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RESEARCH ARTICLE

A new population record and habitat assessment of the endemic fish species *Pseudophoxinus battalgilae* (Teleostei: Leuciscidae) from Central Anatolia

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Abstract

Objective: *Pseudophoxinus battalgilae* is one of two species with the widest distribution of the genus in the Central and Southern Anatolia (Manavgat River) regions, the other being *Pseudophoxinus firati*. The species description was made based on samples from the Beyşehir Lake basin. Records are found from Suğla Lake (Seydişehir); the Akgöl (Ereğli) drainage canals; and the Zengen (Ereğli) and Ilgın (springs and small streams flowing into Çavuşçu Lake); the Kızılca, Akkaya, and Gümüşler reservoirs (Niğde), and the Manavgat River basin. This study attempts to identify new recorded localities of *P. battalgilae* and determines their general distribution areas. In parallel with this, an attempt has been made to evaluate the current state of the species' habitats.

Materials and Methods: *Pseudophoxinus battalgilae* specimens were caught from Gödet Stream (Karaman) with an electrical shock device, with most being released back into the habitat. The metric and meristic characters of the samples were determined. A maximum likelihood phylogenetic tree was created with closely related species and a haplotype network analysis was applied.

Results: The study records the species to have also spread to the Gödet Stream in the Central Anatolia region, thus contributing to identifying the population number and distribution area of the species. The maximum genetic distance of the *P. battalgilae* populations was determined as 0.0061 between Gödet Stream and the Aşıklar (Ereğli) canal populations. In addition to the phylogenetic analysis, the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) results confirm the Gödet Stream population to belong to the *P. battalgilae* species.

Conclusion: As a result, the evaluations in the study show a total of eight populations of the species to have been found. However, many populations of the species are expected to disappear in a short time due to drought and to domestic and agricultural pollution.

Keywords: Central Anatolia, endemic, habitat loss, biodiversity

Introduction

The first study on the phylogeny of the genus *Pseudophoxinus* stated the Anatolian species to have diversified as a result of geological isolation in the early Pliocene period 15 million years ago and the Anatolian and Eastern Mediterranean lineages to have been separated (Hrbek *et al*., 2004). Other molecular study results have found the *Pseudophoxinus* species from the upper Euphrates River and the Levant from the Jordan River north-west to the Seyhan River to form a single phylogenetic group, with the second species group being distributed in all Anatolian inland waters west of Seyhan River (Küçük *et al*., 2012; Geiger *et al*., 2014; Saç *et al*., 2019). Perea *et al*. (2010) differentiated *Pseudophoxinus* species in Anatolia as Central Anatolian species with a very complex morphological structure and as Eastern Mediterranean species with a less complex morphological structure. The genus *Pseudophoxinus* has 22 species in total in Anatolian inland waters according to the latest systematic records, with the most common species being *P. firati* (Eastern Mediterranean) and *P. battalgilae* (Central Anatolia).

This study evaluates the current status of the recorded populations of *P. battalgilae* and adds the presence of a new population in Gödet Stream, which feeds the Karaman Reservoir.

Materials and Methods

Sampling and Morphological Analysis

Pseudophoxinus battalgilae specimens were caught from Gödet Stream with an electrical shock device, with most being released back into the habitat. Some were killed by over-anaesthetization, and then fixed and stored in formaldehyde. Measurements were made with a dial caliper and recorded to an accuracy of 0.1 mm. All measurements were made point to point, never by projections. Methods for counts and measurements follow Kottelat & Freyhof's (2007) methods. Standard length (SL) was measured from the tip of the snout to the end of the hypural complex. The length of the caudal peduncle was measured from behind the base of the posterior anal-fin ray to the end of the hypural complex at the mid-height of the caudal-fin base.

Lateral line scales were counted from the anteriormost scale (the first one to touch the shoulder girdle) to the last scale at the end of the hypural complex. Scales along the lateral line were counted from the first one behind the pectoral girdle to the last one on the caudal-fin base. Gill rakers were counted on the outer margin of the anterior gill arch. The last two branched dorsal and anal-fin rays articulating on a single pterygiophore are counted as 1½. The studied materials were deposited at the Inland Fishes Collection, Isparta University of Applied Sciences, Eğirdir Fisheries Faculty (IFC-ESUF).

