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At the boundary of two biogeographical realms: Palearctic and Oriental leeches (Hirudinea, Glossiphoniidae) of the Ferghana Valley, Uzbekistan

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Glossiphonia ferghanensis Solijonov, Izzatullaev & Kuchboev, 2022, *Alboglossiphonia hyalina* (Müller, 1774), *Alboglossiphonia weberi* (Blanchard, 1897), *Helobdella stagnalis* (Linnaeus, 1758).

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ABSTRACT
The species diversity of glossiphoniid leeches of the Ferghana Valley was studied. Both morphological features and molecular data were employed to identify the leeches and to trace their evolutionary history. The mitochondrial gene cytochrome *c* oxidase subunit I (COI) was used as a molecular marker. The Palearctic leeches *Alboglossiphonia hyalina* (Müller, 1774) and *Helobdella stagnalis* (Linnaeus, 1758) are recorded for the Ferghana Valley based on their morphology and molecular characters. The endemic *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022 differs from typical representatives of the related *G. concolor* (Apáthy, 1888) in the dorsal colouration and forms a well-separated clade confirming the species status of the Ferghana population. *Alboglossiphonia weberi* (Blanchard, 1897) is the first molecularly and morphologically substantiated record of an Oriental leech species in the Ferghana Valley, Uzbekistan and Central Asia.

KEY WORDS
Glossiphoniidae,
Ferghana,
Uzbekistan,
morphological characteristics,
distribution,
DNA barcoding,
leeches,
new record.

RÉSUMÉ
À la frontière de deux domaines biogéographiques : les sangsues paléarctiques et orientales (Hirudinea, Glossiphoniidae) de la vallée de Ferghana, Ouzbékistan.
La diversité des espèces de sangsues Glossiphoniidae Vaillant, 1890 de la vallée de Ferghana a été étudiée. Des caractéristiques morphologiques et des données moléculaires ont été utilisées pour identifier les sangsues et retracer leur histoire évolutive. La sous-unité I du gène mitochondrial de la cytochrome c oxydase (COI) a été utilisée comme marqueur moléculaire. Les sangsues paléarctiques *Alboglossiphonia hyalina* (Müller, 1774) et *Helobdella stagnalis* (Linnaeus, 1758) sont signalées pour la vallée de Ferghana sur la base de leur morphologie et de leurs caractères moléculaires. L'endémique *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022 diffère des représentants typiques de *G. concolor* (Apáthy, 1888) apparentée par la coloration dorsale et forme un clade bien séparé confirmant le statut d'espèce de la population de Ferghana. *Alboglossiphonia weberi* (Blanchard, 1897) est le premier signalement corroboré par des données moléculaires et morphologiques d'une espèce de sangsue orientale dans la vallée de Ferghana, en Ouzbékistan et en Asie centrale.

MOTS CLÉS
Glossiphoniidae,
Ferghana,
Ouzbékistan,
caractéristiques morphologiques,
distribution,
code-barres ADN,
sangsues,
signalement nouveau.

INTRODUCTION

Leeches are freshwater, terrestrial, and marine annelids that are hematophagous ectoparasites of vertebrates or predators feeding on various invertebrates (Sawyer 1986; Sket & Trontelj 2008). As many as 917 species of leeches of the subclass Hirudinea Lamarck, 1818 have been described, including 234 species of the family Glossiphoniidae Vaillant, 1890 (Magalhães *et al.* 2021). Glossiphoniids are mostly small, oval or leaf-shaped, dorsoventrally flattened leeches with 2-8 eyes and a proboscis. They usually feed on the blood of vertebrates or the hemolymph of invertebrates (Lukin 1976; Sawyer 1986) and occasionally swallow prey whole (Kwak *et al.* 2021). Glossiphoniids differ from other leeches by carrying their cocoons and young individuals on the ventral side of a maternal individual (Lukin 1976; Bielecki *et al.* 1999; Light & Siddall 1999; Kutschera & Wirtz 2001; Govedich *et al.* 2019).

Until now, many studies on glossiphoniid DNA barcoding and evolutionary history have been conducted. In particular, their phylogeny was studied and DNA barcoding was performed on the scale of the Palearctic (Kvist *et al.* 2022), individual countries, including Ukraine (Morhun *et al.* 2020) and Russia (Kaygorodova & Mandzyak 2014; Kaygorodova *et al.* 2020), or regions and continents such as the Western Balkans (Grosser *et al.* 2015, 2016; Jovanović *et al.* 2021), East and Southeast Asia (Bolotov *et al.* 2019), and North America (Siddall *et al.* 2005; Moser *et al.* 2012; de Carle 2017; Mack & Kvist 2019; Bolotov *et al.* 2019; Lynggaard *et al.* 2021).

When analysing the previous literature on the biodiversity of the region, it became clear that the first information about the glossiphoniid leeches of the study area was based on materials collected by traveling naturalists in the 20th century. The morphology and biology of *Helobdella stagnalis* (Linnaeus, 1758) were studied by Plotnikov (1907 as *Glossiphonia stagnalis*) in Central Asia for the first time. Later, *Glossosiphonia amudarjensis* (Moltschanov, 1913) was recorded from the Amu Darya delta, and its habitat, morphometric dimensions, and life cycle were studied by Moltschanov (1913). The duck leech *Theromyzon tessulatum* (O. F. Müller, 1774), *Helobdella stagnalis*, *Alboglossiphonia heteroclita* (Linnaeus, 1761), and *Glossiphonia complanata* (Linnaeus, 1758) were recorded from the water bodies of Uzbekistan and its surroundings under the names: *Protoclepsis tessulata* (O. F. Müller, 1774), *Glossosiphonia stagnalis* (Linnaeus, 1758), *Glossosiphonia heteroclita* (Linnaeus, 1761), and *Glossosiphonia complanata* (Linnaeus, 1758) respectively (Shchegolev 1912; Shchegolev & Shchegoleva 1951). Subsequently, *G. complanata*, *A. hyalina*, and *H. stagnalis* were found at two stations on the northeastern coast of the Issyk-Kul in Kyrgyzstan (Jueg *et al.* 2013), and several species of *Glossiphonia* Johnson, 1816 were found in the Irtysh River Basin in Kazakhstan (Fedorova & Kaygorodova 2016). Recently, a new glossiphoniid leech, *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022, was described from Uzbekistan (Solijonov & Izzatullayev 2022).

However, the available information is insufficient to draw a conclusion about the glossiphoniid leeches of the Ferghana Valley, a biogeographically important area of the southern Palearctic. Despite recent findings of glossiphoniid leeches in the study area (Solijonov *et al.* 2023), the leeches of Uzbekistan and Central Asia have been scarcely characterized using molecular methods. Only two species from that area have been studied in this manner: *H. stagnalis* (Bolotov *et al.* 2019) and *Hemiclepsis marginata* (Müller, 1774) (Solijonov *et al.* 2024). Therefore, our aim was to determine morphological characters, phylogenetic relationships, and the species composition of the leeches of the Ferghana Valley.

