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Description of a new species of *Phoxinus* from the Ergene River (Aegean Sea Basin) in Türkiye (Actinopterygii, Leuciscidae)

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Abstract

Phoxinus radeki, a new species, is described from the Ergene River (Aegean Sea Basin). It is distinguished from *Phoxinus* species in Türkiye and the adjacent area by having the scales of the breast, scaled but separated unscaled area anteriorly, short dark rectangular blotches along the lateral line between the lateral line and belly yellowish in both males and females, body depth dorsal fin origin 16–21% SL, caudal peduncle depth 8–10% SL. Additionally, molecular results demonstrated that the new species differed from its closest congeners with a mean genetic distance value of 3.08% (min. 2.82–max. 3.29) and moderately support values in cytochrome *b* (Cyt *b*) gene partial sequences (1064 bp.). Further, the species delimitation analysis identified the new species as a single MOTU independent of other *Phoxinus* species.

Key Words

Cyt *b*, Freshwater fish, minnows, taxonomy

Introduction

The genus *Phoxinus* Rafinesque, 1820 is widely distributed in the Palaearctic region, from the Ebro drainage in Spain eastward to the Anadyr and Amur drainages in Russia and China (basins of the Atlantic, North and Baltic Seas, the Arctic and the northern Pacific Ocean) (Kottelat 2007; Vucić et al. 2018; Bogutskaya et al. 2023; Turan et al. 2023). The fish of the genus *Phoxinus* belong to the family Leuciscidae and are commonly known as minnows. These small freshwater fish are also found in various freshwater habitats, including streams, rivers, lakes, and ponds, in various environments (Banarescu 1992; Kottelat and Freyhof 2007). *Phoxinus* species are typically small fish, with most species reaching lengths of only a few centimeters. They are omnivorous, feeding on various small aquatic invertebrates, algae and plant matter (Billard 1997). Some species of *Phoxinus* are popular among aquarium enthusiasts due to their small size and attractive coloration Froese and Pauly (2023).

All European minnows were previously identified as *Phoxinus phoxinus* (Linnaeus 1758). With the development of molecular and morphological techniques, numerous *Phoxinus* species' designations as synonyms of *P. phoxinus* (such as the list of synonyms published by Kottelat 2007) started to be questioned (Palandačić et al. 2015, 2017, 2020; Vucić et al. 2018; Bogutskaya et al. 2019, 2023; Denys et al. 2020; Turan et al. 2023). The first study on this was by Kottelat (2007), who described three species from Greece and southern France. Following are some studies: Four new *Phoxinus* species were described by Bianco and De Bonis (2015) from Italy and the Western Balkans. Molecular evidence points to a multispecies *Phoxinus* (Cyprinidae) complex in the Western Balkan Peninsula, as reported by Palandačić et al. (2015). Moreover, Vucić et al. (2018) investigated The Western Balkans' distribution of Eurasian minnow. Again, Palandačić et al. (2017) used molecular information in their taxonomic investigations to assign *Phoxinus* species to genetic lineages. The latest studies, *P. krkae* Bogutskaya et al. (2019), described a new minnow from Croatia and initially identified it as a molecular clad and Palandačić et al. (2020) researched museum collections to help with the genetic evaluation of species introductions in freshwater fishes (Cyprinidae: *Phoxinus* species complex): European minnows through time. In 2020, Denys et al. (2020) revised

Phoxinus in France and described two new species (Teleostei, Leuciscidae) and also created zones of coloration in *Phoxinus*. Finally, Turan et al. (2023) described a new species from Türkiye. Presently, the minnows of the genus *Phoxinus* include about 25 species (Eschmeyer et al. 2023; Froese and Pauly 2023; Turan et al. 2023). The geography of Türkiye has become the hub for diversifying many species and taxa currently recognized. Recent genetic-based studies on this topic demonstrate Türkiye's contribution to the planet's biodiversity (Geiger et al. 2014; Bektaş et al. 2019, 2020). Despite this rich biodiversity, the genus *Phoxinus* does not have a wide distribution in Türkiye.

