

Phoxinus abanticus, a new species from the Lake Abant drainage in Türkiye (Teleostei: Leuciscidae)

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Abstract

Phoxinus abanticus, a new species, is described from the Lake Abant basin. It is distinguished from *Phoxinus* species in Türkiye and adjacent waters by the presence of fewer lateral line scales (60–69, vs. 75–91 in *Phoxinus colchicus*, 75–90 in *Phoxinus strandjae*); a deeper caudal peduncle (caudal peduncle depth: 1.8–2.3 times in length, vs. 2.4–2.9 in *P. colchicus*; 2.5–3.2 in *P. strandjae*); the absence of scales in the breast of males (vs. present); and ventral body reddish in nuptial colouration pattern for male (vs. brackish). The new species, *P. abanticus*, is also distinguished from its closest relative, *P. strandjae*, by a minimum of 3.40% genetic distance in the mtDNA cytochrome *b* (*cyt b*) gene.

KEYWORDS

cyt b, freshwater fish, minnow, morphology, taxonomy

1 | INTRODUCTION

The European minnows, genus *Phoxinus* Rafinesque 1820, are widespread in freshwater ecosystems throughout the Palearctic region. Their habitats range from Eurasia to the Ebro drainage in Spain eastward to Anadyr and Amur drainages in Russia (Bianco & De Bonis, 2015; Kottelat, 2007; Kottelat & Freyhof, 2007; Palandačić *et al.*, 2015, 2017; Vucić *et al.*, 2018).

For a long time, the genus *Phoxinus* was represented by a single species *Phoxinus phoxinus* (Linnaeus, 1758) (Palandačić *et al.*, 2017, 2020). Later, Kottelat (2007) raised this issue by comparing 60 different populations in all of Europe and Asia to prove that many individual species were miscategorized as *P. phoxinus*. In addition, the researcher identified three new species from the south of France and Greece (Kottelat, 2007). This study described *Phoxinus bigerri* Kottelat, 2007, from the Adour drainage in south-west France, *Phoxinus septimaniae* Kottelat, 2007, from the Mediterranean coast of France and *Phoxinus*

strymonicus Kottelat, 2007, from the Strymon drainage in Greece and also accepted the previously described species *Phoxinus lumaireul* (Schinz, 1840) from Italy, *Phoxinus colchicus* Berg, 1910, from the Caucasus and *Phoxinus strandjae* Drensky, 1926, from Thrace as valid species. In the early 2000s, the beginning of molecular studies as well as morphological studies revealed the high genetic diversity within the genus *Phoxinus* (Bogutskaya *et al.*, 2019; Denys *et al.*, 2020; Geiger *et al.*, 2014; Palandačić *et al.*, 2015, 2017, 2020; Thaulow *et al.*, 2014; Vucić *et al.*, 2018). Many authors have performed research on both the distribution of species and the discovery of new ones. Bianco and De Bonis (2015) described *Phoxinus ketmaieri* from the Krk Island, *Phoxinus karsticus* from the Popovo Polje in the Trebinje endorheic drainage, *Phoxinus apollonicus* from the Lake Skadar drainage and *Phoxinus likai* from the Oruca River in Croatia. Bogutskaya *et al.* (2019) described *Phoxinus krkae* from the upper Krka River. Most recently, Denys *et al.* (2020) described *Phoxinus fayollarum* from Boron stream in France and *Phoxinus dragarum* from Arrat-Devant stream in France, and also provided information on the distributions of the *Phoxinus csikii*, *P. bigerri*, *P. phoxinus* and *P. septimaniae* species.

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Palandačić *et al.* (2015), Palandačić *et al.* (2017) and Palandačić *et al.* (2020) assigned *Phoxinus* species to genetic lineages in their taxonomic studies based on molecular data.

There are very few genetic and morphological studies, including *Phoxinus* species in Türkiye. The distribution areas and populations of *Phoxinus* species in Türkiye's inland waters, which are known to be naturally distributed in Türkiye and which have not yet been recorded as being carried by various human activities (e.g., fishing and aquaculture), are not known in detail. It has been detected in a few studies on the determination of *Phoxinus* species. Two valid species have been identified in the studies carried out to date. *P. strandjae* was recorded from the Sapanca drainage in molecular studies (Geiger *et al.*, 2014; Palandačić *et al.*, 2020) and from the Thrace region and Biga Peninsula in morphological studies (Kottelat, 2007; Saç & Özuluğ, 2015; Sari *et al.*, 2006, 2019), and *P. colchicus* was recorded from the Çoruh River in morphological studies (Bayçelebi *et al.*, 2015).

According to the aforementioned studies, 23 genetic lineages have now been identified, of which 13 are valid species; 3 lineages have available names, but their species status has not yet been confirmed; and 7 lineages are potentially new species still to be identified, and also some species names have been synonymized (Denys *et al.*, 2020; Palandačić *et al.*, 2017, 2020). As yet, unidentified genetic lineages within *Phoxinus*, distributed over a wide biogeography, are a sign of the possible existence of more *Phoxinus* species and provide a good example of cryptic speciation (Corral-Lou *et al.*, 2019; Palandačić *et al.*, 2017, 2020).

