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Host Range and Phylogenetic Position of *Acipenserobdella volgensis* (Zykoff, 1904) (Hirudinea: Piscicolidae) with a Global Checklist of Bivalve-Associated Fish Leeches

Ivan N. Bolotov [1,2,*], Vadim V. Maryinsky [3], Dmitry M. Palatov [4,5], Alexander V. Kondakov [1,4,6], Tatyana A. Eliseeva [1,4,6], Ekaterina S. Konopleva [1], Mikhail Y. Gofarov [1], Ilya V. Vikhrev [1,2] and Yulia V. Bespalaya [1]

1 N. Laverov Federal Center for Integrated Arctic Research, the Ural Branch of the Russian Academy of Sciences, Northern Dvina Emb. 23, 163069 Arkhangelsk, Russia
2 SSC/IUCN—Mollusc Specialist Group, Species Survival Commission, International Union for Conservation of Nature, Cambridge CB2 3QZ, UK
3 Department of General Ecology and Hydrobiology, M.V. Lomonosov Moscow State University, 119234 Moscow, Russia
4 Laboratory of Macroecology & Biogeography of Invertebrates, Saint Petersburg State University, 7/9 Universitetskaya Emb., 199034 Saint Petersburg, Russia
5 A. N. Severtsov Institute of Ecology and Evolution, the Russian Academy of Sciences, Leninsky Prt. 33, 119071 Moscow, Russia
6 Laboratory for Molecular Ecology and Phylogenetics, Northern Arctic Federal University, Northern Dvina Emb. 17, 163002 Arkhangelsk, Russia
* Correspondence: inepras@yandex.ru

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Abstract: The fish leech *Acipenserobdella volgensis* (Hirudinea: Piscicolidae) is a rare and poorly known freshwater species, which is thought to be an obligate parasite of sturgeons. This leech has a disjunctive range in Europe and Eastern Siberia. Here, we estimate the phylogenetic affinities and host range of *A. volgensis* using a set of DNA sequences (*COI* and *18S rRNA* gene fragments), field observation data, and a review of the body of literature. Based on a time-calibrated Bayesian phylogeny, we show that the European and Siberian lineages of *A. volgensis* have been separated since the latest Pliocene (mean age = 2.7 Ma). The analysis of available host records indicates that this leech is characterized by a broader host range as it was collected from fish belonging to four families (Acipenseridae, Cyprinidae, Salmonidae, and Esocidae). Conversely, only a few suitable primary hosts (six sturgeons, one cyprinid, and one salmonid fish) were confirmed by earlier research. Moreover, this leech could be considered a facultative mussel-associated species that uses bivalves (duck mussel *Anodonta anatina*; Unionidae) as shelter. Globally, three other piscicolid leeches have been recorded from the mantle cavity of bivalve molluscs, that is, the freshwater taxa *Caspiobdella fadejewi* and *Alexandrobdella makhrovi*, and the marine species *Austrobdella coliumicus*.

Keywords: Acipenseridae; *Anodonta anatina*; bivalve-associated leech; Caspian Sea; *Caspiobdella*; fish parasite; freshwater fish leech; Salmonidae; Unionidae

1. Introduction

The family Piscicolidae (fish leeches) is a large group of primarily marine leeches [1,2]. However, there are several freshwater radiations of piscicolids, the largest of which occurs in the Palearctic Region [3]. At least five independent colonization events of piscicolids into freshwater environments have been uncovered on the basis of phylogenetic research [4].

The most species-rich assemblage of freshwater piscicolids was described from Europe [5], although several European species show very low level of genetic divergence from their sister taxa [6]. This assemblage contains a number of rare and poorly known species such as *Acipenserobdella volgensis* (Zykoff, 1904). The holotype (by monotypy) of

the latter species was collected from the Volga River, European Russia [7]. The taxonomic status of this taxon is a matter of doubt [8–10]. Epshtein [11] briefly re-described this leech as a valid species. Later, it was separated to a new monotypic genus, *Acipenserobdella* Epshtein, 1969 [12,13]. Sawyer [14] returned this species to the genus *Piscicola* Blainville, 1818. Nesemann & Neubert [15] transferred this species-group taxon to the genus *Caspiobdella* Epshtein, 1966 based on morphological and anatomical features. Instead, it was again placed in *Acipenserobdella* in the newest identification guide on freshwater leeches from the Palearctic Region [16].

A. volgensis has an unusual, disjunctive range. Most available occurrences are situated within the massive Volga River basin in Russia [17]. A population of this leech was also discovered in a small river on the Baltic Sea coast, northern Poland [15,18]. Furthermore, there are records from the Selenga and Angara rivers, an inlet and the outlet of Lake Baikal in Eastern Siberia [17], as well as from Lake Baikal itself [19,20]. A few recent occurrences of *A. volgensis* from Iraq [21,22] and Iran [23] cannot be accepted as reliable, as they were probably based on misidentified specimens of other piscicolid species.

A. volgensis was considered a specialized parasite of sturgeon fishes (Acipenseridae) [17,24], although the small body of available literature reports host records from other fish families (Cyprinidae, Salmonidae, and Esocidae) as well [18,25,26]. There is a single brief report on the life cycle and feeding ecology of this leech species that were described based on a series of laboratory experiments [27].

It has been shown that freshwater leeches may serve as endosymbionts of freshwater bivalves, being facultative or obligate inhabitants of the mantle cavity of molluscs [28]. Although most mussel-associated leeches belong to the family Glossiphoniidae [28], there are a few records of fish leeches from the mantle cavity of freshwater mussels [4]. However, *A. volgensis* has never been recorded in association with Mollusca. Furthermore, published records of fish leeches from the mantle cavity of bivalve molluscs are yet to be compiled at the global scale.

This study aims to (1) estimate the phylogenetic position and evolutionary origin of *A. volgensis* based on the DNA sequences generated from new samples collected in European Russia; (2) assess the host range of this leech species using a broad compilation of available host records; (3) report on the first record of *A. volgensis* from the mantle cavity of a unionid mussel; and (4) compile a global checklist of bivalve-associated piscicolid leeches.

2. Materials and Methods

2.1. Data Sampling

New samples of *A. volgensis* were occasionally collected by forceps from stones, fish specimens, and from the mantle cavity of freshwater mussels in the Volga River basin, European Russia during the period of 2015–2021 (Table 1). The samples are 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 and the collection of the Department of Zoology and Animal Ecology, V.N. Karazin Kharkiv National University (DZAE KHNU), Kharkiv, Ukraine (Table 1). Images of complete specimens were taken using a Canon EOS 7D digital camera with a Canon EF 100 mm f/2.8 L Macro IS USM lens (Canon Inc., Tokyo, Japan). Morphological traits of preserved specimens were examined and photographed using stereomicroscopes Leica M165C (Leica Microsystems GmbH, Wetzlar, Germany) and Zeiss Axio Zoom.V16 (Carl Zeiss AG, Oberkochen, Germany).

During this study, we collected four geographic occurrences and six host records of *A. volgensis* (Table 1). Five host records were based on visual observations and one host record was uncovered by means of a DNA-based approach. In particular, the crop content of one free-living specimen of *A. volgensis* from the Moscow River (sample RMBH Hir_0461_1) was sequenced for the *COI* gene using a fish-specific primer pair, as described previously [28]. The host species was identified based on that *COI* sequence using NCBI's BLASTn [29] and the Barcode of Life Data System (BOLD IDS) [30] search tools.

