

Taxonomic revision of *Phoxinus* minnows (Leuciscidae) from Caucasus, with description of a new narrow-ranged endemic species

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Abstract

Taxonomic revision of *Phoxinus* from the Caucasus revealed two distinct species. One species, *P. colchicus*, was known from eastern drainage of Black Sea, but was recorded also in the middle reach of the Kuban (Sea of Azov basin), for the first time. The Kuban population represents a genetically unique sub-lineage of *P. colchicus*. Its ancestors might have colonized the Kuban system through the event of ancient river capture. Another species inhabits only the Adagum River basin in the lower Kuban and represents a new narrow-ranged endemic species – *Phoxinus adagumicus* sp. nov. According to mtDNA phylogeny (COI and *cytb*), *P. adagumicus* sp. nov. represents deeply divergent and one of the two early branched lineages of the genus *Phoxinus* being distant to other species (min. *p*-distance = 0.074) including geographical neighbors – *P. chrysoprasius* from Crimean Peninsula and *P. colchicus* from the Caucasus. The new species differs from most *Phoxinus* species by frequently occurring single-row pharyngeal teeth (modal formula 5–4). The narrow geographic range (ca. 55 km in length and 15–20 km in width) and high anthropogenic load on local water systems suggests the new species is under threat and needs protection.

Key Words

Caucasus, DNA barcoding, endemics, freshwater fish, taxonomy

Introduction

Minnows of the genus *Phoxinus* Rafinesque, 1820 are small freshwater fish in the family Leuciscidae Bonaparte, 1835, which prefer rheophilic environment and are widespread in Eurasia from northern Spain eastward to the Anadyr and Amur drainages in Russia and China. Before the implementation of genetic methods to the taxonomy, the genus *Phoxinus* was represented by a few species despite its wide geographic range (Kottelat 1997). Although detailed morphological studies still contribute to identification of new species in *Phoxinus* (Kottelat 2007; Bianco and De Bonis 2015), introduction of genetic methods to *Phoxinus* taxonomy has resulted in a significant increase

in the numbers of newly described species, and taxonomic and geographic range revisions (Palandačić et al. 2015, 2017, 2020; Vucić et al. 2018; Denys et al. 2020; Turan et al. 2023). In particular, recent genetic studies have revealed: i) deep genetic divergences within the genus, and ii) presence of numerous yet undescribed species. On the other hand, discovered patterns of genetic diversity do not always correspond to the morphology-based classification (Palandačić et al. 2017).

The Caucasus is a mountainous transcontinental region between the Black and Caspian seas. It is considered a significant Pleistocene refugium and a hotspot (i.e., the Caucasus Biodiversity Hotspot) of endemism for both plants and animals (Gadzhiev et al. 1979; Kolakovskiy 1980;

Nakhutsrishvili et al. 2015; Mahmoudi et al. 2019; Parvizi et al. 2019; Myers et al. 2000; Gandlin et al. 2022), that remains insufficiently studied from an evolutionary standpoint. The freshwater fish fauna of the Caucasus is rich, highly endemic (up to 68%; Naseka 2010), ecologically diverse, important for fisheries, and despite permanent interest is still significantly underexplored (Kuljanishvili et al. 2020). During the last decade, 15 new species have been described from the Caucasus Biodiversity Hotspot: one species from each of the genera *Rhodeus* Agassiz, 1832 (Esmaili et al. 2020), *Benthophilus* Eichwald, 1831 (Kovačić et al. 2021), *Cobitis* Linnaeus, 1758 (Vasil'eva et al. 2020), and *Gobio* Cuvier, 1816 (Turan et al. 2016); two species from each of the genera *Capoeta* Valenciennes, 1842 (Levin et al. 2019a; Roman et al. 2022) and *Oxynoemacheilus* Bănărescu et Nalbant, 1966 (Çiçek et al. 2018; Freyhof et al. 2021); three species in the genus *Salmo* Linnaeus, 1758 (Turan et al. 2022); and four species in the genus *Ponticola* Iljin, 1927 (Vasil'eva et al. 2015; Eagderi et al. 2020; Zarei et al. 2022a, b; Epatashvili et al. 2023). Species status of some other taxa was re-evaluated (e.g., *Barbus rionicus* Kamensky, 1899 – Levin et al. 2019b; Sevan trout complex *Salmo ischchan* Kessler, 1877 – Levin et al. 2022; *Proterorhinus nasalis* (De Filippi, 1963) – Zarei et al. 2022c), or new species for Caucasus were recorded due to increase of knowledge on the geographic distribution of some species described outside the region — e.g., *Alburnoides samiii* Mousavi-Sabet, Vatandoust & Doadrio, 2015 (Levin et al. 2018).

Minnnows of the genus *Phoxinus* are distributed in the western part of the Caucasus, Black and Azov seas drainage and absent in the eastern part of the Caucasus – in the Caspian Sea basin (Berg 1949; Naseka 2010). Widely distributed *P. colchicus* Berg, 1910 is known from the Eastern drainage of Black Sea – from the Chorokh River system in south (Turkey and Georgia) to small rivers nearby Novorossiysk in north (Russia) (ca. 550 km along coastline). The Kuban River basin (Azov Sea drainage) is locally inhabited by the *Phoxinus* sp., whose taxonomy remains ambiguous for a long time. The Kuban species was referred to *P. phoxinus* (Linnaeus, 1758) (Malyatsky 1930; Berg 1949; Sukhanova and Troitskiy 1949; Reshetnikov et al. 2003), but recent molecular studies (Palandačić et al. 2017, 2020) have shown that they form independent phyletic lineages. In addition, the geographic range of the Kuban *Phoxinus* lineage appears to be discontinuous (Artaev et al. 2021). Some populations were found in the system of the Adagum River (50–130 m a.s.l.), a left tributary of Lower Kuban, while others are located in a narrow riverine segment of the Belaya River (280–580 m a.s.l.), a large left tributary of the Kuban River. Therefore, a discontinuous geographic range as well as difference in the altitude of the identified habitats question the taxonomic identity of these populations.

Using an integrative morphological and molecular framework, this study aims to assess and revise the taxonomic diversity and distribution of *Phoxinus* spp. from the Caucasus, with a special focus on the Kuban basin populations.

Materials and methods

Sampling

Fishes were collected by the authors from different localities using frame and seine nets (mesh size 6–8 mm) (Fig. 1; Suppl. material 1). Fishes 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) equipped with a Nikkor 60 mm f/2.8G lens (Nikon Corporation, Tokyo, Japan). Fin clips (pectoral or pelvic) were collected from some specimens (DNA-vouchers) and fixed in 96% ethanol for subsequent DNA extraction in the laboratory. Most of the collected specimens were preserved in 10% formalin (form.), whereas some smaller 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.

The types (holotype, part of paratypes), additional and comparative material are deposited in the Fish collection of the Papanin Institute for Biology of Inland Waters of Russian Academy of Sciences, Borok, Russia (**IBIW_FS**); the rest of the paratypes are kept in the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (**ZISP**) and the Zoological Museum of the Moscow University, Moscow, Russia (**ZMMU**).