The genus is represented by three species in Türkiye: *Phoxinus abanticus*, *P. colchicus*, *P. strandjae*. Ergene populations in the Thrace (in Türkiye) were defined as *Phoxinus strandjae* in previous studies (Çiçek et al. 2023; Özuluğ et al. 2023). These populations have never been studied in detail and we were able to collect them from six different places. Here, based on the morphological characters and a molecular data set, we describe a new *Phoxinus* species from the Ergene, a tributary of the Meriç River, Aegean Sea Basin, Türkiye.

Materials and methods

Fish sampling and measurements

Fish were collected by Samus 1000 pulsed DC electro-fishing equipment, at thirty-five sampling sites carried out between 2006 and 2017. After anaesthesia, specimens were fixed in 5% formaldehyde and stored in 70% ethanol or directly fixed in 96% ethanol. Measurements were made with a dial calliper and recorded to 0.1 mm. All measurements were made point to point. Methods for counts and measurements follow Kottelat and Freyhof (2007). Standard length (SL) was measured from the tip of the upper lip to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the last anal-fin ray to the end of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins were counted as "1½". lts body measurements were standardized by individuals' SL, and the measurements taken from the head region were standardized by individuals' head length.

The map in Fig. 1 was created using the Qgis software, version 3.22, available at http://diva-gis.org. Occurrence data in the map (Fig. 1) are based on the authors' material. The drawings were made using a Wacom Intuos comıc brand drawing tablet, Adobe ıllustrator and Adobe Photoshop programs (Fig. 7).

Abbreviations used: SL, standard length. **HL**, Head length. **SD**, standard deviation, Collection codes: **FFR**, Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize.

DNA isolation, amplification and sequencing

Total DNA from ethanol-preserved tissue of *Phoxinus* specimens was isolated with the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The Cytochrome *b* (Cyt *b*) gene of vertebrate mitochondrial DNA was amplified using the primers AlbCF (5′-CAACTACAAGAACATGGCAAGCC-3′) and AlbCR (5′-CTTCGGATTACAAGACCGATGC-3′) described by Bektas et al. 2019. The PCR protocol and thermocycler conditions were performed according to

Figure 1. Distribution of *Phoxinus* species in the Aegean and Black Sea basins and Abant Lake.

Turan et al. (2023). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and both directional sequencing of PCR products was performed with the same primers used for amplification at Macrogen Europe using an ABI PRISM 3730×1 Genetic Analyser and a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystem).

Molecular data analyses

The Cyt *b* gene of seven specimens five specimens of stream Buyukdere and two specimens of stream Ahmetbey) from the type locality of the newly identified species was sequenced for molecular comparison. First, the chromatograms of the raw Cyt *b* sequences were examined with the program Bioedit 7.2.5 (Hall 1999) and the detected errors were manually edited. The sequences were then matched with reference data stored in this database using nBLAST (Basic Local Alignment Search Tool) on GenBank. In order to determine the phylogenetic position of the new species and to calculate the sequence difference, the reference Cyt *b* sequences of the topotype samples of the congener species were downloaded from the Genbank. In the absence of sequences of topotype samples of congeners taxa, correct sequences considered representative of the species were included (Also see the "Material used in molecular genetic analysis" section).