Table 1. Samples and hosts of *Acipenserobdella volgensis* (Zykoff, 1904) (Hirudinea: Piscicolidae) examined in this study.

Voucher No.	N	Sampling Locality, Date, and Collector	Latitude	Longitude	Host (Number of Examined Host Specimens, If Available)	Comments on Leech Samples
RMBH Hir_0189	2	European Russia: Moscow River, Volga River basin, Moscow Region, 12 June 2018, V. Maryinsky leg.	55.7038	36.7288	*Anodonta anatina* (Linnaeus, 1758); Unionidae (*N* = 25)	Adult leeches from the mantle cavity of freshwater mussels; formalin-preserved sample
N/A	1	European Russia: Moscow River, Volga River basin, Moscow Region, 30 June 2019, V. Maryinsky leg.	55.7038	36.7288	*Anodonta anatina* (Linnaeus, 1758); Unionidae (*N* = 20)	Adult leech from the mantle cavity of a freshwater mussel; not preserved (examined in the field)
RMBH Hir_0461_1	5	European Russia: Moscow River, Volga River basin, Moscow Region, 21 June 2021, D. Palatov leg.	55.6245	36.4033	*Leuciscus leuciscus* (Linnaeus, 1758); Cyprinidae (host uncovered by crop content sequencing: GenBank acc. No. of the *COI* sequence OP585664)	Free-living sub-adult leeches collected from stones; ethanol-preserved sample; sequenced (*COI* and *18S rRNA*)
DZAE KHNU	15	European Russia: Oka River, Volga River basin, Kaluga Region, 12 June 2015, D. Palatov leg.	54.5078	36.1084	*Acipenser ruthenus* Linnaeus, 1758 (Acipenseridae); *Blicca bjoerkna* (Linnaeus, 1758) (Cyprinidae)	Adult leeches collected from fish hosts; ethanol-preserved sample
DZAE KHNU	2	European Russia: Oka River, Volga River basin, Orel Region, 09 June 2015, D. Palatov leg.	53.5452	36.2289	*Acipenser ruthenus* Linnaeus, 1758 (Acipenseridae)	Adult leeches collected from fish hosts; ethanol-preserved sample

Note(s): N/A—not available.

Additional geographic occurrences (*N* = 16) and host records (*N* = 26) of this species were obtained from the body of available literature (Table S2). The occurrences from published sources were georeferenced and verified using the Google Earth tool (https://www.google.com/intl/ru/earth; accessed on 19 August 2022). To estimate the breadth of host range of this species, we compiled a dataset of raw host records, each of which represents an *A. volgensis* sample from one host that was collected from one locality (Table S2). Based on this dataset, we compiled a generalized table, which contains summary data on its available raw host records per host (Table S3). This summary table was used to visualize the host range of *A. volgensis* (suitable primary hosts vs. uncertain primary hosts vs. shelter hosts) using an online application of the Circos software (http://mkweb.bcgsc.ca/tableviewer accessed on 19 August 2022) [31].

2.2. DNA Sequences and Sequence Alignment of Leeches

Our DNA sequence dataset contains partial sequences of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) and the nuclear *small subunit of 18S ribosomal RNA* (*18S rRNA*) genes (Table S1). New sequences were generated from a European specimen of *A. volgensis* (Tables 1 and S1) using protocols and primers as described in our previous work [28]. Additional sequences were obtained from NCBI's GenBank, including those published by Apakupakul et al. [32], Bolotov et al. [4], Bottari et al. [33], Kaygorodova et al. [34], Siddall & Burreson [35], Truong [36], Tseng et al. [37], Utevsky & Trontelj [1], Utevsky et al. [38], and Williams & Burreson [2]. The final set contains 47 haplotype sequences, including two outgroup taxa, belonging to the family Ozobranchidae (Table S1).

The MUSCLE algorithm of MEGA7 was used to separately align each gene sequence dataset [39]. To exclude large gaps and hypervariable positions from the *18S rRNA* gene alignment, we applied GBlocks v. 0.91b through an online server [40,41] (final length of 1632 bp; 85% of the original 1914 bp). The *COI* and *18S rRNA* alignments were joined to a two-locus alignment using FaBox v. 1.61 (https://birc.au.dk/~palle/php/fabox/accessed on 19 August 2022) [42].

2.3. Phylogenetic Analyses, Divergence Dating, and Ancestral Environment Reconstruction

The combined alignment was applied to reconstruct the maximum likelihood phylogeny with an online version of IQ-TREE v. 1.6.12 (http://iqtree.cibiv.univie.ac.ataccessed on 19 August 2022) using an ultrafast bootstrapping (5000 replications) and an automatic identification of the most appropriate evolutionary models for each partition [43–46]. The time-calibrated Bayesian phylogeny of the Piscicolidae was reconstructed with BEAST v. 1.10.4 [47] using the combined *COI* + *18S rRNA* alignment (see above). We used a strict molecular clock with the Yule speciation process as the tree priors [48]. Available evolutionary rates for the *COI* and *18S rRNA* genes (substitutions/site/year) obtained from a fossil-calibrated Bayesian phylogeny [28] were separately assigned to corresponding partitions as follows: mean *COI* rate = 6.25×10^{-9} (95% HPD = 5.06×10^{-9}–7.53×10^{-9}), and mean *18S rRNA* rate = 1.99×10^{-10} (95% HPD = 1.60×10^{-10}–2.39×10^{-10}). The GTR + G + I model was applied to each gene partition. Three independent BEAST runs were performed through the CIPRES Science Gateway [49]. Each run contained 30,000,000 generations and a tree was sampled every 1000th cycle. The log files were checked with Tracer v. 1.7.2 [50]. The Effective Sample Size (ESS) value for each parameter in the combined runs was found to be >1000. The combined tree set from the three BEAST runs was generated with LogCombiner v. 1.10.4 [47] using a subsequent re-sampling at every 5000th generation and a 10% burn-in (N = 16,200 trees). The final maximum clade credibility tree (median heights) was reconstructed with TreeAnnotator v. 1.10.4 [47].

Ancestral trait modeling was performed with the Bayesian Binary Markov chain Monte Carlo (MCMC) algorithm [51,52]. The analysis was conducted using RASP v. 4.2 software [53] based on the maximum clade credibility tree obtained from our BEAST analyses (see above). Three possible types of environmental preferences for each species were coded as follows: (a) freshwater, (b) marine, and (ab) euryhaline ([4]; see Table S1 for detail). Data on environmental preferences was obtained from the IRMNG database (https://www.irmng.org [54]) and published sources [4,13,17,55,56]. The settings of the MCMC analysis were as follows: F81 + G evolutionary model; 500,000 cycles with sampling every 100th cycle; 10 MCMC chains with temperature = 0.1. Null distribution was not allowed.

3. Results

3.1. New Geographic Occurrences and Distribution Summary of Acipenserobdella volgensis

In total, 20 georeferenced occurrences of *A. volgensis* are available, including the four new occurrences collected in this study (Figures 1–3 and Table 1). The majority of these records are situated within the Volga River basin in European Russia (N = 16), while a few occurrences came from Eastern Siberia (N = 3) and Poland (N = 1). Our data confirm that the range of this species is disjunctive and consists of three distant, isolated freshwater systems: (1) the Volga Basin in European Russia, including the Chusovaya River, a tributary flowing from the Ural Mountains; (2) the Grabowa River in Poland; and (3) Lake Baikal basin with the Selenga and Angara rivers in Eastern Siberia (Figure 1).