MATERIAL AND METHODS

Study area

The Ferghana Valley is located among the mountains in Central Asia and is one of the largest mountain ranges in Central Asia. It is surrounded by the Tien Shan in the north and the Hisar-Alai Mountain ranges in the south. The Ferghana Valley belongs mainly to Uzbekistan and partly to the Republics of Kyrgyzstan and Tajikistan. Its wide part is in the form of a triangle extending to the northern slopes of the Turkestan and Alay mountains, surrounded by the Kurama and Chatkal mountains from the northwest, and the Ferghana mountain from the northeast. Its length is 300 km, its width is 60-120 km, its widest point is 170 km, and its area is 22 000 km^2. Its height is 330 m above sea level in the west and 1000 m in the east. In the west, it is linked to the Mirzachul steppe by the narrow Khujand Gates. Its general structure is elliptical (almond-like). It expands from west to east. The Ferghana Valley is situated in the eastern part of the Republic of Uzbekistan, its geographical coordinates are 39°42'-42°N and 69°30'-74°50'E (Khalikov 2020).

Sampling

The materials were sampled from rivers, canals and springs in the Ferghana Valley in Uzbekistan in March-November 2020-2022 (Fig. 1). The total number of samples was more than 1500, 80% were adult leeches and 20% were young individuals. They were collected manually or with hydrobiological tools. During their fixation, leeches were first paralyzed in 10% ethanol and then stored in 96% ethanol (Jovanović *et al.* 2021). Collections are kept at the Institute of Zoology of the Republic of Uzbekistan, Andijan State University (ASUZM) and at V. N. Karazin Kharkiv National University.

Morphological analysis

External and internal morphological features of the samples (body size, shape, surface, colour; number and location of eyes; annulation; structure of digestive and reproductive systems) were examined. Leeches were identified using previous publications, including keys (Lukin 1976; Nesemann & Neubert 1999; Govedich *et al.* 2019). Classification follows Lukin (1976), Borda & Siddall (2004) and Tessler *et al.* (2018). Distinguishing morphological characters were used to compile a key to the leeches of the family Glossiphoniidae Vaillant, 1890, from the Ferghana Valley (see below).

Molecular analysis

DNA extraction, PCR amplification and sequencing

One specimen of each of *H. stagnalis*, *A. weberi* (Blanchard, 1897), *A. hyalina* and two specimens of *G. ferghanensis* were analysed. A small piece of tissue from a margin of the posterior part of the body was taken to extract DNA. Genomic DNA was isolated using a GeneElute™ Mammalian Genomic DNA Miniprep Kit. The mitochondrial cytochrome *c* oxidase subunit I (COI) fragment was chosen as a standard animal DNA barcode gene region (Hebert *et al.* 2003) and amplified using the following primers 5'–GGTCAACAAATCATAAAGATATTG–3' (forward) and 5'–TAAACTTCAGGGTGACCAAAAAAT–3' (reverse) by applying 5 cycles of 30 s at 94°C, 1 min 30 s at 45°C and 1 min at 72°C, 35 cycles of 30 s at 94°C, 45 s at 51°C and 1 min at 72°C, and 1 cycle of 5 min at 72°C after an initial 3 min denaturation step at 94°C. PCR products were cleaned using two enzymes, Exonuclease I and Shrimp alkaline phosphatase (SAP) (Fermentas, Thermo Fisher Scientific, USA). Exonuclease I (0.2 µl) and SAP (1 µl) were added to 10 µl of the PCR product. The mixture was then incubated for 45 min at 37°C followed by 15 min incubation at 80°C. The

Table 1. — Locations of glossiphoniid leech sampling in the Ferghana Valley, Uzbekistan.

Nr	Collecting biotope	Locality	Coordinates
River			
1	Kara Darya	Kurgantepa district, Andijan region	40°46'30.1"N, 73°03'12.6"E
2	Kara Darya	Izbaskan district, Andijan region	40°52'46.3"N, 72°19'26.8"E
3	Kara Darya	Balikchi district, Andijan region	40°54'54.8"N, 71°50'48.9"E
4	Ak-Buura	Khujaabad district, Andijan region	40°34'59.4"N, 72°45'13.6"E
5	Naryn	Uychi district, Namangan region	41°06'16.6"N, 72°02'39.8"E
6	Naryn	Uychi district, Namangan region	40°56'26.8"N, 71°49'19.3"E
7	Podshootasay	Yangikurgan district, Namangan region	41°17'33.8"N, 71°47'27.0"E
8	Podshootasay	Alihan district, Namangan region	41°14'33.4"N, 71°49'28.7"E
9	Sokh	Sokh district, Ferghana region	40°04'50.3"N, 71°05'21.3"E
10	Isfayramsay	Kuvasay district, Ferghana region	40°15'58.5"N, 72°01'12.3"E
Canal			
11	Shakhrikhansay	Khujaabad district, Andijan region	40°40'36.3"N, 72°29'29.3"E
12	Shakhrikhansay	Asaka district, Andijan region	40°39'38.6"N, 72°09'57.7"E
13	Northern Ferghana canal	Markhamat district, Andijan region	40°30'11.5"N, 72°23'07.1"E
14	Northern Ferghana canal	Ferghana district, Ferghana region	40°19'34.9"N, 71°49'14.5"E
Spring			
15	Uchbulak	Balikchi district, Andijan region	40°53'32.3"N, 71°51'16.8"E
16	Kaynarbulak	Balikchi district, Andijan region	40°53'09.5"N, 71°50'08.6"E
17	Tandirbulak	Andijan district, Andijan region	40°51'13.9"N, 72°22'19.4"E
18	Kushmabulak	Andi jan district, Andijan region	40°51'07.7"N, 72°22'01.3"E
19	Shirmanbulak	Bulakboshi district, Andijan region	40°35'11.5"N, 72°28'59.3"E
20	Kukbulak	Kurgantepa district, Andijan region	40°44'44.3"N, 72°55'31.7"E
21	Fazilmanota	Kurgantepa district, Andijan region	40°48'50.9"N, 72°59'42.2"E
22	Bibiseshanba	Kurgantepa district, Andijan region	40°44'51.8"N, 72°55'48.4"E
23	Kulibukon	Chartak district, Namangan region	41°12'05.9"N, 71°48'55.9"E
24	Uchtepa	Uychi district, Namangan region	40°55'49.3"N, 71°52'49.2"E
25	Balikkul	Chartak district, Namangan region	41°19'24.8"N, 71°50'16.4"E
26	Avval	Ferghana district, Ferghana region	40°18'07.6"N, 71°49'59.0"E
27	Aydin	Kokand district, Ferghana region	40°28'49.1"N, 70°54'55.6"E
28	Chechme	Sokh district, Ferghana region	39°56'18.3"N, 71°12'38.7"E
29	Alibulak	Sokh district, Ferghana region	39°56'24.9"N, 71°08'07.2"E

