonia complanata mollissima* Moore, 1898. The status of Moore's leech was always a matter of doubt, and most authors have considered it a synonym of *G. verrucata* (F. Müller, 1844) both in the Siberian [1,15] and the Nearctic [16,17] parts of its prospective trans-Beringean range.

In our previous work [8], we used *Glossiphonia mollissima* Moore, 1951 as a name for this species but noted that the nomenclatural issues related to this name are still unresolved. In particular, the name *Glossiphonia mollissima* sensu Moore, 1898 (non-Grube, 1871) is based on a misidentification and, according to the International Code of Zoological Nomenclature [18], cannot be used as an available name for that taxon, even if the taxon and the taxon to which the specific or subspecific name correctly applies are in, or are later assigned to, different genera (Article 49).

Based on the considerations outlined above, this study aimed to (1) describe a new *Glossiphonia* species from South Korea; (2) propose a Code-compliant taxonomic name for Moore's *Glossiphonia* species based on information obtained from newly collected samples from Northeast Asian regions, including those from the Bering Island; (3) illustrate digestive and generative systems of both species in detail; (4) clarify the distribution and morphological variability of the two species; and (5) assess the melanism and cryptic coloration patterns in a widespread *Glossiphonia* species.

2. Materials and Methods

2.1. Data Sampling

Samples of glossiphoniid leeches were collected by hand, hydrobiological net, and a sieve from various water bodies throughout Siberia, the Russian Far East, and South Korea. To confirm the identity of Moore's *Glossiphonia* species, one of the coauthors (T.A.E.) managed to collect a *Glossiphonia* sample from the remote Bering Island. The samples were placed into 96% ethanol and were deposited in the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia). Information on the available samples of the two new glossiphoniid species described herein is presented in Tables S1 and S2.

2.2. DNA Data, Phylogenetic, and Phylogeographic Analyses

New sequences of the mitochondrial *cytochrome c oxidase subunit I* (COI) and the nuclear *18S ribosomal RNA* (18S rRNA) gene sequences were generated by applying the standard primers and laboratory manuals [19]. Forward and reverse sequences were obtained using an ABI PRISM® 3730 DNA analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA)

with ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit. The new DNA sequences were manually verified with BioEdit v. 7.2.5 [20].

To reconstruct a two-locus phylogeny, we collected partial sequences of the *COI* and *18S rRNA* genes for each sequenced *Glossiphonia* species (Table 1). Most of these sequences were published in our earlier papers [8,19]. The sequences of each gene were separately aligned using the MUSCLE algorithm of MEGA 11 [21]. The two gene alignments were joined to a combined alignment with FaBox v. 1.61 (<https://birc.au.dk/~palle/ph/p/fabox>; accessed on 1 January 2023) [22]. The maximum likelihood phylogeny (four partitions: 3 codons of *COI* and *18S rRNA*) was calculated with a web server for IQ-TREE v. 1.6.12 (<http://iqtree.cibiv.univie.ac.at>; accessed on 30 March 2023) [23]. The substitution models were automatically chosen for each of the four partitions according to the Bayesian information criterion scores [24], while the node support values were estimated with an ultra-fast bootstrap (5000 replications) [25]. Additionally, a *COI*-based maximum likelihood phylogeny was reconstructed for the subgenus *Boreobdella* Johansson, 1929 using the same approach but with all available haplotypes of the two new glossiphoniid species from Northeast Asia (Table S1).

Table 1. Information on the *COI* and *18S rRNA* gene sequences of the Glossiphoniidae species used in a two-locus phylogenetic reconstruction.

Taxa	Region	<i>COI</i> Acc. No	<i>18S rRNA</i> Acc. No	Reference
Genus <i>Glossiphonia</i> Johnson, 1816 (in-group)				
<i>G. arctica</i> Bolotov et al., 2022	Russia: Polar Urals	ON810735	ON819028	Bolotov et al. [8]
<i>G. baicalensis</i> (Stschegolew, 1922)	Russia: Lake Baikal, Eastern Siberia	AY047329	AY962425	Light and Siddall [26]
<i>G. balcanica</i> Grosser & Pešić, 2016	Russia: Moscow Region	MN295386	MN312176	Bolotov et al. [19]
<i>G. moorei</i> sp. nov. = <i>G. mollissima</i> sensu Moore, 1898, non Grube, 1871	Russia: Khabarovsk Region	MN295375	MN312170	Bolotov et al. [19]
<i>G. complanata</i> (Linnaeus, 1758)	Montenegro	ON841652	OQ067230	Bolotov et al. [8]; this study
<i>G. elegans</i> (Verrill, 1872)	Canada	ON841597	OQ024821	Bolotov et al. [8]; this study
<i>G. sp.1</i> ‘Montenegro’	Montenegro	ON841644	OQ110559	Bolotov et al. [8]; this study
<i>G. nebulosa</i> Kalbe, 1964	Russia: Taymyr Peninsula	ON810698	ON819015	Bolotov et al. [8]
<i>G. verrucata</i> (F. Müller, 1844)	Russia: Yamal Peninsula	MN295411	ON819003	Bolotov et al. [8,19]
<i>G. concolor</i> (Apathy, 1888)	Russia: Taymyr Peninsula	ON810692	ON819012	Bolotov et al. [8]
<i>G. koreaensis</i> sp. nov. = <i>G. sp.2</i> ‘Korea’ sensu Bolotov et al., 2019	South Korea	MN295429	OQ024820	Bolotov et al. [19]; this study
<i>G. taymyrensis</i> Bolotov et al., 2022	Russia: Taymyr Peninsula	ON810706	ON819021	Bolotov et al. [8]
<i>G. sp.3</i> ‘Balkans’	Italy	AY962459	AY962432	Siddall et al. [27]
<i>G. grubei</i> (Lukin & Epstein, 1960) comb. nov.	Russia: Lake Baikal	OM257166	n/a	Bolbat et al. [28]
<i>G. echinulata</i> (Grube, 1871) comb. nov.	Russia: Lake Baikal	OM257165	n/a	Bolbat et al. [28]
Genus <i>Alboglossiphonia</i> Lukin, 1976 (outgroup)				
<i>A. sibirica</i> Bolotov et al., 2022	Russia: Eastern Siberia	MH286267	MH286273	Bolotov et al. [8,19]
<i>A. lata</i> (Oka, 1910)	Russian Far East	MN295414	MN312188	Bolotov et al. [19]

Table 1. Cont.

Taxa	Region	COI Acc. No	18S rRNA Acc. No	Reference
<i>A. pahariensis</i> Neesemann & Sharma, 2007	India	ON548504	ON532907	Bolotov et al. [29]
<i>A. pallida</i> (Verrill, 1872)	USA: Michigan	AF116016	AF115983	Apakupakul et al. [30]
<i>A. bhamoensis</i> Bolotov et al., 2022	Myanmar	ON548507	ON704955	Bolotov et al. [29]

To assess the intraspecific haplotype-level relationships, we reconstructed a median-joining network based on the available *COI* sequences of *Glossiphonia moorei* **sp. nov.** ($N = 44$; see Table S1 for details) using Network v. 4.6.1.3 with the default settings [31]. Sequences were aligned with the MUSCLE algorithm of MEGA 11 [21]. The uncorrected p-distances between *COI* sequences at the intraspecific and interspecific levels were calculated with MEGA 11 [21].

2.3. Morphological and Anatomical Research

The morphological features of the glossiphoniid leeches, such as the ground color, markings pattern, the position and number of eyespots, body shape and size, body annulation, body papillation, as well as the position of genital pores, were examined following standard protocols [2,8,32]. The images of the leech samples and their morphological features were taken using Leica M165C (Leica Microsystems GmbH, Wetzlar, Germany) and Zeiss Axio Zoom.V16 (Carl Zeiss AG, Jena, Germany) stereoscopes and were processed with Adobe Photoshop CS v. 8.0 (Adobe Inc., Adobe World Headquarters, San Jose, CA, USA). Measurements of the individuals of the new species, that is, body length (BL), body width (BW), width of anterior sucker (AW), and width of posterior sucker (PW), were assessed using a Leica M165C stereomicroscope (Leica Microsystems GmbH, Germany) with an ocular-micrometer (see [8] for detail).

The digestive and reproductive systems were investigated based on a series of longitudinal slices processed using hematoxylin and eosin (H&E) stain, as described in our earlier work [8]. The permanent slides were made with a mounting medium (VitroGel, Biovitrum, Saint Petersburg, Russia). Slides were photographed with a digital camera (FLEXACAM C1, Leica Microsystems, Wetzlar, Germany) that was attached to a stereomicroscope Leica M165C (Leica Microsystems GmbH, Wetzlar, Germany). The photos were finalized using Adobe Photoshop CS v. 8.0 software.

2.4. Range Mapping

The distribution map was compiled based on published and original data (Table S1) using ESRI ArcGIS 10 (www.esri.com/arcgis; accessed on 31 December 2022). Occurrences collected from the available literature sources were georeferenced with Google Earth (<https://www.google.com/intl/ru/earth/>; accessed on 15 March 2023).

2.5. Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature (ICZN) [18]; hence, the new names and combinations contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains were registered in ZooBank (<https://zoobank.org>; accessed on 27 May 2023), the online registration system for the ICZN. The Life Science Identifier (LSID) for this publication is <https://zoobank.org/urn:lsid:zoobank.org:pub:E6A7345E-BE26-4CD2-A582-D4F989523E43> (accessed on 27 May 2023). The electronic edition of this paper was published in a journal with an ISSN and has been archived and is available from PubMed Central.

3. Results

3.1. Phylogenetic and Phylogeographic Data

A broad sampling of *Glossiphonia* spp. throughout Northeast Asia revealed that this region is inhabited by at least two species of this genus: *G. koreaensis* **sp. nov.** (South Korea) and *G. moorei* **sp. nov.** (Eastern Siberia, the Russian Far East, and Alaska) (Figure 1). It should be noted that the records of the latter species from Alaska are based on nonsequenced specimens and needs further confirmation with a DNA-based approach. Our two-locus phylogenetic reconstruction (COI + 18S rRNA) of the genus reveals that available species are clustered to two well-supported subclades, corresponding to the subgenera *Glossiphonia* s. str. and *Boreobdella* Johansson, 1929 (Figure 3). The two Asian species are sister taxa, belonging to the *Boreobdella* subclade (i.e., the *verrucata* group of Bolotov et al., 2022 [8]). This subclade also contains *Glossiphonia verrucata* (F. Müller, 1844) and *G. arctica* Bolotov et al., 2022 (Figures 3 and S1).