3.2. Phylogenetic and Ancestral Environment Reconstructions for Acipenserobdella volgensis and Related Taxa

The p-distance between two available *COI* sequences of *A. volgensis* from European Russia and Eastern Siberia is 2.2%, while that between sequences of *A. volgensis* and *Caspiobdella fadejewi* (Epshtein, 1961) is 9.0% (range of 8.6–9.4%). The *Acipenserobdella* and *Caspiobdella* are sister lineages (BPP = 1.00; BS = 98). Our phylogenies (Figures 4 and S1) also reveal the sister relationships between the *Acipenserobdella* + *Caspiobdella* and *Piscicola* + *Baicalobdella* clades (BPP = 0.95; BS = 84) and that the *Baicalobdella* lineage is more closely related to the *Piscicola* clade (BPP = 0.97; BS = 97).

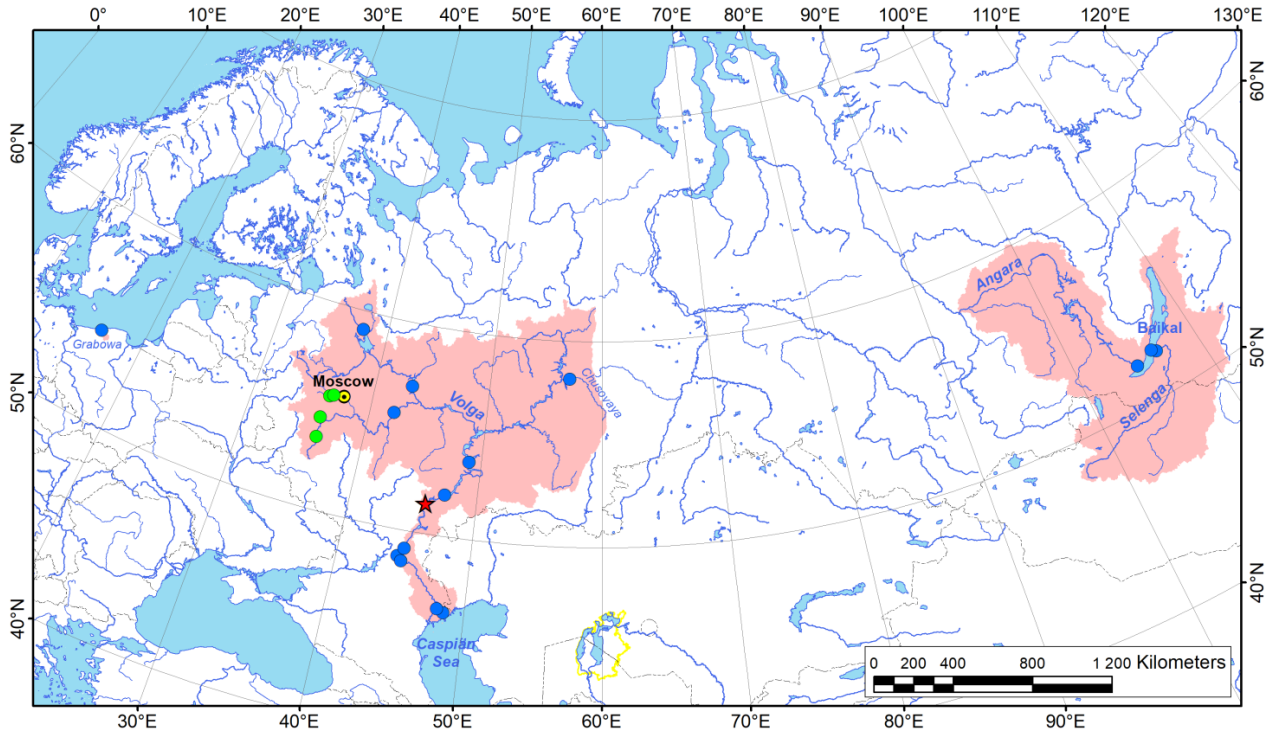

Figure 1. Distribution and occurrences of *Acipenserobdella volgensis* (Zykoff, 1904). The light red areas indicate the range of the species based on freshwater basin boundaries. The red star indicates the type locality of the species; the green circles indicate the localities of specimens that were collected under this study; and the blue circles indicate published records (raw occurrence data is presented in Table S2). The map was created using ESRI ArcGIS 10 software (www.esri.com/arcgisaccessed on 19 August 2022).

Our time-calibrated Bayesian phylogeny indicates that *A. volgensis* and *C. fadejewi* were likely separated near the Oligocene—Miocene boundary (mean age = 23.6 Ma, 95% HPD = 15.9–32.5 Ma) (Figure 4). The divergence event between the European and Siberian lineages of *A. volgensis* is placed in the latest Pliocene (mean age = 2.7 Ma, 95% HPD = 1.2–4.5 Ma). The most recent common ancestor (MRCA) of *Baicalobdella* and *Piscicola* most likely diverged in the latest Eocene (mean age = 38.0 Ma, 95% HPD = 29.1–47.5 Ma).

The ancestral environment reconstruction by means of a Bayesian Binary MCMC approach (Figure 4) reveals that the clade containing *Acipenserobdella*, *Caspiobdella*, *Piscicola*, *Baicalobdella*, and *Cystobranchus* representatives most likely originated from a freshwater ancestor (probability = 98.8%). Our results indicate that the MRCA of the *Acipenserobdella* + *Caspiobdella* clade most likely was a freshwater lineage (probability = 99.9%). However, an addition of saltwater *Caspiobdella* species from the Caspian Sea to the ancestral reconstructions may partly shift the pattern we have reconstructed herein (the DNA sequences of these species are currently not available).