All sequences were aligned using the CLUSTAL-W method (Thompson et al. 1994), trimmed from the ends and converted to FASTA file. The final data set had 1064 nucleotide positions without insertion and deletion. Also, the sequences were translated into protein sequence, and the stop codon was not determined. The interspecific genetic distances were calculated based on the uncorrected *p*-distance in MEGA X version (Kumar et al. 2018). Phylogenetic relationships between the species were estimated using maximum likelihood (ML) algorithm in MEGA X program and the bayesian (BI) algorithm in MrBayes v3.2.1 program (Ronquist et al. 2012). ML tree was generated based on the GTR+I+G model such that the best-fit evolution model was selected by the Akaike information criterion (AIC) in jModeltest 0.1.1 (Posada 2008). The ML tree was generated with 1000 bootstrap replicates to estimate the phylogenetic relationships of the mtDNA lineages. BI tree was generated according to the GTR+I+G model that the best-fit evolution model was selected by the Bayesian Information Criterion (BIC) in jModelTest 0.1.1. For BI, analyses were run for 1×10^6 generations with Metropolis coupled Monte Carlo Markov Chains (MCMC) sampled every 1000 generations. A conservative 25% of the trees were discarded as burn-in. To root the all phylogenetic tree, the authors used *Alburnoides fasciatus*, *Alburnus alburnus*, *Squalius cephalus* and *Rhynchocypris lagowskii* (GenBank accession numbers: MK860065, Bektaş et al. 2019; MT394745, Bektaş et al. 2020; JQ652365, Dubut et al. 2012; MG806688, Schönhuth et al. 2018, respectively) as out-groups.

The species delimitation analysis was carried out using ASAP (Assemble Species by Automatic Partitioning; Puillandre et al. 2012) method based on Cyt *b* data. To implement the ASAP method, we used the Kimura 2-parameter (K2P) distances and transition/transversion ratio (R:7.2) settings at the web address https://bioinfo.mnhn.fr/abi/public/asap/. The transition/transversion ratio (R) for the Cyt *b* data was calculated in MEGA X software.

Results

Molecular data analyses

We used molecular methods to test the validity of the new species, *Phoxinus radeki*, identified in this study. The Cyt *b* gene data of the new species were deposited at NCBI (OR552423–OR552429) and its first genetic record was created. In order to determine the phylogenetic position of the new species, we included the morphologically valid European *Phoxinus* species in our dataset and reconstructed phylogenies with ML and BI methods. Almost similar topologies emerged in the results of both methods, and the genus *Phoxinus* was monophyletic in both. The new species was moderately supported by both methods (BI: 0.55; ML: 72% Fig. 8). The phylogenetic analysis placed the new species as the sister species of *P. abanticus*.

The results based on genetic distance demonstrated that *Phoxinus strandjae* was the closest neighbor species to the new species with a mean *p*-distance of 3.08% (min. 2.82–max. 3.29%), while the most distant species was *P. fayollarum* with a mean *p*-distance of 8.73% (min. 8.46–max. 8.83%).

The species delimitation analysis according to the ASAP method based on Cyt *b* data identified 15 MOTUs (molecular operational taxonomic units) for 15 morphologically valid *Phoxinus* species. This result had the best ASAP score of 1.0 (p = 0.01) at a threshold distance of 0.017644. The new species, *P. radeki*, formed a single MOTU independent of other *Phoxinus* species.

***Phoxinus radeki* sp. nov.**

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Figs 2–4

Materials examined. *Holotype*. FFR 2327, 54 mm SL; Türkiye: Kırklareli prov.: stream Büyükdere about 6 km west of Pınarhisar, 41.6337, 27.5994.

***Paratypes*.** FFR 2301, 12, 45–71 mm SL. –FFR 2304, 20, 42–68 mm SL; –FFR 2314, 19, 38–46 mm SL; same data as holotype. –FFR 2320, 22, 44–60 mm SL; Türkiye: Kırklareli prov.: stream Poyralı about 5 km west of Pınarhisar, 41.6172, 27.5909. –FFR 2311, 1, 49 mm SL; Türkiye: Kırklareli prov.: stream Ahmetbey at Soğurcak, 41.6345, 27.6540. –FFR 2326, 26, 44–65 mm SL; Türkiye: Tekirdağ prov.: Ergene River at Saray, 41.4257, 27.9131.

Figure 2. *Phoxinus radeki* FFR 2327: holotype, 54 mm SL, male; Türkiye: Ergene River.

Figure 3. *Phoxinus radeki* FFR 2326, from top: paratypes, 57–55 mm standard length (SL), possible males; Türkiye: Ergene River.

