Zoosyst. Evol. 100 (3) 2024, 1155–1173 | DOI 10.3897/zse.100.126702

Forgotten for two centuries: redescription of *Phoxinus isetensis* (Georgi, 1775) (Cypriniformes, Leuciscidae) – the most widespread minnow in Europe

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Academic editor: Nicolas Hubert ♦ **Received** 2 May 2024 ♦ **Accepted** 15 July 2024 ♦ **Published** 22 August 2024

Abstract

The morphology, phylogenetic position, and distribution of a recently revalidated species of leuciscid minnow, *Phoxinus isetensis*, were substantially clarified. The species was described in the late 18th century from the Middle Urals but later synonymized with *Phoxinus phoxinus*. As believed, *P. isetensis* is distributed in the Arctic Ocean catchment from the Murman coast via West and East Siberia until the Pacific Ocean catchment in Far East and Northeastern Asia. Our study, with the use of mtDNA markers coupled with extensive morphological data, showed that the distribution of *P. isetensis* is greatly different. Currently, this is the most widespread *Phoxinus* species in Europe, distributed in Northern and Eastern Europe and on the western edge of Siberia (Iset and Ural basins). In particular, *P. isetensis* inhabits the basins of the Caspian, Baltic, White, Barents, and Kara seas, possibly occurring in the North Sea basin. The species was redescribed, and the type locality and neotype were designated. The main morphological difference from other *Phoxinus* spp. is the large total number of vertebrae (39–43, mode 41) due to an increase in the number of caudal vertebrae (16–21, mode 19). Phylogenetically, *P. isetensis* is a sister to the Caucasian species *P. colchicus* (*p*-distance = 5%). The wide distribution of *P. isetensis* within the area of the Last Glacial Maximum suggests rapid colonization of deglaciated regions, probably due to its adaptation to a cold climate.

Key Words

DNA barcoding, Europe-Siberia corridor, freshwater fish, postglacial expansion, taxonomy

Introduction

Minnows of the genus *Phoxinus* Rafinesque, 1820, are predominantly rheophilic small freshwater fish of the family Leuciscidae Bonaparte, 1835, widespread in northern Eurasia from the Pyrenees to the Pacific coast. Initially, the morphology-based taxonomy of the genus *Phoxinus* was controversial due to the complex variability of morphological features and their large overlap. In early comprehensive publications, all *Phoxinus* diversity was reduced either to one species with a number of subspecies (Berg 1949) or to several species (Kottelat and Freyhof 2007). The use of molecular genetic methods has shown a greatly underestimated species diversity in Europe (Palandačić et al. 2015, 2017, 2020), which suggested a new look at the taxonomy of the genus *Phoxinus*. Only two species (*P. bigerri* Kottelat, 2007 and *P. colchicus* Berg, 1910) were supported by morphological and genetic (mitochondrial) data, and six (*P. phoxinus* (Linnaeus, 1758), *P. lumaireul* (Schinz, 1840), *P. karsticus* Bianco & De Bonis, 2015, *P. septimanae* Kottelat, 2007, *P. marsilii* Heckel, 1836, and *P. csikii* Hankó, 1922) were supported by mitochondrial but limitedly corroborated by nuclear data (Palandačić et al. 2017). Then several new species were described using an integrative taxonomy approach: *P. krkae* Bogutskaya, Jelić, Vucić, Jelić, Diripasko, Stefanov & Klobučar, 2019 (Bogutskaya et al. 2019), *P. dragarum* Denys, Dettaï, Persat, Daszkiewicz, Hautecoeur & Keith, 2020, *P. fayollarum* Denys, Dettaï, Persat, Daszkiewicz, Hautecoeur & Keith, 2020 (Denys et al. 2020), *P. abanticus* Turan, Bayçelebi, Özuluğ, Gaygusuz & Aksu, 2023 (Turan et al. 2023), *P. radeki* Bayçelebi, Aksu & Turan, 2024 (Bayçelebi et al. 2024), and *P. adagumicus* Artaev, Turbanov, Bolotovskiy, Gandlin & Levin, 2024 (Artaev et al. 2024).

Along with the productive revision of the taxonomic and genetic diversity of species distributed in Western and Central Europe, the remaining part of the range, including Eastern Europe, was almost unexplored. For instance, taxonomic identification of northern and eastern European minnows with the largest range in Europe was problematic; those were assigned to mitochondrial Clade 17 without species naming (Palandačić at al. 2017, 2020). Our genetic data showed that unnamed *Phoxinus* sp. Clade 17 is conspecific to recently revalidated *Phoxinus isetensis* (Georgi, 1775) (Dyldin et al. 2023) described from the Middle Urals. This study aimed to make taxonomic redescription using the integrative (morphology and genetics) approach, to outline geographic distribution, and to clarify the phylogeny of *P. isetensis*.

Materials and methods

Sampling

Materials for morphological studies and partially for genetic studies were collected by the authors. Fish were caught using a frame net and seine net with a mesh size of 6–8 mm. Fish were euthanized in a solution of clove oil and photographed in an aquarium with artificial lighting using a Nikon D5300 camera (Nikon Corporation, Tokyo, Japan) with a Nikkor 60 mm f/2.8G lens (Nikon Corporation, Tokyo, Japan) using a physical white swatch for color correction. Fin clips (pectoral or pelvic) were taken from some specimens (DNA vouchers) and placed in 96% ethanol for subsequent DNA extraction in the laboratory. Then most fish were preserved in 10% formalin (form.), while some samples (usually small-sized specimens) were preserved in 96% ethanol for molecular analysis. Subsequently, formalin-fixed specimens were washed out in running water and transferred to 70% ethanol for long-term storage.

Neotype and additional material were deposited at the Fish Collection of the Papanin Institute for Biology of Inland Waters of the Russian Academy of Sciences, Borok, Russia (IBIW_FS).

Morphological studies

Morphological material on *Phoxinus* sp. (Clade 17) from seventeen localities (n=272, Fig. 1, Suppl. material 1) was examined. In studying the morphology of *Phoxinus*, we follow Bogutskaya et al. (2019, 2023) and Artaev et al. (2024). In particular, 42 morphometric (Suppl. materials 2, 3), 17 meristic, and two qualitative characters (Suppl. materials 2, 4) were processed. Abbreviations of morphometric characters are seen in Suppl. material 3. Morphometric measurements were taken from the left side of the body using a digital caliper to the nearest 0.1 mm by one operator for the purposes of consistency as recommended by Mina et al. (2005). Meristics (except for axial skeleton) and type of breast scalation (Bogutskaya et al. 2019) were assessed using material stained in an ethanol solution of alizarin red S (Taylor and Van Dyke 1985 with modifications), followed by short exposure to 1–2% potassium hydroxide and preservation in 70% ethanol.

Sex was determined by the shape and size of the pectoral fins, their rays, and the length of the pelvic fins (Frost 1943; Berg 1949; Chen 1996; Bogutskaya et al. 2019). External meristics were counted on the left side. Standard length (SL) was measured from the tip of the upper lip to the end of the hypural complex. The total number of pectoral and pelvic-fin rays was counted on the left fins. The last two branched rays articulated on a single pterygiophore in the dorsal and anal fins are counted as one. Scales above the lateral line were counted between the lateral line and base of the first unbranched ray in the dorsal fin; scales below the lateral line were between the lateral line and base of the first unbranched ray in the anal fin. In both cases, lateral line scales were not taken into account. The number of anterior gill rakers of the first gill arch was counted on the left and right sides of the specimens. Number lines on the scales were counted on the left and right breast patches, and an average value was taken. The counts of meristic characters (except for the axial skeleton) and assessment of qualitative characters were done using the stereomicroscope MC-2-ZOOM (Micromed, Saint Petersburg, Russia). Vertebrae and pterygiophores were counted following Naseka (1996) and Bogutskaya et al. (2019) using radiographs made by X-ray equipment PRDU II (ELTECH-Med, Saint Petersburg, Russia). Images of pharyngeal teeth were obtained