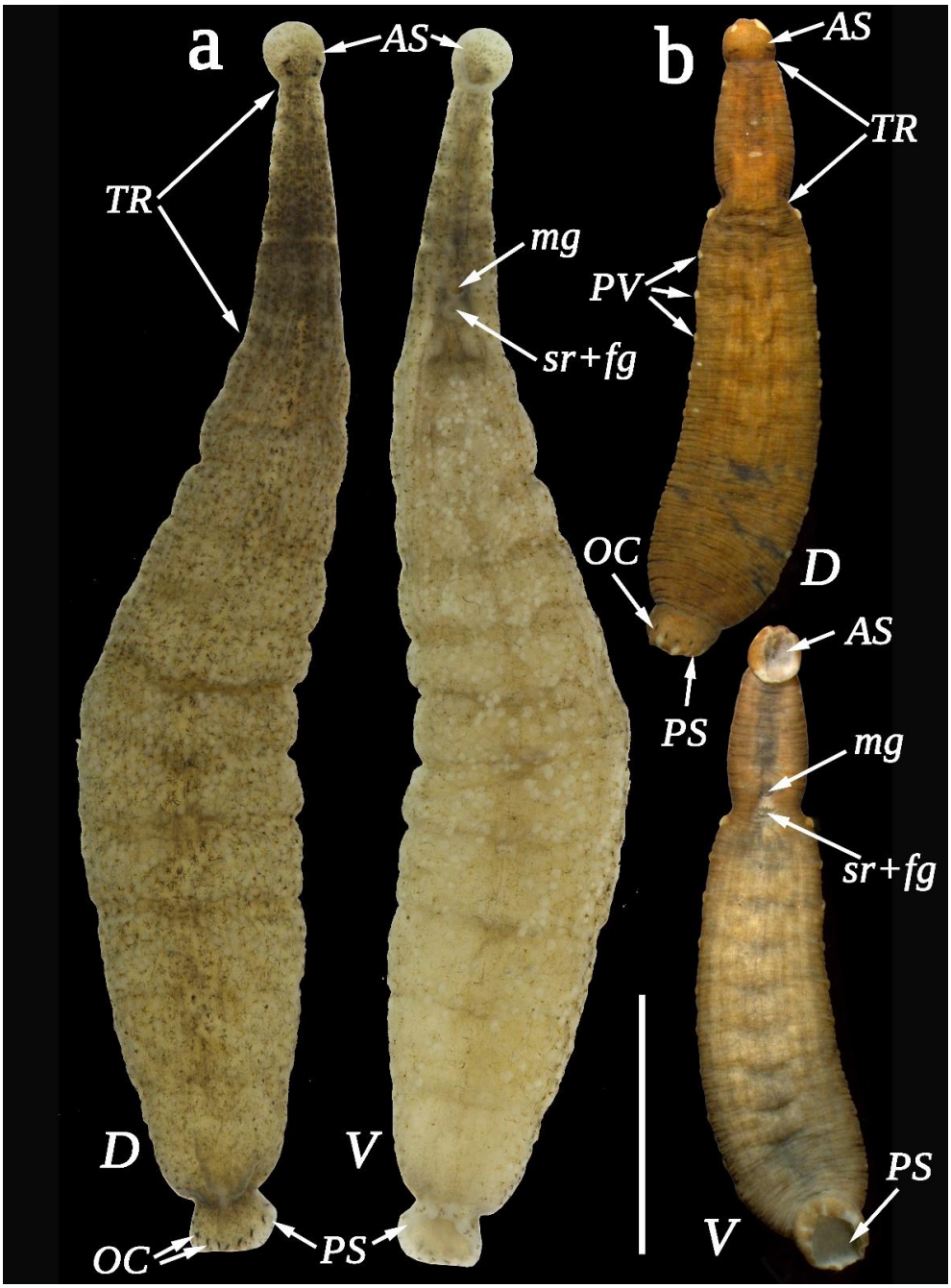

Figure 2. Dorsal (*D*) and ventral (*V*) view of *Acipenserobdella volgensis* (Zykoff, 1904) specimens. (**a**) Large adult specimen RMBH Hir_0189 from the mantle cavity of the duck mussel *Anodonta anatina* (Linnaeus, 1758) (Unionidae), Moscow River, European Russia. (**b**) Smaller sub-adult (immature) specimen RMBH Hir_0461_1 collected from stones, Moscow River, European Russia (sequenced). Abbreviations: *TR*, trachelosome (anterior part of the body); *AS*, anterior sucker; *PS*, posterior sucker; *PV*, pulsatile vesicles; *OC*, ocelli; *mg*, male gonopore; and *sr + fg*, seminal receptacle (spermatheca) opening with female gonopore. Scale bar = 5 mm. Photos: Tatyana A. Eliseeva.

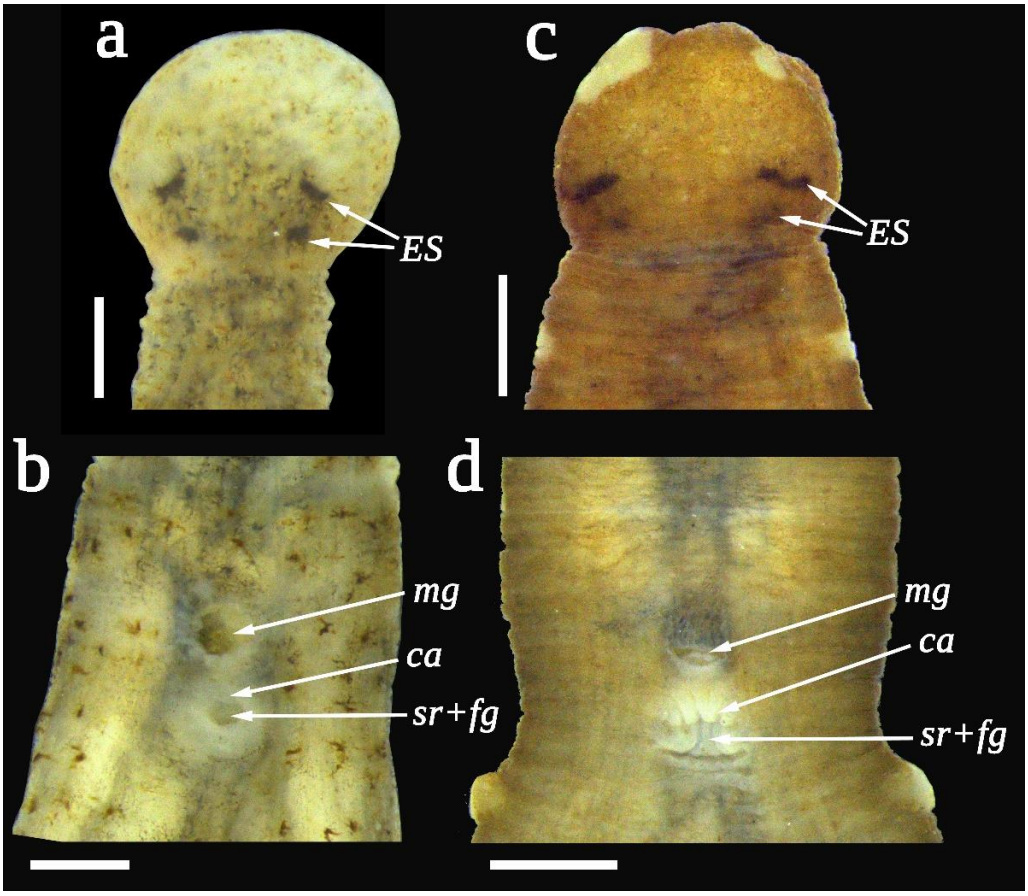

Figure 3. Morphological features of *Acipenserobdella volgensis* (Zykoff, 1904). (**a**,**b**) Specimen RMBH Hir_0189 from the mantle cavity of the duck mussel *Anodonta anatina* (Linnaeus, 1758) (Unionidae), Moscow River, European Russia: head region, dorsal view (**a**); section with genital pores, ventral view (**b**). (**c**,**d**) Free-living specimen RMBH Hir_0461_1, Moscow River, European Russia (sequenced): head region, dorsal view (**c**); section with genital pores, ventral view (**d**). Abbreviations: *ES*, eyespots; *mg*, male gonopore; *sr* + *fg*, seminal receptacle (spermatheca) opening with female gonopore; and *ca*, copulatory area around female genital pore. Scale bars = 0.5 mm. Photos: Tatyana A. Eliseeva.