Genetic material. FFR-DNA-Ph42-43-44-45-46; Türkiye: Kırklareli prov.: stream Büyükdere about 6 km west of Pınarhisar, 41.6337, 27.5994 (GenBank accession numbers: OR552425–OR552426–OR552427–OR552428–OR552429). –FFR-DNA-Ph31-32; Türkiye: Kırklareli prov.: stream Ahmetbey at Soğurcak, 41.6345, 27.6540 (GenBank accession numbers: OR552423–OR552424).

Diagnosis. *Phoxinus radeki* is distinguished from the *Phoxinus* species (*P. strandjae*, *P. abanticus*) in adjacent basins as below. It is immediately distinguished from species *P. strandjae* by body color and pattern (not spawning period and immediately after fixation). *Phoxinus radeki* has short dark rectangular blotches along the lateral line. The area between the lateral line and belly is yellowish in both males and females. There are irregularly-shaped black spots on the upper part of the flank and no dark stripes on the middle part of the flank in males. *Phoxinus strandjae* (Fig. 5) has bars reaching from the dorsal to below the lateral line, hyaline between the lateral line and belly in females, blackish in males and no black spots on the upper part of the flank. It further differs from *P. strandjae* by the scales of the breast. *Phoxinus radeki* has breast scaled but separated unscaled area anteriorly, *P. strandjae* has breast scaled connected or scales not connected anteriorly (Fig. 7).

Phoxinus radeki is distinguished from species *P. abanticus* by the presence of scales on the breast in males (vs. absent, Fig. 7), a slenderer body (body depth dorsal fin origin 16–21% SL vs. 22–25) and a slenderer caudal peduncle (8–10% SL vs. 11–13). It further differs from *P. abanticus* by having more lateral line scales (75–96 vs.60–69).

Description. The general appearance is shown in Figs 2–4, and morphometric data are given in Table 1. The maximum size is 71 mm SL. Body depth at dorsal-fin origin 16%–21% SL.The dorsal body profile more convex than the ventral profile. The head short, its length 24%–27% SL, upper profile straight or slightly convex on the interorbital area and convex on the snout. The snout short and its upper profile markedly convex. The mouth terminal to slightly subterminal, the upper lip not projecting or slightly projecting beyond the tip of the lower lip. The corner of the mouth reaches to level with the lower margin of the eye or pupil.

Lateral line complete, with 75–96 scales, and almost reaching to caudal-fin base; 9–15 scale rows between lateral line and dorsal-fin origin; and 6–9 scale rows between lateral line and anal-fin origin. Dorsal fin with three simple 7½ branched rays, outer margin straight or slightly convex. Pectoral-fin with 16–18 rays, outer margin convex. Pelvic fin with seven to eight branched rays, outer margin convex. Anal fin with three simple 6½–7½ branched rays, outer margin straight or convex. The caudal fin, deeply forked.

Coloration (Not spawning period and immediately after fixation): A short dark rectangular blotch along the lateral line, between the lateral line and belly yellowish in both males and females, irregularly shaped black spots on the upper part of the flank and no dark stipe or very faintly marked narrow stripe on the middle part of flank in males. Dorsal and caudal fins slightly gray or hyaline, pectoral, pelvic and anal fins hyaline (except for some individuals). In some specimens often 10–14 short brownish bars over-imposed on the stripe. Black spot in the middle of the caudal-fin base.

Sexual dimorphism. Males with stronger and longer pectoral fins, and nuptial tubercles on the head, in both species.

Figure 4. *Phoxinus radeki* FFR 2311, from top: paratypes, 46 mm SL, male; 55 mm SL female; Türkiye: Stream Ahmetbey.

Figure 5. *Phoxinus strandjae* FFR 2312, from top: 62 mm SL male; 62 mm SL female; Türkiye: Rezve River.

Etymology. The species is named for Radek Sanda (Prague) for his contribution to the knowledge of the ichthyofaunal of Europe. A noun in genitive, indeclinable.

