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Barbronia borealis sp. nov., the first salifid leech discovered in Russia, with a global checklist of this genus

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Abstract
Freshwater leeches belonging to the family Salifidae (Hirudinea: Erpobdelliformes) are mostly distributed through tropical and subtropical areas of the Old World but a few species occur in warm temperate regions of East Asia. Here, we report on the first record of a salifid leech in Russia. A previously unknown species was discovered from the Razdolnaya (Suifun) River basin in the southern part of the Russian Far East and is described here as *Barbronia borealis* Bolotov, Eliseeva & Kondakov **sp. nov.** based on morphological and molecular evidence. An updated checklist of the genus *Barbronia* Johansson, 1918 with verified information on the type localities, general range, and the presence/absence of the *COI* barcode data on type specimens or topotypes for each valid species-group taxon is compiled. This genus currently contains eight species. We present a taxonomic reappraisal of the *COI* sequences of two widespread species – *Barbronia weberi* (Blanchard, 1897) and *B. gwalagwalensis* Westergren & Siddall, 2004 – in the Barcoding of Life Database (BOLD IDS) to avoid confusion in identification of these species in the future. Finally, a growing body of *B. gwalagwalensis* occurrences in East and Southeast Asia (Myanmar, South Korea, and China) indicates that this species is native to Asia and that its locus typicus in South Africa is situated within the non-native part of the range.

Key words: Hirudinea, Erpobdelliformes, Salifidae, Russian Far East, freshwater leeches, alien species, DNA-based identification, BOLD IDS, *Barbronia weberi*, *Barbronia gwalagwalensis*.

Introduction

The Salifidae Johansson, 1910 (Hirudinea: Erpobdelliformes) is a family of freshwater predacious leeches (Borda and Siddall 2004; Sket and Trontelj 2008; Oceguera-Figueroa *et al.* 2011). The range of this group is mostly confined to tropical and subtropical areas of Eurasia, Africa, Madagascar, Australasia, and Australia, although a few species were recorded in warm temperate regions of East Asia (Sawyer 1986; Westergren and Siddall 2004; Nesemann and Sharma 2012; Nakano and Nguyen 2015). The northernmost occurrences of salifids, attributed to the widespread species *Barbronia weberi* (Blanchard, 1897), were reported from Northeastern China (Moore 1930; Yang 1996). There is a record of *B. weberi formosana* (Oka, 1929) from Japan but the origin and taxonomic status of this population are questionable (Nakano 2017). In contrast, none of this family's representatives was recorded from Russia (Lukin 1976).

The genus *Barbronia* Johansson, 1918 currently attracts the increased attention of scientists because it contains some generalist species, rapidly expanding their ranges throughout the world due to human-mediated introductions (Pamplin *et al.* 2006; Pavluk *et al.* 2011; Iwama and Arruda 2016). Multiple invasions of the Asian species *B. weberi* to Europe, Africa, North America, South America, Australia, and some oceanic archipelagoes are well documented in the large body of literature (Gerlach 1997; Nesemann and Neubert 1999; Govedich *et al.* 2003; Genoni and Fazzone 2008; Oceguera-Figueroa *et al.* 2011; Sawyer & Sawyer 2018; Ludányi *et al.* 2019). Some biological features, including its tolerance to a broad range of habitats (Nesemann *et al.* 2007) and the possibility to reproduce without cross-fertilisation (Sawyer 2020), could explain invasive success of this species. Furthermore, another species of the genus, *B. gwalagwalensis* Westergren & Siddall, 2004, which is known to occur in Asia and South Africa, started to invade Europe (Klass *et al.* 2021).

This study (1) reports on the first discovery of a salifid species in Russia; (2) describes it as a new *Barbronia* species; (3) presents a global checklist of the genus *Barbronia*; and (4) provides a taxonomic framework for reliable identification of some *Barbronia* species within their non-native ranges using the *COI* sequence data.

Materials and methods

A *Barbronia* leech specimen was collected by hydrobiological net as a part of general benthos sampling. The sample was fixed in 96% ethanol and is 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).

New sequences of the mitochondrial *cytochrome c oxidase subunit I* (*COI*) and the nuclear *18S ribosomal RNA* (*18S rRNA*) gene sequences were generated using a small tissue snip of the specimen applying the standard primers and laboratory protocols as described previously (Bolotov *et al.* 2019). Forward and reverse sequence reactions were performed on purified PCR products using the ABI PRISM® BigDye™ Terminator v. 3.1 reagents kit and run on an ABI PRISM® 3730 DNA analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The new sequences were verified visually with BioEdit v. 7.2.5 (Hall 1999). Additionally, the nearest neighbors of the new *COI* and *18S rRNA* sequences were identified through NCBI's BLASTn (Chen *et al.* 2015) and the Barcode of Life Data System (BOLD IDS) (Ratnasingham and Hebert 2007) search tools.

To reconstruct the two-locus phylogeny of the family Salifidae, we sampled available partial sequences of the *COI* and *18S rRNA* genes from GenBank (Appendix 1). Sequences of each gene were separately aligned using the MUSCLE algorithm of MEGA 11 (Tamura *et al.* 2021). The two gene alignments were joined to a combined alignment with FaBox v. 1.61 (https://birc.au.dk/~palle/php/fabox) (Villesen 2007). The maximum likelihood phylogeny (four partitions: 3 codons of *COI* and *18S rRNA*; total length of 2482 bp) was calculated through a web-server for IQ-TREE v. 1.6.12 (http://iqtree.cibiv.univie.ac.at) (Minh *et al.* 2020). The evolutionary models were selected automatically for each partition based on Bayesian information criterion scores (Kalyaanamoorthy *et al.* 2017) as follows: TNe+I (1st codon of *COI*); TPM3u+F+I (2nd codon of *COI*); HKY+F+G4 (3rd codon of *COI*); and TIM2e+G4 (*18S rRNA*). The node support values were estimated with an ultra-fast bootstrap (1000 replications) (Hoang *et al.* 2017). Uncorrected *COI* p-distances between haplotypes were calculated with MEGA 11 (Tamura *et al.* 2021).