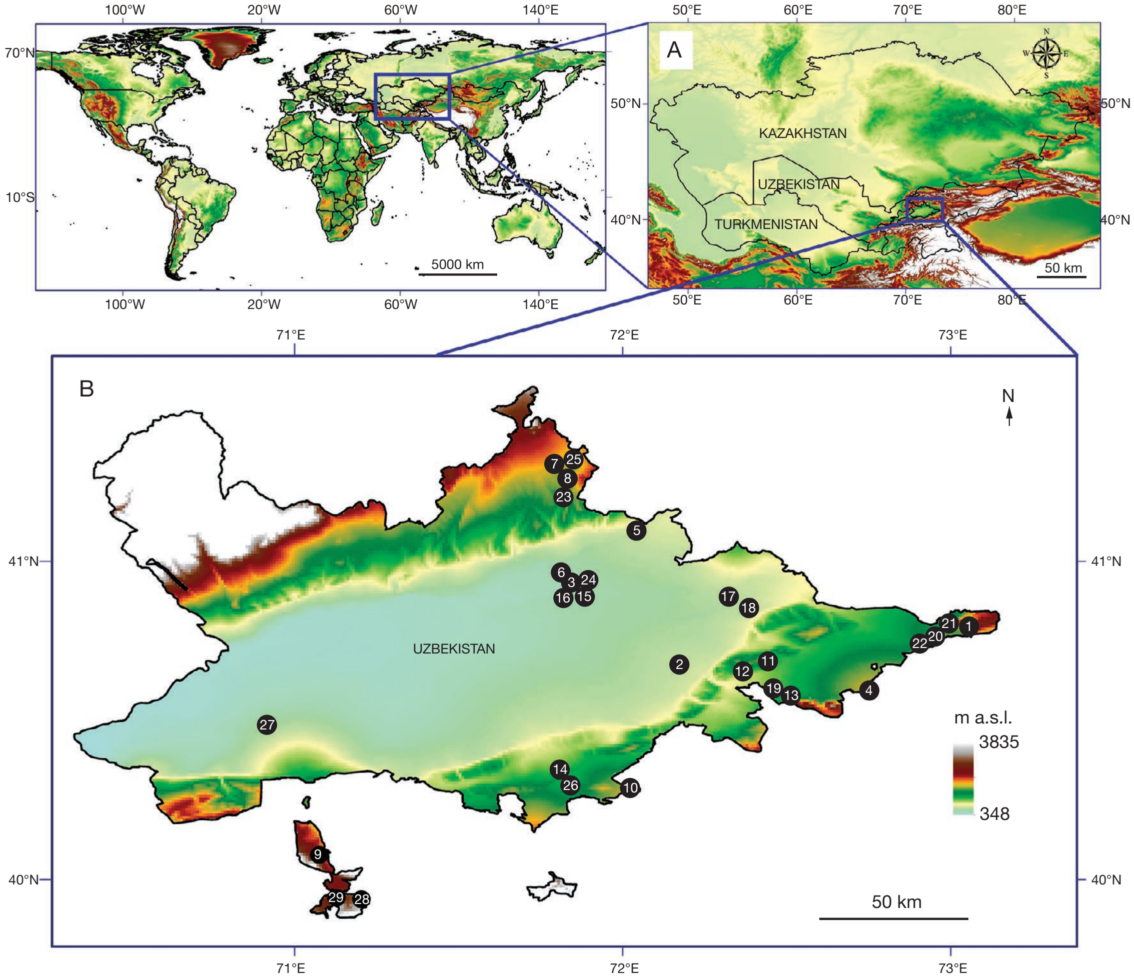


FIG. 1. — **A**, map of Uzbekistan situated in the middle of Central Asia with the Ferghana Valley highlighted in blue; **B**, sampling sites of glossiphoniid leeches in the Ferghana Valley.

cleaned PCR product was then sequenced in both directions by Macrogen Inc. (the Netherlands) using the same primers as at the amplification stage. The chromatograms of sequences were edited using ChromasPro 1.32 (Technelysium Pty., Queensland, Australia): the primer sequences were trimmed and problematic sites were checked. The length of the newly generated COI sequences was 622-661 bp.

The newly generated sequences of the samples marked as d1 (*A. weberi*), d4 and baliqkul1 (*G. ferghanensis*), d9 (*H. stagnalis*) and d10 (*A. hyalina*) were submitted to the GenBank database. Their accession numbers are OQ654110, OQ654111, ON759154, OQ654112, OQ654113, respectively.

Phylogenetic analysis

Two molecular phylogenetic analyses were performed. The COI fragments of leeches collected in the Ferghana Valley and identified as *A. weberi*, *A. hyalina*, and *G. ferghanensis* were added to GenBank sequences of the genera *Glossiphonia* and *Alboglossiphonia* plus *Hyperboreomyzon polaris* Bolotov, Eliseeva, Klass & Kondakov, 2022 chosen as an outgroup (Siddall & Burreson 1998; Light & Siddall 1999; Apakupakul *et al.* 1999; Borda & Siddall 2004; Siddall *et al.* 2005; Kaygorodova & Mandzyak 2014; Park & Oh 2014 [unpublished GenBank record]; Carew *et al.* 2018; Klass *et al.* 2018; Mack & Kvist 2019; Kaygorodova *et al.* 2020; Leerhoei 2020 [unpublished GenBank record]; Behrens-Chapuis *et al.* 2021; Bolotov *et al.* 2019, 2022a, 2022b; Moser *et al.* 2022; Ayhan *et al.* 2024; Kaygorodova *et al.* 2024 [unpublished GenBank record]). The dataset consisted of 149 sequences. The sequences were aligned using the online software MAFFT ver. 7 (Katoh *et al.* 2019) with the L-INS-i strategy selected by the "Auto". The lengths of the aligned COI sequences were 800 bp.