Morphological studies

Morphological analyses included *Phoxinus* spp. materials from fifteen localities (No. 1–2, 4, 6–8, 10, 12, 14, 16–19 and 21–22 on Fig. 1; Suppl. material 1). Morphological methods followed Bogutskaya et al. (2019, 2023). In particular, 28 morphometric measurements (Suppl. material 2), 17 meristic and two qualitative characters (Suppl. material 3), were investigated. Morphological abbreviations of the examined characters are explained in Suppl. material 4. 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 size of the pectoral fins. External meristics were counted on the left side. The total number of the pectoral and pelvic-fin rays was counted on the left fins. Scales above lateral line were counted between lateral line and base of first unbranched ray of dorsal fin; scales below lateral line were counted between lateral line and base of first unbranched ray of anal fin. In both cases, lateral line scales were not taken into account. The number of anterior gill rakers of the first gill arch

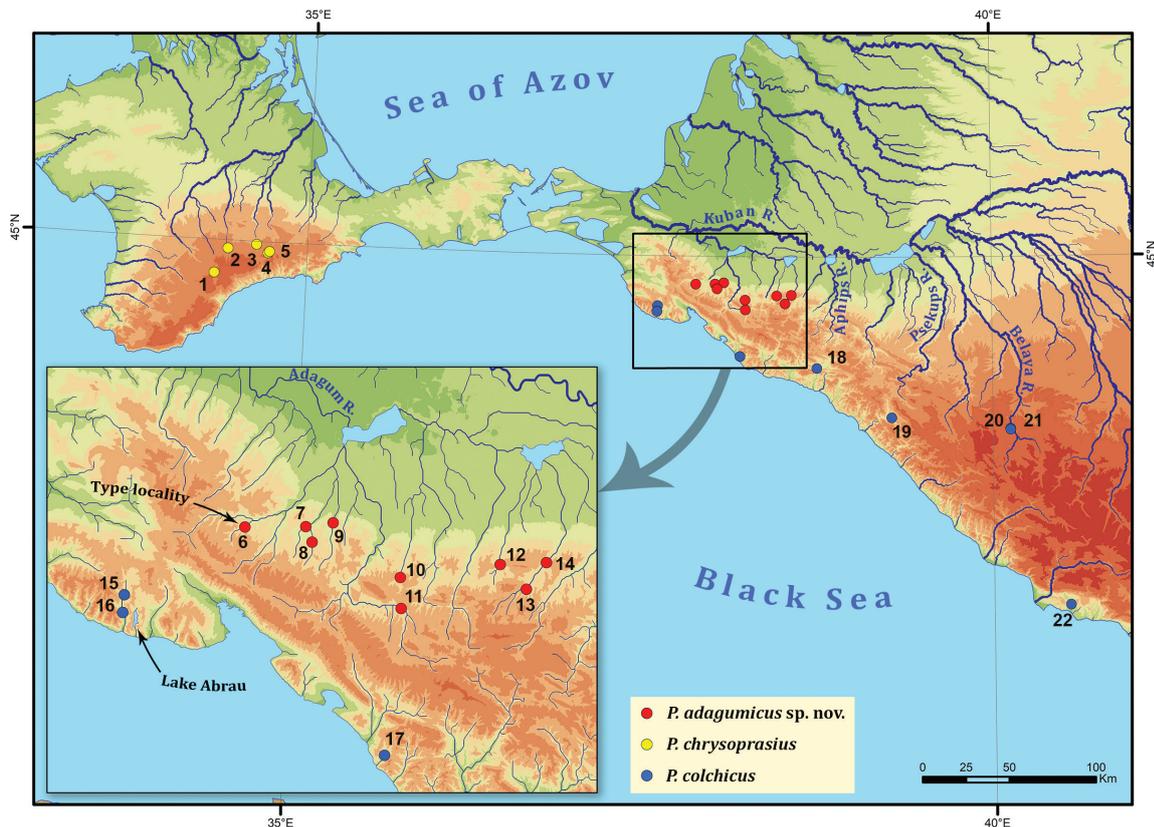


Figure 1. Map of localities of *Phoxinus* spp. sampled for this study. Localities are designated in Suppl. material 1.

was counted on the left and right sides of the specimens. The number of the scale rows was counted on the left and right breast patches and average value was taken according to Bogutskaya et al. (2019). Counts of meristic characters (except for the axial skeleton) and assessment of qualitative characters were done using a MC-2-ZOOM stereomicroscope (Micromed, Saint Petersburg, Russia). Counts of vertebrae and pterygiophores followed Naseka (1996) and Bogutskaya et al. (2019) based on radiographs made by a PRDU (v. II) X-Ray equipment (ELTECH-Med, St. Petersburg, Russia). Images of pharyngeal teeth were taken 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 the 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)]. Differences between sexes were tested using the Mann–Whitney U test in Past 4.12b (Hammer and Harper 2001). Principal component analysis (PCA) was performed using the *ggfortify* (Tang et al. 2016) package in R.

Molecular analyses

Molecular analyses included *Phoxinus* spp. samples from thirteen localities (No. 3–11, 15, 17, 20 and 22 on Fig. 1) (Suppl. material 5).

DNA was isolated by salt-extraction (Aljanabi and Martinez 1997) from ethanol-fixed tissues. Two mitochondrial markers were analyzed. Mitochondrial cytochrome *c* oxidase subunit I (COI) barcode region was amplified using 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, mitochondrial cytochrome *b* fragment was amplified by PCR using the following primers: GluF: 5'-AACCACCGTTGTAT-TCAACTACAA-3' and ThrR: 5'-ACCTCCGATCTTC-GGATTACAAGACCG-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 forward sequencing primer M13F 5'-GTA AAA CGA CGG CCA GT-3' and reverse sequencing primer M13R-pUC 5'- CAG GAA ACA GCT ATG AC-3' (Geiger et al. 2014).

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 *cytb* (1089 bp) concatenated sequences. In addition to the 29 newly determined COI and *cytb* 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; Schönhuth et al. 2018; and unpublished works). Three outgroups representing genera *Pseudaspius*, *Rhynchocypris*, and *Oreoleuciscus* 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 $2^7 \times 10$ 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 v1.2.3 (Zhang et al. 2020; Xiang et al. 2023) under Edge-linked partition model for 1000 ultrafast (Minh et al. 2013) bootstraps. 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 AICc criterion (Suppl. material 6).

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

Results

Phylogenetic placement and genetic distance

Phylogenetic Bayesian tree of the genus *Phoxinus* shows that *Phoxinus adagumicus* sp. nov. has its own cluster representing one of the earliest branches with a position between the earliest branch of *Phoxinus* (*P. tumensis* and *Phoxinus* sp. from Far East) and large clade represented the other species from Europe (Fig. 2). Although ML-tree shows some differences in topology, the early branching of *Phoxinus adagumicus* sp. nov. compared to the most of European species is retained (Suppl. material 7). *Phoxinus adagumicus* sp. nov. shows the lowest genetic distance to *P. cf. morella* (Leske, 1774) from basins of the North and Baltic seas and adjacent upper reaches of the Danube (*p*-distance = 0.074 ± 0.006) (Table 1), although it is almost equally close to *P. marsilii* Heckel,

Table 1. Genetic *p*-distances between species or groups of *Phoxinus* spp. for concatenated COI and *cytb* mtDNA sequences. The averages of interspecies distances are given below diagonal, the standard errors are given above diagonal; the intraspecies divergence is given in a diagonal in bold.