Morphological investigations and measurements of the specimen were performed using a standard approach as described in our previous works (Bolotov *et al.* 2019; Klass *et al.* 2021). Images of the complete holotype and its morphological traits were made and examined using a stereomicroscope Leica M165C (Leica Microsystems GmbH, Wetzlar, Germany). The map of occurrences (Figure 1) was created using ESRI ArcGIS 10 software (www.esri.com/arcgis). Published occurrences were georeferenced using the Google Earth tool (https://www.google.com/intl/ru/earth) and are presented in Appendix 2.

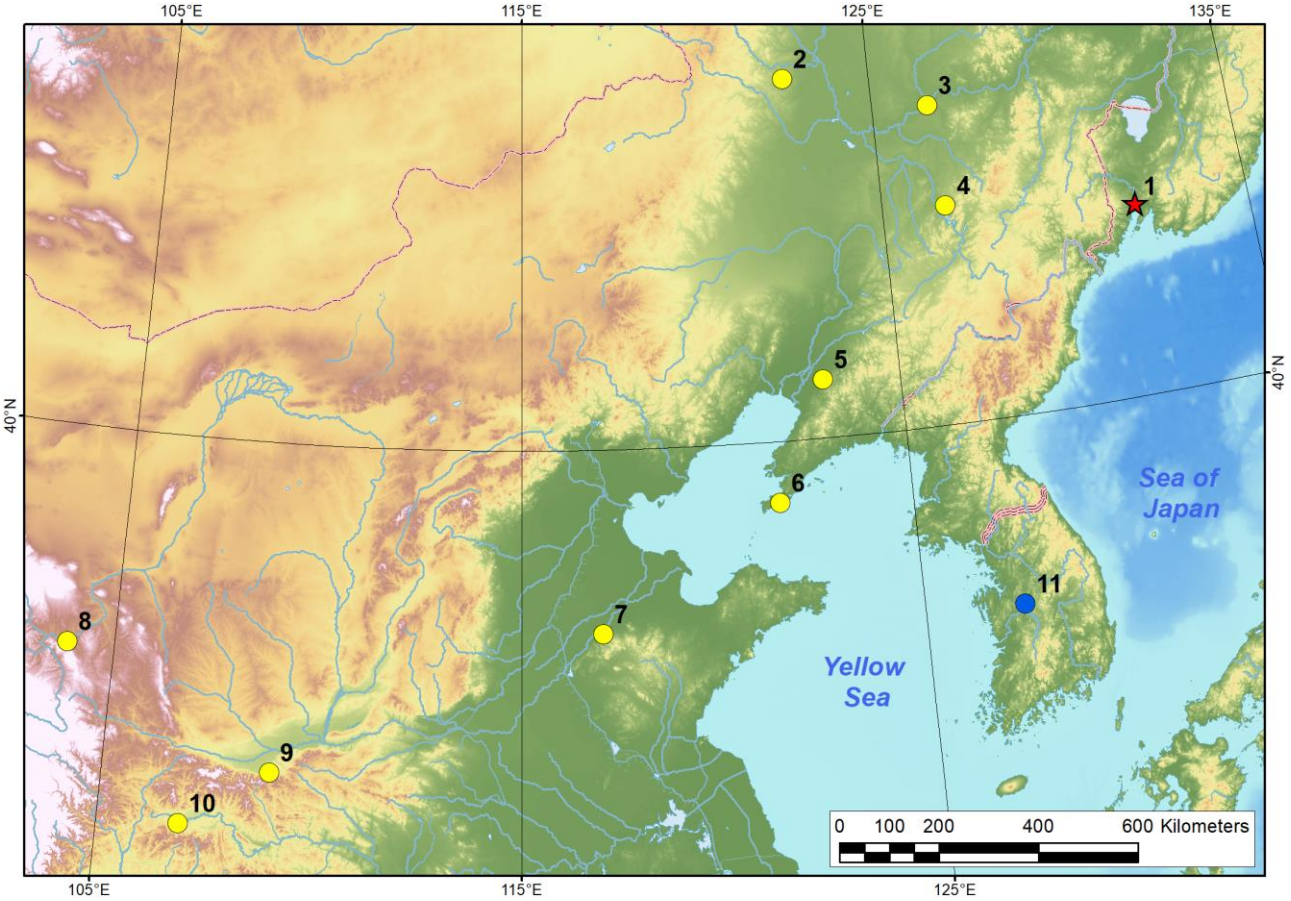

Figure 1. The northernmost occurrences of the Salifidae in their native range in East Asia. The red star indicates the type locality of *Barbronia borealis* **sp. nov.** in Primorye Region, Russia. The yellow circles indicate records of *Barbronia* cf. *weberi* in Northeastern China (Moore 1930; Yang 1996). The blue circle indicates records of *Barbronia gwalagwalensis* and *B.* sp. 'Korea' in South Korea (Kwak *et al.* 2021). Georeferenced occurrence dataset is presented in Appendix 2 (numbers of localities on the map correspond to those in the appendix).

Results

A single *Barbronia* specimen was collected from a riverine pool site in the southern part of the Primorye Region, Russia (Figure 1 and Table 1). Based on the two-locus phylogeny (*COI* + *18S rRNA*), this leech represents a distant phylogenetic lineage that is sister to the clade containing *B. gwalagwalensis*, *B.* sp. 'Korea', and *B. weberi* (Figure 2). The uncorrected *COI* p-distance between it and other sequenced species in this genus varies from 11.6 to 12.6% (Table 2). We consider it as *Barbronia borealis* **sp. nov.**, which is described below.