The COI sequence of *H. stagnalis* collected in the Ferghana Valley was aligned alongside all available GenBank sequences assigned to the genus *Helobdella* and the outgroup sequence of *Hemiclepsis marginata* (O. F. Müller, 1774) (Utevsky & Utevsky 2018). The total number of sequences analysed was 440 with 116 sequences of *H. stagnalis* (Apakupakul *et al.* 1999; Kaygorodova & Mandzyak 2014; Marrone *et al.* 2016; Saglam *et al.* 2018; Kutschera & Butzelar 2018 [unpublished GenBank record]; Bolotov *et al.* 2019; Iwama *et al.* 2019; Leerhoe 2020 [unpublished GenBank record]; Gauthier

Key to the leeches of the family Glossiphoniidae Vaillant, 1890 of the Ferghana Valley

1. Eyes four pairs............ *Theromyzon tessulatum* (Müller, 1974). Recorded from Uzbekistan but not from the Ferghana Valley.
— Eyes one, two or three pairs 2

2. Eyes one pair; dorsal dark scute in trachelosome region .... *Helobdella stagnalis* (Linnaeus, 1758)
— Eyes two or three pairs 3

3. Eyes two pairs; anterior sucker distinct from body .... *Hemiclepsis marginata* (O. F. Müller, 1974)
— Eyes three pairs; anterior sucker not distinct from body 4

4. Eyes of first pair located much closer to each other than eyes of two other pairs .... *Alboglossiphonia* Lukin, 1976) 5
— Eyes of all pairs are equally spaced .... *Glossiphonia* Johnson, 1816 6

5. Tiny tubercles and no black pigment on dorsum.................... *Alboglossiphonia hyalina* (Müller, 1774)
— Large tubercles and black pigment pattern on dorsum.................... *Alboglossiphonia weberi* (Blanchard, 1897)

6. Two conspicuous paramedian dorsal dark lines regularly interrupted by tubercles .... *Glossiphonia complanata* (Linnaeus, 1758). Recorded from Uzbekistan but not from the Ferghana Valley.
— Dorsal dark lines and tubercles absent ...... *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022

et al. 2020; Menabit 2020 [unpublished GenBank record]; Behrens-Chapuis *et al.* 2021; Darabi-Darestani *et al.* 2021; Bolotov *et al.* 2022a; Farzali & Saglam 2022 [unpublished GenBank record]; Ayhan *et al.* 2024). They were processed using the abovementioned methodology with the FFT-NS-2 strategy. The lengths of the aligned COI sequences were 695 bp.

The alignments obtained were examined at the amino acid level and checked for stop codons using MEGA11: version 11 (Tamura *et al.* 2021).

The online version of IQTREE v1.5.5 (Nguyen *et al.* 2015) was used to identify the optimal partitioning scheme and substitution models (Kalyaanamoorthy *et al.* 2017). Best-fit models were determined according to the Bayesian information criterion (BIC): TN+F+G for the first, F81+F+I for the second, and TVM+F+G for the third codon positions of the dataset of *Glossiphonia* and *Alboglossiphonia*; TNe+G for the first, TN+F+G for the second, and TIM2+F+G for the third codon positions of the dataset of *H. stagnalis.*

The phylogenies for all datasets were inferred using the maximum likelihood optimality criterion in IQTREE v1.5.5 with branch support estimated using 1000 replicates of both the ultrafast bootstrapping algorithm (Minh *et al.* 2013) and the SH-like approximate likelihood-ratio test (SH-aLRT; Guindon *et al.* 2010). After the phylogenetic analysis was conducted, the clade consisting of taxa that belong to the genus *Alboglossiphonia* was cut from the resulting tree. Thus, the final dataset consisted of 44 samples. Moreover, the subtree of *G. concolor* and *G. ferghanensis* was extracted from the resulting tree. This clade consisted of 39 samples. The subtree of *H. stagnalis* consisted of 116 samples

Within and between-group mean uncorrected distances and their standard errors and codon-based Z-tests for selection were calculated using MEGA 11. The hypothesis of neutrality was also tested using Tajima's D as implemented in DnaSP v6 (Rozas *et al.* 2017). The latter was also used to calculate the haplotype diversity and the nucleotide diversity of *A. hyalina*, *A. weberi*, *H. stagnalis*, and *G. concolor sensu lato.*

RESULTS

Phylum ANNELIDA Lamarck, 1809
Class CLITELLATA Michaelsen, 1919
Subclass HIRUDINEA Lamarck, 1818
Order HIRUDINIDA Siddall, Apakupakul, Burreson, Coates, Erséus, Gelder, Källersjö & Trapido-Rosenthal, 2001
Suborder GLOSSIPHONIIFORMES Tessler & de Carle, 2018
Family Glossiphoniidae Vaillant, 1890

Genus *Glossiphonia* Johnson, 1816

Glossiphonia Johnson, 1816: 25.

Type species. — *Hirudo complanata* Linnaeus, 1758, designated by R. Blanchard (1894).

Glossiphonia ferghanensis Solijonov, Izzatullaev & Kuchboev, 2022 (Figs 1; 2A)

Glossiphonia ferghanensis Solijonov, Izzatullaev & Kuchboev, 2022: 94.

Material examined. — **Holotype. Uzbekistan** • Ferghana Valley, Andijan region, Balikchi district, Kaynarbulak spring; 8.VI.2020; Kh. Solijonov leg; ASUZM 155.

Additional data. — See Appendix 1.

Diagnosis. — Small-sized leech. Body length of juvenile leeches 7-16 mm, width 2-3 mm; length of adults 17-26 mm, width 3-7 mm.

Body ovate. Dorsal side orange-amber, with very small dark-green spots, body surface smooth, with no papillae and tubercles. Ventral side yellow, with no spots. Three pairs of eyes arranged in 2 parallel rows, round-shaped; 2 posterior pairs larger than anterior one; anteriormost pair of eyes hardly discernible. 69-70 annuli of the same length. Mid-body segment 3-annulate. Gonopores separated by two annuli. Six pairs of crop caeca, posterior crop caeca not branched.

Remark

As for now, it is found that this species is endemic to the Ferghana Valley.

Genus *Alboglossiphonia* Lukin, 1976

Glossiphonia (*Alboglossiphonia*) Lukin, 1976: 255.

Type species. — *Hirudo heteroclita* Linnaeus, 1761, designated by Lukin (1976).

Alboglossiphonia hyalina (Müller, 1774) (Figs 1; 2B)

Hirudo hyalina Müller, 1774: 49 (original species description, type designated, considered holotype).

Clepsine hyalina – Moquin-Tandon 1826: 106 (drawing of the body in pl. 2, fig. 4, p. 105).

Glossobdella hyalina – de Blainville 1827: 565.

Glossosiphonia amudarjensis Moltschanov, 1913: 10. fig. 1, 2.

Glossiphonia heteroclita f. *hyalina* – Pawlowski 1936: 130, fig. 89.

Alboglossiphonia hyalina – Lukin 1976: 256.

Material examined. — **Uzbekistan** • 10 samples; Ferghana Valley, Andijan region; 22.III.2020; Kh. Solijonov leg.; Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University.