3.3. Host Range of Acipenserobdella volgensis and Its Records from the Mantle Cavity of a Freshwater Mussel

Our dataset contains 28 host records from 13 fish and one freshwater mussel species (Figure 5 and Tables 1, S2 and S3). A critical re-analysis of these records reveals that nine fish species could be considered suitable primary hosts: *Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833; *A. baerii* Brandt, 1869; *A. ruthenus* Linnaeus, 1758; *A. stellatus* Pallas, 1771; *A. sturio* Linnaeus, 1758; *Huso huso* (Linnaeus, 1758) (Acipenseridae); *Abramis brama* (Linnaeus, 1758); *Leuciscus leuciscus* (Linnaeus, 1758) (Cyprinidae); and *Salmo trutta* Linnaeus, 1758 (Salmonidae) (N = 14 host records; see Figure 5). Among them, one fish host species (*Leuciscus leuciscus*) was confirmed in the present study based on the *COI* sequence of the crop content of a free-living specimen of *A. volgensis* from Moscow River (GenBank acc. No. OP585664) (Table 1).

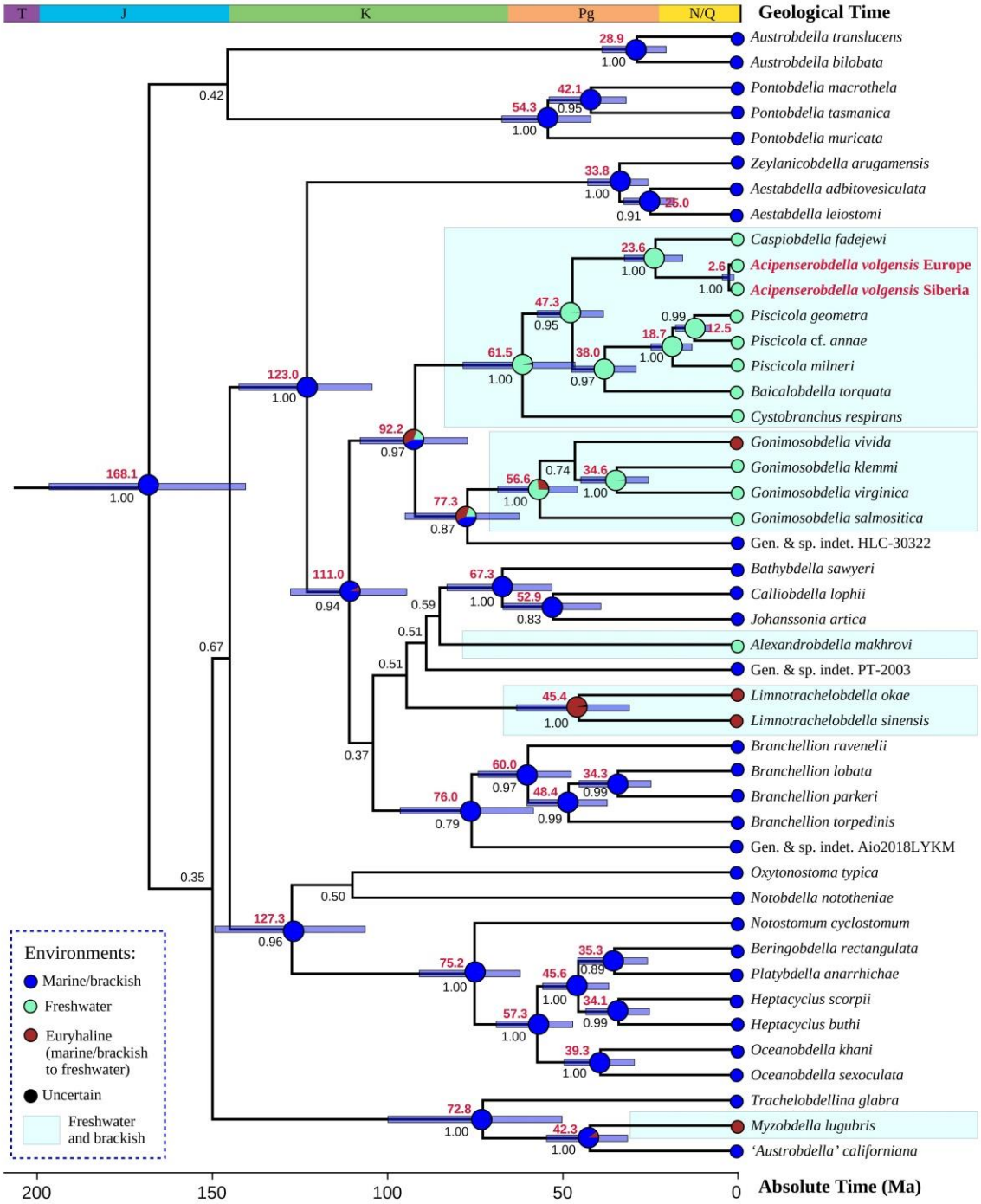

Figure 4. Time-calibrated Bayesian phylogeny (three codons of *COI* + *18S rRNA*) of the Piscicolidae. The black numbers near nodes are Bayesian posterior probabilities (BPP) of BEAST v. 1.10.4. The red bold numbers near nodes are mean node ages (Ma). The nodal circle charts indicate the probabilities of certain ancestral environments based on the Bayesian Binary MCMC reconstruction. Time and ancestral trait reconstructions for weakly supported nodes (BPP < 0.75) are omitted. Two Ozobranchidae taxa were used as an outgroup (not shown). Stratigraphic chart according to the International Commission on Stratigraphy, 2021 (https://stratigraphy.org/chartaccessed on 19 August 2022).