According to the authors' observations and literature, the most important characters that morphologically distinguish *Phoxinus* species are the total number of scales in the lateral line, particularly the scales on the abdomen and breast; the height of the caudal peduncle; and colour and pattern in spawning period in males (Bogutskaya *et al.*, 2019; Denys *et al.*, 2020; Palandačić *et al.*, 2017). Previous molecular studies have revealed that the cytochrome *b* (*cyt b*) gene is an effective gene region in distinguishing *Phoxinus* species. It is observed that most of the genetic analyses in *Phoxinus* genus are based on analysis of mitochondrial genes, and most of the species delimitations are based on *cyt b* and *COI* genes (Palandačić *et al.*, 2015). Even among *Phoxinus* species, genetic lineages were determined using *cyt b* gene distances ranging from 4% to 11% (Corral-Lou *et al.*, 2019; De Santis *et al.*, 2021; Palandačić *et al.*, 2015; Palandačić *et al.*, 2017; Palandačić *et al.*, 2020).

The authors were able to examine *Phoxinus* natural populations from the Thrace region in the south-western Black Sea drainages and the Lake Abant drainages using both morphological and molecular data. Their research presents the existence of a new genetic lineage beyond known genetic lineages among European minnows. The outlet of the Lake Abant population represents an undescribed species, which is discussed.

2 | MATERIALS AND METHODS

Individuals belonging to *Phoxinus* were collected by electrofishing. After anaesthesia, the specimens were fixed in 5% formaldehyde and stored in 70% ethanol or directly fixed in 99% ethanol. Methods for

counts and measurements follow Kottelat and Freyhof (2007) and for nuptial colouration Denys *et al.* (2020). Measurements were made using a dial calliper and recorded to 0.1 mm. All measurements were made point to point. 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."

Denys *et al.* (2020) described five pigmentation zones along the flank between the pectoral-fin origin and anal-fin origin for nuptial colouration of *Phoxinus* species. Those are zone 1 (dorsal pigmentation), zone 2 (a stripe running between the upper edge of the operculum and the upper part of the caudal-fin base), zone 3 (a wide, brown or grey pigmentation field often with iridescent scales), zone 4 (a wide iridescent green or golden pigmentation zone) and zone 5 (a silvery, blackish, red or orange zone lacking iridescent scales, below Z4 and above the pigmentation on the belly, which is usually red, orange, black or silvery). The colours and patterns of *P. strandjae* and *P. colchicus* species were obtained from the images in Kottelat and Freyhof (2007).

Twenty-five measurements of new species ($n = 18$), *P. strandjae* ($n = 44$) and *P. colchicus* ($n = 16$) were analysed with PCA using the software package Past, version 1.8 (Hammer *et al.*, 2001). All metric characters (raw data) were standardized with log after proportioning to SL and head length (HL) and then subjected to PCA.

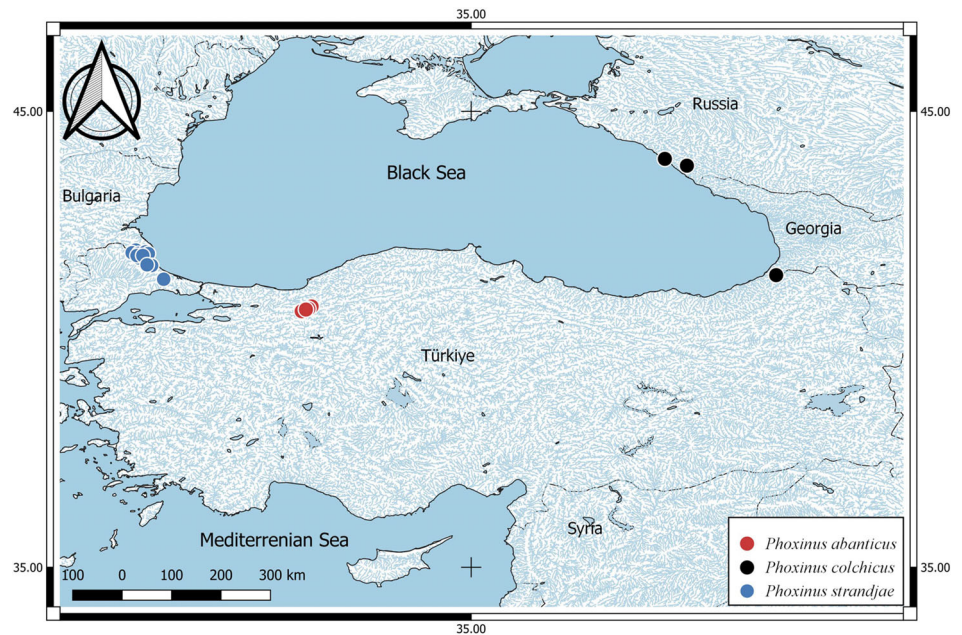
The map in Figure 1 was created using the Qgis software, version 3.22, available at <http://diva-gis.org>. Occurrence data in the map (Figure 1) are based on the authors' material.

The animal welfare laws, guidelines and policies of the Republic of Türkiye approved by the Recep Tayyip Erdogan University Animal Experiments Local Ethics Committee (2014/72) were followed for the care and use of experimental animals.