Previous records. — **Central Asia** • 1 sample; Akmola region, Kazakhstan; 3.VIII.1899; Ignatov leg. (Plotnikov 1907) • 20 samples; Amu Darya River, Turkestan (currently Uzbekistan); 11.VI.1911; Moltschanov leg. (Moltschanov 1913) • 1 sample; Issyk-Kul, Kyrgyzstan; 13.VII.2005; (Jueg *et al.* 2013).

Additional data. — See Appendix 1.

Diagnosis. — Body length of juvenile leeches 2-4 mm, width 1-2 mm, adults 5-12 mm in length, and 4-8 mm in width. When the body is at rest, the leech has a leaf-like shape. Body margins smooth. Many tiny papillae scattered on dorsal surface. Three pairs of eyes: first pair smaller and closer together; posterior two pairs larger, with a greater distance between them. Dorsal and ventral sides yellowish-white. No dark pigmentation. Digestive organs can be seen through body coverings. Six pairs of crop caeca: 1st pair smaller; 2nd-5th pairs longer, with the 5th pair branching into four pouches.

Remark

This species was recorded for the first time in the Ferghana Valley of Uzbekistan based on both morphological characters and molecular data. Collected samples are small.

Alboglossiphonia weberi (Blanchard, 1897) (Figs 1; 2C)

Glossiphonia weberi Blanchard, 1897: 332 (original species description, type designated, considered holotype, fig. 1). — Moore 1924: 351. — Harding & Moore 1927: 62.

Clepsine weberi – Augener 1931: 744.

Glossiphonia weberi weberi – Soós 1969: 397.

Alboglossiphonia weberi – Lukin 1976: 261.

Material examined. — **Uzbekistan** • 6 samples; Ferghana Valley, Andijan region; 22.III.2020; Kh. Solijonov leg.; Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University.

Additional data. — See Appendix 1.

Diagnosis. — Small-sized leech. Body length of young leeches 2-4 mm, width 1-2 mm, adults 5-12 mm in length and their width 3-8 mm. Body elongated oval-shaped at rest. Body margins denticulate. Dorsal body surface with well-developed tubercles. Three pairs of eyes: 1st pair smaller and closer together; 2nd and 3rd pairs larger, with a greater distance between them. Body colour mostly white, almost transparent. Therefore, after feeding, the digestive organs can be seen with the naked eye through the body coverings. Median row of black dots on dorsum present. Live and freshly fixed leeches with segmentally arranged transverse lateral black bands, numerous paramedian black lines, and tiny dots on dorsum. Six pairs of crop caeca: 1st pair smallest; 2nd–5th pairs longer; posterior crop caeca branched into five pouches (Solijonov *et al.* 2023).

Remark

This species, a leech native to the Oriental region, was first recorded in the Ferghana Valley of Uzbekistan and Central Asia based on both morphological characters and molecular data.

Genus *Helobdella* Blanchard, 1896

Helobdella R. Blanchard, 1896: 263.

Type species. — *Hirudo stagnalis* Linnaeus, 1758, designated by R. Blanchard (1896).

Helobdella stagnalis (Linnaeus, 1758) (Figs 1; 2D)

Hirudo stagnalis Linnaeus, 1758: 649 (original species description; type locality and type deposition: not known).

Glossiphonia perata Johnson, 1816: 26.

Erpobdella bioculata Lamarck, 1818: 296.

Clepsine bioculata – Savigny 1822: 102, fig. 2.

Glossobdella pulligera Blainville, 1827: 266, fig. 6.

Clepsine stagnalis – Fillipi 1837: 27.

Glossiphonia bioculata – Moquin-Tandon 1846: 366, fig. 16-26.

Clepsine modesta Verrill, 1872: 126.

Glossiphonia stagnalis – Blanchard 1894: 25.

Fig. 2. — **A**, *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022 (dorsal); **B**, *Alboglossiphonia hyalina* (Müller, 1774) (dorsal); **C**, *Alboglossiphonia weberi* (Blanchard, 1897) (dorsal); **D**, *Helobdella stagnalis* (Linnaeus, 1758) (dorsal). Scale bar: 5 mm. Photograph: Kh. Solijonov, A. Utevsky, and S. Utevsky.

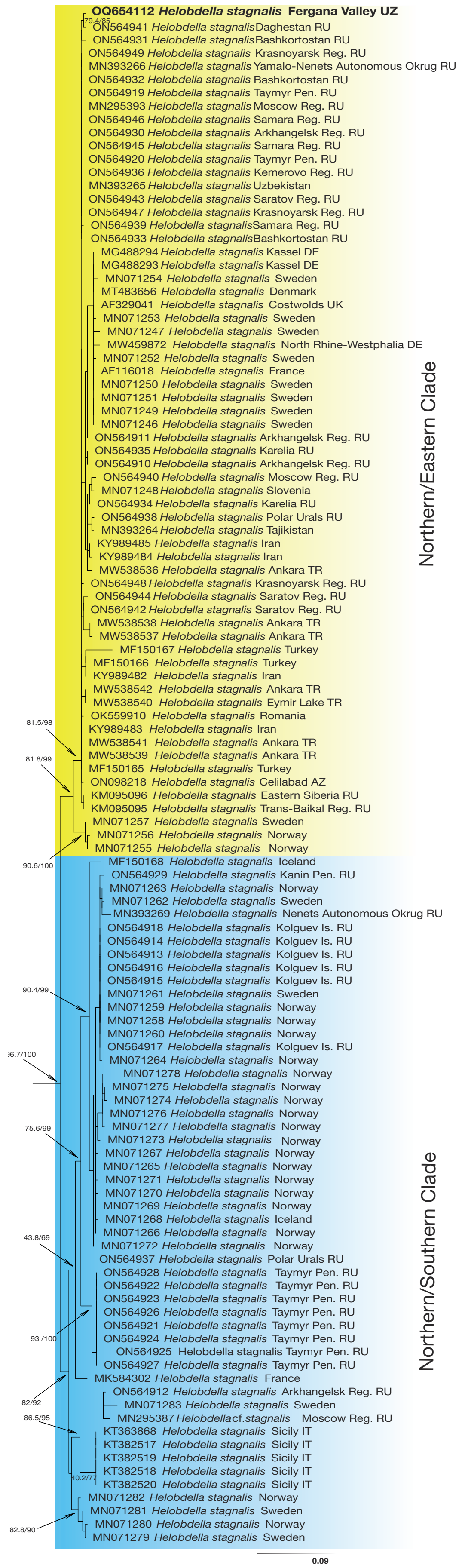
Glossiphonia (*Helobdella*) *stagnalis* – Harding & Moore 1927: 67, fig. 27.