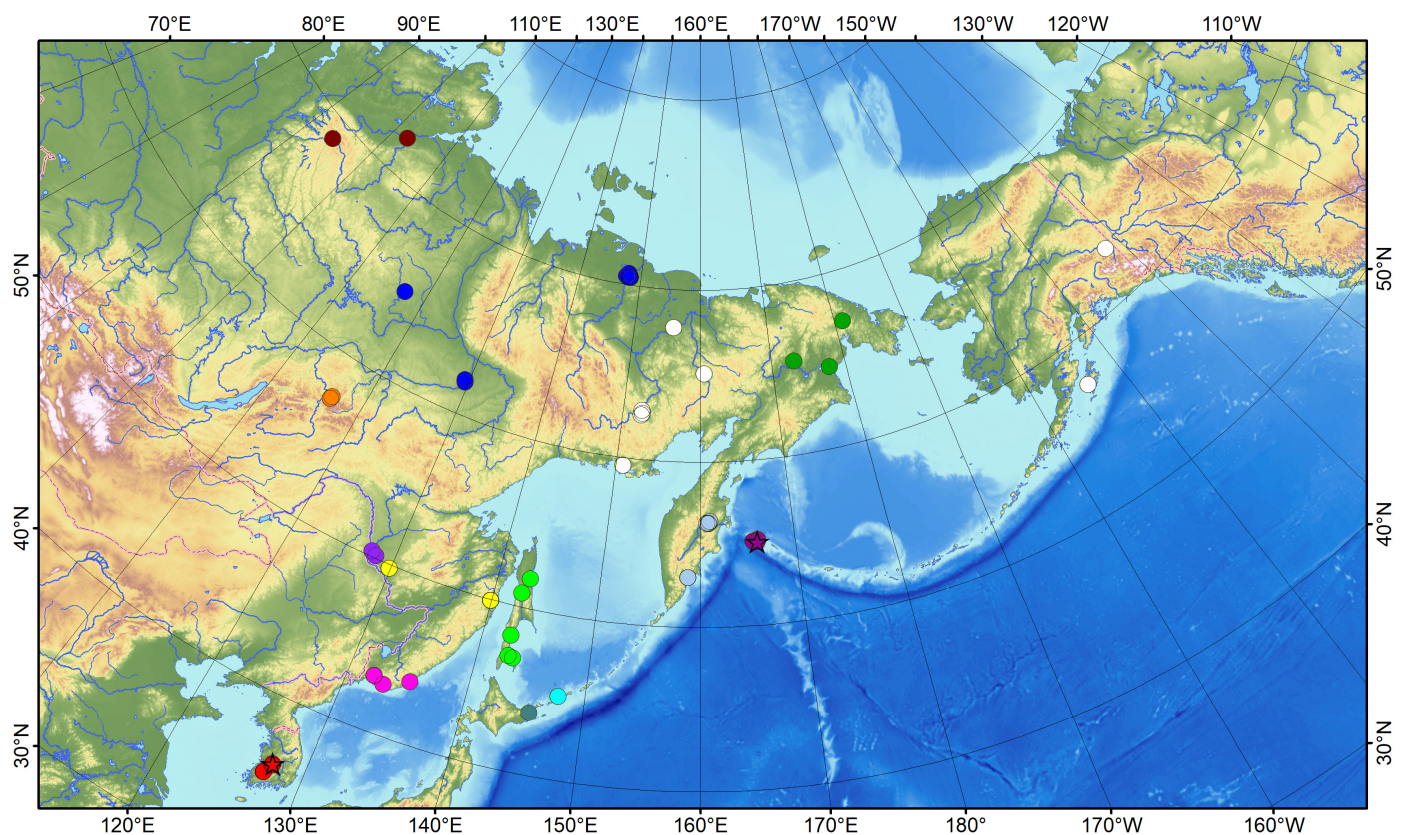


Figure 1. Map of records of the two new *Glossiphonia* species. Stars show the type localities, and circles show occurrences of *G. koreaensis* **sp. nov.** (red) and *G. moorei* **sp. nov.** (explanations of other colors are presented in Figure 2; white circles show nonsequenced records). The raw occurrence data are presented in Table S1.

Glossiphonia koreaensis **sp. nov.** seems to have a low level of genetic diversity ($N = 5$ COI haplotypes) while that of the widespread *G. moorei* **sp. nov.** is much higher ($N = 23$ COI haplotypes) (Figures 2 and S1). The Bering Island population shares the same COI haplotype as those on the Kamchatka and Chukotka peninsulas, which can probably be explained by these regions belonging to the former Beringian Land Bridge.

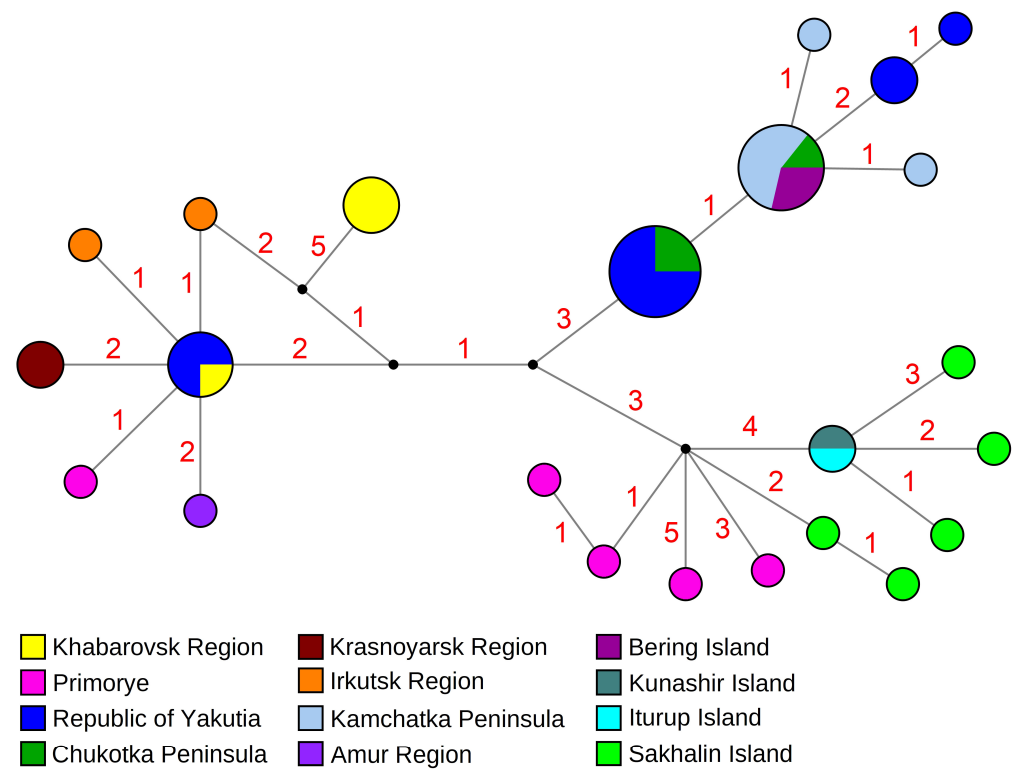


Figure 2. Median-joining network of *Glossiphonia moorei* **sp. nov.** based on available *COI* sequences ($N = 44$; see Table S1 for details). The circle size is proportional to the number of sequences for a given haplotype; the smallest circle = 1 sequence. The small black circles indicate hypothetical ancestral haplotypes. The red values near branches reveal numbers of nucleotide substitutions between haplotypes.

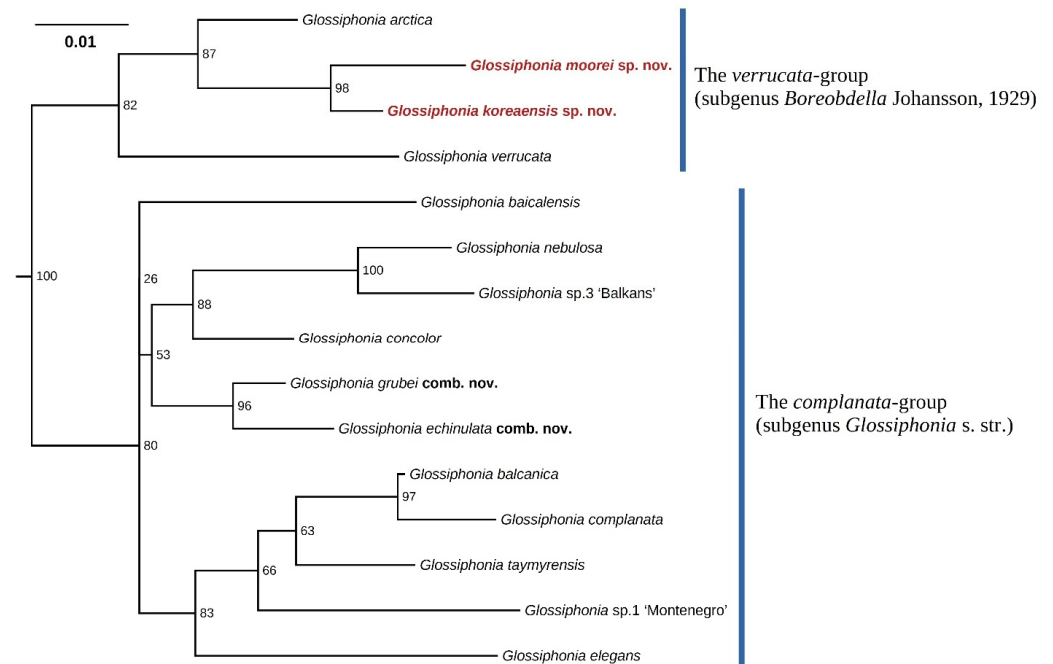


Figure 3. Maximum likelihood phylogeny of the genus *Glossiphonia* based on the combined alignment of the *COI* and *18S rRNA* gene fragments (Table 1). The black numbers near nodes are bootstrap support values from IQ-TREE v. 1.6.12. The scale bar indicates the branch length (substitutions per site). The two new species-group taxonomic names are indicated in red.

3.2. Melanism Patterns in *Glossiphonia moorei* sp. nov.

Our samples of *Glossiphonia koreaensis* sp. nov. do not contain dark-colored (melanic) specimens (Figure 4). In contrast, the samples of *Glossiphonia moorei* sp. nov. contain 25 melanic and 98 light-colored specimens (total $N = 123$) (Table S3 and Figure 5). The proportion of melanic specimens in the samples from the areas north of the Arctic Circle (13 melanic vs. 24 light-colored) is significantly higher than that in the samples from the non-Arctic areas (12 melanic vs. 74 light-colored) (chi-squared test: chi-square statistic = 7.17; $p = 0.0074$).

The available photos of living individuals of *Glossiphonia moorei* sp. nov. show several examples of substrate-associated cryptic coloration (camouflage) (Figure 6), although we did not quantitatively study this phenomenon.