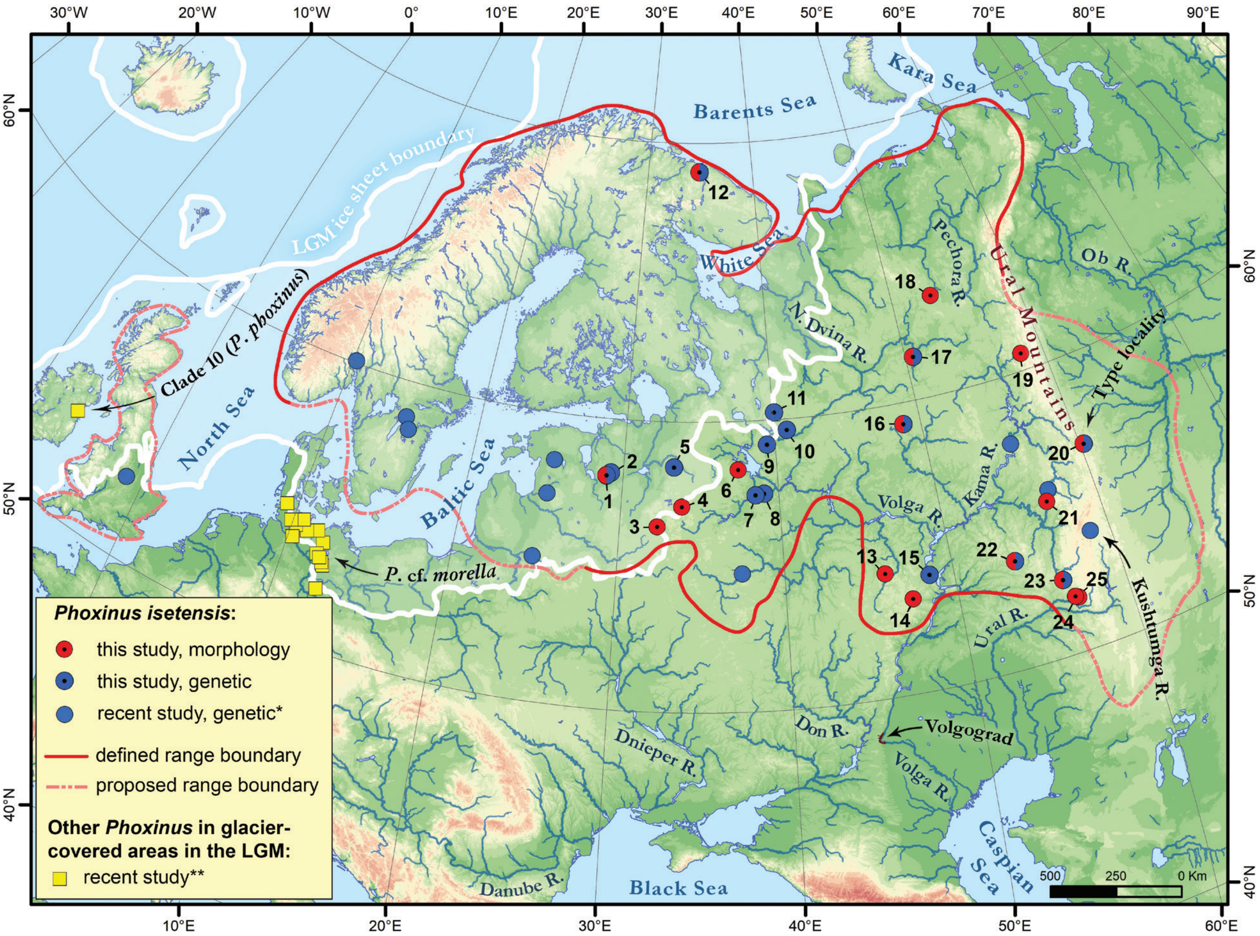

Figure 1. Distribution map and range boundaries of *Phoxinus isetensis* confirmed by morphological and genetic data along with other confirmed *Phoxinus* spp., whose distributions are partially within the glacier-covered area of the last glacial maximum. Locality numbers are designated in Suppl. materials 1, 5. * – by Palandačić et al. (2020); ** – by Palandačić et al. (2020) and Rothe et al. (2019).

using a JEOL JSM-6510LV scanning electron microscope (Jeol, Tokyo, Japan).

Measurement indexes were statistically processed in Microsoft Excel. Comparison of multiple samples was carried out using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction [*rstatix* (Kassambara 2020) and *tidyverse* (Wickham et al. 2019) packages in R version 4.3.1 (Ihaka and Gentleman 1996)]. Principal component analysis (PCA) was performed using the *ggfortify* (Tang et al. 2016) package in R. Differences between sexes were tested using the Mann-Whitney U test in Past 4.13 (Hammer and Harper 2001).

Phylogenetic placement and genetic distance.

DNA was isolated by salt extraction (Aljanabi and Martinez 1997) from ethanol-fixed tissues. Two mitochondrial markers were analyzed. The mitochondrial cytochrome *c* oxidase subunit I (COI) barcode region was amplified using the M13-tailed primer cocktail: FishF2_t1: 5′-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C-3′, FishR2_t1: 5′-CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA A-3′, VF2_t1: 5′-TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C-3′, and FR1d_t1: 5′-CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A-3′ (Ivanova et al. 2007). PCR conditions for COI followed protocols from Ivanova et al. (2007). In addition, the cytochrome *b* (cyt*b*) fragment was amplified by PCR using the following primers: GluF: 5′-AACCACCGTTGTATTCAACTACAA-3′ and ThrR: 5′-ACCTCCGATCTTCGGATTACAAGACCG-3′ (Machordom and Doadrio 2001). PCR amplifications were performed using Evrogen ScreenMix-HS under conditions described by Levin et al. (2017).

Sequencing of the PCR products, purified by ethanol and ammonium acetate (3 M) precipitation, was conducted using the Applied Biosystems 3500 DNA sequencer (Thermo Fisher Scientific, USA), with primers M13F 5′-GTA AAA CGA CGG CCA GT-3′ M13R-pUC 5′-CAG GAA ACA GCT ATG AC-3′ (Geiger et al. 2014) for COI and primers GluF: 5′-AACCACCGTTGTATTCAACTACAA-3′ and ThrR: 5′-ACCTCCGATCTTCGGATTACAAGACCG-3′ (Machordom and Doadrio 2001) for cyt*b*.

DNA chromatograms were checked for errors in FinchTV 1.4.0 (Rothgänger et al. 2006), and the DNA sequences were aligned using the ClustalW algorithm in MEGA7 (Kumar et al. 2016). Phylogenetic analysis was performed on COI (567 bp) and cyt*b* (1089 bp) concatenated sequences. In addition to the 29 newly determined COI and cyt*b* sequences in this study, 294 concatenated sequences of all available *Phoxinus* spp. were mined from the GenBank (derived from the studies of Imoto et al. 2013; Xu et al. 2014; Palandačić et al. 2015, 2017, 2020; Ramler et al. 2016; Xie et al. 2016; Schönhuth et al. 2018; and unpublished works). Three outgroups representing the genera *Pseudaspius* Dybowski, 1869, *Rhynchocypris* Günther, 1889, and *Oreoleuciscus* Warpachowski, 1889, were selected according to the previous phylogenetic studies (Palandačić et al. 2015, 2020) (Suppl. material 5). Only unique haplotypes were used in downstream phylogenetic analyses.

The Bayesian phylogenetic analysis was performed in a Bayesian statistical framework implemented in BEAST v.1.10.4. (Hill and Baele 2019) with 2×10^7 MCMC generations (10% burn-in) and parameters sampled every 2000 steps. The substitution models by codon position for Bayesian analysis were selected in PartitionFinder v.2.1.1 (Lanfear et al. 2016) with the greedy algorithm (Lanfear et al. 2012) (Suppl. material 6).

Maximum likelihood phylogenies were inferred using IQ-TREE v.2.2.0 (Nguyen et al. 2015) in PhyloSuite v.1.2.3 (Zhang et al. 2020; Xiang et al. 2023) under the edgelinked partition model for 1000 ultrafast (Minh et al. 2013) bootstrapping. ModelFinder v.2.2.0 (Kalyaanamoorthy et al. 2017) in PhyloSuite v.1.2.3 was used to select the best-fit partition model (edge-linked) using the AICc criterion (Suppl. material 6).

The average intra-group as well as the average pairwise intergroup *p*-distances using the concatenated COI+cyt*b* sequences data set were calculated using the MEGA7 program (Kumar et al. 2016) with 1000 bootstrap replicas.

Map visualization

The map was created using the QGIS software, v.3.34. Digital elevation model visualized based on GMTED2010, 30 sec. resolution (Danielson and Gesch 2011); river systems – HydroATLAS v.1.0 (Linke et al. 2019); LGM ice sheet boundary according to Batchelor et al. (2019).