Material examined. — **Uzbekistan** • 10 samples; Ferghana Valley, Andijan region; 22.III.2020; Kh. Solijonov leg.; Department of Zoology and Animal Ecology, V. N. Karazin Kharkiv National University

Previous records. — **Central Asia** • 1 sample; Samarkand region, Uzbekistan; 2.XI.1898; Retter leg. • 2 samples; Kurgaldzhin Lake in the Akmola region, Kazakhstan; 14.VI.1899; Ignatov leg. (Plotnikov 1907) • 59 samples; Ashkhabad, Turkmenistan; 9.II.1911; (Shchegolev & Shchegoleva 1951) • 7 samples; Issyk-Kul, Kyrgyzstan; 13.VII.2005; (Jueg *et al.* 2013).

Additional data. — See Appendix 1.

Diagnosis. — Body length at full elongation 8-20 mm, body width 6-12 mm. Body oblong-oval at rest, middle of body wider than anterior end, body slightly tapered posteriorly. During movement, the body can be stretched up to three times more than in a calm state. Body margins denticulate, better expressed in fixed specimens. Dorsal



◀ Fig. 3. — Phylogenetic subtree of *Helobdella stagnalis* (Linnaeus, 1758) showing the relationships of the sample collected in the Ferghana Valley (highlighted in bold). The tree was inferred from COI sequences using the maximum likelihood optimality criterion. Support values from both the SH-like approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrapping are provided for the major clades.

body surface smooth. Body appearing yellowish-grey when stretched and green when shortened due to the presence of dark green spots (dots) on the back surface of the body. They are smaller and more numerous on the posterior portion of the body and larger and less numerous on the anterior portion. Brown chitinous scutum located on trachelosome. One pair of clearly separated eyes.

Remark

This species was recorded for the first time in the Ferghana Valley of Uzbekistan. Collected samples are small.

Molecular genetic analysis

Helobdella stagnalis *(Linnaeus, 1758)*

Helobdella stagnalis formed a distinct clade on the phylogenetic tree of the genus *Helobdella* (log-likelihood: –13015.5363 [s.e. 600.4929]). The Ferghana scute-bearing leech clustered with *H. stagnalis*, occupying a sister position to the Daghestan leech (Fig. 3; Supplementary material 1. Another sequence from Uzbekistan did not group within this clade.

The *Helobdella stagnalis* clade consists of two major monophyletic groups that can be designated as the Northern/Eastern Palearctic and Northern/Southern Palearctic clades to characterise their geographical distribution. The Ferghana leech was assigned to the former monophyletic group. The uncorrected genetic distance between the COI sequence of *H. stagnalis* from the Ferghana Valley and the other sequences of the clade of *H. stagnalis* is 0.0181 ± 0.0031.

Neutrality tests were done to analyse if selection has played a role in the molecular evolution of the COI gene in *H. stagnalis.* The codon-based Z-test revealed purifying selection in the *COI* locus. Tajima's *D* for the entire clade of *Helobdella stagnalis* does not reject the hypothesis of neutrality (Table 2).

Glossiphonia ferghanensis *Solijonov, Izzatullaev & Kuchboev, 2022*

The genus *Glossiphonia* formed a separate clade sister to *Alboglossiphonia* on the phylogenetic tree (log-likelihood of the tree: -5276.9902 [s.e. 268.2413]). Two closely related sequences assigned to *G. ferghanensis* formed a separate clade within the superclade of *G. concolor* (Fig. 4; Supplementary material 2). The uncorrected genetic distance between the two newly generated sequences of *G. ferghanensis* and the rest of the sequences of the clade of *G. concolor* is 0.0344 ± 0.0056 and the distance between the two conspecific sequences is 0.0015 ± 0.0016. Although the codon-based Z-test suggested purifying selection in the COI locus, Tajima's *D* for the entire clade of *G. concolor* does not reject the hypothesis of neutrality (Table 2).

Alboglossiphonia hyalina *(Müller, 1774)*

The COI sequence of *A. hyalina* from the Ferghana Valley was found to be sister to the sequence of the leech from the Tyumen Region in Russia (GenBank: ON631769) identified

TABLE 2. — Polymorphism and selection in populations of *Helobdella stagnalis* (Linnaeus, 1758), *Glossiphonia concolor* (Apathy, 1888) *sensu lato*, *Alboglossiphonia hyalina* (Müller, 1774), and *A. weberi* (Blanchard, 1897): **Hd**, haplotype diversity; **π**, nucleotide diversity; **dN** and **dS**, the numbers of synonymous and nonsynonymous substitutions per site, respectively; Tajima's D, a test statistic of neutrality. *P*-values less than 0.05 do not support the null hypothesis of neutrality.

Species	Number of sequences	Hd	π	dN – dS, P-value	Tajima's D, P-value
Helobdella stagnalis	116	0.933 ± 0.013	0.0184 ± 0.0008	-5.53, < 0.05	-0.818, > 0.10
Glossiphonia concolor s.l.	39	0.798 ± 0.062	0.0204 ± 0.0027	-5.98, < 0.05	-0.311, > 0.10
Alboglossiphonia hyalina	2	1.000 ± 0.500	0.0016 ± 0.0008	-0.98, > 0.05	0.193, > 0.10
Alboglossiphonia weberi	9	0.583 ± 0.183	0.0073 ± 0.0035	-3.93, < 0.05	-1.182, > 0.10

as *A. heteroclita*. This clade is sister, in turn, to the clade of North American leeches (Fig. 5; Supplementary material 2). The uncorrected genetic distance between the COI sequence of *A. hyalina* and its sister sample is 0.0016 ± 0.0016.

Alboglossiphonia weberi *(Blanchard, 1897)*

The COI sequence of *A. weberi* was found to be within the Myanmarese and Indian clade of *A. pahariensis* (Fig. 5; Supplementary material 2). The uncorrected genetic distance between the COI sequence of *A. weberi* from the Ferghana Valley and other members of the clade is 0.0207 ± 0.0056. Although the codon-based Z-test suggested purifying selection in the COI locus, Tajima's *D* for the entire clade of *A. weberi* does not reject the hypothesis of neutrality (Table 2).

DISCUSSION

HELOBDELLA STAGNALIS

Our phylogenetic analysis suggests that scute-bearing leeches of the Ferghana Valley should be assigned to *H. stagnalis*. The species corresponds to a well-supported monophyletic group consisting of a number of subclades. One of the major subclades is restricted to Northern Eurasia, while another one is distributed much wider, from Scandinavia to Anatolia, Central Asia and Iran. The Ferghana sample joined this widespread Palearctic clade. The COI fragment has been under strong purifying or negative selection at the amino acid level, which can be attributed to the high degree of functional constraint acting on the gene. On the other hand, the hypothesis of neutrality was not rejected by the analysis based on Tajima's *D*. This may suggest that the species has not experienced population expansion after a recent bottleneck. Both the complicated phylogenetic structure and the Tajima's *D* neutrality test imply relatively long persistence of a number of populations of *H. stagnalis* within their current ranges.