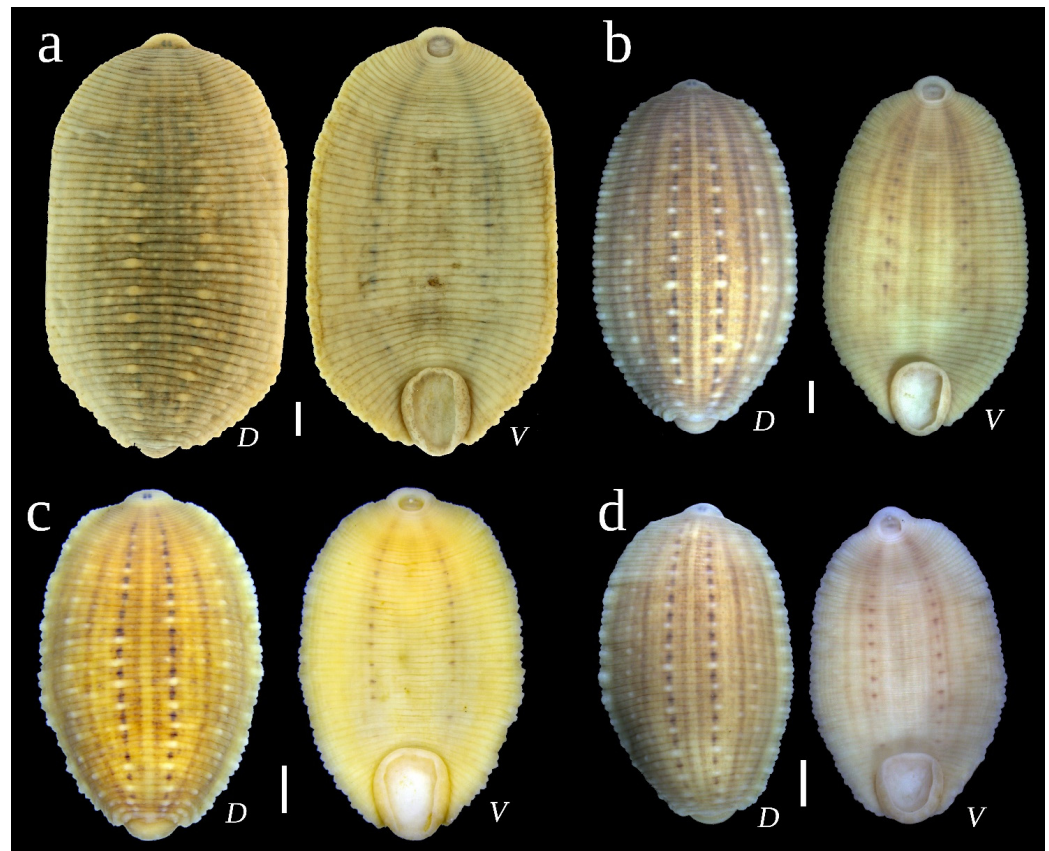


Figure 4. Dorsal (D) and ventral (V) views of the holotype and selected paratypes of *Glossiphonia koreaensis* sp. nov.: (a) holotype RMBH Hir_0110_2-H, Chungcheongnam-do Province, South Korea; (b) paratype RMBH Hir_0113_1, Jeollabuk-do Province, South Korea; (c) paratype RMBH Hir_0110_4, Chungcheongnam-do Province, South Korea; (d) paratype RMBH Hir_0113_1_2, Jeollabuk-do Province, South Korea. Scale bars = 1.0 mm. Photos: Tatyana A. Eliseeva.

3.3. Taxonomic Account

Suborder Glossiphoniiformes Tessler & de Carle, 2018

Family Glossiphoniidae Vaillant, 1890

Subfamily Glossiphoniinae Vaillant, 1890

Genus *Glossiphonia* Johnson, 1816 (type species: *Hirudo complanata* Linnaeus, 1758; by subsequent designation).

= *Baicalocleipsis* Lukin & Epstein (1959): 189 (type species: *Clepsine echinulata* Grube, 1871; by original designation) [33].

= *Paratorix* Lukin & Epstein (1960): 479 (type species: *Torix baicalensis* Stschegolew, 1922; by original designation) [34].

Comments: It was found that the type species of both glossiphoniid genera, which were thought to be endemic to Lake Baikal (*Baicalocleipsis* and *Paratorix*), belong to the *Glossiphonia* clade [27,28]. Hence, these genus-group names are junior synonyms of *Glossiphonia*, and the species they contain must be transferred to the latter genus as follows: *G. baicalensis* (Stschegolev, 1922), *G. echinulata* (Grube, 1871) **comb. nov.**, and *G. grubei* (Lukin & Epshtein, 1959) **comb. nov.** In total, this genus contains 16 species (Table 2), including *G. koreaensis* **sp. nov.** and *G. moorei* **sp. nov.** (Figures 4–11), described below.

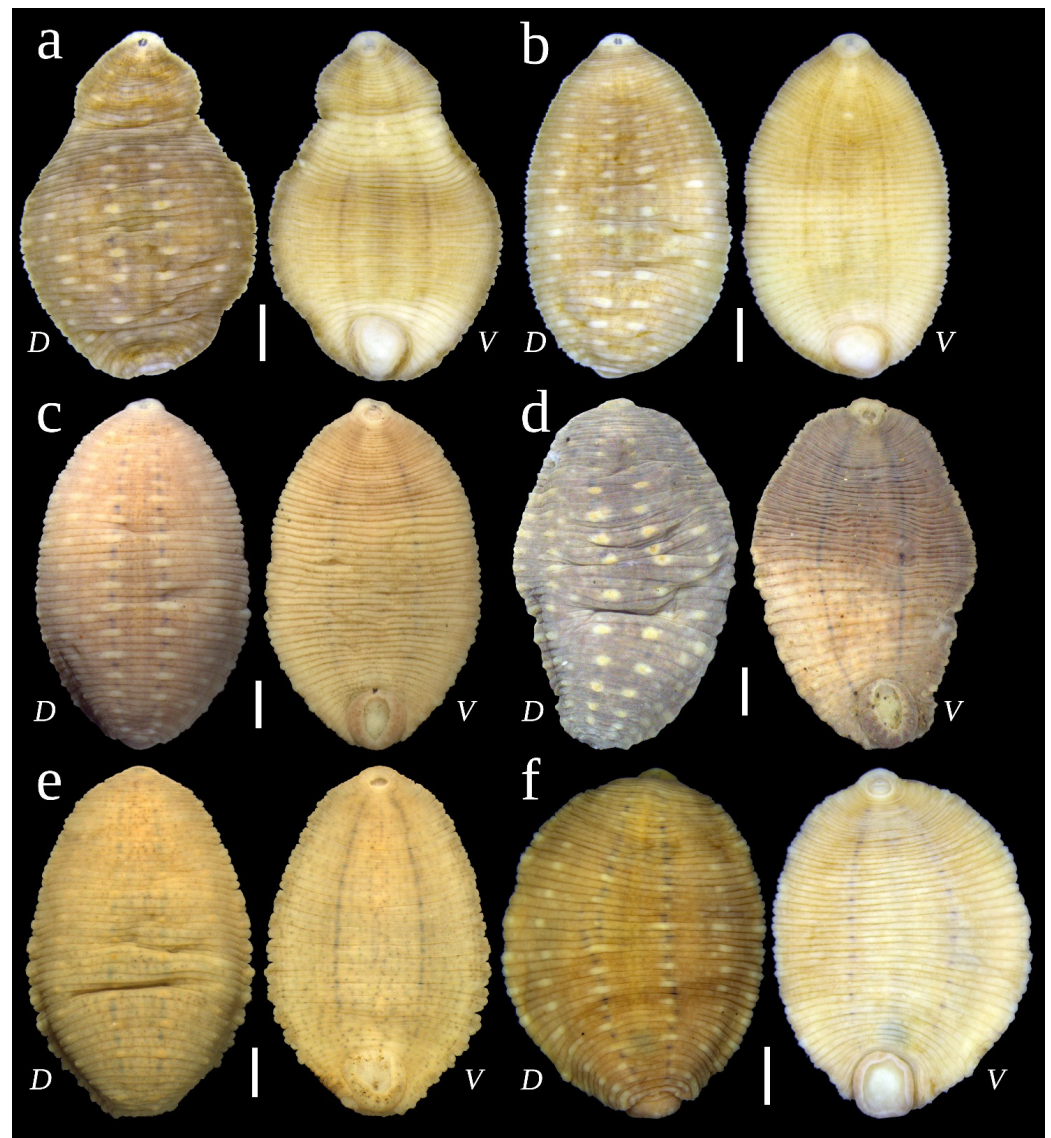


Figure 5. Dorsal (D) and ventral (V) views of the holotype and selected paratypes of *Glossiphonia moorei* **sp. nov.**: (a) holotype RMBH Hir_0627-H (melanic form), Bering Island; (b) paratype RMBH Hir_0628 (light-colored form), Bering Island; (c) paratype RMBH Hir_0480_2 (light-colored form), Arctic Yakutia; (d) paratype RMBH Hir_0483_2 (melanic form), Arctic Yakutia; (e) paratype RMBH Hir_0487 (melanic form), Putorana Plateau; (f) paratype RMBH Hir_0570_2 (light-colored form), Iturup Island. Scale bars = 1.0 mm. Photos: Tatyana A. Eliseeva.

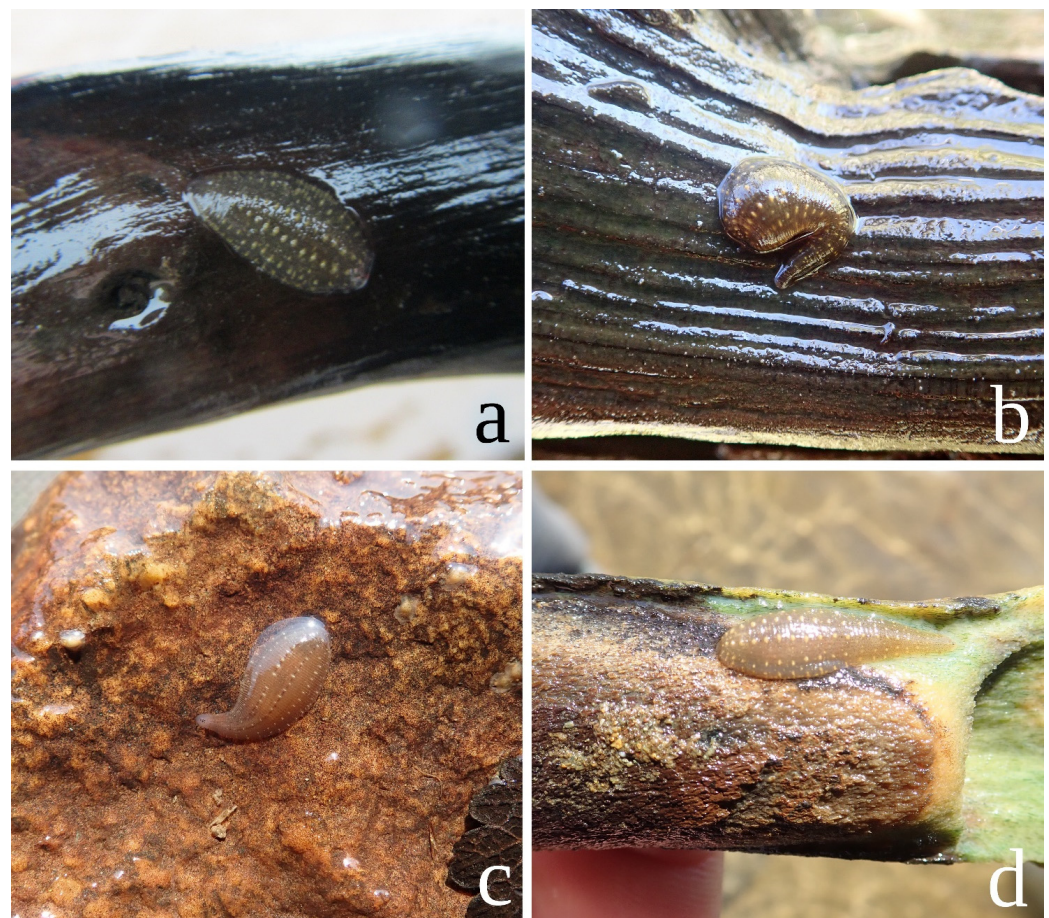


Figure 6. Examples of cryptic coloration (camouflage) of *Glossiphonia moorei* sp. nov. (a,b) Melanic forms on dark wood substrate: Ust-Belaya, Chukotka Peninsula (a); Chokurdakh, Arctic Yakutia (b). (c,d) Light-colored forms on light substrate: light brown flat stone, Lake Khodeevskoye, Chukotka Peninsula (c); light brown reindeer antler, Amguema, Chukotka Peninsula (d). Photos: Olga V. Aksenova.