Our two-locus phylogenetic reconstruction revealed that the genus *Salifa* Blanchard, 1897 in its current understanding is a paraphyletic group, because it contains *Linta be* Westergren & Siddall, 2004 from Madagascar (Figure 2). Moreover, the species *Salifa yunnanensis* (Yang, Wang & Zhang, 1997) takes a separate position outside its genus and sisters to the *Barbronia* + *Odontobdella* + *Mimobdella* clade (Figure 2).

Table 1. Checklist of *Barbronia* species (Hirudinea: Erpobdelliformes: Salifidae).

Taxon	Type locality	General range	*COI* barcode data on type specimens or topotypes	Reference
B. arcana (Richardson, 1971) = *Vivabdella arcana* Richardson, 1971	Australia: above the weir on the lower end of Sullivans Creek in the grounds of the Australian National University, Canberra, Australian Capital Territory [35.2795°S, 149.1181°E, Murray–Darling Basin]	Australia	Not available	Richardson (1971): 226, fig. 1a-g; Sawyer (1986): 696
B. assiuti Hussein & El-Shimy, 1982	Egypt: freshwater canals at the farm of the Faculty of Agriculture, University of Assiut	Lower Nile Basin, Egypt	Not available	Hussein and El-Shimy (1982): 17, figs 1-6; El-Shimy (1996): 100, figs 1a-d, 2a-b
B. borealis Bolotov, Eliseeva & Kondakov **sp. nov.**	Russia: Kiparisovka River, 43.4578°N, 131.9017°E, Razdolnaya (Suifun) River basin, Primorye Region	Not known beyond the type locality	Holotype (OQ940656)	This study
B. gwalagwalensis Westergren & Siddall, 2004	South Africa: Maia's Dam, Gwalagwala, a tented-camp near Hoedspruit [approx. 24.35°S, 30.97°E, Olifants River, Limpopo Basin]	Native to Southeast and East Asia (Myanmar, Korea, China); most likely introduced to South Africa, from which it was described; non-native in Europe (France, Germany)	Paratype (AY786455)	Westergren and Siddall (2004): 3, figs 9-13; Klass *et al.* (2021): 589, figs 3-6
B. nepalensis Nesemann & Sharma, 2007	Nepal: Punyamata near Shree Khandapur, 1450 m a.s.l., Kavre District, Central Zone [Punyamata River near Shreekhandapur City, 27.6162°N, 85.5311°E]	Central Himalaya, Nepal	Not available	Nesemann *et al.* (2007): 190, pl. 62, figs 1-13
B. rouxi Johansson, 19l8	New Caledonia: Oubatche [20.43333°S, 164.6333°E], Canala [21.5203°S, 165.9531°E], and Nouméa [22.2758°S, 166.4580°E]	New Caledonia; probably New Guinea [given by Soós (1966) with a question mark]	Not available	Johansson (1918): 383, pl. 12, figs 2-4; text figs 3-5; Soós (1966): 383
B. shillongensis Nesemann, 2007	India: a stream in Shillong, 1470 m a.s.l., Kashi Hills, Meghalaya [approx. 25.5850°N, 91.8645°E]	Not known beyond the type locality	Not available	Nesemann *et al.* (2007): 191
B. weberi (Blanchard, 1897) s. str. = *Dina weberi* Blanchard, 1897; = *Erpobdella wuttkei* Kutschera, 2004	Indonesia: Bogor on Java, Lake Manindjau on Sumatra, and Bontang on Sulawesi	Native to Indonesia, the Philippines, mainland Southeast and South Asia; non-native in Europe (Austria, England, Italy, Germany, Hungary, Spain, and Serbia), South Africa, Seychelles, Hawaii, North America (USA), South America (Argentina, Brazil, Costa Rica, Mexico), Australia, and New Zealand [all non-sequenced occurrences need to be confirmed by DNA-based approach]	Not available	Blanchard (1897): 353, text fig. 10; Gerlach (1997): 68; Nesemann and Neubert (1999): 150, fig. 79a-f; Kutschera (2004): 148, fig. 1a-c; Nesemann *et al.* (2007): 190, pl. 61, figs 1-10; Genoni and Fazzone (2008): 77; Sawyer and Sawyer (2018): 61; Ludányi *et al.* (2019): 633; Marinković (2020): 37; Klass *et al.* (2021): table 1

Taxon	Type locality	General range	*COI* barcode data on type specimens or topotypes	Reference
B. weberi formosana (Oka, 1929) = *Herpobdella formosana* Oka, 1929	China: environs of Tainan, Taiwan [approx. 23.01°N, 120.28°E]	Native to Taiwan and, probably, to Southeastern China and Korea; a record from Japan needs to be confirmed; a non-native occurrence from Hawaii attributed to this subspecies belongs to *B. weberi* s. str. (see Figure 3)	Not available	Oka (1929): 277; Soós (1966): 383; Sawyer (1986): 746; Nakano (2017): 327

Our identification requests using the BOLD IDS and NCBI's BLASTn indicated that none of previously deposited sequences corresponds to *B. borealis* **sp. nov.** We also noticed that several sequences of other *Barbronia* species are deposited in the BOLD IDS under incorrect names (Figure 3). In particular, this database contains nine sequences belonging to *B. gwalagwalensis*, including those from its non-native range in France and Germany, but all of them are listed as other taxa. Sequences of *B. weberi* are also represented under different names. To avoid confusion in identification of the two species through the BOLD database in the future, we present here a taxonomic reappraisal of these sequences (Figure 3).