Results

Phylogenetic placement and genetic distance

The phylogenetic Bayesian tree of the genus *Phoxinus* shows that *P. isetensis* has its own lineage, being sister to *P. colchicus* distributed in the eastern Black Sea basin and the Kuban system in the Sea of Azov basin (*p*-distance = 0.050±0.005) with a high support in both BI (Fig. 2) and ML (Suppl. material 7) analyses. Three species combined together (*P. isetensis*, *P. colchicus*, and *P. chrysoprasius*) are early branching in the *Phoxinus* tree and represent a sister class to all other European *Phoxinus* spp. apart from *P. adagumicus*, although this was weakly supported. Intraspecies divergence of *P. isetensis* is moderate (0.005) despite its wide distributional range (Suppl. material 8).

Systematics

Class Actinopterygii Klein, 1885
Order Cypriniformes Bleeker, 1859
Family Leuciscidae Bonaparte, 1835
Genus *Phoxinus* Rafinesque, 1820

Phoxinus isetensis (Georgi, 1775)

Figs 3, 4

English name: Northern Minnow; Russian name: Северный гольян

Cyprinus phoxinus – Linnaeus 1758: 322 (Europa (part)); Falk 1786: 432 (Volga, Tsaritsa, Elshanka, Sarpa, etc.); Fischer 1791: 258 (Livonia); Hupel 1777: 467 (Liffland and Estonia).

Cyprinus aphya – Linnaeus 1758: 323 (European rivers (part)); Fischer 1791: 258 (Livonia); Georgi 1775: 881 (Sukhona River); Falk 1786: 429 (Kama R. and its tributaries).

Cyprinus (without Latin species name) – Lepechin 1771: 491 (circa Catharinopolin).

“Галїанъ” or “солдатъ” (without Latin species name) – Lepechin 1772: 309 (upstreams of the Isset, Chusovaya, and Tura rivers).

Cyprinus, “Krasnosobik” or “Soldat” (without Latin species name)– Georgi 1775: 550 (Iset River).

Cyprinus isetensis Georgi 1775: 621 (Chusovaya River).

Cyprinus galian Gmelin 1789: 1421 (vicinities of Yekaterinburg).

Phoxinus rivularis – Wałecki 1864: 50 (Neman River).

Phoxinus laevis – Kessler 1864: 124 (Neva River); Kessler 1870: 268 (Volga, Samara basins, Khmelevka creek near Vasilsursk); Warpachowski 1889: 61 (Volga R. system in Nizhny Novgorod province); Sabaneev 1892: 423 (Yaroslavl and Perm province, near Moscow: rivers Lichoborka and Sinichka (trib. of Jausa R.), Moskva R. at Kamenny most); Dybowski 1862: 105 (Livonia).

Tinca phoxinus – Plater 1861: 37, 63 (Daugava River).

Phoxinus phoxinus – Berg 1912: 260 (Finland, Kola region, European rivers of the Arctic Ocean basin); Berg 1923: 166 (in Russia all over); Berg 1932: 368 (in Arctic Ocean basin from Murmansk eastward, Volga basin upstream Syzran (include Kama and Oka rivers), possible in Ural River); Berg 1949: 588 (same place); Reshetnikov et al. 2003: 301 (widespread in Europe (part)); Kottelat and Freyhof 2007: 228 (Scandinavia and Russia’s northernmost extremity; Upper and middle Volga, Ural).

Phoxinus isetensis – Dyldin et al. 2023: 36 (Arctic Ocean basin, from Murman coast to East Siberian Sea basin (part)).

Phoxinus sp. – Dyldin et al. 2023: 37 (Europe, in North Sea, Baltic Sea basins (including Gulf of Finland and Neva River), and northern Caspian Sea basin (Upper Volga River, including Kama and Oka rivers, probably in Ural River)).

Type material. Neotype, female (SL 63.9 mm, IBIW_FS_422, Genbank Accession numbers PP538745–COI, PP548200–cyt*b*), Russia, Sverdlovsk Region, Ob River basin, Severka River (Tobol River basin) upstream Severka village near Yekaterinburg, 56.8830°N, 60.2716°E,

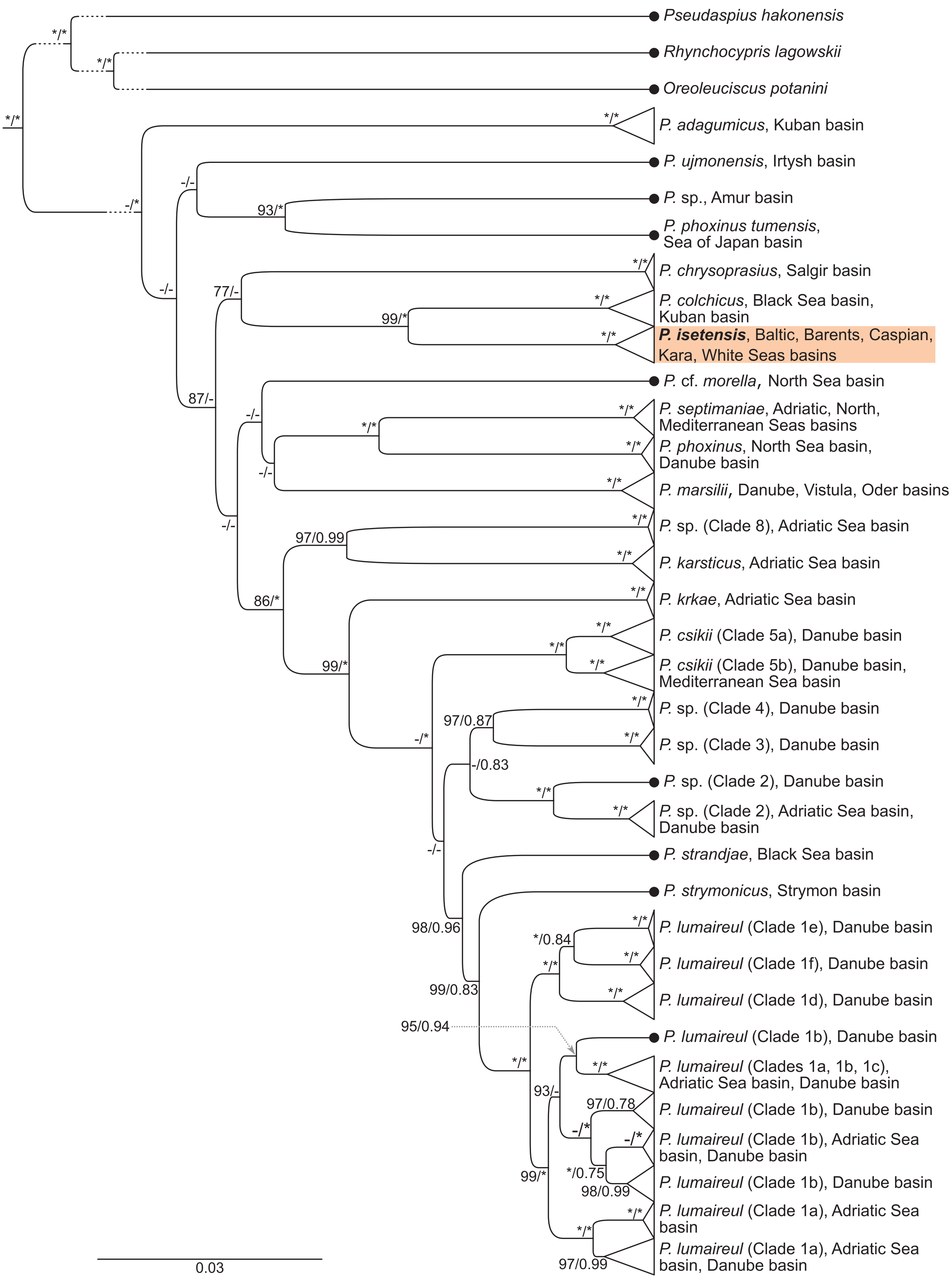

Figure 2. BI consensus tree of concatenated COI and cyt*b* mtDNA sequences representing available *Phoxinus* species in the NCBI data base combined with our data set. The numbers of some yet-unnamed clades are given according to the study of Palandačić et al. (2020). *Phoxinus isetensis* is highlighted with color. Bootstrap values/posterior probabilities above 70/0.7 are shown; asterisks represent 100/1 bootstrap/posterior probabilities values. The scale bar represents the expected substitutions per site. The nodes with multiple specimens were collapsed into a triangle, with the horizontal depth indicating the level of divergence within the node.

Figure 3. Neotype of *Phoxinus isetensis* (SL 63.9 mm, IBIW_FS_422, female). **A.** Live appearance; **B.** General appearance of the preserved specimen; **C.** Radiograph.

21 June 2023, O.N. Artaev, I.S. Turbanov, A.A. Bolotovskiy leg.

Additional material. see Suppl. material 1.