Table 2. Checklist of *Glossiphonia* species (Glossiphoniidae).

Taxon	Type Locality	General Range	DNA-Sequence Data on Type Specimens or Topotypes
Genus <i>Glossiphonia</i> Johnson, 1816			
The <i>complanata</i> group (subgenus <i>Glossiphonia</i> s. str.)			
<i>G. baicalensis</i> (Stschegolev, 1922) = <i>Torix baicalensis</i> Stschegolev (1922): 136 [35]	Russia: Lake Baikal [35]	Endemic to Lake Baikal [1]	Sequenced topotype (COI and 18S rRNA) [26]
<i>G. balcanica</i> Grosser & Pešić, 2016 = <i>G. balcanica</i> Grosser & Pešić in Grosser et al. (2016): 18 [36]	Kosovo: spring Toplla, 42.5719° N, 20.2906° E, KS40 Dečani/Decan, Lebush [36]	Arctic Region from northern Fennoscandia to Taymyr; Iceland; European Russia, and Balkans [8]	Sequenced topotypes (COI) [7]

Table 2. Cont.

Taxon	Type Locality	General Range	DNA-Sequence Data on Type Specimens or Topotypes
<i>G. complanata</i> (Linnaeus, 1758) = <i>Hirudo complanata</i> Linnaeus (1758): 1079 [37]	Not stated [37]	Continental Europe (Austria, France, Germany, Slovenia, Balkans, and European Russia) north to the Moscow Region of Russia, the British Isles, and Morocco [7,8]	Sequenced representatives of this species from Germany (<i>COI</i>) [7] cannot be considered topotypes
<i>G. concolor</i> (Apáthy, 1888) = <i>Clepsine concolor</i> Apáthy (1888): 154 [38]	Hungary: Danube at Dunaharaszti, approx. 47.3627° N, 19.0865° E (based on the neotype) [39]	Arctic Region from Kolguev Island and Polar Urals to Taymyr; Siberia (including Lake Baikal); Kazakhstan; Iran; European Russia; Sweden; Germany; Lithuania, France, and Hungary [8,40–42]	Not available
<i>G. echinulata</i> (Grube, 1871) comb. nov. = <i>Clepsine echinulata</i> Grube (1871): 110 [12]	Russia: Lake Baikal [12]	Endemic to Lake Baikal [1]	Sequenced topotype (complete mitogenome) [28]
<i>G. elegans</i> (Verrill, 1872) = <i>Clepsine elegans</i> Verrill (1872): 132 [43]	USA: West River, New Haven County, CT [43]	Canada and the USA [5]	Sequenced topotypes (<i>COI</i>) [5,44]
<i>G. grubei</i> (Lukin & Epshtein, 1959) comb. nov. = <i>Baicalocleipsis grubei</i> Lukin & Epshtein (1959): 189 [33]	Russia: Lake Baikal [33]	Endemic to Lake Baikal [1]	Sequenced topotype (complete mitogenome) [28]
<i>G. nebulosa</i> Kalbe, 1964 = <i>G. complanata nebulosa</i> Kalbe (1964): 141 [45]	Germany: Nieplitz River near Treuenbrietzen, 52.0911° N, 12.8666° E [45]	Arctic Region from Polar Urals to Taymyr; North Caucasus; Europe (Germany, France); Turkey: Antalya [8]	Sequenced topotype (<i>COI</i>) [7]
<i>G. taymyrensis</i> Bolotov, Eliseeva, Klass & Kondakov, 2022 in Bolotov et al. (2022): 13 [8]	Russia: Dudinka, small lake, 69.4008° N, 86.3384° E, Taymyr Peninsula [8]	Siberia from the Arctic Ocean coast (Taymyr) to Kemerovo Region [8]	Sequenced holotype and paratypes (<i>COI</i> and <i>18S rRNA</i>) [8]
The verrucata group (subgenus <i>Boreobdella</i> Johansson, 1929)			
<i>G. arctica</i> Bolotov, Eliseeva, Klass & Kondakov, in Bolotov et al. (2022): 13 [8]	Russia: a lake near Sob' railway station, 67.0480° N, 65.6316° E, Polar Urals [8]	Polar Urals (unknown beyond the type locality) [8]	Sequenced holotype and paratypes (<i>COI</i> and <i>18S rRNA</i>) [8]
<i>G. koreaensis</i> sp. nov.	South Korea: Geum River 1st site, 36.0708° N, 127.5891° E, Chungcheongnam-do Province	Korean Peninsula	Sequenced paratypes (<i>COI</i> and <i>18S rRNA</i>) (this study)
<i>G. moorei</i> sp. nov. = <i>G. mollissima</i> sensu Moore, 1898, non Grube, 1871 [11]	Russia: Bering Island, small lake near the Kamenka River, 55.1106° N, 166.0123° E, Commander Islands	Eastern Siberia and the Russian Far East from Taymyr and the Lena River basin to Chukotka, Kamchatka, Magadan and Khabarovsk regions, Amur River basin, and Primorye; Commander Islands, Kurile Archipelago, and Sakhalin Island; USA: SE Alaska and Kodiak Island [8]	Sequenced paratypes (<i>COI</i> and <i>18S rRNA</i>) (this study)

Table 2. Cont.

Taxon	Type Locality	General Range	DNA-Sequence Data on Type Specimens or Topotypes
<i>G. verrucata</i> (F. Müller, 1844) = <i>Clepsine verrucata</i> Müller (1844): 23 [46]	Germany: Lake Tegel in Berlin, 52.5790° N, 13.2590° E [47]	Siberia from Yamal and Khanty-Mansi Region up to the Lena River basin; Europe: Scandinavia, the British Isles, Poland, Lithuania, Northern European Russia, Germany, and the Netherlands [8]	Not available (no DNA sequences from Europe)
Incertae sedis (unassigned to species group)			
<i>G. paludosa</i> (Carena, 1824) = <i>Hirudo paludosa</i> Carena (1824): 331 [48]	Italy: ponds near Carmagnola, approx. 44.8518° N, 7.6950° E, Turin Province [48]	Southern part of continental Europe (Italy, Switzerland, France, Austria, Slovakia, Czech, Hungary, Romania, tributaries of the Black Sea and adjacent regions) [32]	Not available
<i>G. pulchella</i> Sket, 1968 = <i>G. complanata</i> f. <i>pulchella</i> Sket (1968): 133 [49]	Littoral of Lake Ohrid [32]	Endemic to Lake Ohrid in north Macedonia and eastern Albania [32]	Not available
<i>G. slovacica</i> (Košel, 1973) = <i>Batracobdella slovacica</i> Košel (1973): 87 [50]	Slovakia: Danube near Bratislava [51]	Slovakia, Slovenia, and the Czech Republic [52,53]	Not available

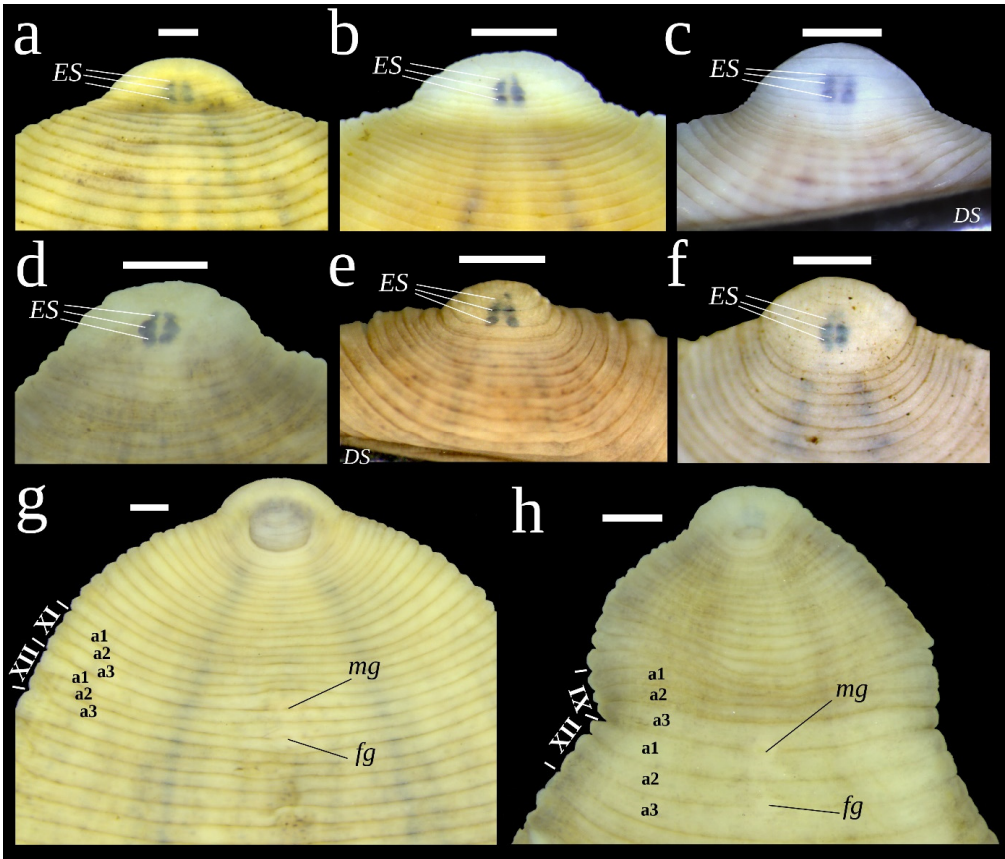


Figure 7. Anterior region with eyespots (dorsal view) and gonopores (ventral view) of the two new *Glossiphonia* species. (a–c) Anterior region of *G. koreaensis* sp. nov.: holotype RMBH Hir_0110_2-H,

Chungcheongnam-do Province, South Korea (a); paratype RMBH Hir_0110_4, Chungcheongnam-do Province, South Korea (b); and paratype RMBH Hir_0113_1_2, Jeollabuk-do Province, South Korea (c). (d–f) Anterior region of *G. moorei* **sp. nov.**: holotype RMBH Hir_0627-H, Bering Island, Russia (d); paratype RMBH Hir_0252, Chukotka, Russia (e); and paratype RMBH Hir_0274_2, Taymyr, Russia (f). (g) Gonopores of *G. koreaensis* **sp. nov.** (holotype RMBH Hir_0110_2-H, South Korea). (h) Gonopores of *G. moorei* **sp. nov.** (holotype RMBH Hir_0627-H, Bering Island). Abbreviations: *ES*, eyespots; *mg*, male gonopore; *fg*, female gonopore; and *DS*, dissecting needle. Body somites are indicated by Roman numerals; bold symbols *a1*, *a2*, and *a3* indicate 1st, 2nd, and 3rd annulus of a somite, respectively. Scale bars = 0.5 mm. Photos: Tatyana A. Eliseeva; graphics: Ivan N. Bolotov.