Description of the new species

Subclass Hirudinea Lamarck 1818

Order Hirudinida Siddall *et al.*, 2001

Suborder Erpobdelliformes Sawyer, 1986

Family Salifidae Johansson, 1910

Genus *Barbronia* Johansson, 1918 (type species: *Barbronia rouxi* Johansson, 19l8; by monotypy)

Barbronia borealis Bolotov, Eliseeva & Kondakov **sp. nov.**
https://zoobank.org/urn:lsid:zoobank.org:act:06EF7905-A09C-4F37-B686-36A0D67A7F7D
Figures 4, 5a-c

Holotype: RMBH Hir_0405 (fixed and stored in 96% ethanol); RUSSIA: Kiparisovka River, 43.4578°N, 131.9017°E, Razdolnaya (Suifun) River basin, Primorye Region, September 05, 2020, O. V. Aksenova, Y. V. Bespalaya, A. V. Kropotin, O. V. Travina & M. V. Vinarski leg.

Etymology: The name of this species reflects its record in the boreal zone of Eurasia.

Differential diagnosis: The new species is externally similar to *Barbronia gwalagwalensis* and *B. weberi*. It differs from *B. gwalagwalensis* by having 5.5 annuli between gonopores (vs 7.5 annuli) and from *B. weberi* by having 6 annuli between accessory pore and corresponding gonopore (vs 5 annuli). However, DNA barcoding should be considered the most reliable approach for identification of these morphologically similar species.

DNA-based diagnosis: The reference DNA sequences of the holotype: OQ940656 (*COI*) and OQ941865 (*18S rRNA*). This species represents a divergent phylogenetic lineage, which is distant from other species in the genus, the sequences of which are available (Table 2).

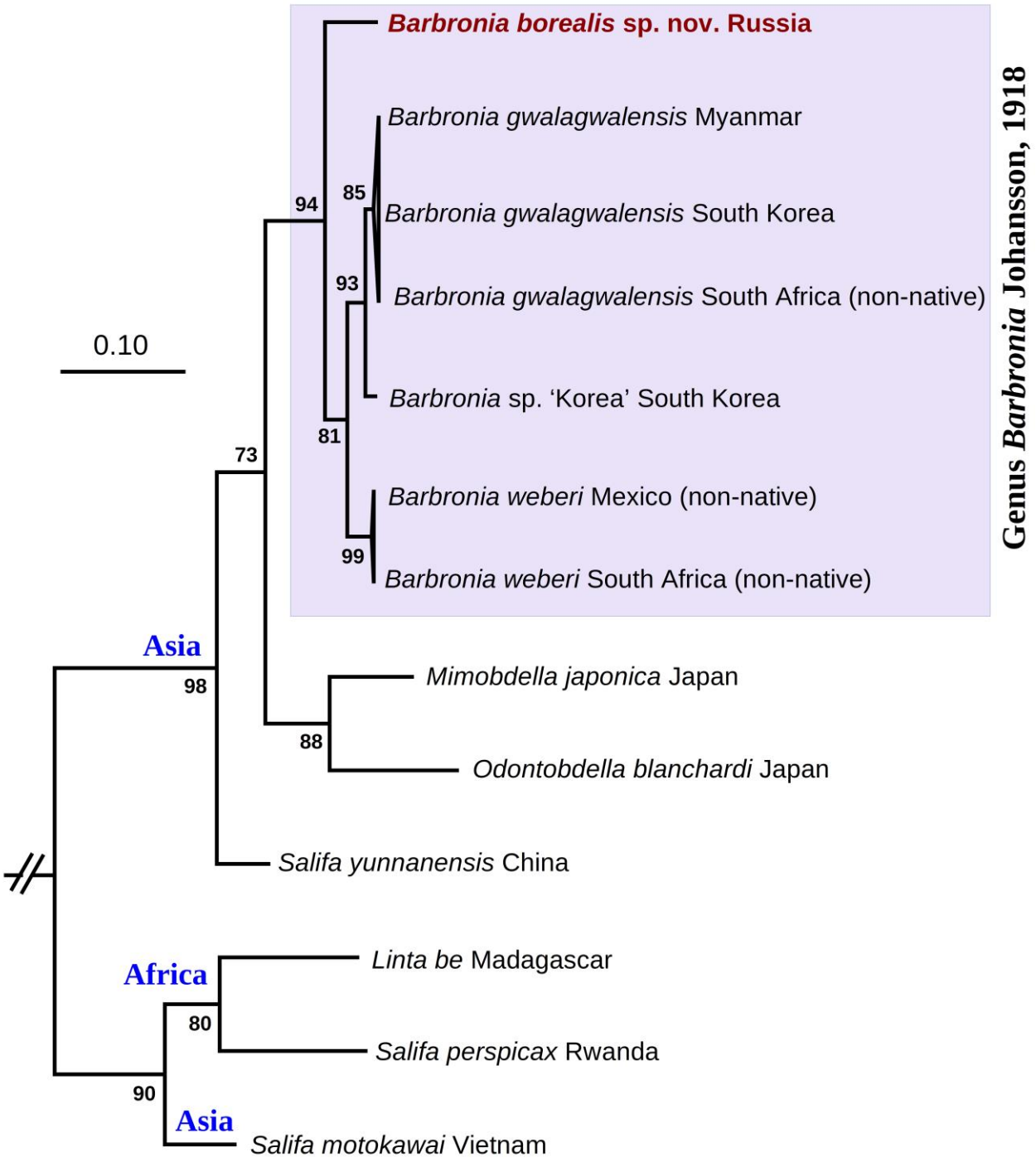

Figure 2. Maximum likelihood phylogeny of the Salifidae based on partial sequences of the *COI* and *18S rRNA* genes (Appendix 1). The black numbers near nodes are bootstrap support values of IQ-TREE v. 1.6.12. The scale bar indicates the branch length. The new species name is red. The geographic affinities of subclades/lineages are blue. Outgroup is not shown.