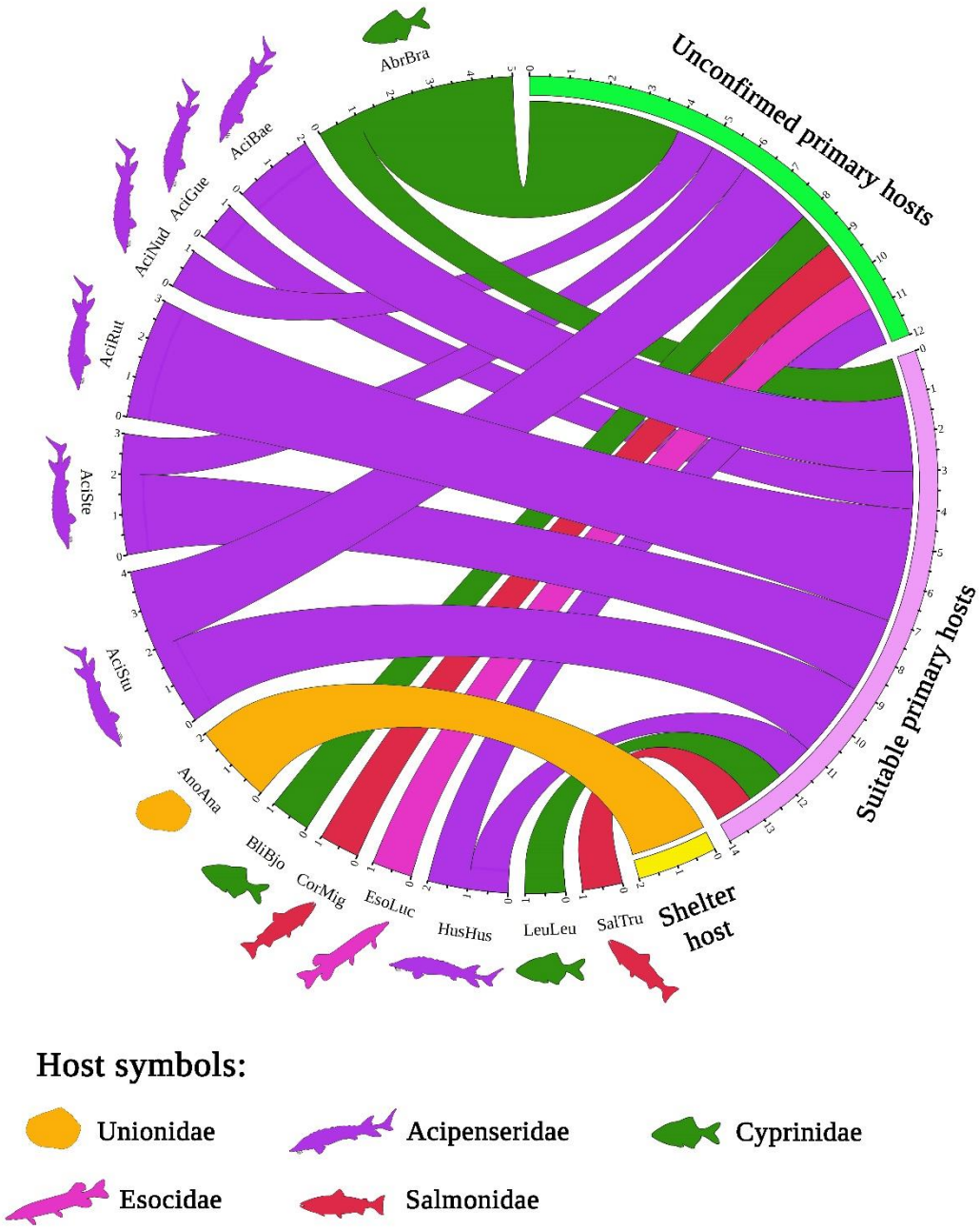

Figure 5. Circos plot of *Acipenserobdella volgensis* (Zykoff, 1904) host range based on the raw host records. Groups of hosts are represented by arcs on the left half of the circle with a size determined by the total number of raw host records for each group (Table S2). Their colors refer to the group of hosts: shelter host (light yellow), suitable primary host (light purple), and unconfirmed primary host (light green). Linking lines between these groups represent shared hosts, with the thickness proportional to the number of available records of this host for each group. The line color refers to the host family: Acipenseridae (purple), Cyprinidae (green), Salmonidae (red), Esocidae (pink), and Unionidae (yellow). Host name abbreviations: AnoAna, *Anodonta anatina*; AciBae, *Acipenser baerii*; AciGue, *Acipenser gueldenstaedtii*; AciNud, *Acipenser nudiventris*; AciRut, *Acipenser ruthenus*; AciSte, *Acipenser stellatus*; AciStu, *Acipenser sturio*; HusHus, *Huso huso*; AbrBra, *Abramis brama*; LeuLeu, *Leuciscus leuciscus*; BliBjo, *Blicca bjoerkna*; CorMig, *Coregonus migratorius*; SalTru, *Salmo trutta*; and EsoLuc, *Esox lucius*. The plot was created with the online application of Circos software (http://mkweb.bcgsc.ca/tableviewer accessed on 19 August 2022).

Conversely, four fish species could be ranked as unconfirmed primary hosts: *Acipenser nudiventris* Lovetsky, 1828 (Acipenseridae); *Blicca bjoerkna* (Linnaeus, 1758) (Cyprinidae); *Coregonus migratorius* (Georgi, 1775) (Salmonidae); and *Esox lucius* Linnaeus, 1758 (Esocidae).

Here, we also report on the first record of *A. volgensis* from the mantle cavity of a freshwater mussel species, *Anodonta anatina* (Linnaeus, 1758) (Unionidae) in the Moscow River, European Russia (Table 1). The mussel samples were collected from a sandy riffle at the midstream section of the river. Two living leeches were obtained from a sample of 25 mussels that was collected on 12 June 2018 (Figures 2a and 3a,b). Each leech was found in the mantle cavity of a separate mussel specimen. One leech was also recorded in a sample of 20 mussels that was collected on 30 June 2019. In contrast, a sample of 50 mussels collected from the same site on 15–20 September 2018 did not contain any leech (V. Maryinsky and D. Palatov, pers. observations).

3.4. Taxonomy

Family Piscicolidae Johnston, 1865

Genus *Acipenserobdella* Epshtein, 1969

Type species: *Piscicola volgensis* Zykoff, 1904 (by original designation)

***Acipenserobdella volgensis* (Zykoff, 1904)**

=*Piscicola podjapolski* Zykoff, 1900 [57] (p. 12) (nomen nudum); Plotnikov [58] (p. 11).

=*Piscicola volgensis* Zykoff, 1904 [7] (pp. 71–74,) (original description).

=*Piscicola volgensis* Zykoff, 1903 (incorrect year of the original description).—Rousseau [8] (p. 264); Behning [10] (p. 167); Epshtein [11] (p. 625, Figure 1549); Bogdanova & Nikolskaya [59] (p. 98); Reshetnikova [60] (p. 314); Ivanov [61] (p. 310); Sawyer [14] (p. 714); Lapkina et al. [25] (p. 133).

=*Piscicola podjapolskii* Plotnikov, 1909 [58] (p. 11) (inadvertent error in the species name).

=*Piscicola wolgensis* Plotnikov, 1909 [58] (p. 11) (inadvertent error in the species name); Stschegolew [9]: pp. 24–25.

=*Piscicola multistriata* Zaika, 1965 [19] (p. 78) (identification error; see Pugachev [20] (p. 110)).

=*Acipenserobdella volgensis* Zykoff, 1903 (new combination; incorrect year of the original description).—Epshtein [12] (p. 287); Lukin [13] (p. 329, Figure 169); Dontzov [62] (p. 23); Epshtein [17] (p. 370, Figures 437a–в and 452a–ж); Bauer et al. [24] (p. 426); Lapkina [27] (p. 110); Lapkina et al. [25] (p. 136); Epshtein [63] (p. 186); Pugachev [20] (p. 110); Bielecki et al. [18] (p. 88); Bielecki et al. [5] (p. 13); Kaygorodova [64] (p. 54); Kaygorodova et al. [65] (p. 2); Govedich et al. [16] (p. 497).

=*Caspiobdella volgensis* Zykoff, 1903 (new combination; incorrect year of the original description).—Nesemann & Neubert [15] (pp. 90–91, Figure 41a–d); Minelli et al. [66] (p. 12).

=*Asipencerobdella* [sic] *volgensis* Lapkina et al. [25] (p. 133) (inadvertent error in the generic name).

=*Piscicolidae* sp.—Kaygorodova et al. [34] (p. 9, Figure 5).

Figures 1–3, Table 1, Table S2

Holotype (by monotypy): specimen of 10 mm long (whereabouts unknown, probably lost); Russia: Volga River near Saratov (approx. 51.5° N, 46.0° E), a pectoral fin of *Acipenser shypa* Lov. (synonym of *Acipenser nudiventris* Lovetsky, 1828), 27 July 1900 [7].

Diagnosis: Medium-sized leech (maximum body length of 30 mm), body smooth; anterior sucker medium-sized, its width corresponds to the maximum width of the trachelosoma; posterior sucker small, its width equal or smaller than the maximum width of the urosoma; pulsatile vesicles small (11 pairs) or not expressed; two pairs of eyespots on anterior sucker; 10 large ocelli on posterior sucker; two annuli between gonopores, spermatheca present, female gonopore situated inside spermatheca; bursa longer than that in *Caspiobdella*; conducting strands and oviducts separate; oviducts cross vector tissue [16,17].