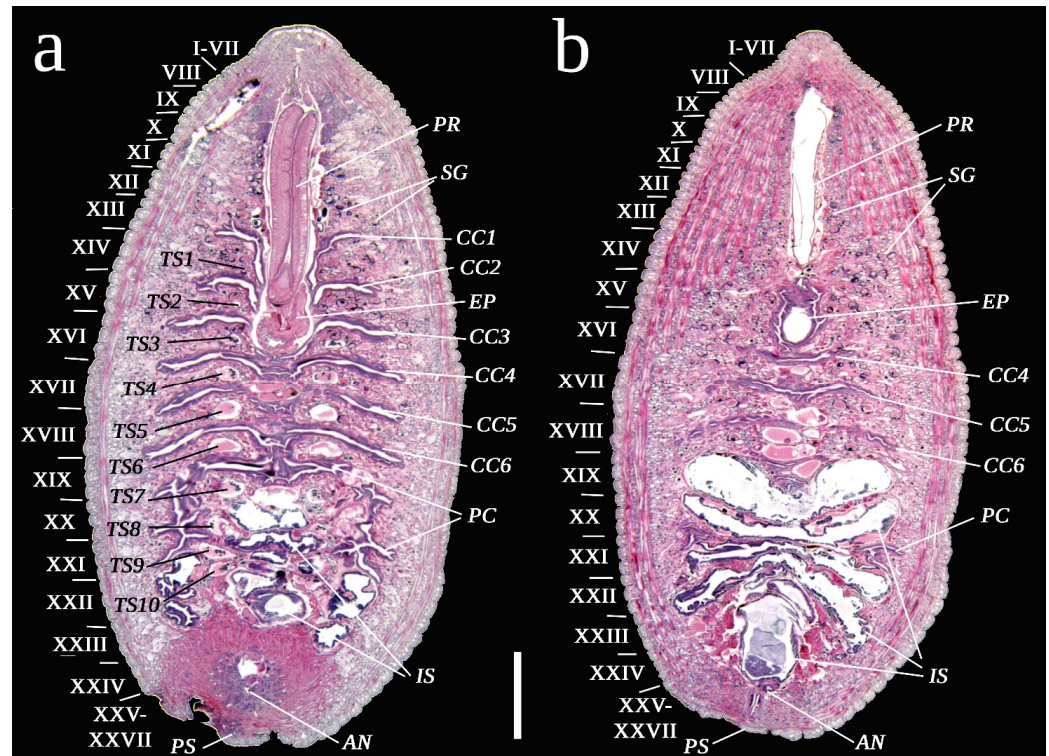


Figure 8. H&E-stained longitudinal slices showing the digestive and generative systems of *Glossiphonia koreaensis* **sp. nov.** (paratype RMBH Hir_0110_4, Chungcheongnam-do Province, South Korea). (a) Closer to the ventral side (slide No. 08); shows the morphology of crop caeca, proboscis sheath, esophagus, salivary glands, and testisacs. (b) Closer to the dorsal side (slide No. 13); shows the structure of intestine. Abbreviations: *PR*, proboscis sheath; *EP*, esophagus; *SG*, salivary glands; *CC*, pairs of crop caeca with their numbers; *PC*, the last pair of crop caeca (posterior caeca); *IS*, intestine; *AN* anus; *TS*, testisacs with their numbers; and *PS*, posterior sucker. Body somites are indicated by Roman numerals. Scale bar = 1.0 mm. Photos: Alexander V. Kropotin; graphics: Ivan N. Bolotov.

Subgenus *Boreobdella* Johansson, 1929 (type species: *Clepsine verrucata* Müller, 1844; by monotypy).

= The *verrucata*-group sensu Bolotov et al. (2022): 13 [8].

Comments: Morphologically, this subgenus differs from *Glossiphonia* s. str. by having larger, broader, and more prominent dorsal papillae (outer paramedian, outer paramarginal, and marginal series). The two new species described herein have 10 pairs of testisacs, but it is unclear whether this feature is characteristic for the entire *Boreobdella* clade or not.

***Glossiphonia koreaensis* Bolotov, Eliseeva, Kondakov, Kim & Lee sp. nov.**

= *Glossiphonia complanata* Huh et al. (2012): 1290 (identification error; reference to *Glossiphonia complanata* (Linnaeus, 1758)) [9]; Shin et al. (2019): 211 [10].

= *Glossiphonia* sp.2 'Korea' Bolotov et al. (2019): 6, Supplementary Table S2 [19]; Bolotov et al. (2022): 3, Table S1 [8].

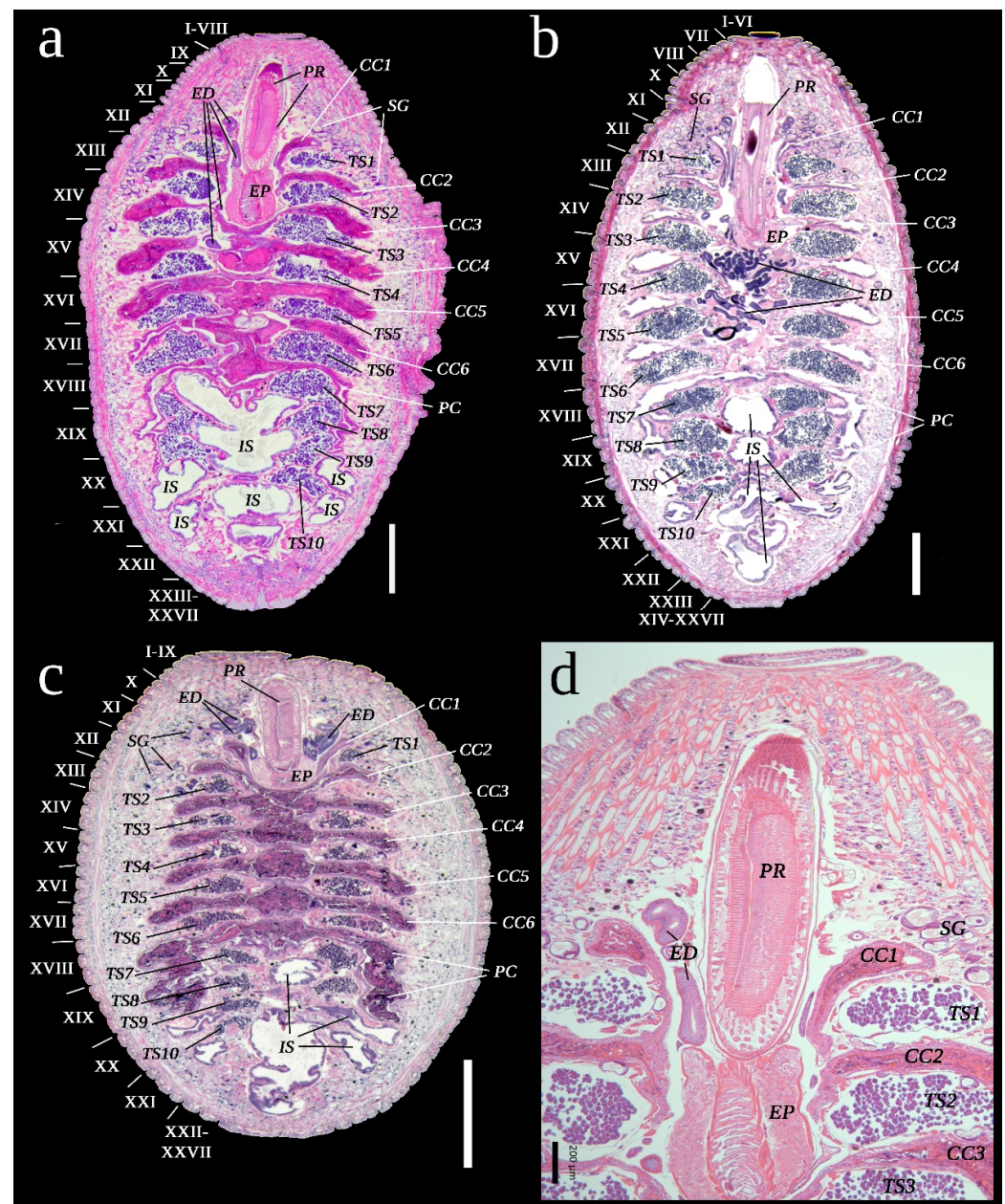


Figure 9. H&E-stained longitudinal slices showing the digestive and generative systems of *Glossiphonia moorei* sp. nov. (a) Paratype RMBH Hir_0512_4, Kamchatka Peninsula (slide No. 13). (b) Paratype RMBH Hir_0581_1, Sakhalin Island (slide No. 13). (c) Paratype RMBH Hir_0626, Bering Island (slide No. 03/2). (d) Longitudinal slice through the anterior part of leech (paratype RMBH Hir_0626; slide No. 03/2). Abbreviations: PR, proboscis sheath; EP, esophagus; SG, salivary glands; CC, pairs of crop caeca with their numbers; PC, the last pair of crop caeca (posterior caeca); IS, intestine; AN, anus; ED, ejaculatory ducts; and TS, testisacs with their numbers. Body somites are indicated by Roman numerals. Scale bars = 1.0 mm (a–c) and 0.2 mm (d). Photos: Alexander V. Kropotin; graphics: Ivan N. Bolotov.

Figures 4a–c, 7a–c,g, 8a,b, 10a and 11a, Table S2.

LSID: <https://zoobank.org/urn:lsid:zoobank.org:act:692335BB-C17F-47AD-8E98-ABCACF5E11E8> (accessed on 27 May 2023).

Holotype. RMBH Hir_0110_2-H; South Korea: Geum River (1st site), 36.0707° N, 127.5891° E, Chungcheongnam-do Province, 8 July 2018, I.N. Bolotov, A.V. Kondakov & I.V. Vikhrev leg.

Paratypes ($N = 7$). South Korea: the type locality, the same collecting date and collectors, four specimens (voucher RMBH Hir_0110); Geum River (second site), 35.9891° N, 127.5836° E, Jeollabuk-do Province, 8 July 2018, the same collectors, one specimen (voucher RMBH Hir_0180); Seomjin River (second site), 35.4217° N, 127.2228° E, Jeollabuk-do Province, 10 July 2018, the same collectors, two specimens (voucher RMBH Hir_0113) (see Tables S1 and S2 for details).