Table 2. Uncorrected *COI p*-distances (%) between *Barbronia* species (Salifidae)

Species	*B. borealis* **sp. nov.**	*B. gwalagwalensis*	*B.* sp. 'Korea'
B. gwalagwalensis	12.2		
B. sp. 'Korea'	12.6	4.6	
B. weberi	11.6	6.9	7.8

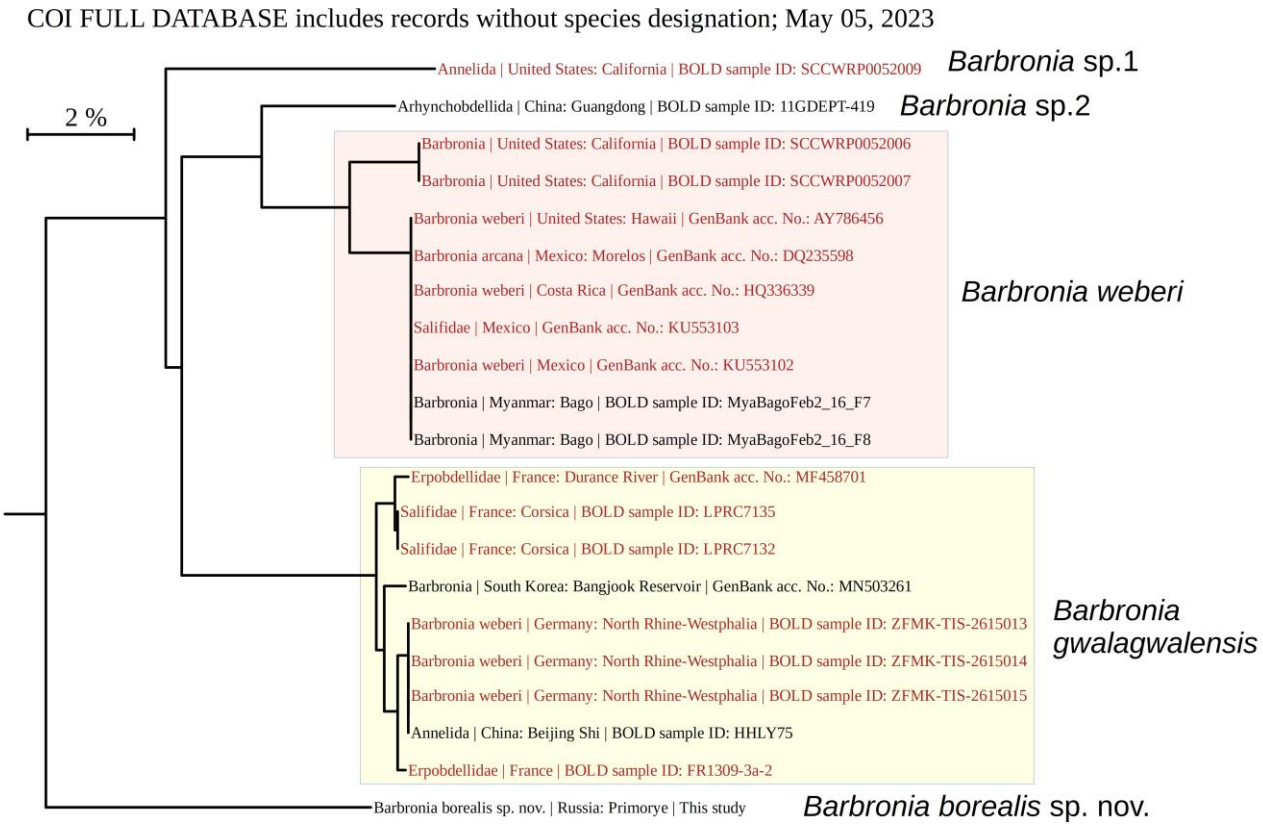

Figure 3. Fragment of the BOLD TaxonID Tree with a taxonomic reappraisal of available *COI* sequences of *Barbronia* species (assessed on May 05, 2023). Titles of sequences from non-native populations are red; those from native populations are black.

Description: Small salifid leech: body length 20.1 mm, maximum body width 3.3 mm, maximum width of anterior sucker 0.9 mm, maximum width of posterior sucker 2.0 mm (Figure 4). Body elongated, vermiform, tapering anteriorly. Body surface smooth, without papillae. Posterior sucker ventrally directed. Dorsum light ochraceous, venter whitish. Anterior region with three pairs of circular eyespots: one labial on 3rd annulus and two buccal on 6-7th annuli (Figure 5a-b). Complete mid-body somite 6 annulate: b1 + b2 + a2 + b5 + c11 + c12 (Figure 5c). Clitellum extends from X b5 to XIV b2. Gonopores large, well visible, separated by 5.5 annuli. Male accessory pore in the furrow X c12/XI b1, male gonopore in XII b1/b2, female gonopore in XIII b1, female accessory pore in XIII c12/XIV b1 (Figure 5c). Anus dorsally at XXVII, two postanal annuli anterior to posterior sucker (Figure 4). Reproductive and digestive systems remain unstudied because only the holotype was available for description.

Distribution: This species is only known from its type locality, situated at the southeastern corner of the Russian Far East. However, numerous occurrences from Northeastern China attributed to *B.* cf. *weberi* (Figure 1) may belong to the new species.

Habitats and ecology: The holotype was collected from a pool site of a small river with clay bottom (Figure 6). This leech specimen was fixed with its prey, an oligochaete, protruding from its mouth. A *COI* sequence was generated from this worm (GenBank acc. No. OQ933549). Searching with the BOLD IDS reveals that the *COI* sequence of the prey item is related to those of oligochaetes identified as *Limnodrilus profundicola* (Verrill, 1871) (Oligochaeta: Naididae). This finding indicates that *Barbronia borealis* **sp. nov.** feeds on small freshwater oligochaetes, as do some other species in this genus (Nesemann *et al.* 2007).