Distribution: Volga River basin in European Russia; Lake Baikal (shallow waters near the Selenga River delta), and Selenga and Angara rivers in Eastern Siberia; Grabowa River in northern Poland (Figure 1). A few occurrences of *A. volgensis* from Iraq and Iran were reported without images and descriptions of voucher specimens [21–23] and, hence, cannot

be accepted as reliable until more convincing evidence (e.g., published photos of sampled specimens) on the presence of this species in the Middle East is available. These records should not be included to the species' range until more convincing evidence on its presence in the Middle East is available.

Hosts: Nine fish species from three families (Acipenseridae, Cyprinidae, and Salmonidae) as confirmed suitable primary hosts; four fish species from four families (Acipenseridae, Cyprinidae, Salmonidae, and Esocidae) as unconfirmed primary hosts (feeding unsuccessful or not observed); one freshwater mussel species (Unionidae) as shelter host (Figure 5 and Tables S2 and S3).

Comments: In an earlier report, Zykoff [57] (p. 12) mentioned this species under another name as follows (translated from Russian): "*Piscicola podjapolski* n. sp. (named in honor of the initiator of the Volga Biological Station, my esteemed student and friend P.P. Podjapolsky). 27.VII. One specimen was found on the ventral side of a left pectoral fin of Ship Sturgeon (*Acipenser shypa* Lov.). Based on the number, shape, and position of eyes in the anterior sucker and based on the number and position of ocelli in the posterior sucker, this leech undoubtedly belongs to the genus *Piscicola*, while the body structure and other features clearly differ from described species of *Piscicola*. The body length in an extended state is approximately 15 mm (detailed description with a picture will be presented in the Proceedings of the Society)". This brief note does not contain a description or a definition of this taxon and is accompanied by a reference to the future paper, which was published in 1904 [7]. Hence, the name *Piscicola podjapolski* fails to conform the requirements of the Article 12 of the ICZN and is here considered a nomen nudum.

All earlier authors concluded that the protologue [7] was published in 1903. However, the statement on the title page indicates that this volume of "Bulletin de la Société impériale des naturalistes de Moscou" (Vol. 1903) was instead printed in 1904.

Epshtein [63] assumed that the nominal taxon *Piscicola conspersa* Grube, 1871 may represent a senior synonym of *A. volgensis*. It was described based on two specimens from the Angara River, Eastern Siberia [67]. However, *P. conspersa* differs from *A. volgensis* by a combination of the following characters: posterior sucker without ocelli (vs. 10 large ocelli in *A. volgensis*); male gonopore represents a wide transverse cleft (vs. a rounded or ovate pore); and female gonopore very small, poorly visible, situated immediately behind the male gonopore (vs. female gonopore situated inside a large and well-distinguishable spermathecal opening). Based on the original description of its general habitus and the gonopores, it may correspond to a separate Asian species belonging to the genus *Piscicola*.

4. Discussion

4.1. Phylogenetic Position and Evolutionary Origin of Acipenserobdella volgensis

Our results reveal that *A. volgensis* is more closely related to *C. fadejewi*. At first glance, the results outlined herein support the morphology-based opinion of Nesemann & Neubert [15] that the genera *Acipenserobdella* and *Caspiobdella* are synonymous. However, *A. volgensis* differs from *Caspiobdella* species by having several specific morphological and anatomical traits such as a much larger size, smaller posterior sucker, and separate conducting strands and oviducts [12,16,17]. The DNA sequences of the marine *Caspiobdella caspica* (Selensky, 1915) and *C. tuberculata* Epshtein, 1966 are needed to better understand the diversification of this peculiar clade, which probably originated in the Caspian refugium [63,68].

Several authors believe that *C. fadejewi* may represent a freshwater form of *C. caspica* because of the high morphological similarity and overlapping ranges of both species [13,69]. If this hypothesis is correct, *C. caspica* should be considered a fully euryhaline lineage (marine to freshwater) similar to *Limnotrachelobdella* spp. and *Myzobdella lugubris* Leidy, 1851 [13,70]. Earlier, an in-depth study showed that several freshwater and brackish-water Nearctic taxa separated on the basis of hosts and environment belong to a single salt-tolerant species, *Myzobdella lugubris*, which uses crustaceans when reaching maturity for cocoon attachment and phoretic reasons and may shift from salt to fresh water [69]. Indeed, future studies should focus on sampling and sequencing of fish leeches from the

Caspian Sea to estimate the phylogenetic affinities of *C. caspica* and *C. tuberculata* and their relationships with *C. fadejewi* and *A. volgensis*. Furthermore, the phylogenetic position of *Caspiobdella hadzii* (Sket, 1985), a local freshwater species endemic to the Balkans [15,16], is yet to be assessed due to the lack of DNA sequence data.

The phylogenetic reconstructions, presented herein, support the morphology-based hypothesis [63] on the sister relationships between the *Acipenserobdella* + *Caspiobdella* and *Piscicola* clades. Conversely, Epshtein's [63] assumption that the Baikalian Piscicolidae and *Limnotrachelobdella* taxa share a common ancestor is not confirmed because *Baicalobdella* sisters to the *Piscicola* clade and is phylogenetically far from *Limnotrachelobdella*. The *Baicalobdella* and *Piscicola* lineages separated after the latest Eocene (mean age = 38.0 Ma), which roughly corresponds to the hypothetical age estimates for several invertebrate radiations endemic to the ancient Lake Baikal, such as amphipods (Crustacea: Gammaridae) and baicaliid molluscs (Gastropoda: Baicaliidae) [71].

Our time-calibrated phylogeny and DNA-based ancestral environment reconstruction indicate that the *Acipenserobdella* + *Caspiobdella* clade evolved within the largest freshwater radiation of the Piscicolidae. At first glance, these results support the hypothesis that these leeches, a portion of which inhabits saltwater environments in the Caspian Sea, were originated from a freshwater ancestor and do not relate to primarily marine groups [63,68], although the DNA sequences of the marine *Caspiobdella* taxa are not available. It was assumed that a large group of the Caspian endemic species, which, among others, contains some rotifers, crustaceans, and cyprinid fishes, was originated from freshwater environments [72]. Previously, freshwater origin was confirmed for the endemic Caspian sponge *Metschnikowia tuberculata* Grimm, 1877 (Spongillida: Metschnikowiidae) based on mitochondrial and nuclear gene sequences [73].

The origin of an isolated population of *A. volgensis* in Eastern Siberia is largely unclear. Tentatively, such a distant isolate may have been originated recently through a human-mediated introduction. However, our time-calibrated phylogeny suggests that there was a natural vicariance event in the latest Pliocene (mean age = 2.7 Ma). Earlier, a Late Pliocene divergence event (mean age = 3.25 Ma) was reconstructed for a pair of sister freshwater snail species, that is, the Siberian *Peregriana dolgini* (Gundrizer & Starobogatov, 1979) and the European *P. peregra* (O. F. Müller, 1774) (Gastropoda: Lymnaeidae) [74]. This event was linked to a hypothetical vicariant barrier for freshwater hydrobionts between Europe and Siberia in the Pliocene, although its physical nature is yet to be established [74].