3 | TOTAL DNA ISOLATION, PCR AMPLIFICATION AND SEQUENCING

Total DNA from fin clips of *Phoxinus* specimens was extracted in the Qiacube Automated DNA isolation device using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The purity of the isolates was assessed on 1% agarose gel, whereas the concentration was quantified on a Nanodrop 2000/c spectrophotometer (Thermo Scientific, Rockford, IL, USA). Dilutions were made based on these quantifications. The *cyt b* gene of vertebrate mtDNA was amplified (1064 bp) using forward primer AlbCF: 5'-CAACTACAAGAACATGGCAAGCC-3' and reverse primer AlbCR: 5'-CTTCGGATTACAAGACCGATGC-3' (Bektas *et al.*, 2019). PCRs were carried out using 50 µl total volumes containing 5 µl of 10× reaction buffer, 5 µl of MgCl₂ (25 mM), 7 µl of dNTPmix (10 mM), 1 µl of forward primer (10 pmol), 1 µl of reverse primer (10 pmol), 0.2 µl of Taq DNA polymerase (1 U), 2 µl of DNA template (50 ng/µl) and 28.8 µl of pure sterilized water. PCRs were performed using a gradient thermal cycler Biorad T100 (Bio-Rad, Hercules, CA, USA). The PCR conditions were as follows: 1 cycle at 95°C for 3 min, followed by denaturation by 35 cycles at 95°C for 45 s,

FIGURE 1 Distribution of *Phoxinus* species in the Black Sea basin and Lake Abant outlet water.



annealing at 55°C for 30 s, extension at 72°C for 1 min and one last cycle at 72°C for 5 min for final extension.

The concentrations and sizes of PCR products were assessed both spectrophotometrically and on 1.2% TAE (Tris-acetate-EDTA)-agarose gel containing 0.5 mg l⁻¹ ethidium bromide (EtBr). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Bidirectional sequencing of PCR products was performed with an ABI PRISM 3730x1 Genetic Analyser (Applied Biosystems; www.appliedbiosystems.com) using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystems) at Macrogen Europe (www.macrogen.com).

4 | MOLECULAR DATA ANALYSES

In the present study, the authors generated mtDNA *cyt b* partial sequences (1064 bp) belonging to *Phoxinus* species from Türkiye. Five individuals of *Phoxinus abanticus* and two of *P. strandjae* were sequenced. The authors included 11 previously published sequences belonging to 11 *Phoxinus* species from GenBank to genetic analysis (refer to the “Material used in molecular genetic analysis” section).

Initially, the chromatograms of the raw *cyt b* sequences were checked. Detected faulty chromatograms were manually corrected using the Bioedit 7.2.5 (Hall, 1999) programme. The *cyt b* data set was created using sequences of the *Phoxinus* species previously published in GenBank and the sequences generated in this study. Then 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 pair-wise 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) algorithms in MEGA X version. ML tree was generated based on the GTR+I+G model such that the best-fit evolution model was selected by the lowest AIC score 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. In phylogenetic analysis, the author used *Alburnoides fasciatus*, *Alburnus alburnus* and *Squalius cephalus* (GenBank accession numbers: MK860065, Bektas *et al.*, 2019; MT394745, Bektas *et al.*, 2020; and JQ652365, Dubut *et al.*, 2012, respectively) as out-groups.

5 | RESULTS

5.1 | PCA data analysis

In PCA, 78 individuals [*Phoxinus anaticus* (*n* = 18), *P. strandjae* (*n* = 44) and *P. colchicus* (*n* = 16)] were log standardized after the 25 metric characters obtained were proportioned to standard height and HL. The results of the PCA further confirm the differences between the new species when compared with the other two species (*P. colchicus* and *P. strandjae*). The plot indicates that the new species is separated from these two closely related species (Table 1; Figure 2). Although the first two extracted components explain 56.17% of the total variation among the examined samples, the first three components were considered due to height eigenvalues, and these account for 34.16%, 22.01% and 9.46% of the total variation, respectively, in the present paper. The loadings on the first principal component (PC I) include seven metric characters (body depth at dorsal-fin origin, distance between pectoral- and pelvic-fin origins, depth of caudal peduncle, dorsal-fin height, pectoral-fin length, pelvic-fin length and depth of caudal peduncle in percentage of caudal peduncle length) (see Table 1, highlighted in bold font).

TABLE 1 Character loadings on principal components I and II (PC I and PC II) for 26 measurements taken on 79 specimens of *Phoxinus abanticus*, *Phoxinus strandjae* and *Phoxinus colchicus*

Metrics features	PC I	PC II
In percentage of standard length		
Head length	0.091	0.024
Body depth at dorsal-fin origin	-0.239	0.277
Predorsal length	-0.002	-0.001
Prepelvic length	-0.076	0.004
Pre-anal length	-0.033	-0.037
Distance between pectoral- and anal-fin origins	-0.141	-0.008
Distance between pectoral- and pelvic-fin origins	- 0.336	0.005
Distance between pelvic- and anal-fin origins	-0.013	-0.031
Length of caudal peduncle	0.073	0.052
Depth of caudal peduncle	-0.261	0.457
Dorsal-fin height	0.218	0.302
Pectoral-fin length	0.321	0.291
Anal-fin height	0.242	0.189
Pelvic-fin length	0.236	0.302
Caudal-fin length	0.257	0.243
In percentage of head length		
Head width at anterior margin of eye	-0.248	-0.039
Head width at posterior margin of eye	-0.130	-0.003
Head width at posterior middle point of opercle	-0.207	0.060
Head depth throughout eye	-0.114	0.110
Head depth at nape	0.147	0.221
Eye diameter	-0.008	0.332
Snout length	-0.049	-0.145
Interorbital distance	-0.181	-0.069
Width of snout at nostrils	-0.224	-0.033
Depth of snout at nostrils	-0.187	0.034
In percentage of caudal peduncle length		
Depth of caudal peduncle	-0.261	0.437

5.2 | Molecular data analyses

The seven newly generated *cyt b* partial sequences were deposited in GenBank accession numbers between OP313690 and OP313696. The pair-wise genetic distance among European minnows ranged from a minimum of 3.40% (*P. abanticus*-*P. strandjae*) to a maximum of 9.02% (*P. phoxinus/Phoxinus marsilii*-*P. karsticus* and *P. septimaniae*-*P. krkae*) (Table 2). The pair-wise genetic distance values of all *Phoxinus* species are presented in Table 2. The European minnows were monophyletic according to the ML tree result and revealed several well-supported (bootstrap values: 75%-100%) lineages (Figure 3). The new species resolved in phylogenetic tree with a high bootstrap value

(100%; Figure 3). The sister taxon of the new species is *P. strandjae* (Figure 3).