Distribution. *Phoxinus radeki* is presently known from the Ergene River (Ageaen Sea Basin) (Fig. 1). It inhabits the cold, well-oxygenated waters of fast-flowing mountain streams and large lowland rivers. A transboundary river known as Meriç-Ergene (Maritsa, Evros) serves as a border between Türkiye, Greece, and Bulgaria. It starts in Bulgaria and flows through Türkiye, where it forms a 203 km-long border with Greece. It is the longest river in the Balkan Region. Before flowing into the Aegean Sea, the river passes through Greece. Bulgaria (upstream), Türkiye (downstream), and Greece (downstream) are the three riparian nations in the basin. The remaining 35% of its catchment area is divided into 28% and 7% within the borders of Türkiye and Greece, respectively, while the remaining 65% of its catchment area is contained within the boundaries of Bulgaria. The border between Türkiye and Greece is formed by a piece of the Meriç River's lower course, while the remaining portion flows in Türkiye. The river emerges into the Aegean Sea from Saros. (https://floodmerg.tarimorman.gov.tr/en/meric-ergene-river-basin). It is known that after the entry of the Ergene River into the system, the water quality of the Meriç River decreased seriously and this situation adversely affected the biota (Elipek et al. 2010; Güher et al. 2011; Tokatlı 2020).

Discussion

Until 2023, only two *Phoxinus* species were known in Türkiye which are *P. colchicus* from Çoruh River (southeast of the Black Sea basin) and *P. strandjae* from the coastal rivers southwest of the Black Sea basin in Thrace region and Lake Sapanca drainage (Geiger et al. 2014; Bayçelebi et al. 2015; Saç and Özuluğ 2015; Sarı et al. 2019). Most recently, Turan et al. (2023) described *P. abanticus* from the Lake Abant drainage. The morphological characters of *Phoxinus radeki* that distinguish it from *P. abanticus* and *P. strandjae* species are given in the identification section. In addition, *Phoxinus radeki* is distinguished from *P. strymonicus*, which is distributed in the Aegean basin, by having fewer scale rows between anal-fin origin and lateral line (7–9 vs.10–13) and a slenderer caudal peduncle (8–10% SL vs. 10–12 Fig. 6). It further differs from *P. strymonicus* by scales of breast. *Phoxinus radeki* has breast scaled but separated unscaled area anteriorly, while *P. colchicus* and *P. strymonicus* have breast scaled and scaled area connected anteriorly.

The COI marker has been frequently used to identify many vertebrate and invertebrate species (Hebert et al. 2003; Hubert et al. 2008, 2015; Geiger et al. 2014). This gene region was preferred in *Phoxinus* species in previous studies, but it was observed that it was less successful in species delimitation for this genus compared to the Cyt *b* gene (Palandačić et al. 2015, 2017, 2020; Vucić et al. 2018; Corral-Lou et al. 2019). Especially within the genus *Phoxinus*, genetic differences based on COI between neighboring species appear to be lower compared to the Cyt *b* gene. (Palandačić et al. 2015, 2017, 2020). Therefore, we performed our analyses based on the Cyt *b* gene.

The findings of analyses based on the mitogenome's Cyt *b* gene proved that it diverged from valid European *Phoxinus* species and developed an independent lineage from them, supporting the morphometric findings.

Figure 6. *Phoxinus strymonicus*, NMP6V88934–88954, from top: 64 mm SL male; 62 mm SL female; Bulgaria: Strymon River.