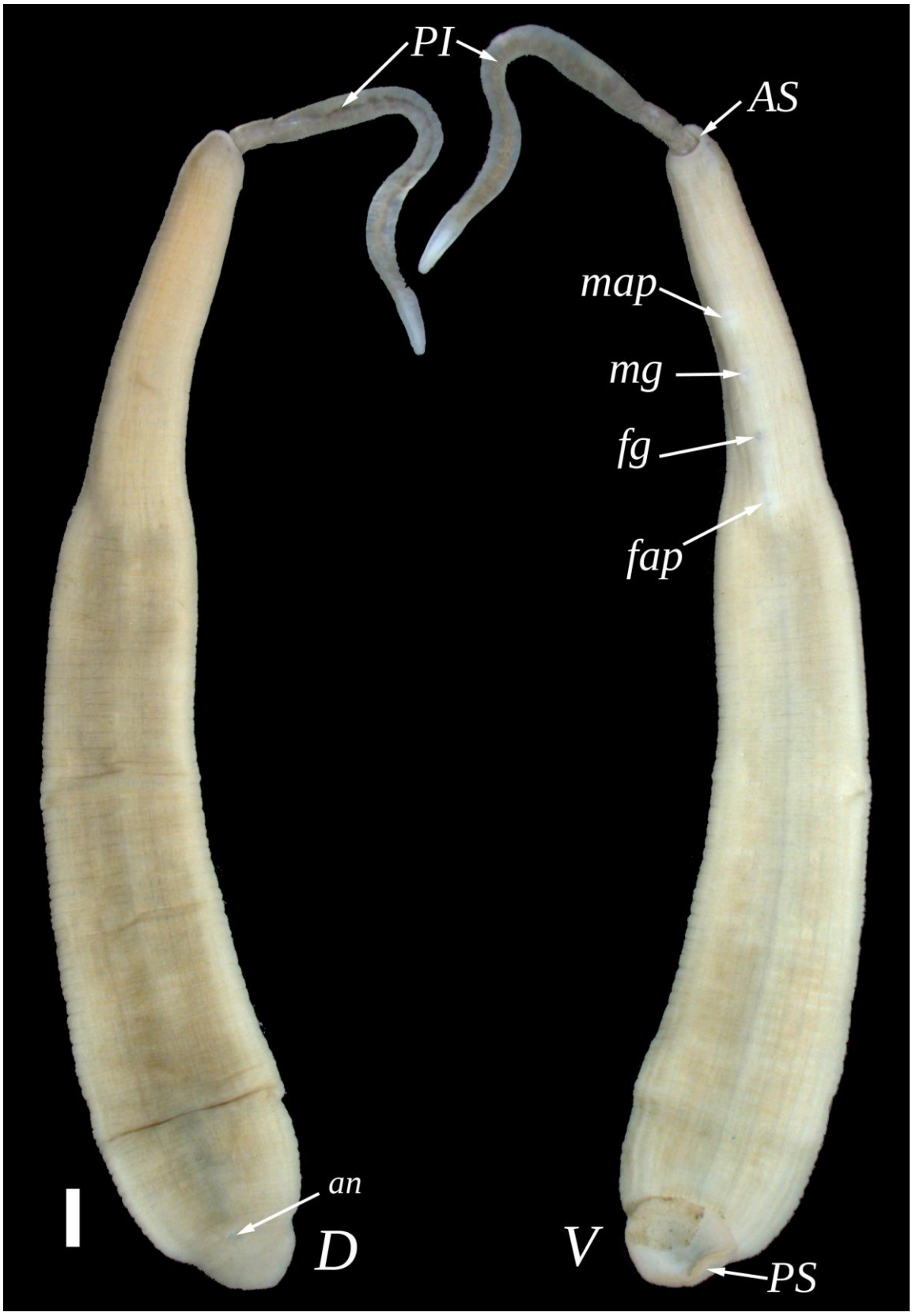

Figure 4. Dorsal (D) and ventral (V) views of the holotype of *Barbronia borealis* **sp. nov.** (RMBH Hir_0405). Abbreviations: *PI*, prey item (an oligochaete specimen); *AS*, anterior sucker; *PS*, posterior sucker; *map*, male accessory pore; *fap*, female accessory pore; *mg*, male gonopore; *fg*, female gonopore; *an*, anus. Scale bar = 1.0 mm. Photos: Tatyana A. Eliseeva.