4.2. A Review of the Host Associations of Acipenserobdella volgensis

A few narrow host specialists have been discovered among freshwater fish leeches. The iconic examples of such taxa are *Calliobdella mammillata* (Malm, 1863) and *Cystobranchus fasciatus* (Kollar, 1842), permanent ectoparasites of Burbot *Lota lota* (Linnaeus, 1758) and Wels catfish *Silurus glanis* Linnaeus, 1758, respectively [15,75]. Traditionally, *A. volgensis* was also thought to be an obligate parasite of fish species from the family Acipenseridae [15,17,24]. It was recorded from *Acipenser baerii*, *A. gueldenstaedtii*, *A. nudiventris* (the type host but feeding on this fish is not confirmed), *A. ruthenus*, *A. stellatus*, *A. sturio*, and *Huso huso* [7,15,17,20,24,61]. Although sturgeons seem to be the preferred and most suitable hosts of this leech, the available data indicates that it is characterized by a broader host range (see Figure 5 for detail). In Poland, this species was found on brown trout *Salmo trutta* (Salmonidae) [18]. In the Selenga River, it was collected from Baikal omul *Coregonus migratorius* (Salmonidae) [34] (Table S2). In the Volga's reservoirs, it frequently occurs on bream *Abramis brama* (Cyprinidae) [25]. There is a single record from *Esox lucius* (Esocidae) in the downstream portion of the Volga River [60]. Finally, under the framework of the present study we found that the crop content of a free-living specimen of *A. volgensis* from the Moscow River contained blood of *Leuciscus leuciscus* (Cyprinidae).

However, there is not much evidence that non-sturgeon hosts are all suitable for this species. A successful shift of *A. volgensis* to a non-typical but abundant cyprinid host such as *Abramis brama* discovered in the Volga reservoirs may be linked to the high infestation rate of this fish by *C. fadejewi* [27]. In particular, *A. volgensis* individuals tend to suck fish

blood from feeding sites of *C. fadejewi* and, hence, may benefit from anticoagulants of the last species' salivary secretions [27]. In general, *C. fadejewi* is known to use a wide array of fish hosts belonging to six families [18,26,76], but *Abramis brama* seems to be the preferred and most suitable host for this leech [25,27,77]. At first glance, the hypothesis on commensal relationships between the two leech species very well explains the frequent occurrences of *A. volgensis* on *Abramis brama* in the Volga reservoirs since the 1980s, which coincides with the establishment of an abundant population of *C. fadejewi* there [25,77]. However, it needs to be confirmed experimentally in the future.

4.3. Associations of Fish Leeches with Bivalve Molluscs

Earlier, a diverse assemblage of freshwater mussel-associated glossiphoniid leeches was discovered [28,78–80]. In contrast, only four members of the family Piscicolidae may occur in association with bivalve molluscs globally, including one marine and three freshwater species (Table 2).

Table 2. Global checklist of bivalve-associated fish leeches (Hirudinea: Piscicolidae) that are known to occur in the mantle cavity of mussels and clams in freshwater and marine environments.

Leech Species	Bivalve Host	Type of Association with Bivalves	Primary Fish Host	Environment	Region
Acipenserobdella volgensis (Zykoff, 1904)	*Anodonta anatina* (Linnaeus, 1758) (Unionidae)	Facultative shelter-like	Host generalist: Acipenseridae (preferred hosts), Cyprinidae, Salmonidae; Esocidae (one host record) [18,26]	Freshwater	Russia: Moscow River, Volga River basin
Caspiobdella fadejewi (Epshtein, 1961)	*Dreissena polymorpha* (Pallas, 1771) (Dreissenidae) [81]	Facultative shelter-like [81]	Host generalist: Acipenseridae, Cyprinidae (preferred hosts), Esocidae, Lotidae, Percidae, Salmonidae [18,26]	Freshwater [18,26,81]	Russia: Volga River basin [81]
Alexandrobdella makhrovi Bolotov et al., 2020	*Cristaria plicata* (Leach, 1814) (Unionidae) [4]	Probably facultative shelter-like [4]	*Silurus asotus* (Linnaeus, 1758) (Siluridae) [4]	Freshwater [4]	Russia: Lake Khanka, Prymorie Region, Far East [4]
Austrobdella coliumicus Williams, Urrutia & Burreson, 2007	*Ensis macha* (Molina, 1782) (Pharidae) [82]	Probably obligate shelter-like [82]	Unknown [82]	Marine [82]	Chile: Coliumo Bay, Region VIII [82]

Among freshwater species, *A. volgensis* and *C. fadejewi* are facultative bivalve-associated leeches that use mollusc hosts as shelter. The same hypothesis was proposed for the recently described *Alexandrobdella makhrovi* Bolotov et al., 2020 from East Asia, although this unusual leech is only known from three type specimens, all of which were collected from the mantle cavity of a freshwater mussel species, *Cristaria plicata* (Leach, 1814) (Unionidae) [4].

To the best of our knowledge, the Chilean species *Austrobdella coliumicus* Williams, Urrutia & Burreson, 2007 is the single bivalve-associated leech in marine waters known to date (Table 2). This leech was exclusively collected from the mantle cavity of the razor clam *Ensis macha* (Molina, 1782) (Pharidae) and may represent a host-specific obligate endosymbiont of this bivalve species because it was not found in other clams at the same locality [82]. Moreover, the presence of two bivalve-associated species belonging to the *Acipenserobdella* + *Caspiobdella* clade in freshwater environments suggests that marine species from this clade (i.e., *C. caspica* and *C. tuberculata*) may also occur in association with bivalves in the Caspian Sea. This interesting issue needs to be checked in the future.

5. Conclusions

Our results reveal that *Acipenserobdella* represents a sister lineage to *Caspiobdella* based on sequences of *COI* and *18S rRNA* gene fragments. The range of *A. volgensis* is disjunctive and consists of three distant, isolated freshwater systems in European Russia, Poland, and Eastern Siberia. The European and Siberian lineages of *A. volgensis* have been separated since the latest Pliocene (mean age = 2.7 Ma).

Although specimens of *A. volgensis* were collected from fish belonging to four families (Acipenseridae, Cyprinidae, Salmonidae, and Esocidae), only a few suitable primary hosts (six sturgeons, one cyprinid, and one salmonid fish) were confirmed by earlier research.

New findings, described above, indicate that this leech is a facultative mussel-associated species that uses bivalves (duck mussel *Anodonta anatina*; Unionidae) as shelter. Our global review of the body of available literature reveals that three other piscicolid leeches were recorded from the mantle cavity of bivalve molluscs: *Caspiobdella fadejewi* and *Alexandrobdella makhrovi* (freshwater species), and *Austrobdella coliumicus* (marine species).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14244010/s1, Table S1: List of *COI* and *18S rRNA* gene sequences of the Hirudinea used in this study; Table S2: Georeferenced occurrences and host records of *Acipenserobdella volgensis* (Zykoff, 1904) (Hirudinea: Piscicolidae); Table S3: Summary data on available raw records of *Acipenserobdella volgensis* (Zykoff, 1904) (Hirudinea: Piscicolidae) per host; Figure S1: Maximum likelihood phylogeny (three codons of *COI* + *18S rRNA*) of the Piscicolidae. The tip color circles indicate the environmental preference of each species. The black numbers near nodes are bootstrap support (BS) values of IQ-TREE v. 1.6.12. Two Ozobranchidae taxa were used as an outgroup (not shown). The scale bar indicates the branch lengths (substitutions per site).

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Data Availability Statement: The DNA sequences generated in this study are available from NCBI's GenBank (https://www.ncbi.nlm.nih.gov/genbank; accessed on 1 September 2022).

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