Care for the experimental animals was consistent with the Republic of Türkiye's animal welfare laws and guidelines, alongside the policies approved by Isparta University of Applied Sciences Local Ethics Committee for Animal Experiments (Date: 12.03.2020, No: 001).

DNA isolation, PCR and Sequence Analysis

Genomic DNA extraction was made from the fin tissue of the samples which were kept in 70% ethanol. The DNA extraction was performed using the Hibrigen DNA Purification kit in accordance with manufacturer's instructions. Polymerase chain reaction (PCR) was performed on the Biorad Thermal Cycler using the mitochondrial cytochrome c oxidase subunit I (COI) gene universal barcoding primers Fish-F1 (5'- TCA ACC AAC CAC AAA GAC ATT GG CAC -3') and Fish-R1 (5'- TAG ACT TCT GGG TGG CCA AAG AAT CA -3'; Ward *et al*., 2005). PCR was performed using the New England Biolabs Taq DNA polymerase in a 50 µl reaction volume. The reaction mixture used 100 ng DNA, 5 µl 10X PZR buffer, 0.25 mM dNTP mix, 1.5 mM $MgCl_2$, 0.25 µM each primer, and 0.25 U Taq DNA polymerase. PCR product purification and sequence analysis were performed in a commercial laboratory (Macrogen Europe Inc.). The raw data from sequence analysis were edited by visually checking the peaks with the software Bioedit 7.2.5 (Hall, 1999). Sequences were compared with the databases of the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The molecular identification analysis used 20 newly produced cytochrome c oxidase I (COI) barcodes and 6 sequences (KJ554376, KJ554454, KJ554116, KJ554089, KJ554385, KJ554428) from a previously published study (Geiger *et al*., 2014). *Alburnoides smyrnae* (GenBank number: MZ539436) was chosen as the outgroup taxa for rooting the phylogenetic tree. Haplotype network inference was constructed using a median-joining (MJ) algorithm (Bandelt *et al*., 1999) and Farris's maximum-parsimony (MP and implemented in the software Network (version 10.0; www.fluxus-engineering.com). The software MEGA X was used for the nucleotide substitution model, the maximum likelihood (ML) analysis, and the pairwise genetic distance. The nucleotide substitution model K2+ G was selected for the

ML analysis, and *p* distance was selected for determining genetic distance.

Comparative materials

IFC-ESUF 03-0962: 17 specimens, 40.0-149.8 mm SL (Konya, Türkiye: Çarşamba canal, Seydişehir)

IFC-ESUF 03-0965: 4 specimens, 43.94-49.30 mm SL (Karaman, Türkiye: Bozkır Stream)

IFC-ESUF 03-0960: 12 specimens, 71.73-123.20 mm SL (Antalya, Türkiye: Manavgat Reservoir)

IFC-ESUF 03-0961: 9 specimens, 70.38-113.45 mm SL (Antalya, Türkiye: Manavgat Reservoir)

IFC-ESUF 03-0963: 12 specimens, 49.24-88.72 mm SL (Antalya, Türkiye: Manavgat River and Manavgat Reservoir)

IFC-ESUF 03-1010: 4 specimens, 66.3-73.4 mm SL (Konya, Türkiye, Tatlıkuyu Village Ereğli)

IFC-ESUF 03-1018: 9 specimens, 29.05-51.8 mm SL (Konya, Türkiye: Aşıklar Village-Ereğli)

IFC-ESUF 03-0967: 3 specimens, 37.32-39.90 mm SL (Konya, Türkiye: Zengen Village-Ereğli)

IFC-ESUF 03-0966: 11 specimens, 33.05-82.81 mm SL (Niğde, Türkiye: Akkaya Reservoir)

IFC-ESUF 03-1025: 17 specimens, 43.30-59.28 mm SL (Niğde, Türkiye: Gümüşler Reservoir)

IFC-ESUF 03-0964: 10 specimens, 28.1-57.8 mm SL (Konya, Türkiye: Springs on the shores of Çavuscu Lake)

IFC-ESUF 03-1016: 3 specimens, 43.69-53.83 mm SL (Konya, Türkiye: Canal near Çavuşcu Lake)

IFC-ESUF 03-1027: 26 specimens, 41.43-61.16 mm SL (Konya, Türkiye: Bulasın Creek, near Ilgın)