	<i>P. adagumicus</i> sp. nov.	<i>P. chrysoprasius</i>	<i>P. colchicus</i> (Black Sea)	<i>P. colchicus</i> (Kuban basin)	<i>P. csikii</i> (Clade 5a)	<i>P. csikii</i> (Clade 5b)	<i>P. karsticus</i>	<i>P. krkae</i>	<i>P. lumaireul</i> (Clade 1a)	<i>P. lumaireul</i> (Clade 1b)	<i>P. lumaireul</i> (Clade 1c)	<i>P. lumaireul</i> (Clade 1d)	<i>P. lumaireul</i> (Clade 1e)
<i>P. adagumicus</i> sp. nov.	0.007	0.007	0.007	0.007	0.006	0.006	0.006	0.007	0.006	0.006	0.006	0.006	0.006
<i>P. chrysoprasius</i>	0.084	0.001	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.006
<i>P. colchicus</i> (Black Sea)	0.089	0.073	0.005	0.002	0.007	0.007	0.007	0.006	0.006	0.006	0.006	0.006	0.006
<i>P. colchicus</i> (Kuban basin)	0.091	0.074	0.012	0.001	0.007	0.006	0.007	0.006	0.006	0.006	0.006	0.006	0.006
<i>P. csikii</i> (Clade 5a)	0.086	0.072	0.085	0.086	0.006	0.003	0.006	0.005	0.005	0.005	0.005	0.005	0.005
<i>P. csikii</i> (Clade 5b)	0.085	0.071	0.084	0.084	0.019	0.008	0.006	0.005	0.005	0.005	0.005	0.005	0.005
<i>P. karsticus</i>	0.090	0.073	0.091	0.092	0.074	0.076	0.003	0.006	0.005	0.005	0.005	0.005	0.006
<i>P. krkae</i>	0.089	0.071	0.082	0.085	0.063	0.062	0.070	0.001	0.005	0.005	0.005	0.005	0.005
<i>P. lumaireul</i> (Clade 1a)	0.081	0.068	0.078	0.078	0.047	0.048	0.073	0.060	0.007	0.002	0.002	0.004	0.003
<i>P. lumaireul</i> (Clade 1b)	0.082	0.068	0.079	0.080	0.046	0.047	0.073	0.059	0.021	0.013	0.002	0.003	0.003
<i>P. lumaireul</i> (Clade 1c)	0.078	0.068	0.079	0.078	0.047	0.046	0.070	0.058	0.019	0.017	0.004	0.003	0.003
<i>P. lumaireul</i> (Clade 1d)	0.083	0.066	0.081	0.081	0.046	0.045	0.070	0.064	0.028	0.028	0.026	0.004	0.003
<i>P. lumaireul</i> (Clade 1e)	0.079	0.067	0.079	0.079	0.047	0.046	0.067	0.057	0.026	0.025	0.023	0.022	0.002
<i>P. lumaireul</i> (Clade 1f)	0.082	0.064	0.077	0.077	0.043	0.046	0.069	0.059	0.026	0.026	0.025	0.022	0.019
<i>P. marsilii</i>	0.075	0.066	0.074	0.076	0.071	0.071	0.076	0.070	0.071	0.067	0.066	0.071	0.066
<i>P. cf. morella</i>	0.074	0.066	0.072	0.070	0.074	0.074	0.071	0.068	0.066	0.068	0.067	0.069	0.062
<i>P. phoxinus</i>	0.086	0.077	0.082	0.084	0.078	0.079	0.080	0.078	0.077	0.075	0.074	0.081	0.074
<i>P. septimaniae</i>	0.086	0.071	0.078	0.078	0.067	0.066	0.073	0.081	0.067	0.066	0.066	0.069	0.064
<i>P. sp.</i> (Amur basin)	0.090	0.079	0.090	0.086	0.090	0.087	0.086	0.082	0.086	0.085	0.084	0.089	0.081
<i>P. sp.</i> (Clade 2)	0.078	0.062	0.077	0.078	0.044	0.045	0.065	0.052	0.041	0.039	0.038	0.041	0.041
<i>P. sp.</i> (Clade 3)	0.081	0.069	0.083	0.083	0.045	0.042	0.069	0.061	0.046	0.046	0.043	0.043	0.043
<i>P. sp.</i> (Clade 4)	0.080	0.067	0.078	0.080	0.043	0.044	0.070	0.056	0.043	0.043	0.041	0.044	0.045
<i>P. sp.</i> (Clade 8)	0.089	0.070	0.079	0.079	0.077	0.075	0.066	0.072	0.070	0.072	0.073	0.075	0.068
<i>P. strandjae</i>	0.077	0.066	0.081	0.081	0.043	0.044	0.069	0.062	0.039	0.036	0.035	0.042	0.040
<i>P. strymonicus</i>	0.081	0.065	0.078	0.078	0.047	0.046	0.072	0.062	0.035	0.038	0.036	0.038	0.040
<i>P. tumensis</i>	0.092	0.076	0.089	0.090	0.075	0.077	0.085	0.082	0.083	0.081	0.084	0.083	0.082

Table 1. Continued.

	<i>P. lumaireul</i> (Clade 1f)	<i>P. marsilii</i>	<i>P. cf. morella</i>	<i>P. phoxinus</i>	<i>P. septimaniae</i>	<i>P. sp.</i> (Amur basin)	<i>P. sp.</i> (Clade 2)	<i>P. sp.</i> (Clade 3)	<i>P. sp.</i> (Clade 4)	<i>P. sp.</i> (Clade 8)	<i>P. strandjae</i>	<i>P. strymonicus</i>	<i>P. tumensis</i>
<i>P. adagumicus</i> sp. nov.	0.006	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
<i>P. chrysoprasius</i>	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.006	0.006	0.005	0.006	0.006	0.006
<i>P. colchicus</i> (Black Sea)	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.006	0.006	0.006	0.006	0.007
<i>P. colchicus</i> (Kuban basin)	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.007
<i>P. csikii</i> (Clade 5a)	0.005	0.006	0.006	0.006	0.006	0.006	0.005	0.005	0.005	0.006	0.005	0.005	0.006
<i>P. csikii</i> (Clade 5b)	0.005	0.006	0.006	0.006	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.006
<i>P. karsticus</i>	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.005	0.006	0.007
<i>P. krkae</i>	0.005	0.006	0.005	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.005	0.006	0.006
<i>P. lumaireul</i> (Clade 1a)	0.003	0.005	0.006	0.006	0.006	0.006	0.004	0.005	0.005	0.005	0.004	0.004	0.006
<i>P. lumaireul</i> (Clade 1b)	0.003	0.005	0.005	0.006	0.005	0.006	0.004	0.005	0.005	0.005	0.004	0.004	0.006
<i>P. lumaireul</i> (Clade 1c)	0.003	0.005	0.006	0.006	0.006	0.006	0.004	0.005	0.005	0.006	0.004	0.004	0.006
<i>P. lumaireul</i> (Clade 1d)	0.003	0.006	0.006	0.006	0.006	0.006	0.004	0.005	0.005	0.006	0.004	0.005	0.007
<i>P. lumaireul</i> (Clade 1e)	0.003	0.006	0.006	0.006	0.006	0.006	0.005	0.005	0.005	0.005	0.004	0.005	0.006
<i>P. lumaireul</i> (Clade 1f)	0.000	0.005	0.006	0.006	0.006	0.006	0.004	0.005	0.005	0.005	0.004	0.004	0.006
<i>P. marsilii</i>	0.067	0.004	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.006
<i>P. cf. morella</i>	0.065	0.065	—	0.006	0.006	0.006	0.005	0.006	0.006	0.006	0.006	0.005	0.006
<i>P. phoxinus</i>	0.076	0.072	0.069	0.001	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
<i>P. septimaniae</i>	0.064	0.066	0.064	0.059	0.003	0.006	0.006	0.006	0.006	0.005	0.005	0.006	0.007
<i>P. sp.</i> (Amur basin)	0.085	0.078	0.085	0.088	0.085	—	0.006	0.006	0.006	0.006	0.006	0.006	0.006
<i>P. sp.</i> (Clade 2)	0.039	0.066	0.065	0.070	0.068	0.083	0.004	0.004	0.004	0.005	0.004	0.004	0.006
<i>P. sp.</i> (Clade 3)	0.044	0.068	0.065	0.075	0.070	0.087	0.036	0.002	0.004	0.005	0.004	0.005	0.006
<i>P. sp.</i> (Clade 4)	0.041	0.068	0.066	0.076	0.069	0.086	0.033	0.035	0.001	0.006	0.005	0.005	0.006
<i>P. sp.</i> (Clade 8)	0.073	0.073	0.069	0.073	0.069	0.081	0.065	0.067	0.072	0.001	0.005	0.006	0.006
<i>P. strandjae</i>	0.037	0.064	0.068	0.072	0.067	0.085	0.035	0.039	0.042	0.067	—	0.004	0.006
<i>P. strymonicus</i>	0.037	0.065	0.063	0.073	0.069	0.086	0.038	0.044	0.041	0.069	0.038	—	0.007
<i>P. tumensis</i>	0.077	0.081	0.079	0.088	0.080	0.073	0.074	0.077	0.075	0.084	0.078	0.079	—

1836 (0.075±0.005) from the Danube basin (Black Sea drainage) and rivers of Baltic Sea basin. The new species is not close in genetics to geographically neighbor species – *P. colchicus* and *P. chrysoprasius* (Pallas, 1814) (Table 1). At the same time, another population of *Phoxinus* sp. from the Kuban basin, inhabiting the middle reach of Belaya basin, is characterized by unique haplotypes and has a minimum distance (0.012±0.002) to *P. colchicus* from the Black Sea basin (Fig. 2) and currently is considered a divergent population of *P. colchicus* (Fig. 2). Intraspecific divergence of *Phoxinus adagumicus* sp. nov. (0.007) is comparable to such in other species (Table 1).