Comparative material. see Suppl. material 1.

Etymology. Since Lepechin (1771: 491) described species from the vicinity of Yekaterinburg, it can be assumed that Georgi (1775: 621) (see taxonomic remarks) gave its name to the Iset River, flowing through Yekaterinburg.

Diagnosis. *Phoxinus isetensis* is distinguished from other European minnows (*P. adagumicus*, *P. chrysoprasius*, *P. colchicus*, *P. csikii*, *P. krkae*, *P. lumaireul* (Clade 1a and Clade 1b), *P. marsilii*, *P. septimaniae*, *P. strandjae*, and *Phoxinus* sp. (Clade 2) by having a number of total vertebrae (39–43, mean 41.0, mode 41) and a number of caudal vertebrae (16–21, mean 18.9, mode 19).

Phoxinus isetensis is further distinguished from minnows from Eastern Europe (*P. adagumicus*, *P. chrysoprasius*, and *P. colchicus*) by a longer caudal peduncle (caudal peduncle length 2.5–3.7, mean 3.1 times caudal peduncle depth); fewer circumpeduncular scales (28–45, mean 35.3); fewer scale rows above the lateral line (10–21, mean 15.1); and a combination of characters, none of which is unique, as follows: eye horizontal diameter 5.9–8.8% SL, mean 7.2 and eye horizontal diameter 23.4–33.8% HL, mean 28.1; depth of caudal peduncle 6.6–9.0% SL, mean 7.7 in females and 7.2–9.5, mean 8.2 in males; caudal peduncle length 20.5–26.9% SL, mean 23.9 in females and 22.9–27.7 mean 25.1 in males; 8–16 scale rows below lateral line (mean 11.2, mode 11) (Suppl. materials 3, 4).

Description. The live and preserved appearance as well as radiograph of neotype is shown on Fig. 3, general appearance of live specimens of *Phoxinus isetensis* from different basins is shown on Fig. 4, morphometrics of neotype and additional material from the type locality with level of significance of sex-related differences are given in Table 1, meristic and qualitative characters for specimens from the type locality are given in Table 2, and primary morphological data for specimens from the type locality (neotype and additional material) are given in Suppl. material 2, the meristic and qualitative characters of *P. isetensis* and *Phoxinus* spp. are given in Suppl. material 4, the morphometrics of *P. isetensis*, *P. adagumicus*, *P. chrysoprasius*, and *P. colchicus* and their comparison are given in Suppl. material 3.

Figure 4. Live appearance of *Phoxinus isetensis* from different basins. **A.** Male in pre-spawning coloration, SL 48.5 mm, Nataleyka R. (Middle Volga basin), 53.9781°N, 45.6530°E, 05 May 2022; **B.** Female in pre-spawning coloration, SL 48.8 mm, same location and date; **C.** Female, SL 64.3 mm, Okhomlya R. (Baltic Sea basin), 58.7078°N, 33.5199°E, 12 September 2021; **D.** Female, SL 62.4 mm, Kyltymyu River (Northern Dvina basin), 61.4981°N, 50.5831°E, 21 September 2022; **E.** Male, SL 59.5 mm, Marat Bai River (Ural basin), 54.0668°N, 58.8038°E, 4 June 2023; **F.** Female, SL 63.6 mm, Karnasyavryok River (Barents Sea basin), 68.9314°N, 34.9318°E, 27 May 2023.

Table 1. Morphometrics of *Phoxinus isetensis* from the type locality (Severka River) (mean±SD - bold, and ranges - narrow) with level of significance of sex-related differences (primary data see in Suppl. material 2). * Difference between females and males, Mann-Whitney U test: ns ($p > 0.05$), + ($p < 0.05$), ++ ($p < 0.01$).

Morphometric characters	Neotype (female)	females, n=11	males, n=9	p*
SL	63.9	**56.5±3.7** 50.9–63.9	**50.5±2.7** 46.2–54.9	
In percentage of standard length (% SL)				
Body depth at dorsal-fin origin	18.0	**18.3±1.4** 15.9–20.2	**18.7±1.0** 16.7–20.0	ns
Body width at dorsal-fin origin	12.6	**13.4±0.8** 11.8–14.4	**12.6±1.2** 11.1–14.8	ns
Minimum depth of caudal peduncle	6.9	**7.7±0.4** 6.9–8.3	**8.3±0.4** 7.7–8.8	ns
Caudal peduncle width	8.7	**9.0±0.4** 8.3–9.6	**9.1±0.6** 7.5–9.7	+
Predorsal length	56.2	**56.5±0.7** 55.5–58.3	**55.6±1.3** 54.3–58.7	+
Postdorsal length	34.2	**33.8±1.3** 32.1–36.2	**34.2±0.9** 32.7–35.7	ns
Prepelvic length	47.8	**48.6±1.2** 47.1–51.1	**47.5±0.8** 46.1–48.4	ns
Preanal length	64.6	**65.0±1.5** 62.1–67.7	**63.4±0.9** 62.0–65.2	ns
Pectoral – pelvic-fin origin length	23.6	**24.8±1.2** 23.2–26.7	**23.0±1.1** 21.3–25.0	++
Pelvic – anal-fin origin length	17.7	**17.8±0.7** 16.5–18.9	**17.6±0.8** 16.6–19.0	ns
Caudal peduncle length	24.1	**23.9±1.0** 22.0–25.1	**25.3±0.8** 24.1–26.4	ns
Dorsal-fin base length	11.0	**11.1±0.6** 10.4–12.5	**11.6±0.6** 10.7–12.5	ns
Dorsal-fin depth	18.6	**18.9±0.7** 17.8–20.1	**20.8±1.0** 18.9–21.9	+
Anal-fin base length	11.4	**10.6±0.5** 9.7–11.4	**10.8±0.6** 10.0–11.6	ns
Anal-fin depth	18.6	**18.7±0.7** 17.5–19.9	**19.8±0.8** 18.7–21.1	++
Pectoral-fin length	17.1	**17.6±1.1** 16.3–20.1	**19.8±0.9** 18.1–20.7	+
Pelvic-fin length	13.2	**13.7±0.7** 12.9–15.4	**15.8±0.8** 14.7–17.1	+
Head length	25.6	**25.2±0.5** 24.4–25.8	**26.0±0.7** 24.8–27.5	ns
Head depth at nape	15.5	**15.5±0.5** 14.4–16.4	**16.4±0.4** 15.7–16.9	ns
Maximum head width	13.9	**13.6±0.5** 12.7–14.4	**13.6±0.6** 12.4–14.5	ns
Snout length	7.3	**7.6±0.3** 7.2–8.1	**7.8±0.3** 7.5–8.4	ns
Eye horizontal diameter	6.7	**6.9±0.3** 6.5–7.4	**7.1±0.3** 6.6–7.6	+
Interorbital width	8.5	**8.8±0.5** 8.2–9.7	**8.7±0.6** 7.6–9.6	ns
In percentage of head length (% HL)				
Maximum head width	54.3	**54.0±1.6** 51.5–56.0	**52.5±2.8** 47.7–55.9	ns
Snout length	28.4	**30.1±1.1** 28.4–31.6	**30.1±1.3** 28.3–32.6	ns
Head depth at nape	60.4	**61.3±1.8** 58.6–64.4	**63.2±1.8** 60.6–65.3	ns
Head depth through eye	48.3	**48.7±1.0** 47.4–50.8	**49.9±1.5** 48.1–52.2	ns
Eye horizontal diameter	26.2	**27.4±0.8** 26.2–28.8	**27.4±1.3** 25.3–29.6	++