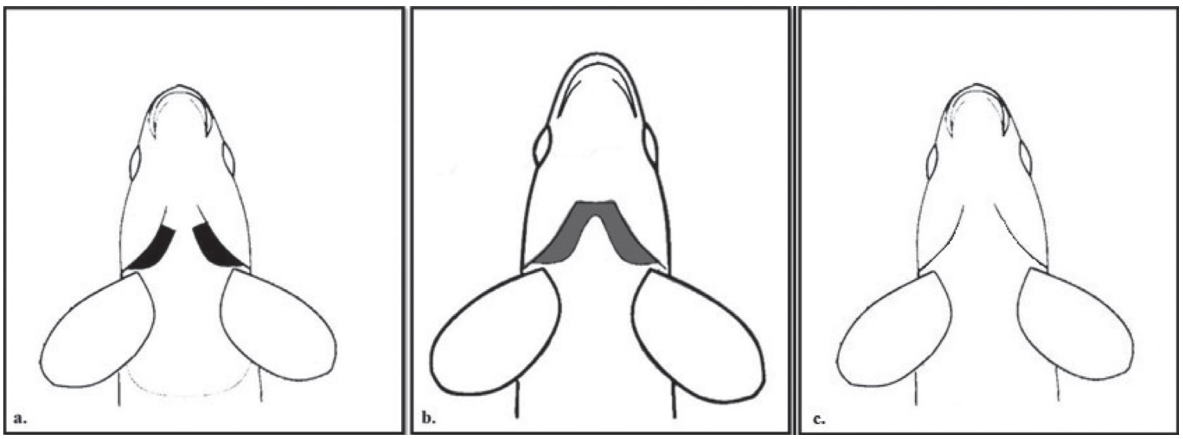

Figure 7. Breast scale shape of a. *Phoxinus radeki*,b. *P. strandjae* and c. *P. abanticus*.

The genetic characterization of the new species was carried out in the current investigation, and its first genetic record was created. This could be important for upcoming research on the genus *Phoxinus*. With the initial data of type samples, inaccurate identifications can be avoided. Previous investigations have evaluated the effectiveness of the mtDNA Cyt *b* gene in identifying genetic lineages in the genus *Phoxinus*, and those investigations have produced positive outcomes (Palandačić et al. 2015, 2017, 2020; Vucić et al. 2018; Corral-Lou et al. 2019; Turan et al. 2023). Furthermore, our findings agreed with those of previous investigations (Palandačić et al. 2015, 2017, 2020; Vucić et al. 2018; Corral-Lou et al. 2019; Turan et al. 2023).

Comparative material

Materials examined are already listed by Turan et al. (2023), except the materials listed below:

Phoxinus strymonicus NMP6V88934-88954, 17, 42–66 mm SL; Bulgaria: Elešnica River, upstream of Vaksevo Strymon River basin, 42.1364, 22.8521.