GLOSSIPHONIA FERGHANENSIS

The clade of *G. concolor sensu lato* was found to have a deep and complicated structure suggesting cryptic diversity within the formally recognised species. The Ferghana samples formed a well-separated clade in the species tree, which implies the species taxonomic status of that population. The genetic differences are paralleled by the morphological features of the Ferghana *Glossiphonia*, including the complete lack of the typical colouration pattern of *G. concolor* (see Lukin 1976; Nesemann & Neubert 1999). The phylogenetic position of

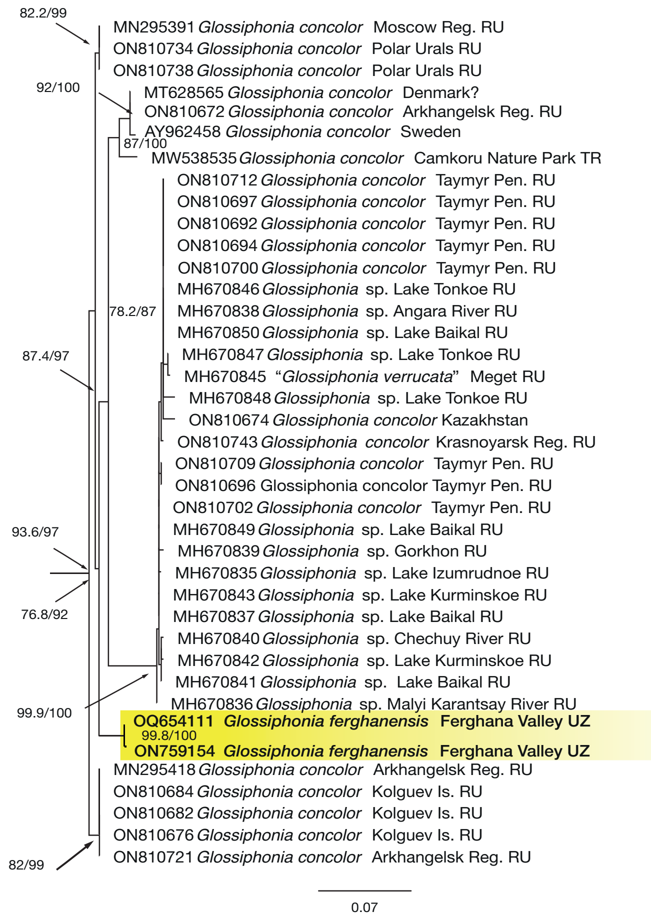


FIG. 4. — Phylogenetic subtree of *Glossiphonia concolor* (Apathy, 1888) *sensu lato* illustrating the relationships of two *Glossiphonia ferghanensis* Solijonov, Izzatullaev & Kuchboev, 2022 samples collected in the Ferghana Valley. The tip labels of the Ferghana leeches are highlighted in **bold**, and their clade is marked in yellow. The tree was inferred from *COI* sequences using the maximum likelihood optimality criterion. Support values from the SH-like approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrapping are shown for the major clades.

the Ukrainian samples of *G. concolor* (Kaygorodova & Mandzyak 2014), which entered the other clade of Baikal endemic glossiphoniids (Supplementary material 2), needs serious substantiation based on fresh material. In *G. concolor sensu lato*, the COI fragment has undergone purifying or negative selection at the amino acid level. On the other hand, the hypothesis of neutrality was not rejected by the analysis based on Tajima's *D*. Therefore, the same demographic scenario may be hypothesised for *G. concolor sensu lato* analogously to the previous species.

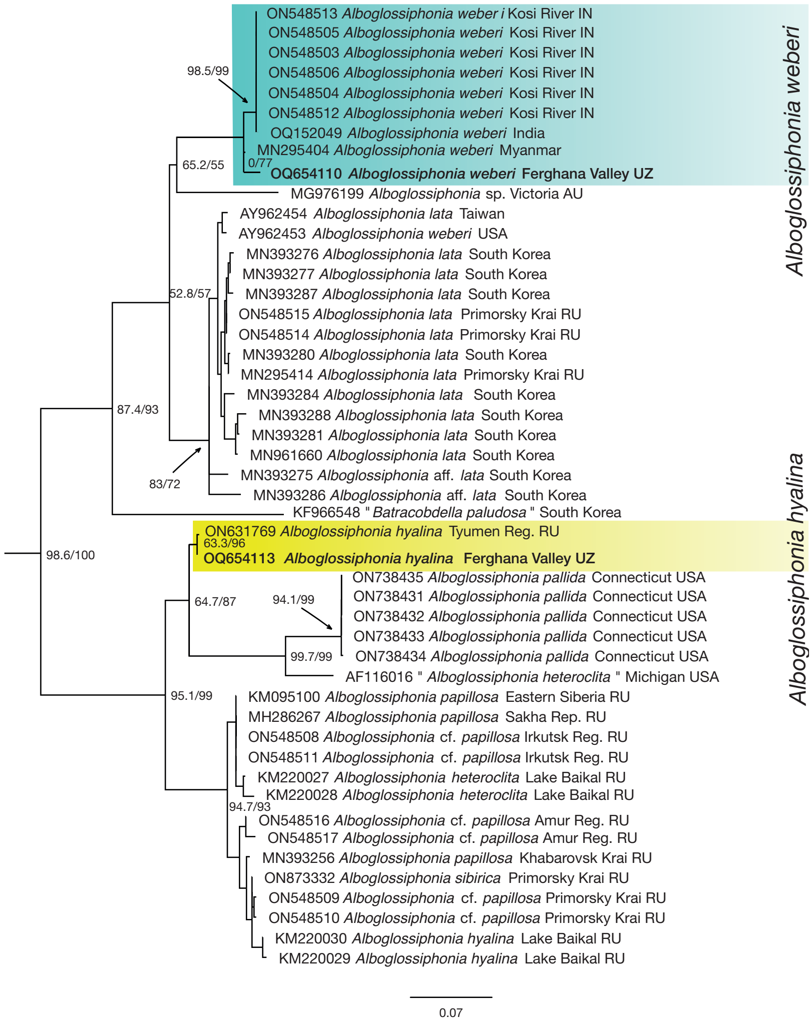


Fig. 5. — Phylogenetic subtree of the genus *Alboglossiphonia* showing the relationships of two samples collected in the Ferghana Valley (their tip labels are highlighted in **bold**). The clades of *A. weberi* (Blanchard, 1897) and *A. hyalina* (Müller, 1774) are marked in **light blue** and **yellow**, respectively. The tree was inferred from COI sequences using the maximum likelihood optimality criterion. Support values from the SH-like approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrapping are provided for the major clades.