Morphometric characters	Neotype (female)	females, n=11	males, n=9	p*
Postorbital distance	45.6	**44.5±1.1**	**44.8±2.1**	+
		42.4–45.9	41.9–47.6	
Interorbital width	33.4	**34.9±2.0**	**33.6±1.9**	ns
		31.7–38.6	29.5–35.4	
In percentage of caudal peduncle length				
Minimum depth of caudal peduncle	28.7	**32.4±2.0**	**32.8±2.2**	ns
		28.7–35.0	29.4–36.5	
In percentage of body depth				
Head length	142.5	**138.3±10.6**	**139.5±8.9**	+
		127.1–159.6	129.6–155.2	
In percentage of interorbital width				
Eye horizontal diameter	78.4	**79.0±5.2**	**81.8±4.1**	+
		72.4–91.0	75.5–85.8	
Ratios:				
Interorbital width/eye horizontal diameter	1.3	**1.3±0.1**	**1.2±0.1**	+
		1.1–1.4	1.2–1.3	
Snout length/eye horizontal diameter	1.1	**1.1±0.0**	**1.1±0.1**	ns
		1.0–1.2	1.0–1.2	
Head depth at nape/eye horizontal diameter	2.3	**2.2±0.1**	**2.3±0.1**	++
		2.0–2.4	2.2–2.4	
Head length/caudal peduncle depth	3.7	**3.3±0.2**	**3.1±0.2**	+
		3.1–3.7	2.9–3.4	
Length of caudal peduncle/caudal peduncle depth	3.5	**3.1±0.2**	**3.1±0.2**	ns
		2.9–3.5	2.7–3.4	
Pectoral fin length/pectoral – pelvic-fin origin distance	0.7	**0.7±0.1**	**0.9±0.1**	++
		0.7–0.9	0.7–0.9	
Predorsal length/head length	2.2	**2.2±0.0**	**2.1±0.1**	++
		2.2–2.3	2.0–2.3	
Body width at dorsal-fin origin/Caudal peduncle depth	1.8	**1.7±0.1**	**1.5±0.1**	+
		1.6–1.8	1.4–1.7	

Morphometrics (Table 1, Suppl. material 3). The maximum size among studied specimens 76.3 mm SL. The species has a slender and elongated caudal peduncle. The caudal peduncle depth 6.9% SL in neotype, 6.9–8.8% SL in additional material from type locality, and 6.6–9.5% SL in other additional materials (here and further – from basins of Caspian, Baltic, Barents, and Kara seas); caudal peduncle depth 28.7% in caudal peduncle length in neotype, 28.7–36.5 in additional material from type locality, and 26.9–40.6 in other additional material; caudal peduncle depth 3.5 times the caudal peduncle length in neotype, 2.9–3.4 in additional material from type locality, and 2.5–3.7 in other additional material. The species has a slender body, body depth at dorsal-fin origin 18.0% SL in neotype, 15.9–20.0 in additional material from type locality, and 14.7–21.6 in other additional material. Eyes larger (horizontal eye diameter: 26.2% HL in neotype, 25.3–29.6 in additional materials from type locality, 23.4–33.8 in other additional).

Meristics (Table 2, Suppl. material 4). Dorsal fin with 3 (very rarely 2) unbranched and (8) 7½ (6) branched rays. Anal fin with 3 unbranched and (6) 7½ (8) branched rays. Pectoral fin with 14–20, commonly 16–18 rays. Pelvic fin with (7) 8 (9) rays. Caudal fin with (18) 19 (20) rays.

Among 135 individuals, the most common pharyngeal teeth formula is classic for the genus 2.5–4.2 (n=102) (Fig. 5A; Suppl. material 4). Other variants are 2.5–4.1 (n=10), 2.4–4.2 (n=8), 1.5–4.2 (n=4), 2.5–5.2 (n=3), 1.5–4.1 (n=2), 2.4–4.1 (n=2), 2.3–4.2 (n=1), 2.5–4.3 (n=1), 2.3.5–4.3.2 (n=1). Among those one is exceptionally rare for phoxinin fishes – three-rowed formula 2.3.5–4.3.2 that was recorded in an individual from the Tsna R., Baltic Sea basin.

Forty-two total vertebrae in neotype, 40–42 in additional material from type locality, and 39–43 in other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 41 vertebrae. Twenty-one abdominal vertebrae in neotype, 21–23 in additional material from type locality, and 21–24 in other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 21–23 vertebrae. Twenty-one caudal vertebrae in neotype, 17–21 in additional material from type locality, and 16–21 in other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 18–20 vertebrae. Fourteen predorsal vertebrae in neotype, 14–16 in additional material from type locality, and 13–16 for other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 14–15 vertebrae. Three anal-fin pterygiophores in front of the first caudal vertebrae in neotype, 3–6 in additional material from type locality, and 3–7 in other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 4–6 pterygiophores. Difference between numbers of abdominal and caudal vertebrae zero in neotype, zero to 6 in additional material from type locality, and zero to 7 for other additional material from basins of Baltic and Barents seas, Volga and Ob rivers, commonly 2–5.

Table 2. Meristic characters of *Phoxinus isetensis* from the type locality (Severka River) (primary data see in Suppl. material 2).

Characters	Mean±SD (or mode) range	n
Total number of scales in lateral series (sql)	**86.1±4.4** 80–93	10
Total number of lateral-line (pored) scales (llt)	**50.8±15.1** 23–66	10
Number of pored scales in first complete (non-interrupted) section of lateral line (llcs)	**20.8±12.7** 1–45	10
Relative number of total lateral-line scales, quotient llt: sql (lltr)	**0.60±0.19** 0.26–0.83	10
Mean number of scale rows on left and right breast patches (BrPScale)	**8.4±1.0** 7.5–10.5	8
Number of circumpeduncular scales (cps)	**35.2±1.6** 33–39	10
Scales above lateral line (between lateral line and base of first unbranched ray in D) (all)	**15.4±1.3** 14–18	10
Scales below lateral line (between lateral line and base of first unbranched ray in A) (bll)	**11.4±1.6** 9–14	10
Pattern of scalation on the breast and anterior belly (cstyp)	4 4–6	8
Total number of pectoral-fin rays (P)left	**16.4±0.8** 15–18	10
Total number of pelvic-fin rays (V)	**8.0±0.5** 7–9	10
Number of branched dorsal-fin rays (D)	**7.0±0.0** 7–7	10
Number of branched anal-fin rays) (A)	**7.0±0.0** 7–7	10
Number of rays in caudal fin (C)	**18.7±0.5** 18–19	10
Total number of vertebrae (tv)	**40.9±0.6** 40–42	30
Number of abdominal vertebrae (abdv)	**21.9±0.7** 21–23	30
Number of caudal vertebrae (caudv)	**19.0±0.9** 17–21	30
Number of predorsal abdominal vertebrae (preDv)	**14.7±0.5** 14–16	30
Number of anal-fin pterygiophores in front of the first caudal vertebrae (preAp)	**4.9±0.9** 3–6	30
Difference between numbers of abdominal and caudal vertebrae (dac)	**2.9±1.5** 0–6	30
Gill rakers in first arch	**8.5±0.8** 7–10	10

Seventy-one to 103 (mean 85.7) total number of scales in the lateral series. Lateral line incomplete and interrupted. The relative number of total lateral-line (pored) scales varies greatly from 12% to 99%, mean 61%. Five to 11 (commonly 6–9) scale rows on breast patches. 28–45 (mean 35.2) circumpeduncular scales. Ten to 21 (mean 15.1) scale rows above lateral line. Eight to 14 (16), mean 11.2 scale rows below lateral line.

Seven to 10 (mode 8) gill rakers (in series from type locality) on first arch.

Qualitative characters. Pectoral fins do not reach the beginning of pelvic fins in females and most of the males (ca. 75%). In the most specimens (ca. 85%) tip of the upper lip above horizontal level of lowest point of the eye and in about 5% of specimens – at this level. Origin of anal fin is mainly ahead or at vertical level of posterior insertion of the dorsal fin (ca. 45% for each form), rarely behind (ca. 8%). Free margin of the dorsal fin is mainly straight or slightly convex, rarely slightly concave; anal fin most often slightly concave and straight, rarely slightly convex. 3rd–6th type of breast scalation (mode 4th type, often 3th type, 5th and 6th type are less common) (Fig. 5B, Suppl. material 4).

Coloration. Males and females outside of spawning have predominantly brown coloring of the upper half of the body and light lower part in males and white in females (Fig. 4). Juveniles often show a large contrast: the black horizontal stripe and the white belly. During spawning, color of both sexes becomes much brighter, the color of the sides is dominated by green (many males become dark green, almost black), in front, it is mixed with golden, less often purple and red. In males, as well as some females, the lips and lower jaw, as well as body at the bases of the pectoral, pelvic, and anal fins, become red. The operculum is blue and the suboperculum is yellow in both sexes, but coloration is much more pronounced in males. In both sexes, the bases of ventral and anal fins are light blue. The specimens preserved in formalin had a yellowish color, which is somewhat darker with a brown tint in the upper parts.

Sexual dimorphism. Significant differences are observed in 18 out of 41 morphometric characters (Table 1). In general, females have smaller relative anal (anal-fin depth), dorsal (dorsal-fin depth), pelvic (pelvic-fin length), and pectoral fins (pectoral-fin length), a greater predorsal length, and pectoral – pelvic-fin origin length. In females, the pectoral fins never reach the pelvic fins, while in ca. 25% of males, reach.