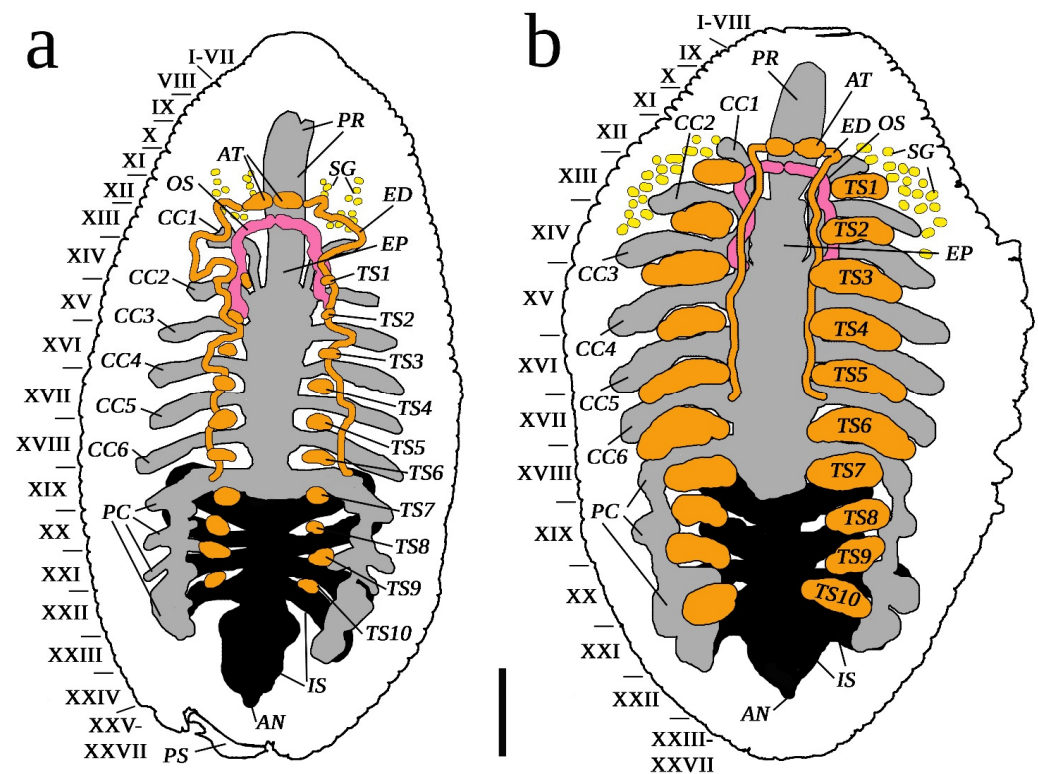


Figure 10. General scheme of the digestive and generative systems of the two new *Glossiphonia* species from Northeast Asia (dorsal view; line contours of body and posterior sucker are shown). (a) *G. koreaensis* sp. nov. (b) *G. moorei* sp. nov. Abbreviations: PR, proboscis sheath; EP, esophagus; SG, salivary glands; CC, pairs of crop caeca with their numbers; PC, the last pair of crop caeca (posterior caeca); IS, intestine; AN, anus; AT, atrium; ED, ejaculatory ducts; TS, testisacs with their numbers; OS, ovisacs; and PS, posterior sucker. Body somites are indicated by Roman numerals. Scale bar = 1.0 mm. Graphics: Ivan N. Bolotov.

Etymology. This species is named after the Korean Peninsula, on which its type locality is situated.

Differential diagnosis. Medium-sized leech, which could be distinguished from other congeners by a combination of the following characters: six rows of dorsal papillae (outer paramedian, outer paramarginal, and marginal series), dorsal markings pattern with 2 blackish dotted outer paramedian lines accompanied by 10–12 brown longitudinal lines, ventral markings pattern with 2 outer paramedian series of dark spots (one per somite on a2). Externally, the new species is similar to *G. arctica* and *G. moorei* sp. nov. but differs from these species by having a characteristic markings pattern with several dark, solid, longitudinal lines dorsally and two outer paramedian series of dark spots ventrally.

Molecular diagnosis. The new species represents a separate genetic lineage belonging to the *verrucata* group (Figure 3). The pairwise uncorrected *COI* *p*-distance between the new species and other congeners varies from 6.2 to 13.3% (the nearest neighbor: *G. moorei* sp. nov.). The intraspecific pairwise *COI* *p*-distance ranges from 0.15 to 0.91% (mean $\pm$ s.e.m. = $0.42 \pm 0.10\%$; $N = 5$ sequences and 10 pairwise distance values). The

GenBank acc. numbers of reference DNA sequences (*COI* and *18S rRNA*) are given in Tables 1, S1 and S2.

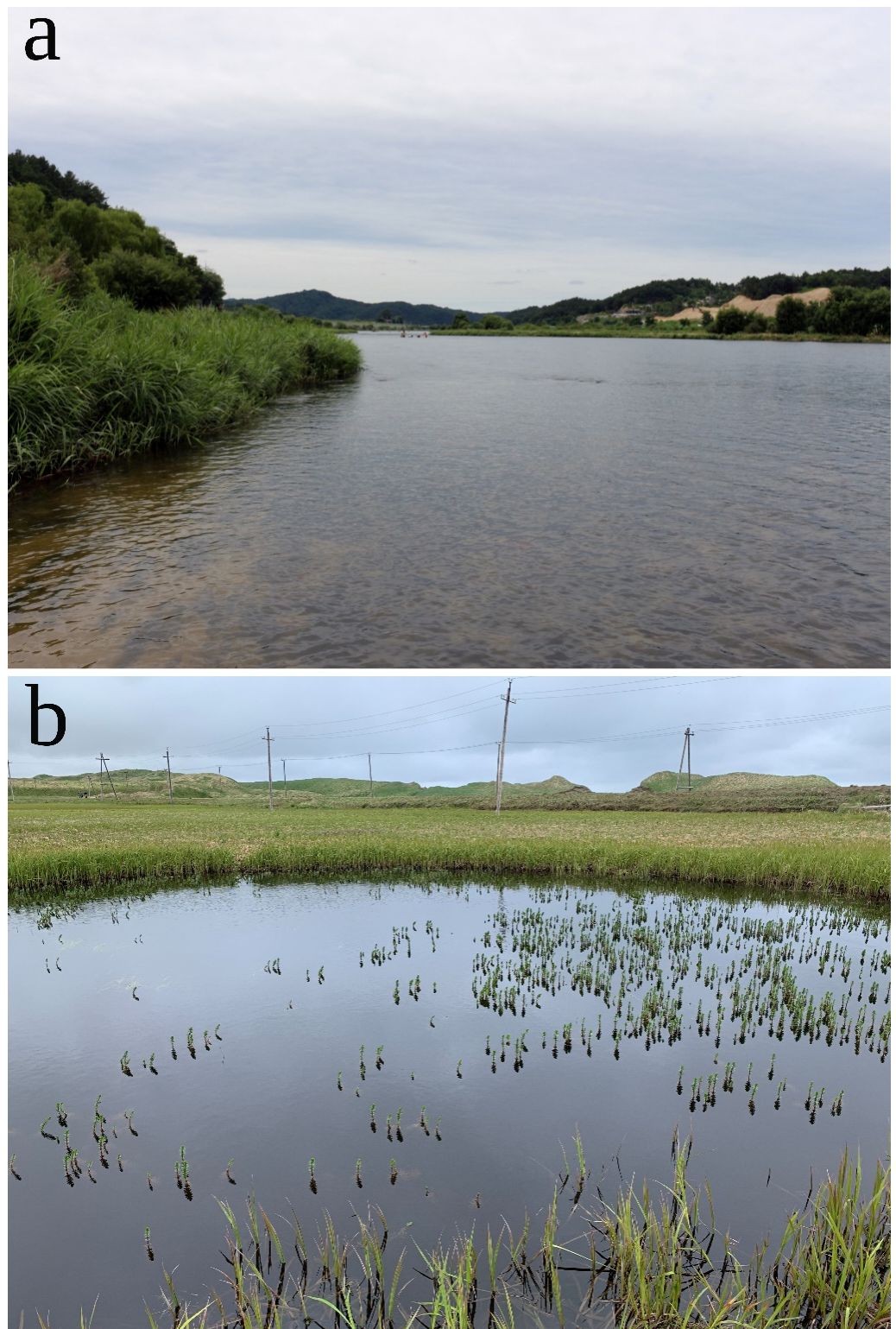


Figure 11. Type localities of the two new *Glossiphonia* species. (a) *G. koreaensis* **sp. nov.**: Geum River, 36.0707° N, 127.5891° E, Chungcheongnam-do Province, South Korea, 8 July 2018. (b) *G. moorei* **sp. nov.**: a small lake near the Kamenka River, 55.1106° N, 166.0123° E, Bering Island, Commander Islands, Russia, 14 June 2022. Photos: Alexander V. Kondakov (a) and Tatyana A. Eliseeva (b).

Description. Medium-sized leech (body length up to 15.8 mm). The measurements of the holotype and paratypes are presented in Table S2. Body broad, elongate-ovate (Figure 4a–d). Dorsum with six rows of rounded papillae of moderate size on annulus a2 (outer paramedian, outer paramarginal, and marginal series). Posterior sucker small, circular (maximum diameter of 3.3 mm), and ventrally directed. Proboscis pore in the anterior part of anterior sucker. Coloration of living animals: not examined. Coloration of ethanol-preserved animals: dorsum light yellow (light-colored forms) to brown (melanic forms), with 2 dark outer paramedian lines (inconspicuous in melanists) accompanied by 10–12 brown longitudinal lines; each dorsal papilla bears rounded small yellow or whitish spot (outer paramedian, outer paramarginal, and marginal series) (Figure 4a–d). Three pairs of circular eyespots arranged to two parallel rows and usually joined to a single comma-like or elongate-ovate large spot at each side (Figure 7a–c). Venter yellowish to light brown, with two outer paramedian series of dark spots (one per somite on a2) and sometimes with several unclear brownish longitudinal lines (Figure 4a–d). Total number of annuli: 70. Somites I–III uniannulate, IV biannulate, V–XXIV triannulate, XXV biannulate, XXVII uniannulate. The male and female genital pores are separated by two annuli and are located in furrows XIa3/XIIa1 and XIIa2/a3, respectively (Figure 7g). Reproductive system: 10 pairs of rather small, spherical testisacs intersegmentally from XIII/XIV to XXI/XXII; atrium spherical, the atrial cornua twisted anteriorly; paired ejaculatory ducts long, extending to XIX; paired ovisacs long, arranged as loops (Figures 8a,b and 10a). Digestive system: proboscis sheath massive, thick, elongated; esophagus short, broad; salivary glands diffuse; crop with 7 pairs of crop caeca: 1st–6th rather narrow, elongate bag-like, usually with 2–3 very small processes distally, 7th pair (posterior caeca) with 4 blind processes; intestine enlarged, with 4 pairs of large, long, bag-like processes, slightly expanding distally; a large circular extension after the last pair of processes (Figures 8a,b and 10a).

Distribution. This species is known from a few localities in South Korea and is probably endemic to the Korean Peninsula.

Habitats and ecology. This species seems to be a habitat specialist because it was collected only from rivers (Table S1 and Figure 11a). However, this pattern needs future research because it could reflect limited sampling efforts. The life cycle and feeding behavior of this species are unknown.

***Glossiphonia moorei* Bolotov, Eliseeva & Kondakov sp. nov.**

= *Glossiphonia mollissima* Moore (1898): 547 (identification error; reference to *Clepsine mollissima* Grube, 1871) [11].