5.3 | *P. abanticus*, new species

urn:lsid:zoobank.org:act:3FD91E33-900B-458E-A2AE-8EFCAFB49820.

5.3.1 | Holotype

Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize (FFR), 2322, 1, 63 mm SL; female, Türkiye: Bolu Province: outlet of Abant Lake, 40.664722 N, 31.425000 E.

5.3.2 | Paratypes

FFR 2309, 2, 42-48 mm SL, female; FFR 2309, 2, 55-57 mm SL, male; same data as holotype. FFR 2321, 16, 32-47 mm SL, female; FFR 2325, 6, 39-53 mm SL, female; Türkiye: Bolu Province: outlet of Abant Lake, 40.648420 N, 31.371780 E. Istanbul University, Science Faculty, Hydrobiology Museum, Istanbul (IUSHM) 2022-1469, 1, 52 mm SL, male; Bolu Province: outlet of Abant Lake near Dereceören village, 40.648420 N, 31.371780 E. IUSHM 2022-1468, 3, 20-33 mm SL, female; IUSHM 2014-1158, 3, 30-33 mm SL, male; Bolu Province: stream Büyüksu at Yumrukaya, 40.716480 N, 31.496540 E.

5.3.3 | Diagnosis

P. abanticus is distinguished from species *P. colchicus* and *P. strandjae* in adjacent waters by the absence of scales on the breast in males (Figure 4) [vs. breast scaled and scales continuously across the breast in *P. colchicus* (Figure 5) and breast scaled and scales not connected anteriorly in *P. strandjae* (Figure 6)]; fewer lateral line scales (60-69, vs. 75-91 in *P. colchicus*, 75-90 in *P. strandjae*); and a short and deeper caudal peduncle (caudal peduncle depth 1.8-2.3 times its length, vs. 2.4-2.9 in *P. colchicus*; 2.5-3.2 in *P. strandjae*); a deeper body depth at dorsal-fin origin (22%-25% SL, mean 23.6, vs. 18-23, mean 20.7 in *P. colchicus*; 16-23, mean 19.5 in *P. strandjae*). *P. abanticus* is further distinguished from *P. colchicus* and *P. strandjae* by colour pattern in spawning period in males. In *P. abanticus*, Z1 brownish with small irregularly shaped blackish spots (vs. dark brown in *P. strandjae*, light brown with vertically elongated pale blotches in *P. colchicus*); Z2 disappeared in the front of the body, slightly distinct in the posterior part of the body (vs. distinct in both anterior and posterior parts of body in *P. strandjae*, indistinct in both posterior and anterior parts of body in *P. colchicus*); Z3 distinct only in anterior part of body (vs. absent); and Z5 and belly with orange (vs. yellowish).