Taxonomic remarks. According to the early literary sources reviewed in Berg (1912), the first name within the range of species is *Cyprinus isetensis*, given by Georgi (1775: 621), which lists species (without description) for the Chusovaya River with reference to Lepechin (1771: 491). Lepechin gives a description of the species but does not give the species name, designated as “CYPRINVS” with the type locality “circa Catharinopolin” (now Yekaterinburg). Although Georgi does not provide a description of species when mentioning the name *isetensis*, he makes a reference to the description of this species in Lepechin’s study, which makes this name valid since it complies with Art. 12.1 and 12.2 of the International Code of Zoological Nomenclature (ICZN 1999).

Type locality. The type locality from the original description (Lepechin 1771: 493) is “… habitat in rivis scopulosus circa Catharinopolin,” which means “… lives in the rocky streams around Catharinopolin (now Yekaterinburg).” Probably Lepechin meant the upper reaches of the Iset, Chusovaya, and Tura rivers, which were specified in further publication (Lepechin 1772: 311).

Type locality for the neotype: Severka River (56.8830°N, 60.2716°E) upstream of Severka village near Yekaterinburg, Sverdlovsk Oblast, Russia (Fig. 6). A tributary of the Reshotka River → Iset River → Tobol River → Irtysh River → Ob River → Kara Sea.

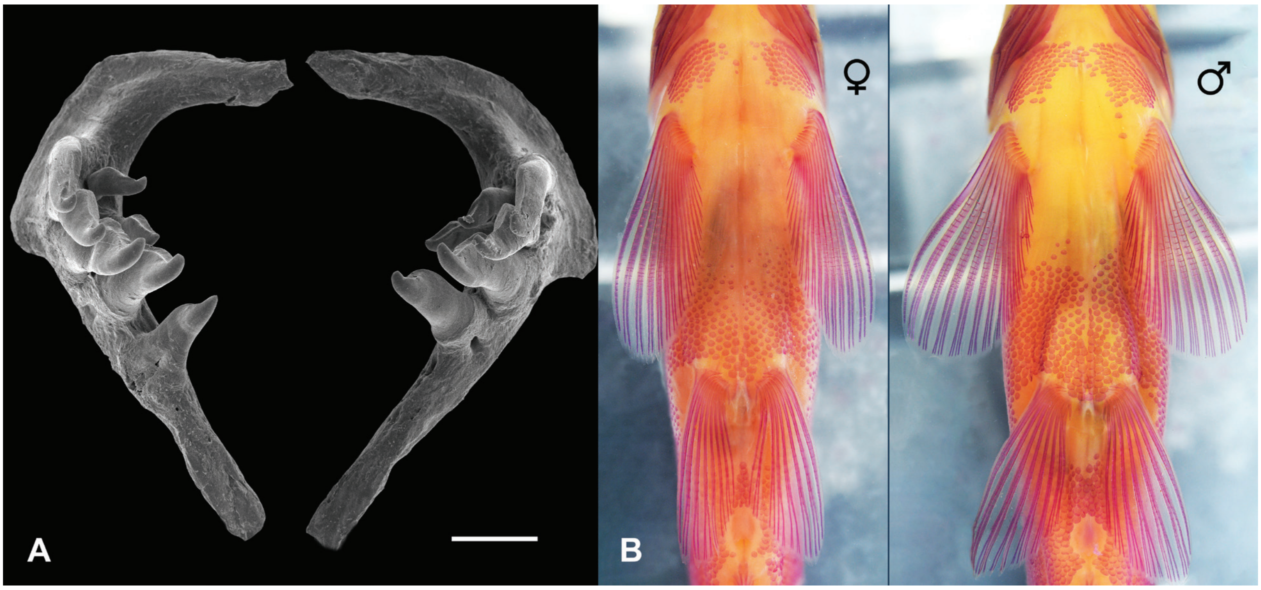

Figure 5. Morphological features of *Phoxinus isetensis*. **A.** Most frequent variant of the formula of pharyngeal bones: double-rowed formula 2.5–4.2, scale bar 0.5 mm; **B.** Ventral view of alizarin-stained female and male from the Bekshanka River (Volga basin). Female had 5th type scalation on breast and belly, male had 6th type.

Figure 6. Example of habitat in the Severka River, type locality for *Phoxinus isetensis*, 21 June 2023.

Nomenclatural and taxonomic actions. The need to designate a neotype for *P. isetensis* is determined by the following considerations: first, our attempts to find a type specimen at the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (ZISP), where the largest and oldest ichthyological collection in Russia is stored, were unsuccessful. At the end of the 18th century, at the time of the description of *P. isetensis*, it was the only scientific organization in Russia where type specimens were deposited. The type specimens of *P. isetensis* were absent in the ZISP already at the beginning of the 20th century (Berg 1912). Second, no type specimens for all the fish species described by the naturalist and explorer Johann Gottlieb Georgi (1729–1802) were designated

(Fricke et al. 2024). Thus, we conclude that Georgi did not designate a type series for this species. Third, in addition to *P. isetensis*, at least one more species of this genus inhabits the Ob River basin—*P. ujmonensis* Kashchenko, 1899 (see Discussion), and their possible sympatric co-occurrence requires further clarification.

Thus, based on the above-mentioned circumstances and in accordance with Article 75 of the ICZN, we designate a neotype for *P. isetensis*. Our nomenclatural actions do not contradict the statements of Article 75.3 (qualifying conditions), and the designation of a nomenclatural type (neotype) for *P. isetensis*, as a widespread species living in Europe and Asia, will make it possible to clearly describe both morphological and genetic differences from other species of the genus *Phoxinus* (Articles 75.3.1 and 75.3.2).

Distribution and habitat. Widespread in northern and eastern Europe and in the western edge of Siberia (Iset and partially Ural basin). *Phoxinus isetensis* inhabits the basins of the Caspian, Baltic, White, Barents, and Kara seas, possibly occurring in the North Sea basin. In the Caspian Sea basin, it is widely distributed in the upper and middle Volga, Kama, in the mountainous part of the Ural basin. In the Baltic Sea basin, it is widespread in the northern and eastern parts. In the Kara Sea basin, it is known in the Iset basin (Ob basin). According to Palandačić et al. (2017, 2020), mtDNA of this species (Clade 17) was detected in Scandinavia and the British Isles, suggesting that minnows from these areas also belong to the species *P. isetensis*.

Dyldin et al. (2023: 671) pointed out the distributional range of *P. isetensis* as “Arctic Ocean basin, from Murman coast to East Siberian Sea basin (Kolyma basin); rivers of northern and western Sea of Okhotsk basin (Ola and Uda rivers); rivers of Peter the Great Bay drainage, probably Amur River basin, and northwestern Sakhalin Island.” This data only partially corresponds to the above-mentioned range. See further explanations in the discussion.

Phoxinus isetensis prefers rivers with fast-flowing water that are rheophilic. In the northern regions, it also inhabits riverbeds of large rivers, lakes, and brackish waters (Berg 1949: 590; Tsvelev 2007: 277; our data).

Morphology comparison. PCA of 41 morphometric characters shows differences between *P. isetensis* and *P. adagumicus*, *P. chrysoprasius*, and *P. colchicus* from the Crimean Peninsula and the Caucasus (Fig. 7). The greatest difference is from *P. colchicus* (no overlap), while the remarkable overlap with the other two species (*P. adagumicus* and *P. chrysoprasius*) is noted.

Compared to *P. abanticus* from the Lake Abant basin in Türkiye (Turan et al. 2023), *P. isetensis* has scales on the breasts in both sexes (vs. absence of scales on the breast in males); 18–20 rays in the caudal fin (vs. 15–16 rays); and a more slender caudal peduncle (6.6–9.5, mean 8.0 vs. 11.0–12.7, mean 12.0).

Compared to *P. adagumicus* from the Kuban basin (Artaev et al. 2024), *P. isetensis* has fewer scale rows above the lateral line (10–21, mean 15.1, vs. 15–24, mean 18.4); more total vertebrae (39–43, mean 41.0, mode 41, vs. 39–42, mean 40.4, mode 40); more caudal vertebrae (16–21, mean 18.9, mode 19, vs. 16–19, mean 18.0, mode 18); double-row pharyngeal teeth with modal formula 2.5–4.2 (vs. single-row pharyngeal teeth with modal formula 5–4) (Suppl. material 4).

Compared to *P. bigerri* from the Adour and Ebro basins in France and Spain (Kottelat 2007), *P. isetensis* has fewer scale rows above the lateral line (10–21, mean 15.1, vs. 19–23).