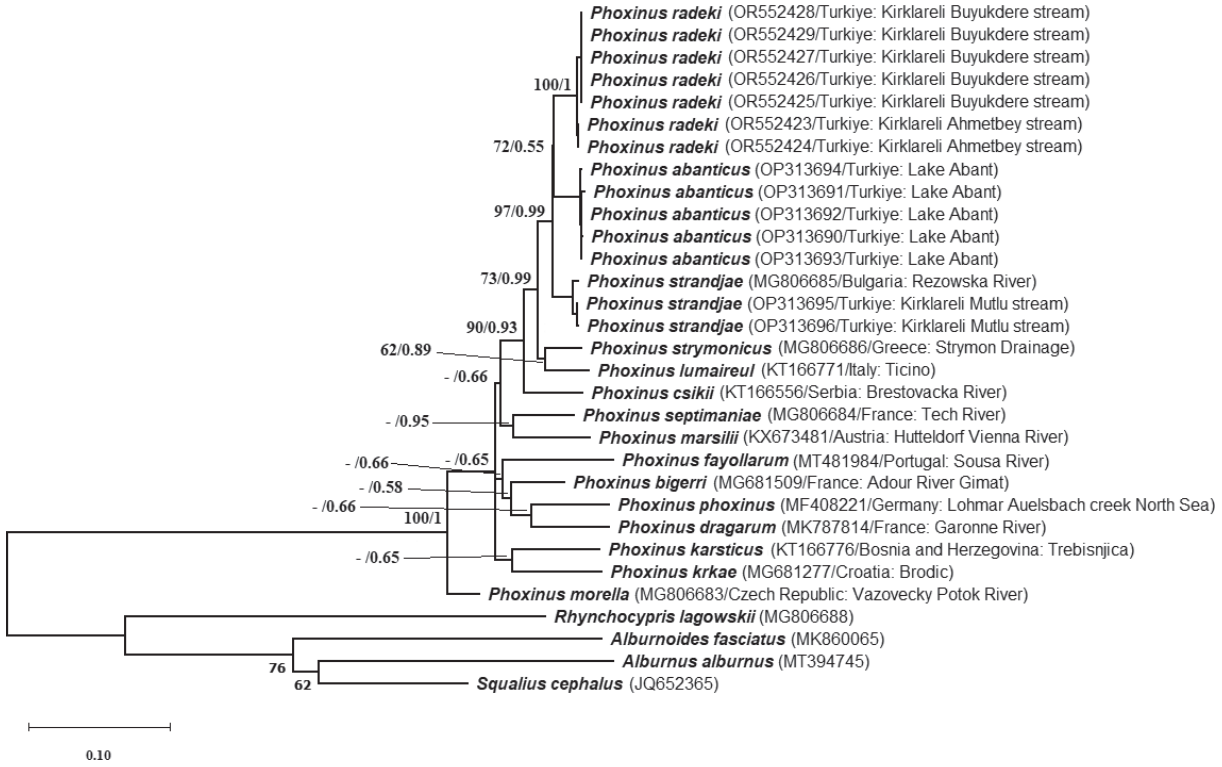

Figure 8. Maximum Likelihood (ML) phylogenetic tree reconstructed based on the Cyt *b* gene. ML and BI methods yielded the same topologies, and therefore only the ML tree is indicated. The bootstrap values of ML and posterior probability values of BI are indicated on nodes (ML/BI). The bootstrap and posterior probability values are indicated above nodes on tree if 50% and 0.5 or higher.

Table 1. Morphometry of *Phoxinus radeki* and *P. strymonicus* species. Mean values are given in parentheses.

	***P. radeki* n=38**			***P. strymonicus* n=19**	
	Aegean Sea			**Aegean Sea**	
	Ergene River			**Strymon River**	
	Range	**SD**	**H**	**Range**	**SD**
Standard length (mm)	46–61		54	47–64	
In per cent of standard length					
Head length	24.4–26.7 (25.7)	0.07	25.5	23.9–28.3 (25.8)	0.12
Body depth at dorsal-fin origin	16.0–21.1 (18.8)	0.11	19.4	18.8–21.6 (20.0)	0.08
Caudal peduncle depth	8.1–10.2 (9.2)	0.05	9.9	9.7–12.0 (10.7)	0.05
Head $width_1$ (ant. margin of the eye)	30.4–39.9 (36.2)	0.20	39.6	31.6–49.7 (38.3)	0.04
Head $width_2$ (post. margin of the eye)	47.7–55.2 (50.6)	0.16	51.0	38.6–57.6 (51.5)	0.48
Head $width_3$ (at opercle)	48.8–58.1 (53.3)	0.26	57.0	46.2–64.6 (57.2)	0.40
Head $depth_1$ at the interorbital region	44.2–53.1 (48.9)	0.21	53.1	40.7–60.1 (50.4)	0.46
Head $depth_2$ (at occiput)	57.6–69.8 (64.1)	0.28	67.7	56.9–72.0 (64.5)	0.39
Eye diameter	20.6–30.5 (25.2)	0.20	26.7	24.7–31.7 (27.6)	0.19
Snout length	25.1–32.4 (29.0)	0.19	29.7	22.3–31.6 (28.1)	0.27
Interorbital width	23.9–30.1 (27.6)	0.16	28.7	27.1–42.7 (32.6)	0.43
Snout width at nostrils	24.6–35.9 (31.2)	0.23	29.8	26.4–39.0 (32.1)	0.36
Snout depth at nostrils	25.6–35.6 (30.9)	0.23	35.6	30.1–42.9 (36.2)	0.34
Predorsal length	52.2–57.7 (55.0)	0.11	54.0	52.3–57.0 (54.3)	0.15
Prepelvic length	44.0–50.2 (46.6)	0.16	44.5	41.3–46.8 (44.6)	0.16
Preanal length	61.8–67.1 (64.2)	0.13	62.3	58.2–65.0 (62.0)	0.20
Pectoral-fin origin to anal fin	36.7–44.0 (41.0)	0.19	39.7	37.7–44.6 (41.0)	0.20
Pectoral-fin origin to pelvic fin	19.4–27.2 (23.1)	0.18	21.3	19.7–25.1 (22.3)	0.19
Pelvic-fin origin to anal fin	16.3–20.7 (17.8)	0.09	17.1	16.4–22.1 (18.6)	0.12
Caudal peduncle length	22.7–28.0 (25.3)	0.12	25.5	22.4–28.6 (26.1)	0.14
Dorsal fin height	17.0–24.3 (20.7)	0.14	21.7	17.9–23.3 (21.3)	0.15
Pectoral-fin length	16.7–22.3 (19.3)	0.15	20.7	15.6–23.4 (18.2)	0.17
Pelvic-fin length	13.1–19.6 (15.3)	0.14	16.6	13.0–17.5 (15.3)	0.13
Anal-fin length	18.9–23.2 (20.4)	0.11	22.1	16.2–21.0 (18.9)	0.13
Upper caudal-fin lobe	19.1–24.4 (21.8)	0.12	20.9	21.3–26.1 (23.6)	0.12