Systematics

Class Actinopterygii Klein, 1885

Order Cypriniformes Bleeker, 1859

Family Leuciscidae Bonaparte, 1835

Genus *Phoxinus* Rafinesque, 1820

Phoxinus adagumicus sp. nov.

<https://zoobank.org/67F76C90-DC80-48CE-974B-B4A3DEB39263>

Figs 3, 4

Phoxinus phoxinus – Berg 1949: 590 (Khabl, Abin, Shibik, Psebeps rivers and Abrau Lake); Sukhanova and Troitskiy 1949: 154, 164–165 (Khabl, Adagum, Shibik, Abin and Ayuk rivers); Emtyl et al. 1994: 137–141, figs 1–2 (Ayuk and Dyurso rivers); Reshetnikov et

al. 2003: 301–302 (Kuban River); Otrishko and Emtyl 2013a: 20 (Kuban River).

Phoxinus phoxinus kubanicum Emtyl et Ivanenko, 2002 *unavailable name*: 90–92, fig. 69 ex Berg 1949 not *P. adagumicus* sp. nov. (Psekups River basin; Aphips, Il, Ubin, Abin, Adagum rivers and Abrau Lake); Otrishko and Emtyl 2013a: 20 (Kuban River).

Phoxinus phoxinus kubanicus [sic] – Karnaukhov 2020: 76–77 (Adagum and Psebeps rivers).

Phoxinus kubanicus [sic] – Otrishko and Emtyl 2013b: 22 (Adagum River); Otrishko and Emtyl 2013c: 69–70 (Adagum, Ubin, Aphips and Ayuk rivers).

Phoxinus colchicus – Kottelat and Freyhof 2007: 226 (southern tributaries of lower Kuban).

Phoxinus sp. – Artaev et al. 2021 (Zybza, Il, Psyzh, Shibik and Neberd-jai rivers).

Phoxinus sp. Kuban Clade 19 – Palandačić et al. 2020: figs 1–2 (Kuban River); Bogutskaya et al. 2023: 6, Fig. 2 (Adagum River).

Type material. *Holotype*, IBIW_FS_385, female, (57.5 SL mm, Genbank Accession numbers **OR713923** - COI, **PP351730** - cytb), Russia, Krasnodar Krai, Kuban River drainage, Pryamaya Shchel River (Adagum River drainage) upstream Nizhnebakanskaya, 44.8538°N, 37.8417°E, 22 May 2023, I.S. Turbanov leg. **Paratypes**: 3 females, 2 males (IBIW_FS_386), SL 45.7–51.1 mm, 3 females, 2 males (ZISP 57031), SL 42.2–51.1 mm, 3 females, 2 males (ZMMU P-24612), SL 45.3–51.7 mm, all from the same locality and date as holotype.

Additional material. Suppl. material 1.

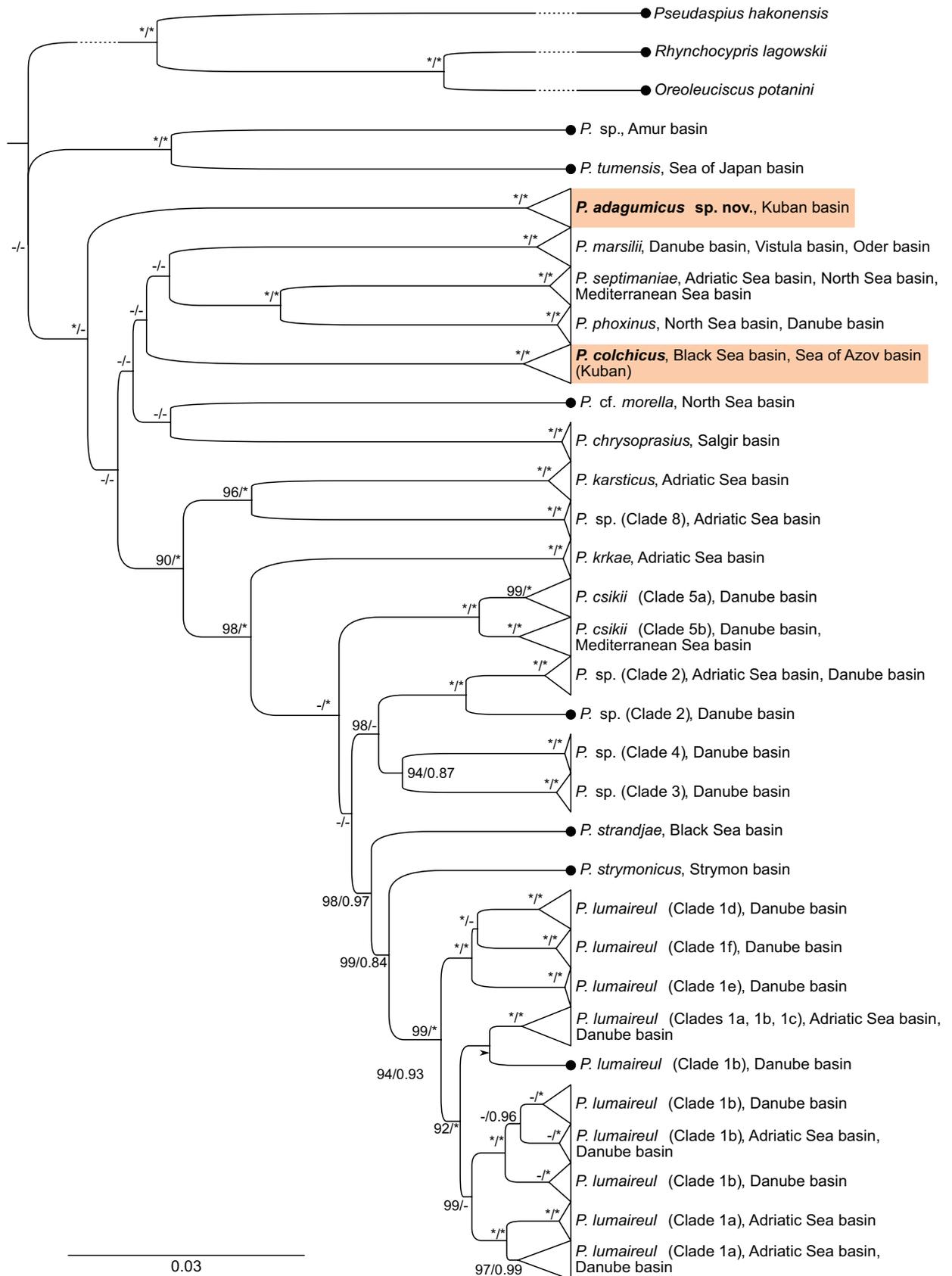


Figure 2. BI consensus tree of concatenated COI and *cytb* mtDNA sequences representing all available *Phoxinus* species in Genbank combined with our data set. Number of some clades is given according to the study of Palandačić et al. (2020). Species from the Kuban basin are highlighted with color. Bootstrap values/posterior probabilities above 80/0.8 are shown; asterisks represent of 100/1 bootstrap/posterior probabilities values. Scale bar is in expected substitutions per site. The nodes with multiple specimens were collapsed to a triangle, with the horizontal depth indicating the level of divergence within the node.