IFC-ESUF 03-1037: 25 specimens, 74.44-91.53 mm SL (Karaman, Türkiye: Gödet Stream)

Results and Discussion

The species was first recorded in Beyşehir Lake as *Acanthorutilus maeandricus* by Kosswig (1952; *nomen nudum*). Later, a second sampling was made from the same lake in 1964 (Bogutskaya, 1997). Its scientific identification was made by Bogutskaya (1997) based on ZMH examples (Haplotype ZMH 8861, Beyşehir Lake, August-September, Collector C. Kosswig, 1964; Paratypes: ZMH 2701 (3), ZMH 6634 (3), ZMH 1080 (2) same date and same collector as holotype).

The scientific identification distinguished the species from other *Pseudophoxinus* species, with its complete lateral line, greater number of branched rays in the dorsal fin (D: III-IV, 8-9) and anal fin (A: III-IV, 8-9), significantly flattened lateral body structure, and the presence of an uninterrupted keel between the pelvic-fin and the anal fin particularly notable.

Apart from the records of Kosswig (1952; 1964) recording its type locality as Lake Beyşehir, no other scientific records of *P. battalgilae* are found in any other scientific studies conducted to date. The current study encountered no specimens in the samplings conducted in Beyşehir Lake and its surrounding spring waters-streams (Eflatunpınarı, Deliktaş spring, karst springs mixing into the lake on the western shores of the Lake Beyşehir, Sarıöz Canal, Üstünler, Soğuk and İli Stream) between 1997-2021.

Meanwhile, we encountered a few samples between 2000-2014 in the Taşağıl and Çarşamba canals flowing into the Suğla Reservoir in Beyşehir Lake basin and in some small springs around Seydişehir. However, the recently conducted field studies (2018-2021) found no *P. battalgilae* samples due to heavy pollution (sewage and agricultural fertilizer and pesticide inputs) in the above-mentioned habitats of the Taşağıl and Çarşamba canals, as well as due to seasonal drying in some springs near Seydişehir and damage caused by construction equipment.

Recently, Atalay (2005), Küçük (2007), Freyhof & Özuluğ (2010), Küçük *et al*. (2016), Bayçelebi *et al*. (2020) and Küçük *et al*. (2020) have provided information regarding the morphology and zoogeography of the species. The molecular phylogenetic analysis of the species is available in the studies conducted by Hrbek *et al*. (2004), Perea *et al*. (2010), and Geiger *et al*. (2014). The existence of eight populations has been reported so far (Atalay, 2005; Freyhof & Özuluğ, 2010; Küçük *et al*., 2016; Küçük *et al*., 2020). This study has evaluated the current status of the populations given to date and added the presence of a new population in Gödet Stream, which feeds Karaman Reservoir (Fig. 1). The coordinates for the locations of the *P. battalgilae* are given in Table 1.

The current field studies have determined *P. battalgilae* to be distributed over 5 basins: the spring waters, rivers, and lakes in Central Anatolia (3); Manavgat River in the Mediterranean basin (1), and the streams and spring waters around Ilgın (1) adjacent to Sakarya River. Evaluations regarding these inland waters and *P. battalgilae* populations are given below:

1-Beyşehir Lake basin: In the current population of this region, the species is encountered to a limited extent in the drainage canals where the spring waters flow into the Suğla Reservoir (Kuğu Park surroundings, Seydişehir) are collected. In the early 2000s, the outflow canal of Beyşehir Lake, known as Çarşamba canal, used to host a population