Compared to *P. chrysoprasius* from the rivers of the Crimean Peninsula (Artaev et al. 2024; Bogutskaya et al. 2023), *P. isetensis* has a slightly selender caudal peduncle (minimum depth of caudal peduncle (6.6–9.0% SL, mean 7.7 in females and 7.2–9.5, mean 8.2 in males (vs. 8.4–9.9, mean 9.1 in females and 8.1–11, mean 9.9 in males); slightly fewer circumpeduncular scales–28–45, mean 35.3 (vs. 41–55, mean 46.2); more total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 38–42, mean 40.4, mode 40); more number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 16–20, mean 18.0, mode 18) (Suppl. material 4).

Compared to *P. colchicus* from the Black Sea coast of the Caucasus and Kuban basin (Artaev et al. 2024; Bogutskaya et al. 2023), *P. isetensis* in both sexes have a slenderer caudal peduncle (minimum depth of caudal peduncle in percentage of caudal peduncle length 26.9–40.6, mean 32.5; vs. 42.0–58.6, mean 49.5); (Suppl. material 3); less number of circumpeduncular scales – 28–45, mean 35.3 (vs. 36–48, mean 40.9); less number of scales above lateral line – 10–21, mean 15.1 (vs. 16–23, mean 18.8); fewer scale rows below lateral line – 8–16, mean 11.2 (vs. 11–17, mean 13.4); different patterns of scalation on the breast and anterior belly – 3rd –6th types, mode 4th (vs. 3rd–10th, 13th and 14th, modal 6th); more total number of vertebrae – 39–43, mean 41.0, mode 41 (vs. 39–42, mean 40.1, mode 40); more number of caudal vertebrae – 16–21, mean 18.9, mode 19 (vs. 16–19, mean 17.6, mode 18) (Suppl. material 4).

Compared to *P. csikii* from the Danube River basin, Montenegro, and Bulgaria (Bogutskaya et al. 2019, 2023), *P. isetensis* has a different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 3rd–9th types, 11th, mode 7th); more total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 38–42, mean 40.1, mode 40); more number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 15–19, mean 17.4, mode 17); smaller difference between numbers of abdominal and caudal vertebrae–0–7, mean 3.2 (vs. 2–9, mean 5.4) (Suppl. material 4).

Compared to *P. krkae* from the Krka River, Croatia (Bogutskaya et al. 2019), *P. isetensis* has a greater number of lateral-line scales (pored) 12–94, mean 53.7 (vs. 11–54, mean 32.6); different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 3rd–7th types, mode 6th); more total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 37–40, mean 38.4, mode 39); more number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 15–18, mean 16.8, mode 17) (Suppl. material 4).

Compared to *P. lumaireul* Clades 1a and 1b from rivers in Adriatic and Black Sea basins in Italy, Slovenia, and Croatia (Bogutskaya et al. 2019), *P. isetensis* has a

Figure 7. PCA based on 41 morphometric characters for *Phoxinus* spp. – *P. isetensis*, *P. chrysoprasius* from the Crimean Peninsula, *P. colchicus*, and *P. adagumicus* from the Caucasus – and loading plot showing how strongly each character influences principal components. Samples of *P. isetensis* from the type locality (Severka River) are encircled by black.

different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 2nd–7th types, mode 3rd); more total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 37–41, mean 39.3, mode 39); more number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 16–19, mean 17.5, mode 18) (Suppl. material 4).

Compared to *P. marsilii* from the Danube River basin, Austria, and Croatia (Bogutskaya et al. 2019, 2023), *P. isetensis* has a different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 3rd–8th types, mode 6th); more total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 38–42, mean 40.1, mode 40) (Suppl. material 4).

Compared to *P. radeki* from the Ergene River (Aegean Sea basin) in Türkiye (Bayçelebi et al. 2024), *P. isetensis* has a higher number of scales above the lateral line-10–21 (vs. 9–15) and below the lateral line–8–16 (vs. 6–9).

Compared to *P. septimaniae* from the Herault River, France (Bogutskaya et al. 2019), *P. isetensis* has a different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 12th–14th types, mode 14th); a greater total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 37–41, mean 39.3, mode 39); a greater number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 16–19, 21, mean 17.6, mode 18) (Suppl. material 4).

Compared to *P. strandjae* from the rivers of the Black Sea basin, Bulgaria, and the rivers of the Marmara Sea, Türkiye (Bogutskaya et al. 2019, 2023), *P. isetensis* has a different pattern of scalation on the breast and anterior belly–3rd–6th types, mode 4th (vs. 3rd–12th types, mode 11th); a greater total number of vertebrae–39–43, mean 41.0, mode 41 (vs. 38–42, mean 39.8, modal 40); a greater number of caudal vertebrae–16–21, mean 18.9, mode 19 (vs. 16–19, mean 17.7, mode 18) (Suppl. material 4).

Compared to *P. strymonicus* from the Strymon basin in Greece and Macedonia (Kottelat, 2007), *P. isetensis* has a smaller number of scales above the lateral line: 10–21 (vs. 21–24).

Compared to *P.* cf. *ujmonensis* from the Mundybash River in Altai part of the Ob basin (Suppl. material 4), *P. isetensis* has a smaller number of scales above the lateral line: 10–21, mean 15.1 (vs. 15–19, mean 17.3); and a number of scales below the lateral line: 8–16, mean 11.2 (vs. 12–15, mean 12.9).

Discussion

Our study recognized the unnamed *Phoxinus* sp. with the largest range in Europe (Clade 17 sensu Palandačić et al. 2017) as *P. isetensis*, the species that was described from the upper reach of the Chusovaya River (Kama-Volga basin) and the Iset River basin (Ob basin) in the Middle Urals. We redescribed the morphology and significantly clarified the distributional range of this species. According to our results, *P. isetensis* can be distinguished from other neighbor species by the large total number of vertebrae – 39–43 (mode 41) (vs. 37–42), primarily due to an increase in the number of caudal vertebrae – 16–21 (mode 19) (frequency distribution see in Suppl. material 4) and in sequences of COI mtDNA (minimal *p*-distance = 0.05 to closely related *P. colchicus*). Remarkably, there is some discrepancy between the genetic and morphological similarities of *P. isetensis*. Being close genetically to *P. colchicus* from the West Caucasus, it has more significant morphological differences with this species compared to other phylogenetically more distant species, *P. adagumicus* and *P. chrysoprasius* (Figs 2, 7). In particular, these species are more similar to *P. isetensis* in total number of vertebrae and number of caudal vertebrae (Suppl. material 4). Of the eighteen populations of *P. isetensis*, seventeen had modes of 41 or 42 vertebrae, and only one population from the Baltic Sea basin had 40 vertebrae. This main morphological difference – an increased number of vertebrae and northernmost range relative to other European *Phoxinus* – is consistent with Jordan's rule (vertebral number in fish increases with latitude) (McDowall 2008). However, it is noteworthy that within the species range extended in the latitudinal direction for more than 2000 km within 51–69 northern latitudes, there was no correlation.

Phoxinus isetensis redescribed in this study was recently revalidated in Dyldin et al. (2023), but the authors outlined its range mistakenly for the whole of West and East Siberia as well as for Northeast Asia and the Far East due to the absence of genetic and morphological data. According to our genetic results, the *P. isetensis* described from the rivers near Yekaterinburg belonging to both the upper stream of left tributaries of the Ob River and the Chusovaya River itself (Kama-Volga basin) are identical or very similar (conspecific) to other samples from the Volga basin, the Ural basin, and the remaining range as outlined for Clade 17 sensu Palandačić et al. (2017). Therefore, *P. isetensis* has a huge range in northern and eastern Europe, extending approximately 3.800 km from west to east and 2.000 km from north to south. Its range in Europe includes the basins of the Caspian, Baltic, White, Barents, and North Seas. In the Caspian Sea basin, it is widespread in the Upper and Middle Volga and Kama basins. In the Baltic Sea basin, it is widespread in the northern and eastern parts. In the North Sea basin, it is noted for Scandinavia and the British Isles (Palandačić et al. 2017).