Figure 3. Holotype of *Phoxinus adagumicus* sp. nov. (IBIW_FS_385): **A.** General appearance of preserved specimen; **B.** Radiograph.

Comparative material. Suppl. material 1.

Material used in the genetic analysis. Suppl. material 5.

Etyymology. The new species is named after the Adagum River, left tributary of the lower reach of the Kuban River, where the species occurs; *adagumicus* – an adjective.

Diagnosis. *Phoxinus adagumicus* sp. nov. is distinguished from geographically close species (*P. chrysoprasius* and *P. colchicus*) by the presence and predominance of specimens with single-row pharyngeal teeth on one or both sides and a combination of characters, none of which is unique, as follows: head depth at nape 54.1–64.8% HL (mean 60.5), and head depth through eye 45.3–51.6% HL (mean 48.0); head length 2.7–3.7 (mean 3.1) times caudal peduncle depth in females and 2.7–3.2 (mean 2.9) times in males; body width at dorsal-fin origin 1.4–2 (mean 1.6) times caudal peduncle depth in females and 1.3–1.6 (mean 1.4) times in males; mean number of scale rows on left and right breast patches 3–9 (mean 6.1); scales below lateral line 8–14 (mean 11.8); number of circumpectuncular scales 37–50 (mean 41.5); 3rd–6th type of breast scalation (mode 4th type).

Description. The general appearance of *P. adagumicus* sp. nov. is shown in Figs 3–4. Morphometric measurements for the holotype and type series with level of significance of sex-related differences are given in Table 2; meristic and qualitative characters for specimens from the type locality are given in Table 3. Primary morphological data for specimens from the type locality (holotype, paratypes and additional material) are given in Suppl. material 2; meristic and qualitative characters of *P. adagumicus* sp. nov. and other *Phoxinus* spp. are given in Suppl. material 3; morphometric measurements of *P. adagumicus* sp.

nov., *P. chrysoprasius* and *P. colchicus* and their comparison are given in Suppl. material 4.

Morphometrics (Table 2, Suppl. materials 2, 4). The new species has a medium size – the maximum SL is 59.4 mm (male from the Il River). The new species has elongated head – head depth at nape (% HL) (holotype: 62.2, paratypes: 58.5–63.9, add. material 54.1–64.8); and head depth through eye (% HL) (holotype: 49.8, paratypes: 46.4–50.0, add. material 45.3–51.6). Head length 3.0 times caudal peduncle depth in the holotype, 2.8–3.2 in the paratypes, and 2.7–3.7 in the add. material.

Meristics (Table 3, Suppl. material 3). Dorsal fin with 3 (sometimes 2, rarely 4) unbranched and 7½ branched rays. Anal fin with 3 unbranched and 7½ (rarely 6½) branched rays. Pectoral fin with 14–18 rays, often 16–17. Pelvic fin with 8 rays (rarely 7 or 9). Caudal fin with 19 rays (sometimes 18, rarely 20). Number of dorsal procurrent caudal-fin rays 9–13, often 10–12. Number of ventral procurrent caudal-fin rays 7–12, often 9–10.

The most common pharyngeal teeth formulae are 5–4 (n = 11), 5–4.1 (n = 6), 1.5–4 (n = 5) and 1.5–4.1 (n = 7) (Fig. 5, Table 4). Total number of vertebrae in the holotype 41, 39–41 in the paratypes, and 39–42 in the add. material, commonly 40 or 41. Number of abdominal vertebrae in the holotype 22, 22–23 in the paratypes, and 21–24 in the add. material, commonly 22 or 23. Number of caudal vertebrae in the holotype 19, 17–19 in the paratypes, and 16–19 in add. material, commonly 18. Number of predorsal abdominal vertebrae in the holotype 15, 14–15 in the paratypes, and 14–16 in the add. material, commonly 15. Number of anal-fin pterygiophores in front of the first caudal vertebrae in the holotype 3, 3–6 in the paratypes,



Figure 4. Live coloration of *Phoxinus adagumicus* sp. nov. from the Zybza River. Side and ventral views are of the same specimens (IBIW_FS_339). Fish of spawning or post-spawning coloration were caught on 8th May 2022.

and 3–7 in the add. material, commonly 4 or 5. Difference in the number of abdominal and caudal vertebrae in the holotype 3, 3–6 in the paratypes, and 2–7 in the add. material, commonly 4 or 5.

Total number of scales in the lateral series 74–94, mean 84.5. Lateral line incomplete and discontinuous. Relative number of total lateral-line (pored) scales in specimens of 41–57 mm SL varies greatly from 26 to 92%, mean

58.5%. Number of scale rows on breast patches 3–6, commonly 4. Number of circumpeduncular scales 37–44, mean 41.3. Number of scales above lateral line 16–22, mean 18.4. Number of scales below lateral line 9–14, mean 11.8.

Gill rakers (in series from the type locality) on the first left arch 7–8 (mode 8), on the first right arch 7–9 (modes 7, 8, and 9) (Suppl. material 2).

Table 2. Morphometric measurements of *Phoxinus adagumicus* sp. nov. (type series) (primary data see in Suppl. material 2); p^* - difference between females (including the holotype) and males, Mann–Whitney U test: ns ($p > 0.05$), + ($p < 0.05$), ++ ($p < 0.01$).