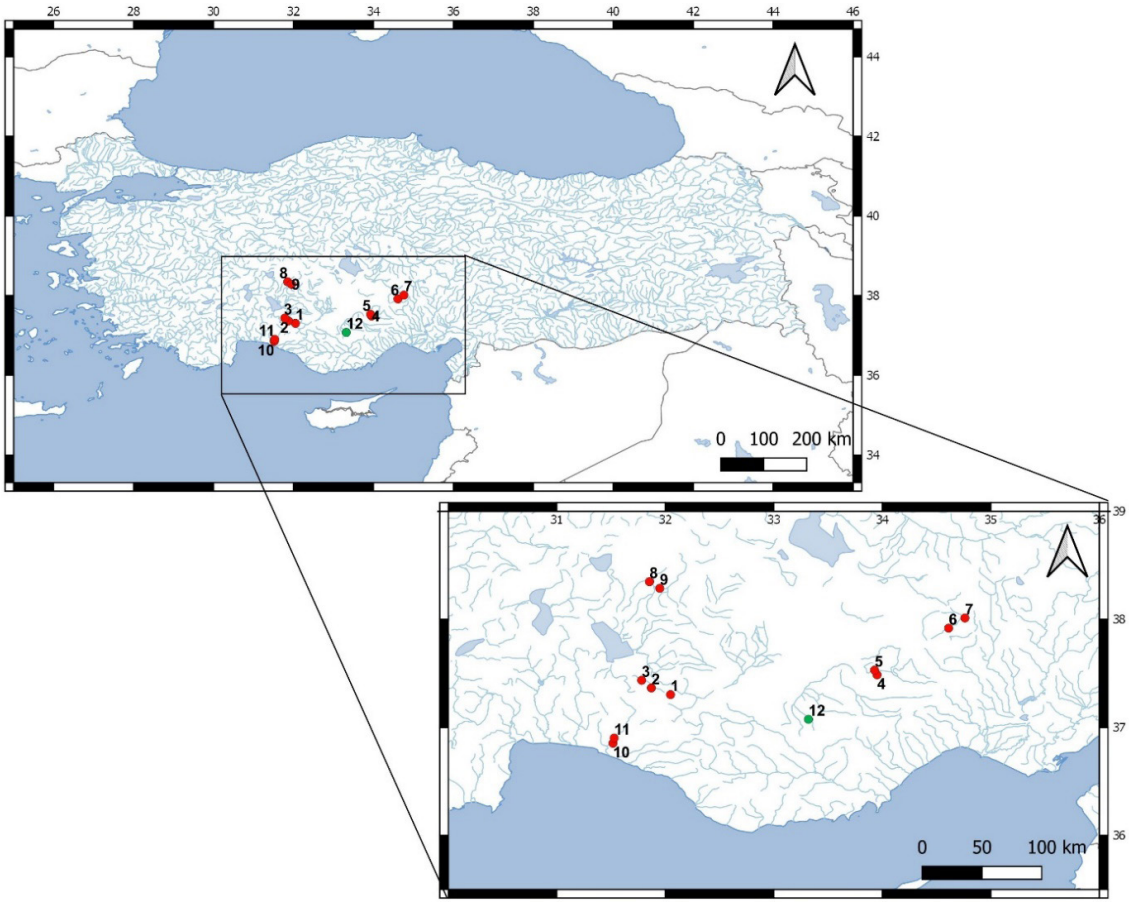

Figure 1. Distribution areas of *Pseudophoxinus battalgilae* (● = new record; ● = old records).

of this species in a relatively healthy state. However, due to intense agricultural, domestic and industrial pollution over the last 20 years, this species is no longer found in this habitat. Similarly, in Bozkır Stream, specimens were last encountered in 2005, but recent studies have failed to find any specimens due to intense domestic pollution and water drying in recent years. However, it has been reported that in 2023, a very few examples were found during fishing with very porous nets on the Yeşildağ shores of Beyşehir Lake (Dr. Vedat Yeğen personal opinion). For this reason, it is important to monitor the population of the species in Lake Beyşehir.

2-Manavgat River basin: The species forms dense populations along the coastal areas of the Manavgat and Oymapınar reservoirs (especially in the spring), as well as in the cold river waters of the river section where the Manavgat River flows into the dam lake. However, irregular flow in the dam lakes, water level instability in the lakes, and the effects of exotic species (e.g., *Carassius gibelio, Squalius anatolicus*, and *Alburnus escherichii*) are external factors pressuring their populations.

3-Akgöl (Ereğli) basin: The region is under severe drought pressure. The canals flowing from the above localities to Akgöl (near Tatlıkuyu and Aşıklar villages) have dried up over the past 8-10 years. The species has not been recently detected in the Akkaya and Gümüşler reservoirs around Niğde. In this basin, *P. battalgilae* individuals are encountered to a limited extent only in the agricultural irrigation canals near Taşağıl Village (Ereğli), which are supplied by the İvriz Reservoir. All recorded populations in the region are undergoing extinction.