Material used in molecular genetic analysis

***Phoxinus abanticus*:** FFR-DNA-Ph26-27-28-29-30; Türkiye: Bolu prov., Lake Abant, 40.6647, 31.4250 (GenBank accession numbers: OP313690–OP313691–OP313692–OP313693–OP313694; Topotype samples; Turan et al. 2023).
***P. strandjae*:** FFR-DNA-Ph34-36; Türkiye: Kırklareli prov.: stream Mutlu (Rezve), 41.9422, 27.6202 (GenBank accession numbers: OP313695–OP313696; Topotype samples; Turan et al. 2023). – Bulgaria: Rezowska River (GenBank accession number: MG806685; Topotype sample; Schönhuth et al. 2018).
***P. strymonicus*:** Greece: Strymon Drainage (GenBank accession number: MG806686; Topotype sample; Schönhuth et al. 2018).
***P. csikii*:** Serbia: Brestovacka River (GenBank accession number: KT166556; Palandačić et al. 2015).
***P. septimaniae*:** France: Tech River (GenBank accession number: MG806684; Schönhuth et al. 2018).
***P. lumaireul*:** Italy: Ticino, Po River (GenBank accession number: KT166771; Topotype sample; Palandačić et al. 2015).
***P. marsilii*:** Austria: Hutteldorf Vienna River (GenBank accession number: KX673481; Topotype sample; Ramler et al. 2016).
***P. bigerri*:** France: Adour River Gimat (GenBank accession number: MG681509; Topotype sample; Vucić et al. 2018).
***P. karsticus*:** Bosnia and Herzegovina: Trebisnjica (GenBank accession number: KT166776; Topotype sample; Palandačić et al. 2015).
***P. phoxinus*:** Germany: Lohmar Auelsbach creek North Sea (GenBank accession number: MF408221; Palandačić et al. 2017).
***P. morella*:** Czech Republic: Vazovecky Potok River (GenBank accession number: MG806683; Topotype sample; Schönhuth et al. 2018).
***P. krkae*:** Croatia: Brodic (GenBank accession number: MG681277; Topotype sample; Vucić et al. 2018).
***P. fayollarum*:** Portugal: Sousa River (GenBank accession number: MT481984; Garcia-Raventós et al. 2020).
***P. dragarum*:** France: Garonne River (MK787814; Topotype sample; Corral-Lou et al. 2019).

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