Characters	Holotype (female)		Females, n=10			Males, n=6		p^*
		mean	range	SD	mean	range	SD	
Standard length (SL, in mm)	57.5	48.9	42.2–57.5	3.2	47.1	45.3–51.1	2.5	
In percentage of standard length (% SL)								
Body depth at dorsal-fin origin	20.0	21.1	19.4–23.4	1.3	20.5	19.1–22.2	1.2	ns
Body width at dorsal-fin origin	12.7	13.4	12.6–14.9	0.8	13.5	12.9–14.2	0.5	ns
Minimum depth of caudal peduncle	8.1	8.7	8.0–9.2	0.4	9.5	8.7–10.0	0.6	ns
Caudal peduncle width	8.3	8.6	7.6–10.1	0.7	9.2	8.7–9.7	0.4	ns
Predorsal length	55.2	57.2	55.2–60.5	1.6	55.4	53.9–57.0	1.1	+
Postdorsal length	34.1	33.9	32.9–35.6	0.9	33.0	31.9–34.3	1.0	ns
Prepelvic length	47.3	50.9	47.3–54.8	1.6	49.1	46.6–51.1	1.5	+
Preanal length	65.1	67.9	65.1–70.8	1.3	65.7	63.2–69.1	2.4	ns
Pectoral – pelvic-fin origin length	23.8	25.8	23.8–27.2	1.2	23.7	22.4–24.3	0.7	++
Pelvic – anal-fin origin length	19.0	19.0	17.8–20.7	0.9	18.0	16.5–19.3	1.2	ns
Caudal peduncle length	23.5	21.7	20.1–23.5	1.0	23.1	22.3–24.7	0.8	+
Dorsal-fin base length	11.8	11.8	11.1–12.4	0.5	12.2	11.1–13.1	0.8	ns
Dorsal-fin depth	20.6	20.8	18.8–22.4	1.2	22.2	21.5–23.8	0.9	+
Anal-fin base length	9.2	10.5	9.2–11.5	0.5	10.8	9.6–11.3	0.6	ns
Anal-fin depth	18.6	19.6	17.7–21.3	1.1	21.0	20.5–21.6	0.4	++
Pectoral-fin length	18.3	19.0	17.2–21.9	1.5	21.1	20.1–21.6	0.6	+
Pelvic-fin length	14.1	14.4	12.5–15.9	1.1	16.2	15.1–17.2	0.7	++
Head length (HL)	24.0	26.5	24.0–27.9	0.9	27.4	25.4–28.7	1.3	ns
Head depth at nape	14.9	16.1	14.9–17.1	0.6	16.7	16.2–17.3	0.4	ns
Maximum head width	12.6	13.8	12.6–14.8	0.7	13.9	13.0–14.8	0.7	ns
Snout length	7.7	8.3	7.3–9.4	0.7	8.2	7.4–9.3	0.7	ns
Eye horizontal diameter	6.3	7.2	6.3–7.7	0.3	6.8	6.4–7.1	0.3	+
Interorbital width	8.6	9.1	8.3–10.0	0.5	9.3	8.6–9.9	0.5	ns
In percentage of head length (% HL)								
Maximum head width	52.5	52.0	46.6–54.4	2.8	50.7	45.4–54.9	3.1	ns
Snout length	31.9	31.2	28.9–33.6	1.8	30.0	27.6–32.5	1.8	ns
Head depth at nape	62.2	60.8	58.5–63.0	1.7	61.0	58.5–63.9	2.0	ns
Head depth through eye	49.8	48.2	46.4–50.0	1.5	48.4	47.4–49.2	0.7	ns
Eye horizontal diameter	26.3	27.2	26.3–28.5	0.7	24.9	23.8–25.7	0.7	++
Postorbital distance	51.8	45.6	41.9–51.8	1.8	48.0	46.6–49.2	1.3	ns
Interorbital width	35.8	34.4	31.0–36.7	1.6	34.1	32.1–35.4	1.3	ns
In percentage of caudal peduncle length								
Minimum depth of caudal peduncle	34.3	40.1	34.3–43.8	2.9	41.3	38.1–44.9	3.0	ns
In percentage of body depth								
Head length	120.0	126.0	116.7–139.9	7.9	133.9	128.2–143.4	5.6	+
In percentage of interorbital width								
Eye horizontal diameter	73.4	79.3	71.8–90.1	5.0	73.3	71.0–75.6	1.6	+
Ratios								
Interorbital width/eye horizontal diameter	1.4	1.3	1.1–1.4	0.1	1.4	1.3–1.4	0.0	+
Snout length/eye horizontal diameter	1.2	1.1	1.0–1.2	0.1	1.2	1.1–1.3	0.1	ns
Head depth at nape/eye horizontal diameter	2.4	2.2	2.1–2.4	0.1	2.4	2.3–2.6	0.1	++
Head length/caudal peduncle depth	3.0	3.1	2.8–3.2	0.1	2.9	2.8–3.1	0.1	+
Length of caudal peduncle/caudal peduncle depth	2.9	2.5	2.3–2.9	0.2	2.4	2.2–2.6	0.2	ns
Pectoral fin length/pectoral – pelvic-fin origin distance	0.8	0.7	0.6–0.9	0.1	0.9	0.9–0.9	0.0	++
Predorsal length/head length	2.3	2.2	2.1–2.3	0.1	2.0	1.9–2.1	0.1	++
Body width at dorsal-fin origin/caudal peduncle depth	1.6	1.5	1.4–1.7	0.1	1.4	1.3–1.5	0.1	+

Qualitative characters. Pectoral fins do not reach beginning of pelvic fins, except for a few males (ca. 7% in total). In most specimens (ca. 70%), the tip of upper lip above the horizontal level of lowest point of the eye, in some specimens (ca. 25%) at the level, and in 5% of the specimens below the level. Origin of the anal fin is mainly behind the vertical of the posterior insertion of the dorsal fin. (ca. 53%), often at the vertical (39%), rarely ahead (8%). Free margin of the dorsal fin mainly straight or slightly convex, anal fin slightly convex or rarely slightly concave. 3rd–6th type of breast scalation (mode 4th type).

Coloration. Live coloration of females outside the spawning period is brown, gray or light golden hues (see Fig. 4). In males, the color is similar, but with a greater dominance of golden or greenish hues. In spawning coloration of females, golden hue increases significantly and the coloration becomes more contrasting, in general. The spawning coloration of males is also more contrasting, with dominance of green color with variations towards yellow-green or black-green. The operculum stains are blue and the suboperculum is yellow in both sexes, but this is much more pronounced in males. Red hues appear in spawning coloration and



Figure 5. Most frequent variants of formula of pharyngeal bones of *Phoxinus adagumicus* sp. nov.: **A.** Single-row formula 5-4 (in 33% of specimens); **B.** Double-row formula 1.5-4.1 (in 21% of specimens). Scale bar: 0.5 mm.

are concentrated at the base of the pelvic, pectoral and anal fins, as well as around the mouth. 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 were observed in 18 out of 41 morphometric characters (Table 2). In addition to some classical sex characteristics in *Phoxinus* minnows (e.g., narrower pectoral fins and less bright colors in females), females of the new species generally have shorter anal and pelvic fins, smaller eye diameter, and a higher ratio of predorsal length to head length.

Table 3. Meristics and scalation pattern of *Phoxinus adagumicus* sp. nov. from type series and additional material from the type locality (primary data see in Suppl. material 2). Additional material from non-type localities is given in Suppl. material 3.

Nos.	Characters	mean	range	SD	n
1	Total number of scales in lateral series (sql)	84.9	81-90	3.0	7
2	Total number of lateralline (pored) scales (llt)	44.2	24-67	17.7	6
3	Number of pored scales in first complete (non-interrupted) section of lateral line (llcs)	29.7	19-57	15.0	7
4	Relative number of total lateral line scales, quotient llt:sql (lltr)	0.5	0.3-0.8	0.2	6
5	Mean number of scale rows on left and right breast patches (BrPScale)	6.4	6-7	0.4	7
6	Number of circumpeduncular scales (cps)	41.1	39-43	1.5	7
7	Scales above lateral line (ScAboveLL)	17.3	15-20	1.6	7
8	Scales below lateral line (ScBelowLL)	11.7	10-13	1.0	6
9	Pattern of scalation on the breast and anterior belly (cstyp)	5	4-6		7
10	Total number of pectoral fin rays (P)	16.6	15-18	1.0	7
11	Total number of pelvic fin rays (V)	8.0	8-8		7
12	Number of branched dorsal fin rays (with 1/2) (D)	7.0	7-7		7
13	Number of branched anal fin rays (with 1/2) (A)	7.0	7-7		7
14	Number of rays in caudal fin (C)	18.9	18-20	0.4	7
15	Total number of vertebrae (tv)	40.4	39-42	0.8	23
16	Number of abdominal vertebrae (abdv)	22.6	21-24	0.7	23
17	Number of caudal vertebrae (caudv)	17.9	17-19	0.7	23
18	Number of predorsal abdominal vertebrae (preDv)	14.8	14-16	0.5	23
19	Number of anal fin pterygiophores in front of the first caudal vertebrae (preAp)	4.5	3-6	0.8	23
20	Difference between numbers of abdominal and caudal vertebrae (dac)	4.6	3-7	1.1	23

Table 4. Frequency of different pharyngeal teeth formulas in *Phoxinus adagumicus* sp. nov., *P. colchicus* and *P. chrysoprasius*.