= *Glossiphonia mollissima* Livanow (1902): 353 (assumption that this taxon represents an undescribed species: “Moore’s *Gl. mollissima* aber betrachte ich als eine vielleicht neue Art von *Glossosiphonia*”) [13].

= *Glossosiphonia complanata* Miyadi (1937): 450 (identification error; reference to *Glossiphonia complanata* (Linnaeus, 1758)) [54].

= *Glossiphonia complanata* Miyadi (1938): 134 (identification error; reference to *Glossiphonia complanata* (Linnaeus, 1758)) [55].

= *Glossiphonia complanata mollissima* Moore in Moore & Meyer (1951): 59 (new combination with reference to the identification error-based name *Glossiphonia mollissima* Moore, 1898) [14].

= *Glossiphonia camplanata mullissimi* Keith (1955): 104 (erroneous spelling) [56].

= *Boreobdella verrucata* Klemm (1982): 110 (identification error) [16].

= *Glossiphonia* sp.3 ‘Tumnin River’ Bolotov et al. (2019): 6, Supplementary Table S2 [19]. Figures 5a–f, 6a–d, 7d–f,h, 9a–d, 10b and 11b, Table S2.

LSID: <https://zoobank.org/urn:lsid:zoobank.org:act:C77498B1-390B-42FC-BBA8-4DEAF5332856> (accessed on 27 May 2023).

Holotype. RMBH Hir_0627-H; Russia, Commander Islands, a small lake near the Kamenka River, 55.1106° N, 166.0123° E, Bering Island, 12 June 2022, T. A. Eliseeva leg.

Paratypes (*N* = 62). Russia: the type locality, 14 June 2022, the same collector, one specimen (voucher RMBH Hir_0628); a small lake, 55.2450° N, 165.9586° E, Bering Is-

land, Commander Islands, 9 June 2022, S. E. Sokolova & T. A. Eliseeva leg., two specimens (voucher RMBH Hir_0626); Tumnin River, 49.9451° N, 139.9181° E, Khabarovsk Region, 14 July 2014, I. N. Bolotov & I. V. Vikhrev leg., four specimens (vouchers RMBH Hir_0004 and RMBH Hir_0005); a floodplain (oxbow) lake of Avvakumovka River near Permskoe village, 43.7578° N, 135.1861° E, Primorye, 31 May 2017, I. N. Bolotov leg., three specimens (voucher RMBH Hir_0087); a tributary of Partizanskaya River situated upstream of a bridge near Lozoviy railway station, 43.0586° N, 133.1562° E, Primorye, 27 May 2017, I. N. Bolotov leg., one specimen (voucher RMBH Hir_0091_2); a small lake near Nakyn settlement, 64.9928° N, 117.0253° E, Yakutia Republic, 7 July 2018, collector unknown, one specimen (voucher RMBH Hir_0188_2); Anadyr River, 65.5175° N, 173.1046° E, Chukotka Peninsula, 20 July 2019, O. V. Aksenova, A. V. Kondakov & I. V. Vikhrev leg., one specimen (voucher RMBH Hir_0238); a lake near Ugolnye Kopi village, 64.7501° N, 177.7771° E, Chukotka Peninsula, 27 July 2019, O. V. Aksenova, A. V. Kondakov & I. V. Vikhrev leg., four specimens (voucher RMBH Hir_0251_2); a small lake in the Amguema River basin, 67.1428° N, 178.7370° E, Chukotka Peninsula, 10 July 2019, Aksenova, A. V. Kondakov & I. V. Vikhrev leg., two specimens (voucher RMBH Hir_0252); Pike Lake, Khatanga, 71.9562° N, 102.6251° E, Taymyr Peninsula, 24 July 2018, S. E. Sokolova & A. R. Shevchenko leg., one specimen (voucher RMBH Hir_0274_2); a puddle near railway in Artem city, 43.3794° N, 132.2188° E, Primorye, 10 September 2020, O. V. Aksenova et al. leg., three specimens (voucher RMBH Hir_0403_1); a small ditch near road, Knevichi settlement, 43.3886° N, 132.1880° E, Primorye, 10 September 2020, O. V. Aksenova et al. leg., two specimens (voucher RMBH Hir_0409_2); Temie Lake near Yakutsk, 62.0519° N, 129.4778° E, Yakutia Republic, 26 July 2020, O. V. Aksenova et al. leg., three specimens (voucher RMBH Hir_0439_2); Gilchinskoe reservoir near Kozmodemyanovka settlement, 50.1538° N, 128.2342° E, Amur Region, 2 July 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0472_1); Tunnekh Lake, 70.4902° N, 147.5736° E, Chokurdakh, Yakutia Republic, 24 July 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0478); Sette-Tala Lake, 70.4343° N, 148.2141° E, Chokurdakh, Yakutia Republic, 26 July 2021, O. V. Aksenova et al. leg., two specimens (vouchers RMBH Hir_0479 and RMBH Hir_0480); small lake, 70.4353° N, 148.2094° E, Chokurdakh, Yakutia Republic, 26 July 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0481); Sette-Tala Lake, 70.4344° N, 148.2071° E, Chokurdakh, Yakutia Republic, 26 July 2021, O. V. Aksenova et al. leg., four specimens (vouchers RMBH Hir_0482 and RMBH Hir_0483); small lake, 68.8992° N, 94.1593° E, Putorana Plateau, 1 July 2021, E. S. Chertoprud leg., one specimen (voucher RMBH Hir_0487); an unnamed small lake by the road Klyuchi—Ust-Kamchatsk, 56.4461° N, 161.0064° E, Kamchatka Peninsula, September 9, 2021, A. V. Kondakov leg., one specimen (voucher RMBH Hir_0490); a lake near the village of Klyuchi, 56.3187° N, 160.8767° E, Kamchatka Peninsula, 10 September 2021, A. V. Kondakov & A. A. Lyubas leg., one specimen (voucher RMBH Hir_0511_2); Khalaktyrskoye Lake, 53.0274° N, 158.7362° E, Kamchatka Peninsula, 12 September 2021, A. V. Kondakov & A. A. Lyubas leg., four specimens (voucher RMBH Hir_0512); Bureya River, 49.7885° N, 129.8907° E, Khabarovsk Region, 4 July 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0514_1); swamp in Vitim Nature Reserve, 57.3904° N, 116.4936° E, Irkutsk Region, 21 August 2020, E. S. Babushkin leg., two specimens (voucher RMBH Hir_0558_1); a small lake on Kunashir Island, 43.7655° N, 145.5008° E, Kurile Archipelago, 8 September 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0561_2); Lake Maloe on Iturup Island, 45.0813° N, 147.7020° E, Kurile Archipelago, 18 September 2021, O. V. Aksenova et al. leg., two specimens (voucher RMBH Hir_0570_2); stream in Yuzhno-Sakhalinsk city, 46.9396° N, 142.7268° E, Sakhalin Island, 21 August 2021, O. V. Aksenova, M. V. Vinarski & A. V. Kropotin leg., two specimens (voucher RMBH Hir_0576_1); Tym River, 50.8589° N, 142.6459° E, Sakhalin Island, 24 August 2021, O. V. Aksenova, M. V. Vinarski & A. V. Kropotin leg., one specimen (voucher RMBH Hir_0578); Tym River, 51.8169° N, 143.1461° E, Sakhalin Island, 25 August 2021, O. V. Aksenova, M. V. Vinarski & A. V. Kropotin leg., two specimens (voucher RMBH Hir_0581_1); Lake Sedykh, 46.8564° N,

143.1496° E, Sakhalin Island, 1 September 2021, O. V. Aksenova et al. leg., one specimen (voucher RMBH Hir_0595); an unnamed lake, 59.5572° N, 150.8408° E, Magadan Region, 18 July 2022, Sokolova, O. V. Travina & A.V. Kropotin leg., one specimen (voucher RMBH Hir_0725); a swampy lake near the Petlya River, Seymchan, 62.9036° N, 152.4148° E, Magadan Region, 26 July 2022, Y. V. Beshpalaya, O. V. Aksenova & A. S. Aksenov leg., one specimen (voucher RMBH Hir_0726); Grieve Lake, Seymchan, 62.6460° N, 152.3598° E, Magadan Region, 27 July 2022, Y. V. Beshpalaya, O. V. Aksenova & A. S. Aksenov leg., one specimen (voucher RMBH Hir_0727); swamp, Seymchan, 62.7411° N, 152.4924° E, Magadan Region, 31 July 2022, Y. V. Beshpalaya, O. V. Aksenova & A. S. Aksenov leg., one specimen (voucher RMBH Hir_0728); a lake, Omolon, 65.2287° N, 160.5224° E, Chukotka Peninsula, 25 July 2022, S. E. Sokolova, O. V. Travina & A.V. Kropotin leg., one specimen (voucher RMBH Hir_0729) (see Tables S1 and S2 for detail and reference sequence accession numbers).

Etymology. This species is named in memory of Dr. John Percy Moore (1869–1965), who discovered it in samples from the Bering Island.

Differential diagnosis. Medium-sized leech, which could be distinguished from other congeners by a combination of the following characteristics: six rows of broadly ovate but shallow dorsal papillae (outer paramedian, outer paramarginal, and marginal series), each dorsal papilla bears yellow or whitish spot; dorsal markings pattern with two blackish dotted outer paramedian lines (invisible in darker melanic forms), ventral markings pattern with two outer paramedian dark lines. Externally, the new species is similar to *G. arctica* and *G. koreaensis* **sp. nov.** It differs from the first species by having larger papillae and a well-developed black markings pattern dorsally and from the second species by the lack of multiple dark longitudinal lines on the dorsum.

Molecular diagnosis. The new species represents a separate genetic lineage belonging to the *verrucata* group (Figure 3). The pairwise uncorrected *COI* *p*-distance between the new species and other congeners varies from 6.2 to 14.5% (the nearest neighbor: *G. koreaensis* **sp. nov.**). The intraspecific pairwise *COI* *p*-distance ranges from 0.0 to 2.6% (mean $\pm$ s.e.m. = $1.11 \pm 0.02\%$; $N = 44$ sequences and 946 pairwise distance values). The GenBank acc. numbers of reference DNA sequences (*COI* and *18S rRNA*) are given in Tables 1, S1 and S2.