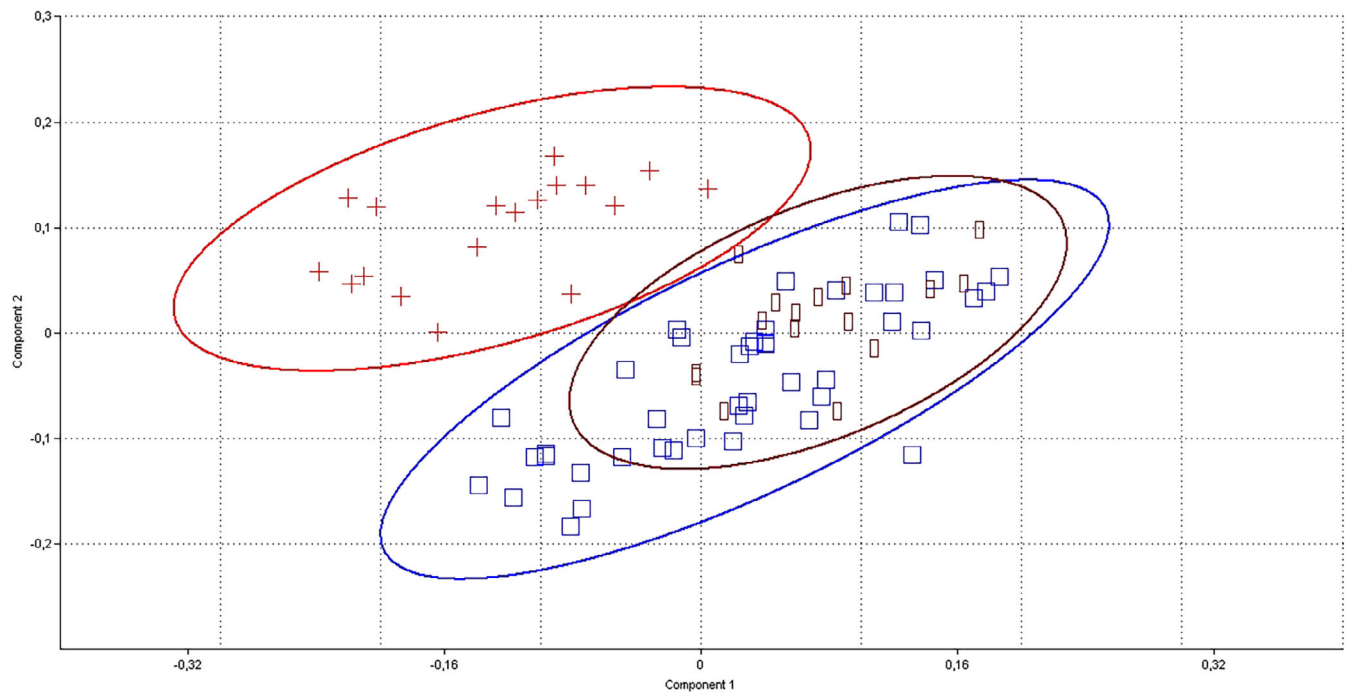


FIGURE 2 A scatter plot of the scores of the first two principal components (PC I and PC II) for 80 specimens of 3 species *Phoxinus abanticus* (+), *Phoxinus strandjae* (□) and *Phoxinus colchicus* (▢) based on 26 morphometric characters

TABLE 2 Pair-wise genetic distances between European minnows under uncorrected *p*-distance

Species	1	2	3	4	5	6	7	8	9	10	11
1 <i>Phoxinus abanticus</i>											
2 <i>Phoxinus strandjae</i>	0.0340										
3 <i>Phoxinus lumaireul</i>	0.0513	0.0482									
4 <i>Phoxinus strymonicus</i>	0.0500	0.0479	0.0451								
5 <i>Phoxinus csikii</i>	0.0594	0.0539	0.0602	0.0592							
6 <i>Phoxinus bigerri</i>	0.0729	0.0711	0.0733	0.0695	0.0724						
7 <i>Phoxinus Phoxinus</i>	0.0836	0.0805	0.0855	0.0827	0.0846	0.0761					
8 <i>Phoxinus septimaniae</i>	0.0763	0.0686	0.0686	0.0724	0.0686	0.0667	0.0677				
9 <i>Phoxinus Marsilii</i>	0.0773	0.0742	0.0780	0.0752	0.0818	0.0808	0.0865	0.0695			
10 <i>Phoxinus Morella</i>	0.0829	0.0774	0.0808	0.0724	0.0855	0.0752	0.0855	0.0695	0.0780		
11 <i>Phoxinus krkae</i>	0.0765	0.0764	0.0742	0.0808	0.0827	0.0799	0.0893	0.0902	0.0827	0.0808	
12 <i>Phoxinus karsticus</i>	0.0867	0.0796	0.0855	0.0874	0.0893	0.0780	0.0902	0.0808	0.0902	0.0818	0.0827

5.3.4 | Description

The general appearance is shown in Figures 7–10, and morphometric data are given in Table 3. The maximum size is 70 mm SL. Body deep, its depth at dorsal-fin origin 22%–25% SL. Dorsal profile of the body convex, ventral profile less convex than the dorsal profile. The head short, its length 24%–26% SL, upper profile straight or slightly convex on the interorbital area and markedly convex on the snout. The snout short and rounded, its length 27%–32% HL, its upper profile markedly convex. The mouth subterminal, upper lip not projecting or slightly projecting beyond tip of the lower lip. Tip

of upper lip about level with the lower margin of eye. The eye large, its eye diameter 23%–30% HL. No scales on the breast in both males and females.

Lateral line complete, with 60–69 scales, and almost reaching to caudal-fin base; 11–14 scale rows between lateral line and dorsal-fin origin; and 7–10 scale rows between lateral line and anal-fin origin. Dorsal fin with three simple 7½ branched rays, outer margin convex. Pectoral-fin with 15–17 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 markedly convex. Caudal fin with 15–16 branched rays, deeply forked.