Species	5-4	1.5-4	5-4.1	5-5.1	1.4-4.2	1.5-4.1	1.5-4.2	1.5-5.1	2.4-4.2	2.5-4.1	2.5-4.2	2.5-5.2	n
<i>P. adagumicus</i> sp. nov.	11	5	6	1	0	7	1	1	0	1	0	0	33
<i>P. chrysoprasius</i>	0	0	0	0	0	0	0	0	0	1	12	2	15
<i>P. colchicus</i>	0	0	0	0	1	1	3	0	1	3	5	1	15

Taxonomic remarks. The presence of *Phoxinus* minnow in the left lower tributaries of the Kuban has been documented since the first half of the 20th century (Malyatsky 1930; Berg 1949; Sukhanova and Troitsky 1949), and all previous researchers have attributed this population to *P. phoxinus*. Emtyl and Ivanenko (2002) used the name '*Phoxinus phoxinus kubanicum* sp. nov.' for the minnows from Trans-Kuban rivers (Aphips, Il, Ubin, Abin, and Adagum) and Lake Abrau (Black Sea basin). Even though the name is accompanied by a comparative description, it cannot be considered as valid because it does not comply with the criteria stipulated in art. 16.4 of the International Code of Zoological Nomenclature (Ride et al. 1999) for species-group names proposed after 1999, as it is not accompanied by an explicit preservation of a holotype or syntypes for the nominal taxon (art. 16.4.1.) and a statement of deposit in a collection (art. 16.4.2.) (Bogutskaya et al. 2023). The names *P. phoxinus kubanicum*, *P. phoxinus kubanicus* [sic], and *P. kubanicus* [sic], after an attempt to describe this species (Emtyl and Ivanenko 2002), were subsequently used several times (Otrishko and Emtyl 2013a, 2013b, 2013c; Karnaukhov 2020); however, according to Bogutskaya et al. (2023), an ICZN commissioner, Nikita Kluge (pers. comm.), taxonomists Pyotr Petrov (pers. comm.) and Boris Kataev (pers. comm.), they cannot be considered as available names.

It is worth noting that the original description of '*P. phoxinus kubanicum*' does not correspond to the

morphological diagnosis of minnows from the Adagum basin rivers obtained in this research. For example, the two-row formula (2.5–4.2) of the pharyngeal teeth is indicated (Emtyl and Ivanenko 2002), but here, none out of 33 examined specimens had such a formula (Table 4). Also, it is necessary to emphasize that the given image of ‘*P. phoxinus kubanicum*’ (fig. 69 in Emtyl and Ivanenko 2002) does not relate to this species but is borrowed from the monograph of L.S. Berg (1949, fig. 447) and refers to the minnows inhabiting Lovozero (Northern Russia), which most probably belong to *Phoxinus* sp. clade 17 of Palandačić et al. (2020). Thus, ‘*P. phoxinus kubanicum*’ should be considered as an *unavailable name*.

Type locality. Pryamaya Shchel River (44.8538°N, 37.8417°E) upstream of Nizhnebakanskaya, Krasnodar Krai, Russia. A tributary of the Bakanka River → Adagum River → Kuban River → Sea of Azov.

Distribution and habitats. An endemic species living in the northwestern Caucasus in the Adagum River basin, a tributary of the Kuban (Fig. 1). The species has a rather limited range with only 55 km between most distant known occurrences. The species has been found only in small streams located in mountainous and foothill areas – in a zone of a width about 15–20 km along the northern slope of the western part of the Main Caucasian Range. Example of habitat for this species (Abin River) is shown in Fig. 6. Habitat of *P. adagumicus* sp. nov. in other parts of the Kuban basin and on the Black Sea coast of the Caucasus (Lake Abrau) indicated according to literature data (Berg 1949; Sukhanova and Troitskiy 1949; Emtyl et al. 1994; Emtyl and Ivanenko 2002; Karnaukhov 2020) was not confirmed by our research and may require additional study (see Discussion).

Morphological comparisons. PCA of morphometric characters shows that *P. adagumicus* sp. nov. is more overlapping with *P. chrysoprasius* than with *P. colchicus* (Fig. 7). The highest loadings for *P. adagumicus* sp. nov.

are: head length/caudal peduncle depth, body width at dorsal-fin origin/caudal peduncle depth and eye horizontal diameter (% interorbital width). Sex differences in all three species are divergent in the second component.

The occurrence of single-row pharyngeal teeth, frequent in *P. adagumicus* sp. nov. (Fig. 5, Table 4), is rare in the genus *Phoxinus*. In comparison with the geographically neighboring species, *P. colchicus* and *P. chrysoprasius*, *P. adagumicus* sp. nov. has unique formulas of pharyngeal teeth: 5–4, 1.5–4, 5–4.1, 5–5.1 and 1.5–5.1. According to our data, *P. colchicus* and *P. chrysoprasius* do not show single-row formulae even on one side, while in *P. adagumicus* sp. nov. formula 5–4 is found in 1/3 of all studied specimens. In *P. colchicus* and *P. chrysoprasius*, formula 2.5–4.2 is modal, but this is absent among individuals of *P. adagumicus* sp. nov. Single-row pharyngeal teeth were indicated as a unique feature for *P. apollonicus* Bianco et De Bonis, 2015 from the basin of Lake Skadar in Montenegro (Bianco and De Bonis 2015).

Compared to *P. chrysoprasius* from the rivers of the Crimean Peninsula (Bogutskaya et al. 2023; this study), *P. adagumicus* sp. nov. has a more elongated head – head depth at nape (% HL) 54.1–64.8, mean 60.5 (vs. 58.9–70.7, mean 64.0) in both sexes; larger eyes relative to head height in females – head depth at nape/eye horizontal diameter 2.1–2.6, mean 2.3 (vs. 2.4–3.4, mean 2.7) (Suppl. material 4); less anal fin pterygiophores in front of the first caudal vertebrae (3–7, mean 4.4 vs. 4–8, mean 5.4); less total number of scales in the lateral series (74–94, mean 84.5 vs. 78–104, mean 90.5); less total number of scales in the lateral series (pored) (21–81, mean 58.5 vs. 46–84, mean 70.0); less mean number of scale rows on left and right breast patches (3–9, mean 6.1 vs. 6–11, mean 8.0); less number of circumpeduncular scales (37–44, mean 41.3 vs. 41–55, mean 46.2); and less number of scales below lateral line (8–14, mean 11.8 vs. 11–16, mean 13.8) (Suppl. material 3).



Figure 6. Abin River (44.7210°N, 38.2051°E; 18 Aug. 2016) – example of biotope of *Phoxinus adagumicus* sp. nov.

in Italy, Slovenia, and Croatia (Bogutskaya et al. 2019), *P. adagumicus* sp. nov. has a smaller number of anal-fin pterygiophores in front of the first caudal vertebrae (3–7, mean 4.4 vs. 3–8, mean 5.5 and 3rd–6th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 2nd–7th types with predominance of 3rd type) (Suppl. material 3).

Compared to *P. krkae* Bogutskaya, Jelić, Vucić, Jelić, Diripasko, Stefanov et Klobučar, 2019 from the Krka River, Croatia (Bogutskaya et al. 2019), *P. adagumicus* sp. nov. has more total number of vertebrae (39–43, mean 40.4 vs. 37–40, mean 38.4); more number of abdominal vertebrae (21–24, mean 22.5 vs. 21–22, mean 21.6); more number of caudal vertebrae (16–18, mean 18 vs. 15–18, mean 16.8); more number of predorsal abdominal vertebrae (14–16, mean 14.8 vs. 13–15, mean 14.0) and 3rd–6th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–7th types with predominance of 5th and 6th types) (Suppl. material 3).

Compared to *P. marsilii* Heckel, 1836 from the Danube River basin, Austria and Croatia (Bogutskaya et al. 2019, 2023), *P. adagumicus* sp. nov. has 3rd–6th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–8th types with predominance of 6th type) (Suppl. material 3).