Description. Medium-sized leech (body length up to 18.2 mm). Measurements of the holotype and paratypes are presented in Table S2. Body broad, ovate (Figure 5a–f). Dorsum with six rows of ovate, broad, but shallow papillae on annulus a2 (outer paramedian, outer paramarginal, and marginal series). Posterior sucker small, circular (maximum diameter of 2.6 mm), and ventrally directed. Proboscis pore in the anterior part of anterior sucker. Coloration of living animals: body light brown (light-colored forms) to dark brown (melanic forms), dorsum with six rows of ovate yellow to whitish spots on each papilla at annulus a2 (outer paramedian, outer paramarginal, and marginal series) (Figure 6a–d). Coloration of ethanol-preserved animals: dorsum light brown (light-colored forms) to blackish (melanic forms), with two dark outer paramedian lines (inconspicuous in melanists); each dorsal papilla bears large, ovate light yellow or whitish spot (outer paramedian, outer paramarginal, and marginal series) (Figure 5a–f). Three pairs of circular eyespots arranged to two parallel rows; in some specimens, eyespots on each side are joined to a single comma-like or elongate-ovate large spot (Figure 7d–f). Venter yellowish to light brown with two dark outer paramedian lines (Figure 5a–f). Total number of annuli: 70. Somites I–III uniannulate, IV biannulate, V–XXIV triannulate, XXV biannulate, XXVII uniannulate. The male and female genital pores are separated by two annuli and are located in furrows XIa3/XIIa1 and XIIa2/a3, respectively (Figure 7h). Reproductive system: 10 pairs of very large, ovate testisacs intersegmentally from XIII/XIV to XXI/XXII; atrium spherical, the atrial cornua twisted anteriorly; paired ejaculatory ducts long, extending to XIX; paired ovisacs long, arranged as loops (Figures 9a–c and 10b). Digestive system: proboscis sheath massive, thick, elongated; esophagus broad; salivary glands diffuse; crop with 7 pairs of crop caeca: 1st–6th uniform, bag-like, expanding distally, 7th pair (posterior caeca)

with 4 blind processes; intestine enlarged, with 4 pairs of large, long, bag-like processes, expanding distally, each with several short lobes; a large circular extension after the last pair of processes (Figures 9a–d and 10b).

Distribution. This species is widespread throughout Northeast Asia (Eastern Siberia and the Russian Far East) from the Lena River basin and Taymyr to Chukotka, Kamchatka, Magadan and Khabarovsk regions, Amur River basin, and Primorye; Commander Islands, Kurile Archipelago, and Sakhalin Island (confirmed by DNA sequences); USA: SE Alaska and Kodiak Island (based on nonsequenced specimens) [8,11,54,56].

Habitats and ecology. This species is a habitat generalist, which is known to occur in a broad range of water bodies such as small to large natural lakes, reservoirs, oxbow lakes, rivers and creeks, swamps, ditches, and puddles (Table S1 and Figure 11b). The life cycle and feeding behavior of this species are unknown.

4. Discussion

4.1. Taxonomic Novelties

Our new findings, presented above, contribute to the systematics of the glossiphoniid leeches from Northeast Asia. Here, we described a new *Glossiphonia* species from South Korea, that is, *G. koreaensis* **sp. nov.** Furthermore, a valid name (*G. moorei* **sp. nov.**) was introduced for *Glossiphonia mollissima* sensu Moore, 1898, non Grube, 1871, a little-known but widespread (putative trans-Beringean) species, which was incorrectly referred to the specific names *mollissima* Grube, 1871 [8,11,13,14] and *complanata* Linnaeus, 1758 [54,55].

G. koreaensis **sp. nov.** seems to be the only species in this genus on the Korean Peninsula. In turn, *G. moorei* **sp. nov.** may be the single representative of the genus throughout the vast territory of the Russian Far East [8], including the Amur Basin; Kolyma Highlands; Chukotka and Kamchatka peninsulas; Magadan, Khabarovsk, and Primorye regions; Sakhalin Island; Commander Islands; and the Kurile Archipelago. Our dataset does not contain samples from China, Japan, or North Korea; and records of these and additional *Glossiphonia* species from the areas, outlined above, are expected. In particular, the available samples from the South Kurile Islands (Kunashir and Iturup) and published records [57] indicate that *Glossiphonia moorei* **sp. nov.** may also be distributed in Japan (Hokkaido to Kyushu). Moreover, its occurrences from the Amur, a transboundary river system, and published data [3] suggest that the range of this species should also include northeast China.

Our new results together with a review of the body of literature [5,7,8,28,32] indicate that the genus *Glossiphonia* represents a rather species-rich clade of freshwater leeches, which currently contains no less than 16 valid species (Table 2). The taxonomic status of these species was already confirmed by means of a DNA-based approach, except for *Glossiphonia paludosa*, *G. pulchella*, and *G. slovaca*, whose sequences are unavailable. The maximum species richness of this clade is observed in the Palearctic, with only two species recorded from the Nearctic (*Glossiphonia elegans* and *G. moorei* **sp. nov.**). A few species-level phylogenetic lineages discovered in Southern Europe [7,8] are probably undescribed species, lacking available Linnean names, indicating that the number of *Glossiphonia* species will increase in the future.

From a phylogenetic perspective, the genus *Glossiphonia* comprises two large clades: the *complanata* group and the *verrucata* group (see Figure 3). Taxonomically, these groups correspond to the subgenera *Glossiphonia* s. str. and *Boreobdella* Johansson, 1929. In our earlier work [8], we also separated the *concolor* group, but its delineation is weakly supported in our expanded two-locus phylogeny. Both Asian species described in this study belong to the *verrucata* group.

4.2. Biogeographic Issues

Once again, we confirm that the trans-Palearctic species of freshwater leeches, delineated by earlier scholars [1–3], do not exist in nature, being complexes of cryptic species with more restricted distribution as well as phylogenetically distant species with similar

external traits, resulting from convergent evolution. Nesemann [58] was first to start the discussion on this issue based on a comprehensive morphology-based study of *Glossiphonia paludosa* (Carena, 1824) sensu lato. The latter taxon was thought to be common in Northeast Asia, but its populations in Japan were found to represent a species belonging to the genus *Ancyrobdella* Oka, 1917, indicating that the distribution of the widespread Palearctic leeches needs an intense revision [58]. Later, the Northeast Asian populations of what was thought to be *Hemiclepsis marginata* (O. F. Müller, 1774) sensu lato [1,2] were reconsidered as belonging to several separate species such as *H. schrencki* Bolotov et al., 2019 and *H. tumminiana* Bolotov et al., 2019 [19,59]. The former Holarctic taxa *Helobdella stagnalis* (Linnaeus, 1758) and *Alboglossiphonia heteroclita* (Linnaeus, 1761) [1,2] were also subdivided into several species with narrower ranges [8,29]. A similar biogeographic pattern was recovered in the fish leech *Piscicola geometra* (Linnaeus, 1758) (Piscicolidae), which was thought to have a Holarctic range [60]. It represents a complex of several cryptic, deeply divergent phylogenetic lineages, including *P. khubsugulensis* Kaygorodova, 2023, *P. nordica* Kaygorodova, 2023, and *P. sibirica* Kaygorodova, 2023 from the Eastern Palearctic [60].

It was thought that the ancient Lake Baikal housed two endemic genera of the glossiphoniid leeches [1,61]. However, a body of recent phylogenetic research [27,28] clearly indicates that the level of endemism of this family in Baikal should be downgraded to the species level, with three endemic species: *Glossiphonia baicalensis*, *G. echinulata*, and *G. grubei*. Epshtein [61] assumed that all the three species may have originated from a common ancestor. While a comprehensive phylogenetic reconstruction based on complete mitochondrial genomes recovered *Glossiphonia echinulata* and *G. grubei* as a pair of sister species [28], strongly supporting their common origin within the lake, the phylogenetic position of *G. baicalensis* is still uncertain (see Figure 3). The ancient Lake Ohrid housed one endemic species (*Glossiphonia pulchella*), but its origin and phylogenetic affinities are yet to be estimated.

4.3. Melanism and Cryptic Coloration in Glossiphoniid Leeches

It has been shown that melanism commonly occurs in glossiphoniid leech populations from Arctic areas (e.g., *Glossiphonia* and *Theromyzon*) but the causes of this phenomenon are unclear (see review [8]). Here, we showed that the samples of *Glossiphonia moorei* **sp. nov.** collected north of the Arctic Circle contain a significantly larger proportion of melanic individuals compared with those from the non-Arctic areas (35.1 vs. 14.0%, respectively). There are a few examples of cryptic coloration (camouflage) in this species when melanic individuals were recorded on darker substrate, and vice versa. Considering these occasional observations, we could assume that light-colored and melanic forms in this species may reflect substrate-induced cryptic coloration, but this preliminary hypothesis needs to be statistically checked in the future. Conversely, Sawyer and Dierst-Davies [62] showed that individual leeches darken in the light and lighten in the dark and that they do not adapt to the background. From this perspective, a larger proportion of melanic individuals in the Arctic could be linked to the polar day (midnight sun) phenomenon, i.e., extended period of daytime occurring in areas north of the Arctic Circle during the summer months.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15060756/s1>, Figure S1: Maximum likelihood phylogeny of the subgenus *Boreobdella* Johansson, 1929 based on the COI gene sequences; Table S1: Reliable occurrences and information on samples and DNA sequences of *Glossiphonia moorei* **sp. nov.** and *G. koreansis* **sp. nov.** (Glossiphoniidae); Table S2: Voucher numbers, accession numbers of reference DNA sequences, and measurements for the type series of the new *Glossiphonia* species; Table S3: Number of melanic and light-colored specimens in *Glossiphonia moorei* **sp. nov.** samples from Arctic and non-Arctic areas. References [8,11,14,19,56] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, I.N.B., T.A.E., A.V.K. (Alexander V. Kondakov) and M.V.V.; Methodology, I.N.B., T.A.E., A.V.K. (Alexander V. Kondakov) and A.V.K. (Alexander V. Kropotin); Software, M.Y.G.; Literary Survey, I.N.B.; Molecular Analysis, T.A.E. and A.V.K. (Alexander V.

Kondakov); Statistical Analysis, I.N.B.; Fieldwork, I.N.B., T.A.E., A.V.K. (Alexander V. Kondakov), A.V.K. (Alexander V. Kropotin), O.V.A., Y.V.B., O.V.T., M.Y.G., S.K.K., J.H.L. and M.V.V.; Species Identification, Specimen Measurements, I.N.B. and T.A.E.; Writing—Original Draft Preparation, I.N.B.; Writing—Review and Editing, all authors; Visualization, I.N.B., T.A.E., A.V.K. (Alexander V. Kropotin) and M.Y.G.; Supervision, I.N.B.; Project Administration, I.N.B.; Funding Acquisition, M.V.V. and I.N.B. All authors have read and agreed to the published version of the manuscript.

Funding: The general financial support for this study was obtained through project No. 19-14-00066/P funded by the Russian Scientific Foundation (RSF). Work on phylogenetic reconstructions and mapping was supported by the Ministry of Science and Higher Education of Russia (project No. FUUW-2022-0056 to I.N.B., M.Y.G., and A.V.K.). The Nakdonggang National Institute of Biological Resources (NNIBR) supported S.K.K. under project No. NNIBR202201101. The fieldwork was carried out with partial financial support from the Ministry of Science and Higher Education of Russia (project No. FUUW-2022-0039 to Y.V.B., O.V.A., A.V.K., O.V.T., and T.A.E.).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The type series of the two new leech species were deposited to the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences (Arkhangelsk, Russia). The new sequences, generated in this study, are available through NCBI's GenBank. Additional data, used and collected during this research, are available from the authors upon reasonable request.

Acknowledgments: We are thankful to the three anonymous reviewers for their insightful comments on earlier versions of this paper. We are grateful to Clemens Grosser for his valuable advice during the preparation of this study. Special thanks to Arsen Trofimov (Chokurdach, Yakutia), Elena Linnik, and Alexander Yakovlev (Kurilsky Nature Reserve, Kurile Archipelago) and to the staff of the Gidrostroj Tour Company for their invaluable help with the fieldwork.

Conflicts of Interest: The authors declare no conflict of interest.

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