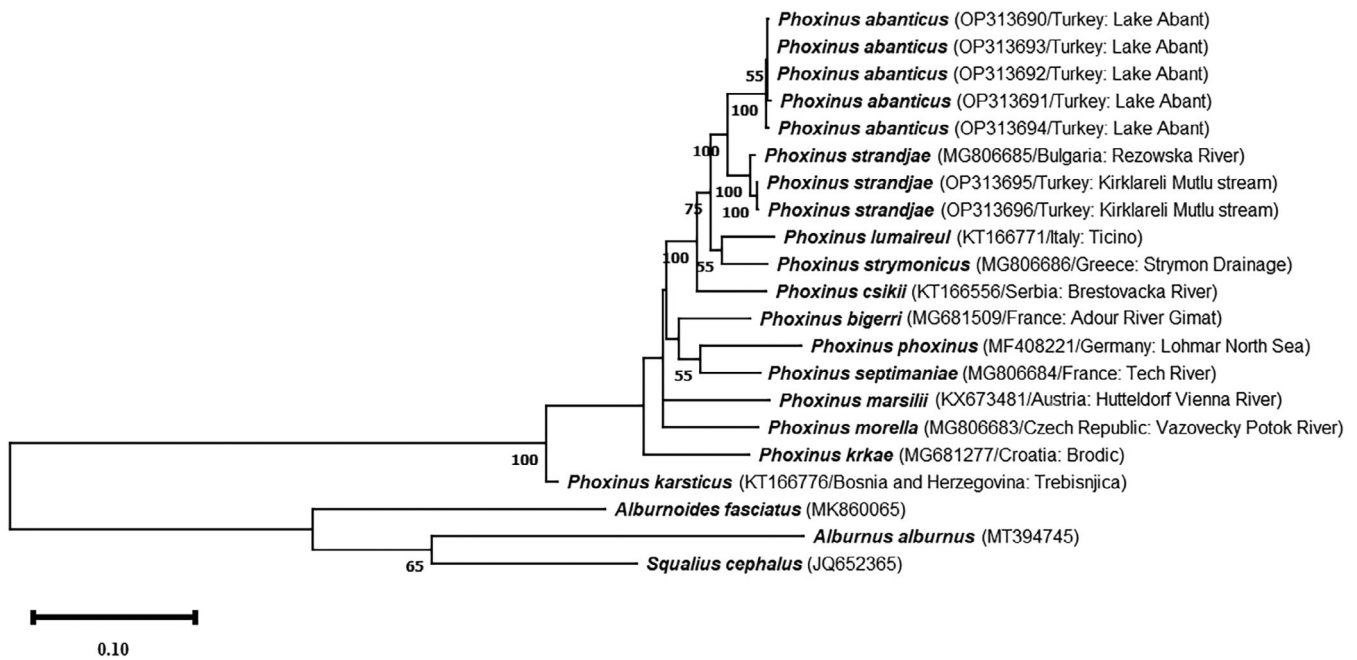


FIGURE 3 The phylogenetic tree generated by using maximum likelihood method based on the mtDNA *cyt b* (cytochrome *b*) gene. The bootstrap values are indicated above nodes on tree if 50% or higher.



FIGURE 4 Ventral view of the breast: *Phoxinus abanticus* FFR 2309, paratypes, from left: 57 mm standard length (SL) male, 63 mm SL, female; Türkiye: Lake Abant outlet



FIGURE 5 Ventral view of the breast: *Phoxinus colchicus*, FSJF 816, from left: 62 mm standard length (SL) male, 69 mm SL, female; Russia: Middle Shakhe River

5.3.5 | Colouration

The specimens were fixed in formalin: back and upper parts of flank brown or dark brown, the lower part of flank and belly yellowish. There are 14–19 short and dark-brown rectangular bars along the lateral line. Dorsal and caudal fins grey, pectoral, pelvic and anal fins yellowish. In live specimens in spawning period in males: Z1 brownish with small irregular-shaped blackish spots; Z2 disappeared in the front of the body, slightly distinct in the

posterior part of the body; Z3 and Z4 yellowish and only distinct in anterior part of the body; and Z5, belly, lips, the base of dorsal, pectoral, pelvic and anal fins red (Figure 8). In females: Z1 and Z3 light brown, Z2 slightly distinct from posterior edge of the operculum to the base of the caudal fin as a stripe, Z4 and Z5 yellowish; all fins yellowish to greyish.



FIGURE 6 Ventral view of the breast: *Phoxinus strandjae*, FFR 2313, from left: 60 mm standard length (SL) male, 63 mm SL, female; Türkiye: Mutlu (Resowska) stream



FIGURE 7 *Phoxinus abanticus*, FFR 2322, holotype, 63 mm standard length (SL), female; Türkiye: Lake Abant outlet

5.3.6 | Sexual dimorphism

Male with stronger and longer pectoral fins and nuptial tubercles on the head in spawning periods in males.

5.3.7 | Etymology

The species is named for the Abant Lake, an adjective.

5.3.8 | Distribution

P. abanticus is presently known from the Lake Abant basin (Figure 1). It inhabits the cold and well-oxygenated waters of fast-flowing mountain streams and large lowland rivers (Figure 11). The Abant Lake lies between the latitudes and longitudes, 40.606560 N–31.280612 E, in the Black Sea region of Türkiye. The lake is one of the significant (1.28 km²) natural reservoirs in Türkiye's north-west Black Sea region and is located at 1298 m a.s.l. The Abant was formed as a result of drainage disruption by a landslide (Erinç *et al.*, 1961), and the lake is



FIGURE 8 *Phoxinus abanticus*, USHM 2020-1419, from top, paratypes, 52 mm standard length (SL), male; Türkiye: Lake Abant outlet; IUSHM 2014-1158, 33 mm SL, female; Türkiye: stream Büyüksu



FIGURE 9 *Phoxinus abanticus*, FFR 2309, from top: paratypes, 57–55 mm standard length (SL), males; Türkiye: Lake Abant outlet



FIGURE 10 *Phoxinus abanticus*, FFR 2321, from top: paratypes, 60–65 mm standard length (SL), females; Türkiye: Lake Abant outlet

located in a tectonic depression (Lahn, 1948), controlled by the North Anatolian Fault (Neugebauer *et al.*, 1997). Fed by spring waters, the lake has a maximum depth of 45 m (Doğan & Kızılkaya, 2010).

The outlet water of Abant Lake is the drainage of the Filyos River and drains into the Black Sea basin. There is no study on the genus *Phoxinus* in the studied area, but there are some studies on new species belonging to other genus from the Filyos River, *e.g.*, *Gobio kizilirmakensis* and *Alburnoides turani* (Kaya, 2020; Turan *et al.*, 2016).