4-Çavuşcu Lake Basin: The study conducted sampling from several small spring waters flowing into Çavuşcu Lake and from Bulasın Creek in the southeastern part of the lake. *Pseudophoxinus* has been known since the early 2000s to be widespread in this region. This study's samplings encountered very few *P. battalgilae* specimens in the habitats. Çavuşcu Lake (Ilgın) is reported as being isolated from Sakarya River; therefore, the fish fauna are similar in both regions (Turan & Kaya, 2019). Unlike Central Anatolia and the Manavgat River basin, the distribution of *P. battalgilae* in this lake basin is considered interesting from a zoogeographical point of view.

Table 1. Locality and coordinates for the distribution areas of *P. battalgilae.*

No	Locality	Coordinates (DMS)
1	Suğla reservoir-Seydişehir	37°18'11.55"N; 32°02'50.29"E
2	Kuğu Park-Seydişehir	37°21'53.65"N; 31°52'15.94"E
3	Gökçehüyük Pond-Seydişehir	37°26'08.63"N; 31°46'53.71"E
4	Taşağıl village- Ereğli Marshes	37°29'10.13"N; 33°57'03.91"E
5	Aşıklar village- Ereğli Marshes	37°31'44.77"N; 33°55'38.34"E
6	Akkaya reservoir- Niğde	37°55'03.83"N; 34°36'37.53"E
7	Gümüşler reservoir- Niğde	38°00'42.40"N; 34°45'37.49"E
8	Çavuşcu Lake Spring-Ilgın	38°20'50.28"N; 31°51'11.96"E
9	Bulhasan Creek-Ilgın	38°17'11.32"N; 31°56'54.56"E
10	Manavgat reservoir-Manavgat	36°51'11.53"N; 31°31'02.02"E
11	Manavgat River-Manavgat	36°54'04.04"N; 31°31'41.03"E
12	Gödet Stream- Karaman	37°04'29.91"N; 33°19'07.46"E

5-Gödet Stream (Karaman) drainage: The Gödet Stream originates as two separate streams from springs near Güldere and Paşabağı villages and goes on to forms the Gödet Reservoir near Karaman after the confluence of both streams (Fig. 2). Its approximate length is 20 km. The stream is used primarily for drinking water and agricultural irrigation. *Pseudophoxinus* specimens (Fig. 3) were caught from a location near where the stream empties into the reservoir.

Figure 3. *Pseudophoxinus battalgilae* (IFC-ESUF 03-1037: 6.73 mm SL [Karaman, Türkiye: Gödet Stream]).

The Gödet Stream specimens' lateral line have a total of between 52-64 scales, with 12-15 scale rows found between the lateral line and the origin of the dorsal fin, 4-5 (4-5.5) scale rows are found between the origin of the lateral line anal-fin, and 11-13 gill rakers are found on outer side of the first gill arch. The dorsal fin has 7 branched rays, while the anal-fin mostly has 8-9 branched rays (Tables 2, 3). The taxonomic characteristics of the Gödet Stream samples are similar to other *P. battalgilae* populations in Anatolia.

According to the results from the phylogenetic analyses, the *Pseudophoxinus* specimens from Gödet Stream clearly inhabit the same monophyletic group as the other *P. battalgilae* populations found in Türkiye (Aşıklar canal in Ereğli, Akkaya Reservoir in Niğde, Seydişehir's surroundings, Manavgat River, Tuz drainage, and Ilgın surroundings; see Figs. 4 and 5). Furthermore, *P. iconii* is a

Figure 2. A new record habitat of *Pseudophoxinus battalgilae* (Gödet Stream-Karaman).

Table 2. Frequency distribution of certain meristic characteristics of the *P. battalgilae* from the Central Anatolia and the Manavgat River Basin