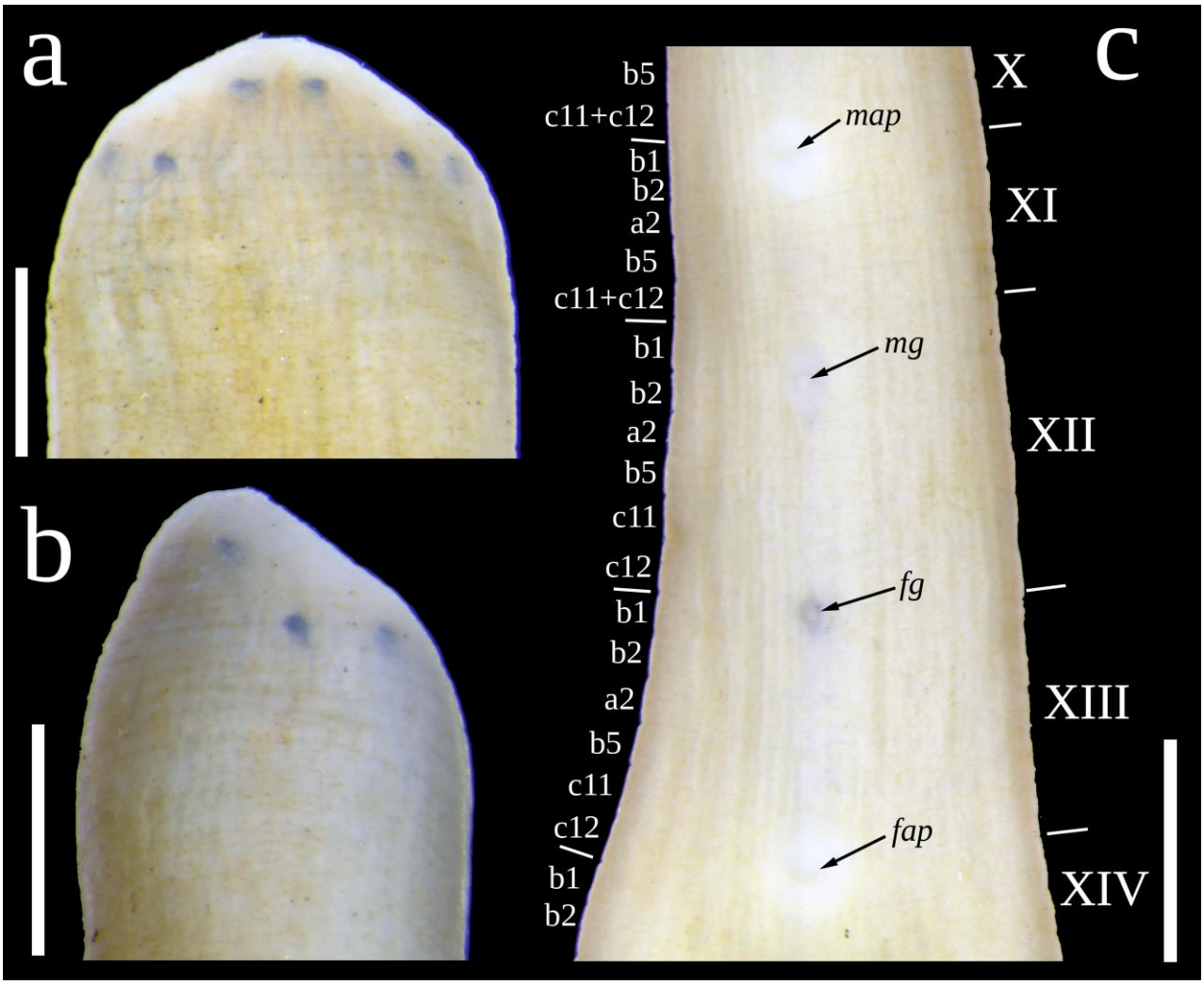

Figure 5. External morphological features of the holotype of *Barbronia borealis* sp. nov. (RMBH Hir_0405). (**a-b**) Arrangement of three pairs of eyespots: dorsal view (**a**) and lateral view from right side (**b**). (**c**) Ventral region of clitellum, with male (*mg*) and female (*fg*) gonopores, and male and female accessory copulatory pores (*map* and *fap*, respectively). Body somites are indicated by roman numerals; symbols b1, b2, a2, b5, c11, and c12 indicate the number of annulus. Scale bars = 0.5 mm (a-b) and 1.0 mm (c). Photos: Tatyana A. Eliseeva; graphics: Ivan N. Bolotov.

Discussion

Our checklist of the genus *Barbronia* contains eight species, including a new species described here (Table 1). Two more nominal species, that is, *Barbronia yunnanensis* Yang, Wang & Zhang, 1997 and *Barbronia zhejiangica* Yang, 1996, were initially described in this genus (Yang 1996; Yang *et al.* 1997). However, Nesemann and Sharma (2012) transferred them to the genus *Salifa* based on the lack of accessory copulatory pores. These pores were considered a primary diagnostic feature of the genus (Johansson, 1918; Sawyer 1986; Nakano and Nguyen 2015). In our phylogeny, *Salifa yunnanensis* was expectedly placed outside the *Barbronia* clade. The phylogenetic results, presented here, support the conclusion of Nakano and Nguyen (2015) that the morphology-based classification of salifid leeches did not receive full confirmation by means of a molecular approach. In particular, the genus *Salifa* in its current understanding does not represent a monophyletic clade and definitely needs an integrative revision in the future.

The discovery of a salifid leech, *Barbronia borealis* **sp. nov.**, in the Primorye Region of Russia largely aligns with general biogeographic patterns, caused by environmental and paleogeographic reasons (Bolotov *et al.* 2020). In particular, this area is considered a biogeographic transitional zone between boreal and (sub)tropical aquatic faunas (Garibian *et al.* 2021; Chertoptud *et al.* 2023). It houses a number of species belonging to freshwater invertebrate clades having more southern affinities, including an orobdellid leech

(Nakano and Prozorova 2019), some glossiphoniids (Bolotov *et al.* 2017; Bolotov *et al.* 2019), freshwater mussels (Bolotov *et al.* 2020), as well as a *Protohermes* dobsonfly (Liu *et al.* 2016).

Finally, we present here a framework for reliable identification of two widespread *Barbronia* species, that is, *B. gwalagwalensis* and *B. weberi*, based on the *COI* sequences through the BOLD IDS database (see Figure 3). Interestingly, none of the *COI* sequences of *B. weberi* is available from Europe, except for that of a specimen obtained from a German aquarium and attributed to *B. wuttkei* (GenBank acc. No. DQ009666; Pfeiffer *et al.* 2005). Instead, all available European sequences of *Barbronia* belong to *B. gwalagwalensis* (see Figure 3). This finding indicates that the species-level identification of *B. weberi* recorded in several countries of Europe (e.g., Nesemann and Neubert 1999; Genoni and Fazzone 2008; Sawyer and Sawyer 2018; Ludányi *et al.* 2019) needs to be confirmed by means of a molecular approach. Furthermore, now *B. gwalagwalensis* is known to occur in Myanmar, South Korea, and China. These findings indicate that it is an Asian species and that its population in South Africa was likely established through introduction event from Asia.