5.3.9 | Remarks

P. strandjae was identified by Drensky (1926, 1951) in the Istranca Mountains of Bulgaria. Later, studies also show that these species

are found in the streams of Resowska and Veleka in Bulgaria and in the southern Black Sea of Türkiye (Kottelat, 2007; Saç & Özuluğ, 2015). *P. colchicus* was originally discovered in the Achvis Tzchali River in Ozurgety District, of Georgia, by Berg (1910). The distribution area of *P. colchicus* are various streams in Georgia, Russia, and southern tributaries of lower Kuban (Kottelat & Freyhof, 2007). *P. colchicus* is found only in the streams of Borçka (Çoruh River drainage) in Türkiye. The detailed distribution of these two species is shown in Figure 1. *P. abanticus*, a new minnow species, is found in the Lake Abant basin (Filyos River drainage), which is a very important area with three endemic species, *Salmo abanticus*, *G. kizilirmakensis* and *A. turani*. (Kaya, 2020; Turan *et al.*, 2014, 2016, 2017). No *Phoxinus* species are reported between the Filyos River and the Borçka stream.

TABLE 3 Morphometry of *Phoxinus abanticus*, *Phoxinus strandjae* and *Phoxinus colchicus*

	<i>P. abanticus</i> n = 18			<i>P. strandjae</i> n = 47			<i>P. colchicus</i> n = 16		
	Range	s.d.	H	Range	s.d.	Range	s.d.		
Basin	Lake Abant			Black Sea			Black Sea		
Stream	Lake Abant outlet water			Stream Mutlu (Resowska)			River BzychMiddle		
Standard length (mm)	43–70		63	40–73		50–70			
In percentage of standard length									
Head length	23.7–25.8 (24.9)	0.06	25.5	23.7–28.5 (25.8)	0.10	23.7–27.3 (25.4)	0.08		
Body depth at dorsal-fin origin	21.6–25.4 (23.6)	0.11	22.4	16.4–23.1 (19.5)	0.12	18.1–22.6 (20.7)	0.10		
Caudal peduncle depth	11.0–12.7 (12.0)	0.05	11.8	8.2–10.5 (9.3)	0.05	8.6–10.5 (9.7)	0.05		
Predorsal length	53.0–57.6 (55.4)	0.12	54.4	52.4–61.7 (55.6)	0.14	53.1–58.1 (55.3)	0.14		
Prepelvic length	45.8–50.2 (47.9)	0.13	49.6	42.4–49.7 (46.8)	0.15	43.7–47.9 (46.0)	0.13		
Pre-anal length	62.1–67.9 (65.1)	0.18	67.7	60.1–69.3 (63.7)	0.18	62.0–66.9 (64.9)	0.15		
Pectoral-fin origin to anal fin	40.8–47.2 (43.3)	0.18	47.2	37.5–45.4 (41.1)	0.20	37.5–47.7 (42.2)	0.24		
Pectoral-fin origin to pelvic fin	23.8–27.9 (25.8)	0.13	27.5	20.0–27.3 (23.5)	0.19	19.9–25.3 (22.7)	0.19		
Pelvic-fin origin to anal fin	15.5–19.9 (17.5)	0.12	19.9	14.7–20.3 (17.3)	0.11	15.9–22.3 (18.9)	0.15		
Caudal peduncle length	23.0–27.2 (25.4)	0.12	23.0	22.3–28.4 (25.6)	0.14	23.6–29.0 (25.3)	0.14		
Dorsal-fin height	17.0–23.1 (20.7)	0.18	19.2	16.9–28.0 (20.8)	0.22	17.7–22.6 (20.2)	0.14		
Pectoral-fin length	16.0–22.4 (18.8)	0.20	16.7	15.2–24.4 (19.4)	0.23	17.1–22.6 (20.2)	0.14		
Pelvic-fin length	11.9–16.8 (14.3)	0.13	13.4	11.8–18.6 (14.9)	0.16	13.2–18.2 (15.7)	0.15		
Anal-fin length	15.9–22.4 (19.2)	0.15	16.7	14.4–23.5 (19.8)	0.21	18.0–21.7 (20.4)	0.10		
Upper caudal-fin lobe	18.6–23.8 (21.2)	0.15	19.3	18.1–27.8 (21.9)	0.23	19.8–25.9 (23.3)	0.16		
In percentage of head length									
Head width ₁ (anterior margin of eye)	35.6–43.6 (40.7)	0.26	39.3	34.3–45.1 (38.7)	0.27	31.6–38.3 (34.0)	0.20		
Head width ₂ (posterior margin of eye)	50.2–58.6 (54.8)	0.23	52.8	47.3–57.7 (53.2)	0.24	46.7–53.8 (49.8)	0.20		
Head width ₃ (at opercle)	56.3–66.1 (60.6)	0.27	56.6	50.3–60.1 (55.4)	0.25	48.6–58.5 (54.4)	0.28		
Head depth ₁ at interorbital region	45.8–55.5 (52.5)	0.28	52.3	43.9–54.2 (48.8)	0.23	44.9–53.6 (49.2)	0.25		
Head depth ₂ (at occiput)	67.9–79.7 (73.9)	0.29	69.7	59.4–72.0 (64.8)	0.25	61.0–71.7 (66.2)	0.25		
Eye diameter	22.9–30.2 (26.5)	0.21	25.2	20.8–29.4 (24.2)	0.24	20.5–27.6 (23.8)	0.23		
Snout length	26.7–31.5 (29.5)	0.13	27.6	26.4–34.3 (30.8)	0.19	26.4–32.1 (29.7)	0.16		
Interorbital width	25.4–37.3 (31.2)	0.31	30.8	25.6–36.0 (30.3)	0.24	27.8–34.3 (29.9)	0.16		
Snout width at nostrils	30.6–40.2 (35.4)	0.28	33.2	29.5–39.8 (34.2)	0.23	24.9–34.9 (29.9)	0.16		
Snout depth at nostrils	31.3–39.0 (34.8)	0.22	35.8	29.9–37.4 (33.0)	0.17	27.8–36.0 (30.0)	0.20		

Note: Mean values are given in parentheses.