Alboglossiphonia hyalina

This research presents the first molecular characterisation of *A. hyalina* recognised as a separate species on the basis of its distinguishing morphological features, including tiny tubercles on the dorsum, yellow colouration and the complete lack of dark pigment. The sister sequence from Western Siberia, which was identified by its authors (Bolotov *et al.* 2022b) as *A. heteroclita*, is conspecific to the Ferghana sample. More surveys are needed to clarify the identity of bearers of the morphological characters throughout the range of *A. hyalina* in the Western Palearctic. The low genetic distance between the Ferghana and Western Siberian samples suggests a relatively recent range expansion. However, given the low number of nucleotide sequences studied, it is too early to hypothesise about the demographic history of this species.

Alboglossiphonia weberi

The Ferghana leech, whose morphological characters are in agreement with the descriptions of the Indian *A. weberi* by Kaburaki (1921) and Moore (1924), joined the samples identified as *A. pahariensis* Nesemann & Sharma, 2007 (see Bolotov *et al.* 2022b) to form a well-supported clade of Indian, Myanmarese and Ferghana leeches. Recently the species identity of Indian leeches of the genus *Alboglossiphonia* has been reconsidered given the fact that Blanchard (1897) did not record the dorsal median pigment stripe in the Sumatran *A. weberi*. Therefore, Indian leeches bearing that stripe were identified as *A. pahariensis* (Bolotov *et al.* 2022b). However, Kaburaki (1921) and Moore (1924) stressed the presence of well-developed tubercles on the dorsum of both Indian and Sumatran leeches, which is, in their opinion, a distinguishing feature of *A. weberi*. Taking into account that stability is a major property of taxonomic names and the fact that both the continental and Sumatran leeches possess well-developed dorsal tubercles, we retain the traditional naming of the Southern Asian leeches and assign them and the Ferghana leech to *Alboglossiphonia weberi*. Obviously, more morphological and molecular comparative studies of the Central Asian, Indian, Indo-Chinese and Sumatran leeches of the genus

Alboglossiphonia should be done to resolve this issue. The shallow phylogenetic structure of the clade of *A. weberi* and its low nucleotide diversity may be explained by a relatively recent range expansion amid negative selection at the amino acid level driving the evolution of the COI gene.

Biogeographically, the Ferghana Valley may be characterised as a southern frontier of common Palearctic species or species groups with some local populations that have evolved to separate subspecies or species such as *G. ferghanensis.* On the other hand, a common Oriental species, *A. weberi*, occurs in this region, which is the most northerly border of the species range known so far.

The Ferghana Valley is inhabited predominantly by Palearctic species of leeches. Their identity was revealed using both morphological characters and molecular data. A population of *Glossiphonia ferghanensis* has evolved into a taxon of the species level with its own morphological features. An Oriental species, *Alboglossiphonia weberi*, penetrates the Ferghana Valley and probably adjacent areas of Central Asia.

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APPENDIX AND SUPPLEMENTARY MATERIALS

Appendix 1. — Information about leeches recorded for the first time in the Fergana Valley of Uzbekistan as a result of research. It shows the name of the identified species, the locality where it was recorded, the samples and the date.

Species	Locality	Samples	Date
G. ferghanensis	Uzbekistan, Ferghana Valley, Andijan region	26	8.VI.2020
A. hyalina	Uzbekistan, Ferghana Valley, Andijan region	10	22.III.2020
		26	16.IV.2020
		40	12.VII.2020
		30	2.V.2021
		20	26.VI.2022
		42	20.VI.2020
		20	4.VIII.2021
		10	5.VIII.2020
		30	8.IV.2020
		34	08.VI.2020
		23	9.V.2022
		22	9.V.2022
		29	19.VII.2021
		23	14.IV.2022
		33	10.VI.2021
		26	12.IV.2022
		12	13.V.2021
		36	13.VIII.2020
		42	2.IX.2021
		32	1.VII.2020
		20	16.VII.2021
		22	22.IX.2021
		37	22.IX.2021
A. weberi	Uzbekistan, Ferghana Valley, Andijan region	6	22.III.2020
		12	12.VII.2020
		20	20.VI.2020
		10	4.VIII.2021
		15	19.VII.2021
		28	10.VI.2021
H. stagnalis	Uzbekistan, Ferghana Valley, Andijan region	10	22.III.2020
H. stagnalis		20	16.IV.2020
		16	27.IX.2021
		80	12.VII.2020
		34	11.IV.2020
		34	4.VIII.2021
		14	2.V.2021
		18	26.VI.2022
		6	14.IX.2020
		12	23.V.2021
		40	20.VI.2020
		36	4.VIII.2021
		20	5.VIII.2020
		12	4.IV.2021
		40	8.IV.2020
		40	8.VI.2020
		26	9.V.2022
		22	9.V.2022
		22	19.VII.2021
		20	12.IV.2022
		42	10.VI.2020
		26	12.IV.2022
		7	13V.2021
		42	13.VII.2020
		76	2.IX.2021
		60	1.VII.2020
		26	16.VII.2021
		20	22.IX.2021
		32	22.IX.2021

Supplementary material 1. — Phylogenetic relationships of the *Helobdella stagnalis* (Linnaeus, 1758) sample collected in the Ferghana Valley, inferred using the maximum likelihood optimality criterion. The tree is constructed from all available COI sequences of *Helobdella* Blanchard, 1896 species, with *Hemiclepsis marginata* (Müller, 1774) selected as the outgroup. Support values from the SH-like approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrapping are indicated on the branches using a colour gradient. Log-likelihood of the tree: -13015.5363 (s.e. 600.4929). https://sciencepress.mnhn.fr/sites/default/files/documents/en/zoosystema2026v48a2s1.svg; https://doi.org/10.5852/zoosystema2026v48a2_s1

Supplementary material 2. — Phylogenetic relationships of the *Glossiphonia* Johnson, 1816 and *Alboglossiphonia* Lukin, 1976 samples collected in the Ferghana Valley, inferred using the maximum likelihood optimality criterion. The tree is constructed from COI sequences, with *Hyperboreomyzon polaris* Bolotov, Eliseeva, Klass & Kondakov, 2022 selected as the outgroup. Support values from the SH-like approximate likelihood-ratio test (SH-aLRT) and ultrafast bootstrapping are indicated on the branches using a colour gradient. Log-likelihood of the tree: -5276.9902 (s.e. 268.2413). https://sciencepress.mnhn.fr/sites/default/files/documents/en/zoosystema2026v48a2s2.svg; https://doi.org/10.5852/zoosystema2026v48a2_s2