Figure 6. Type locality of *Barbronia borealis* **sp. nov.**: Kiparisovka River, 43.4578°N, 131.9017°E, Razdolnaya (Suifun) River basin, Primorye Region, Russia, September 05, 2020. Photo: Olga V. Aksenova.

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Appendix 1. Information on the *COI* and *18S rRNA* sequences of freshwater leeches used in this study (numbers of new sequences are bold).

Taxon	Locality	Status of population	*COI* acc. No.	*18S rRNA* acc. No.	Reference
Barbronia borealis **sp. nov.** (holotype)	Russia: Kiparisovka River, 43.4578°N, 131.9017°E, Razdolnaya (Suifun) River basin, Primorye Region	Native	**OQ940656**	**OQ941865**	This study
B. gwalagwalensis Westergren & Siddall, 2004	Myanmar: Kyee Phyu Lake, Lake Inle basin, Salween River drainage	Native	MN295405	**ON854145**	Bolotov *et al.* (2019); Klass *et al.* (2021); this study
B. gwalagwalensis [=*B.* sp. HJK-2020]	South Korea: Bangjook Reservoir	Native	MN503261	MT010330	Kwak *et al.* (2021)
B. gwalagwalensis (paratype)	South Africa: Hoedspruit (type locality)	Most likely non-native	AY786455	AY786462	Borda and Siddall (2004); Oceguera-Figueroa *et al.* (2011)
B. gwalagwalensis [=*B.* sp. T-P54]	France: Durance River, Rhône River drainage	Non-native	MF458701	n/a	Corse *et al.* (2017)
B. weberi (Blanchard, 1897)	South Africa: Kruger National Park	Non-native	AY786457	AY786463	Borda and Siddall (2004)
B. weberi [=*B. weberi formosana* sensu Borda & Siddall, 2004]	USA: Kauai, Hawaii	Non-native	AY786456	AY786461	Borda and Siddall (2004)
B. weberi	Mexico	Non-native	KU553102	n/a	Garduno-Montes de Oca *et al.* (2016)
B. weberi	Mexico	Non-native	KU553103	n/a	Garduno-Montes de Oca *et al.* (2016)
B. weberi [=*B. arcana* sensu Figueroa *et al.*, 2005]	Mexico: Rio Amacuzac, Morelos	Non-native	DQ235598	DQ235608	Oceguera-Figueroa *et al.* (2005)
B. weberi [=*Erpobdella wuttkei* Kutschera, 2004]	Germany: aquarium	Non-native	DQ009666	n/a	Pfeiffer *et al.* (2005)
B. sp. 'Korea'	South Korea	Native	KF966549	n/a	Klass *et al.* (2021)
Linta be Westergren & Siddall, 2004 (paratype)	Madagascar: Esana, 5 km west of Tolagnaro (type locality)	Native	AY786460	AY786466	Borda and Siddall (2004)
Mimobdella japonica Blanchard, 1897	Japan: Kagoshima, Tatsugo, Akina, 28.44°N, 129.56°E	Native	AB675014	AB663650	Nakano *et al.* (2012)
Odontobdella blanchardi (Oka, 1910)	Japan:Kyoto, Kyoto, Sakyo-ku, Iwakura-muramatsu, 35.09°N, 135.79°E	Native	AB675016	AB663651	Nakano *et al.* (2012)
Salifa motokawai Nakano & Nguyen, 2015	Vietnam: Kon Tum, Ngoc Linh Nature Reserve, 15.19°N, 107.78°E	Native	LC029431	LC029434	Nakano and Nguyen (2015)
Salifa perspicax Blanchard, 1897	Rwanda: Lake Ihema	Native	HQ336343	HQ336377	Oceguera-Figueroa *et al.* (2011)
Salifa yunnanensis (Yang, Wang & Zhang, 1997)	China	Native	OQ076772	n/a	GenBank
Alexandrobdella makhrovi Bolotov *et al.*, 2019 (Piscicolidae; outgroup)	Russia: Primorye Region, Far East	Native	MN295413	MN312187	Bolotov *et al.* (2019)

Appendix 2. The northernmost occurrences of salifid leeches in their native ranges.

Species	Locality with its number on the map (see Figure 1) in square brackets	Latitude	Longitude	Reference
Barbronia borealis **sp. nov.**	Far East of Russia: Kiparisovka River, Razdolnaya (Suifun) River basin, Primorye Region [1]	43.4578	131.9017	This study
Barbronia cf. *weberi*	NE China: Bayangol, Inner Mongolia [2]	46.5351	122.5343	Yang (1996)
Barbronia cf. *weberi*	NE China: Sun Island, Harbin, Heilongjiang Province [3]	45.7933	126.5964	Yang (1996)
Barbronia cf. *weberi*	NE China: Jiangmifengzhen Town, Longtan District, Jilin Province [4]	43.9567	126.7514	Yang (1996)
Barbronia cf. *weberi*	NE China: Qianshan District, Anshan City, Liaoning Province [5]	41.0664	122.9542	Yang (1996)
Barbronia cf. *weberi*	NE China: Dalian, Liaoning Province [6]	38.9000	121.6000	Moore (1930)
Barbronia cf. *weberi*	NE China: Jinan City, Shandong Province [7]	36.6717	117.0160	Yang (1996)
Barbronia cf. *weberi*	NE China: Yantan, Lanzhou, Gansu Province [8]	36.0603	103.8577	Yang (1996)
Barbronia cf. *weberi*	NE China: Taiyigong Town, Chang'An, Shaanxi Province [9]	34.0321	109.0014	Yang (1996)
Barbronia cf. *weberi*	NE China: Nanhu, Nanzheng District, Shaanxi Province [10]	32.9939	106.9306	Yang (1996)
B. gwalagwalensis	South Korea: Bangjook Reservoir [11]	36.6208	127.4548	Kwak *et al.* (2021)
Barbronia sp. ‘Korea’	South Korea [11]	36.3100	127.4370	GenBank