To the best of our knowledge, the occurrence of *P. isetensis* in Asia is restricted to the Ural basin (Caspian Sea basin) and the Iset basin (Tobol River → Irtysh River → Ob River, which flows into the Kara Sea). The Ural Mountains are a well-known biogeographic barrier between Europe and Asia (Abell et al. 2008), which, however, is characterized by the corridor linking Europe and Asia in the Middle Ural via the Chusovaya River Valley. It was assumed this corridor might facilitate aquatic fauna exchange between Siberia (Ob basin) and European Russia (Volga basin) (Kostarev 1973; Makhrov et al. 2021). The Chusovaya River belongs to the Kama-Volga basin, but its upper reaches are located in Siberia – eastward the main Ural ridge and sharing a watershed with the Ob basin through the Iset river basin. We have to note that in the upper reaches of the Chusovaya River, there are a number of man-made reservoirs. One of those, the Volchikha Reservoir, has a connection through a small artificial canal to the Ob basin via the Reshotka River, a tributary of the Iset River. The reservoir and canal are dated to the mid-1940s (Korlyakov and Nohrin 2014). Although it can be assumed that an artificial canal could facilitate the dispersal of *P. isetensis* from the Kama-Volga system to the Ob basin, populations of minnow, at least in the Iset basin, apparently colonized the Ob basin rather earlier. First, it is supported by early evidence of the presence of conspecific populations in both the Ob and Chusovaya basins by Lepechin (1772) and Georgi (1775). This is further supported by the fact that not only the Severka (type locality), but other rivers in the Iset basin, inhabited just one species of minnow, *P. isetensis* – for instance, in the Kushtumga River (genetic data), which is located ca. 750 km along the riverbed from the canal connecting the Volga and Ob basins near Yekaterinburg (Fig. 1).

Our finding in the distributional pattern of *P. isetensis* is additional evidence of faunal exchange between Siberia and Europe via the Chusovaya River Valley. Contrary to previous observations and suggestions on fish migrations from Siberia to Europe (Kostarev 1973; Marić et al. 2014; Perdices et al. 2015; Levin et al. 2017; Zinoviev and Bogdanov 2017; Vinarski et al. 2021), the case of *P. isetensis* suggests that fish migrations took place in the reverse direction too. Remarkably, the eastward direction of the recent (seemingly postglacial) colonization pattern of *P. isetensis* is also corroborated by its presence in the Upper and Middle Ural systems.

Noteworthy, one more *Phoxinus* species inhabits the Ob basin – *P. ujmonensis*, described from the upper tributaries of the Ob in the Altai Mountains. This species is genetically distant from *P. isetensis* (Fig. 2, Suppl. material 7). Our unpublished molecular data indicate that the distribution of *P. ujmonensis* in the Ob basin is not restricted by the Altai Mountains. The distribution of both species in the Ob basin needs further clarification.

Based on the current distribution of *P. isetensis* (Fig. 1), this species might rapidly colonize large northern areas following the deglaciation of the last Pleistocene glaciers. This is in line with the previously proposed hypothesis about the colonization of Scandinavia by freshwater fish from the southeast (Museth et al. 2007). Within the boundaries of the Last Glacial Maximum (LGM), the habitation of two other *Phoxinus* species (clades) is confirmed – *P.* cf. *morella* and Clade 10 (*P. phoxinus*) from Ireland, with an unknown origin of population that might be introduced – Denys et al. (2020) (Fig. 1). However, northern populations of those species are located on the southern periphery of the LGM, while *P. isetensis* inhabits most of the deglaciated region. One may suggest that during the colonization of new habitats after LGM, *P. isetensis* could hybridize with other minnow species. This hypothesis may be supported by a decrease in the total number of vertebrae (mean 40.5 vs. 41.1 in the rest of the range, Suppl. material 4) in the potential contact zone – the east of the Baltic Sea basin—and it should be tested in the future. It is noteworthy that in northern Norway, the *Phoxinus* minnow has been a significant expansion of the range as a result of introductions since the 1900s (Museth et al. 2007); its ancient DNA was not found in the sediments dating to 5.6–13 thousand years ago from the Storsteinhola cave, while the ancient DNA of species currently sharing biotopes with *Phoxinus* (*Barbatula* sp. and *Gobio gobio*) was found (Boilard et al. 2024). Seemingly, along with the post-glacial expansion of *P. isetensis* northward, a shrinking of the range from the south can be suggested. For example, Falk (1786: 432) recorded *Cyprinus phoxinus* for the Volga and small rivers (“Zariza, Jelschanka. Sarpa u. f.”) near Volgograd city in Russia (Fig. 1). This is > 450 km southward from the most southern contemporary records in the upper reaches of the Tereshka (Artemyeva and Selishchev 2005) and Sura basins within the Volga system. Although the right drainage of the Volga north of Volgograd is steep and rich in small tributaries with fast-flowing water, minnows were not recorded in this region. Probably populations in these small rivers were extinct, being vulnerable to changes in the environment, including general climate warming.

Acknowledgements

The authors are very grateful to taxonomist Boris Kataev for his valuable advice on zoological taxonomy and nomenclature. We are very thankful to Alexandra Komarova, who helped with sampling. This study was supported by the Russian Science Foundation, grant no. 24-44-20019, “Fishes of the Caspian Sea basin: genetic diversity, evolution, and biogeography”.

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Supplementary material 1

Additional and comparative materials

Authors: Oleg N. Artaev, Aleksey A. Bolotovskiy, Ilya S. Turbanov, Alexander A. Gandlin, Aleksey V. Kutuzov, Marina A. Levina, Danila A. Melentev, Ivan V. Pozdeev, Mikhail Ya. Borisov, Boris A. Levin

Data type: docx

Explanation note: Additional material on *Phoxinus isetensis* and comparative material on *P. adagumicus*, *P. chrysoprasius* and *P. colchicus*.

Link: https://doi.org/10.3897/zse.100.126702.suppl1

Supplementary material 2

Primary morphological data from type locality

Authors: Oleg N. Artaev, Aleksey A. Bolotovskiy, Ilya S. Turbanov, Alexander A. Gandlin, Aleksey V. Kutuzov, Marina A. Levina, Danila A. Melentev, Ivan V. Pozdeev, Mikhail Ya. Borisov, Boris A. Levin

Data type: xlsx

Explanation note: Primary morphological data of *Phoxinus isetensis* from type locality (Severka River).

Link: https://doi.org/10.3897/zse.100.126702.suppl2

Supplementary material 3

Comparsion of morphometrics

Authors: Oleg N. Artaev, Aleksey A. Bolotovskiy, Ilya S. Turbanov, Alexander A. Gandlin, Aleksey V. Kutuzov, Marina A. Levina, Danila A. Melentev, Ivan V. Pozdeev, Mikhail Ya. Borisov, Boris A. Levin

Data type: xlsx

Explanation note: Morphometrics of *Phoxinus isetensis*, *P. adagumicus*, *P. chrysoprasius*, *P. colchicus* and its comparison.

Link: https://doi.org/10.3897/zse.100.126702.suppl3

Supplementary material 4

Meristic and qualitative characters

Authors: Oleg N. Artaev, Aleksey A. Bolotovskiy, Ilya S. Turbanov, Alexander A. Gandlin, Aleksey V. Kutuzov, Marina A. Levina, Danila A. Melentev, Ivan V. Pozdeev, Mikhail Ya. Borisov, Boris A. Levin

Data type: xlsx

Explanation note: Meristic and qualitative characters of *Phoxinus isetensis* and other *Phoxinus* species published in the literature.

Link: https://doi.org/10.3897/zse.100.126702.suppl4

Supplementary material 5

Material for genetic studies

Authors: Oleg N. Artaev, Aleksey A. Bolotovskiy, Ilya S. Turbanov, Alexander A. Gandlin, Aleksey V. Kutuzov, Marina A. Levina, Danila A. Melentev, Ivan V. Pozdeev, Mikhail Ya. Borisov, Boris A. Levin

Data type: xlsx

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Link: https://doi.org/10.3897/zse.100.126702.suppl5

Supplementary material 6

Best partition schemes

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Data type: docx

Explanation note: The best partition schemes generated by ModelFinder v.2.2.0 (ML) and PartitionFinder v.2.1.1 (BI).

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Link: https://doi.org/10.3897/zse.100.126702.suppl6

Supplementary material 7

ML tree

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Data type: docx

Explanation note: ML phylogenetic tree of concatenated COI and cyt*b* mtDNA sequences.

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Link: https://doi.org/10.3897/zse.100.126702.suppl7

Supplementary material 8

Genetic *p*-distances

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Data type: xls

Explanation note: estimates of average evolutionary divergence over sequence pairs within and between *Phoxinus* species and clades.

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Link: https://doi.org/10.3897/zse.100.126702.suppl8

Zeitschrift/Journal: Zoosystematics and Evolution

Jahr/Year: 2024

Band/Volume: 100

Autor(en)/Author(s): diverse

Artikel/Article: Forgotten for two centuries: redescription of Phoxinus isetensis (Georgi, 1775) (Cypriniformes, Leuciscidae) – the most widespread minnow in Europe 1155-1173