Compared to *P. strandjae* from the rivers of the Black Sea basin, Bulgaria and the rivers of the Marmara Sea, Turkey (Bogutskaya et al. 2019, 2023), *P. adagumicus* sp. nov. has a smaller number of anal-fin pterygiophores in front of the first caudal vertebrae (3–7, mean 4.4 vs. 4–8, mean 5.6); and 3rd–6th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–12th types with predominance of 6th, 7th, 9th and 11th types) (Suppl. material 3).

Discussion

This study clarified the taxonomy, morphology, genetics, and distribution of the *Phoxinus* minnows inhabiting the Caucasus including the Kuban basin, a large riverine system in the Northern Caucasus that is the richest in endemic species compared to fish fauna in remaining European Russia (Abell et al. 2008). Two distinct species, *P. adagumicus* sp. nov. and *P. colchicus*, were identified. Geographic ranges of *P. adagumicus* sp. nov. and *P. colchicus* in the Kuban basin are separated from each other by at least 145 km in a straight line, and also differ in altitude that is ranging within ca. 50–130 m above sea level for *P. adagumicus* sp. nov. and within ca. 280–580 m for *P. colchicus*. *Phoxinus adagumicus* sp. nov. was found only in the Adagum basin, while another species, inhabiting the middle reach of the Belaya River, refers to *P. colchicus* – species widely distributed in the rivers draining the east coast of the Black Sea. *Phoxinus adagumicus* sp. nov. represents deeply divergent lineage that is earlier branched than other European species. Therefore, *P. adagumicus* sp. nov. may represent a relic lineage of

European minnows, the ancestors of which were among first colonizers of Europe from the East.

The population of *P. colchicus* in the Belaya River system is apparently a result of the past river capture event. The Kuban population of *P. colchicus* is the single population recorded outside the Black Sea basin. The uniqueness of haplotypes from the Kuban basin may indicate rather long isolation of this population or be a result of founder effect. The upper reaches of the Belaya River system share watershed with upper reaches of the rivers Shakhe, Sochi and Achipse belonging to the Black Sea basin. River captures with naturally translocated fish individuals between the Kuban and Black Sea tributaries might be a common phenomenon. For example, the recent discovery of the Black Sea populations of *Barbus tauricus* Kessler, 1877 in some tributaries of the Lower Kuban might be a result of past colonization through main channel of the Kuban River or be an event of river captures (Levin et al. 2019b).

Large morphological variation and overlap between species are a challenge for *Phoxinus* taxonomy. However, *Phoxinus adagumicus* sp. nov. differs from other closely-related or geographically neighboring species in pharyngeal teeth formula, having reduced number of teeth and rows. The *Phoxinus* spp. usually have two-rowed pharyngeal teeth (Berg 1949) while *P. adagumicus* sp. nov. represents one-rowed formula as strictly dominating over two-rowed. Remarkably, one-rowed teeth formula was found in only one another species – *P. apollonicus* from the basin of Lake Skadar in Montenegro (Bianco and De Bonis 2015), which was suggested to be synonymized with *P. karsticus* Bianco et De Bonis, 2015 (Palandačić et al. 2017). A decrease in the number of teeth (4.2 and 4.1) is also observed in another Western Balkan species – *P. ketmaieri* Bianco et De Bonis, 2015, synonymized subsequently with *P. lumaireul* (Palandačić et al. 2017). Traditionally, the phylogenetic value of pharyngeal tooth numbers and their organization (formula) was high (Chu 1935; Tao et al. 2019) but some recent studies showed great phenotypic plasticity of this character from one side (Shkil et al. 2010; Bolotovskiy and Levin 2011) as well as inheritance of the teeth row numbers from another side (Shkil' and Levin 2008). Nevertheless, in our opinion, future studies should pay more attention to the pharyngeal teeth formula since it is often neglected in the current taxonomical studies.

The geographic distribution of *P. adagumicus* sp. nov. might be wider than its known distribution range in the Adagum River basin. Emtyl et al. (1994) stated that minnows from the rivers Dyrurso (Black Sea basin) and Ayuk (tributary of the Psekups River, Kuban basin) are different from “common minnow” (authors did not use Latinian name of species using common name instead, we assume that this is some *Phoxinus* from the European part of the USSR) and “Colchis minnow” (which refers to *P. colchicus*). The habitat of minnows in the Ayuk basin was noted earlier (Sukhanova and Troitskiy 1949), where it was identified as ‘*P. phoxinus*’. This species was previously reported for the Dyrurso River (Luzhnyak 2003). Our examination of the *Phoxinus* material from this river

and nearby (Ozereyka River), only confirmed the presence of *P. colchicus*. Emtyl and Ivanenko (2002), when described '*P. phoxinus kubanicum*', indicated Lake Abrau in the Black Sea basin as the habitat for this species in addition to the rivers of the Kuban basin. The *Phoxinus* was recorded in the lake previously (Malyatsky 1930; Berg 1949), but current studies could not confirm its presence in both Lake (Luzhnyak 2003) and its tributary, the Abrau River (our field survey in 2023). What *Phoxinus* species lived there is unknown, but given the presence of *P. colchicus* in adjacent rivers, one may suggest that this is the same species. In addition, Emtyl and Ivanenko (2002) noted the following locations of the Kuban *Phoxinus* outside the Adagum and Psekups basins: the Aphips and Ubin Rivers. It is worth noting that the Aphips River basin including its left tributary, the Ubin River, are located between the Adagum and Psekups basins (Fig. 1). At the same time, *Phoxinus* was never recorded in the Aphips basin by our numerous field surveys during 2015–2023, or by earlier researchers (Sukhanova and Troitskiy 1949), which also pointed to a large gap in the distributional range between the Adagum and Psekups basins. From all of the above, we can conclude that the entire or the main range of *P. adagumicus* sp. nov. is located within the Kuban River in its sub-basin – the Adagum River system. The taxonomic status of *Phoxinus* from the Ayuk River in the Psekups basin, should be clarified in future research.

We have to consider other peculiarities of the distribution of *P. adagumicus* sp. nov. in the Kuban basin in the light of recent report on its finding (clade 19 by Palandačić et al. 2020) in the Belaya River (tributary of the Kuban). Clade 19 was also reported by Bogutskaya et al. (2023) for “Adagum River at Krymsk, Kuban drainage”. Habitat of the *Phoxinus* in the Belaya basin is confirmed by our studies but according to genetic results (Fig. 2) and morphological analysis (Fig. 7), this population belongs to *P. colchicus*. The latter is common in the Belaya River and its tributaries at least from Khamyshki vil. down to Tulsy town along ca. 60 km (Artaev et al. 2021). Thus, we have to conclude that either two species of the genus *Phoxinus* coexist in the Belaya basin, or the coordinates in Palandačić et al. (2020) are indicated incorrectly. We are inclined to the second opinion.

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Supplementary material 1

Additional material on *Phoxinus adagumicus* sp. nov. and comparative material on *Phoxinus chrysoprasius* and *P. colchicus*

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Aleksandr A. Gandlin, Boris A. Levin
Data type: docx

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Link: <https://doi.org/10.3897/zse.100.115696.suppl1>

Supplementary material 2

Primary morphological data of *Phoxinus adagumicus* sp. nov. from type locality (Pryamaya Shchel River)

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Data type: xlsx

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Supplementary material 3

Meristic and qualitative characters of *Phoxinus adagumicus* sp. nov., *P. chrysoprasius*, *P. colchicus* and other *Phoxinus* species published in the literature

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Aleksandr A. Gandlin, Boris A. Levin
Data type: xlsx

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Supplementary material 4

Morphometrics of *Phoxinus adagumicus* sp. nov., *P. chrysoprasius*, *P. colchicus* and its comparison

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Aleksandr A. Gandlin, Boris A. Levin

Data type: xlsx

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Supplementary material 5

Material for genetic studies

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Supplementary material 6

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

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Supplementary material 7

ML phylogenetic tree of concatenated COI and cytb mtDNA sequences representing all available species in Genabnk combined with our data set

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