Abbreviations: H, holotype; n, number of samples.



FIGURE 11 Türkiye: stream Büyüksu and Lake Abant outlet, habitat of *Phoxinus abanticus*.

6 | DISCUSSION

The presence or absence of scales and their arrangement in the breast are the main characters used in the distinction of the species. Nonetheless, in all the examined *Phoxinus* species, there are no scales on the breast of the females. Also, regarding *P. abanticus*, there are no scales on the breast of both males and females. This is the principal characteristic that distinguishes *P. abanticus* from that found in other two species (*P. strandjae* and *P. colchicus*) in Türkiye and adjacent waters.

Results of the molecular analysis were in accordance with those of the morphometric analysis. In the present study, the genetic characterization of the new species was performed. *Cyt b*, a protein-coding gene of mtDNA, was preferred in the analyses. The efficacy of the mtDNA *cyt b* gene in identifying genetic lineages in the genus *Phoxinus* has been tested in previous studies and has yielded successful results (Corral-Lou *et al.*, 2019; Palandačić *et al.*, 2015, 2017, 2020; Vucić *et al.*, 2018). The new species was compared with all European minnows based on the mtDNA *cyt b* gene. The topotype samples of the species were preferred in the sequences included in the molecular analysis from GenBank. Otherwise, sequences considered to represent the species were preferred. Only *P. colchicus* species could not be included in the analysis because *cyt b* data were not available in GenBank.

The new species, *P. abanticus*, differed from other *Phoxinus* species by its minimum 3.40% genetic distance value (Table 2). Some researchers cite mtDNA *cyt b* differences between 2% and 11% when trying to delimitate fish species (Gilles *et al.*, 2010; Schönhuth *et al.*, 2012; Tsoumani *et al.*, 2014). Even similarly, in the Palandačić *et al.* (2015) study based on the partial sequence of the *cyt b* gene, genetic lineages were determined among *Phoxinus* species, with distances ranging from 4% to 11%. The new species is distinguished from all European minnows by its eight unique and distinctive nucleotide positions. The new species formed an independent and well-supported lineage in the phylogenetic tree (Figure 3). *P. abanticus* is genetically most closely related to *P. strandjae*.

For a long time, *Phoxinus* was known as a single species. Nonetheless, recent studies reveal both the discovery of cryptic species and the genetic lineage map of *Phoxinus* with the contribution of molecular analyses. With the discovery of new genetic lineages, the evolutionary process of the genus *Phoxinus* will be more accurately interpreted, and thus its taxonomy will be understood more clearly.

7 | COMPARATIVE MATERIAL

Additional materials of *P. strandjae* species examined other than those that follows are listed by Saç and Özuluğ (2015).

P. colchicus: FFR 2303, 1, 64 mm SL; Türkiye: Artvin Province: stream Aralık at Borçka, 41.404017 N, 41.695817 E. Fischsammlung J. Freyhof, Berlin (FSJF) 816, 3, 48–53 mm SL; Russia: Lazarevsky: Middle Shakhe River at Khartsyz-2 village, 43.805833 N, 39.609000 E. FSJF 861, 13, 27–70 mm SL; Russia: Lazarevsky: stream BzychMiddle River of Bzogu village, 43.807333 N, 39.736500 E. FSJF 886, 16, 36–50 mm SL; Russia: Lower River Ashe, at Ashe village, under road bridge, 43.956667 N, 39.252500 E.

P. strandjae: FFR 2302, 9, 46–50 mm SL; Türkiye: İstanbul Province: stream Madara at İğneada, 41.877940 N, 27.907606 E. FFR 2306, 12, 53–66 mm SL; Türkiye: Kırklareli Province: stream Yenesu at northwestern Balkaya, 41.622222 N, 27.9349000 E. FFR 2312, 14, 54–73 mm SL; Türkiye: Kırklareli Province: stream Velika at Balaban, 41.781683 N, 27.708833 E. FFR 2313, 26, 34–66 mm SL; Türkiye: Kırklareli Province: stream Mutlu (Resowska) at Yiğitbaş village, 41.942233 N, 27.620233 E. FFR 2317, 17, 31–57 mm SL; Türkiye: Kırklareli Province: stream Mutlu (Resowska) at Demirköy 41.944970 N, 27.608993 E.

8 | MATERIAL USED IN MOLECULAR GENETIC ANALYSIS

P. abanticus: FFR-DNA-Ph26-27-28-29-30; Türkiye: Bolu Province, Lake Abant, 40.6647 N, 31.4250 E (GenBank accession numbers: OP313690–OP313691–OP313692–OP313693–OP313694; topotype samples).

P. strandjae: FFR-DNA-Ph34-36; Türkiye: Kırklareli Province: stream Mutlu (Rezve), 41.9422 N 27.6202 E (GenBank accession numbers: OP313695–OP313696; topotype samples). 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).

Phoxinus 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).

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