		Number of rows of scales between						Number of rows of scales between					
	N	**11**	**12**	**13**	**14**	**15**	**X̄**	**4**	**$4_{1/2}$**	**5**	**$5_{1/2}$**	**6**	**X̄**
Gödet Stream	26	-	4	14	7	1	**13.2**	12	5	4	5	-	**4.5**
Ereğli basin	13	-	4	7	2	-	**12.8**	11	-	2	-	-	**4.2**
Akkaya Reservoir	5	-	2	2	1	-	**12.8**	-	-	5	-	-	**5.0**
Beyşehir basin	10	2	7	-	-	1	**12.1**	2	-	7	-	1	**4.9**
Manavgat basin	12	3	3	5	1	-	**12.0**	5	-	6	-	1	**4.7**
Ilgın basin	5	4	1	-	-	-	**11.2**	2	-	3	-	-	**4.6**
	Branched dorsal-fin rays				**Branched anal-fin rays**								
	N	**7**	**8**	**X̄**	**8**	**9**	**X̄**						
Gödet Stream	26	26	-	7.0	18	8	8.3						
Ereğli basin	13	13	-	7.0	13	-	8.0						
Akkaya Reservoir	5	5	-	7.0	5	-	8.0						
Beyşehir basin	8	7	1	7.1	8	-	8.0						
Manavgat basin	12	12	-	7.0	7	5	8.4						
Ilgın basin	5	5	-	7.0	1	4	8.8						

Table 3. Frequency distributions for the lateral line scales and gill rakers of the *P. battalgilae* from Central Anatolia and the Manavgat River basin.

		Lateral Line													
	N	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**63**	**64**	**65**	**X̄**
Gödet Stream	**25**	-	2	1	-	5	1	2	2	2	5	3	2	-	**59.7**
Ereğli basin	**13**	-	1	2	-	4	2	2	2	-	-	-	-	-	**57.4**
Akkaya Reservoir	**5**	-	-	-	-	1	1	-	-	1	1	-	1	-	**60.4**
Beyşehir basin	**10**	3	1	1	1	1	1	-	2	-	-	-	-	-	**56.0**
Manavgat basin	**13**	-	-	1	1	-	3	1	1	1	-	4	-	1	**60.2**
Ilgın basin	**5**	3	1	1	-	-	-	-	-	-	-	-	-	-	**53.6**
		Gill Rakers													
	N	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**X̄**						
Gödet Stream	**11**	4	6	1	-	-	-	-	11.7						
Ereğli basin	**13**	4	7	2	-	-	-	-	11.8						
Akkaya Reservoir	**5**	1	3	1	-	-	-	-	12.0						
Beyşehir basin	**4**	-	1	1	-	-	1	1	14.5						
Manavgat basin	**9**	-	1	7	-	1	-	-	13.1						
Ilgın basin	**5**	1	-	3	1	-	-	-	12.8						

sister species to *P. battalgilae*, with the other *Pseudophoxinus* species (e.g., *P. hitittorum*, *P. firati*) inhabiting nearby basins being in different monophyletic groups (Fig. 4).

The maximum genetic distance among the *P. battalgilae* populations was identified as 0.0061 between the specimens from Gödet Stream and Aşıklar canal specimens in the

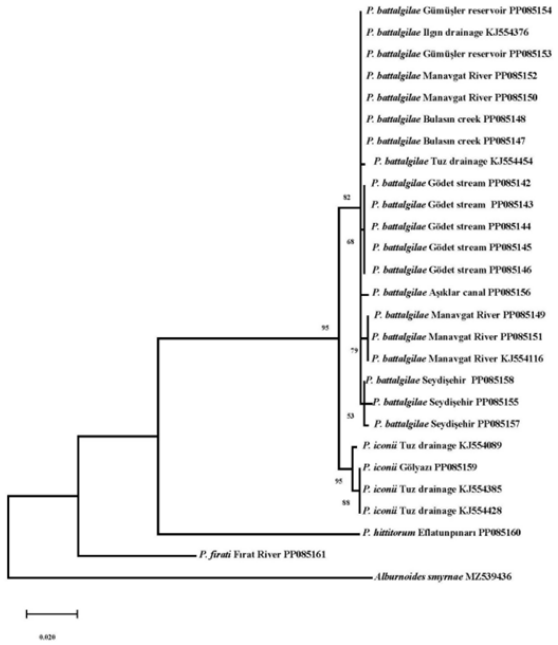

Figure 4. Maximum likelihood phylogenetic tree of *Pseudophoxinus* populations..

Figure 5. *Pseudophoxinus battalgilae* (IFC-ESUF 03-0961: 96.0 mm SL [Antalya, Türkiye: Manavgat Reservoir]).

Figure 6. *Pseudophoxinus iconii* (IFC-ESUF 03-1026: 63.8 mm SL [Konya, Türkiye: Gölyazı village]).

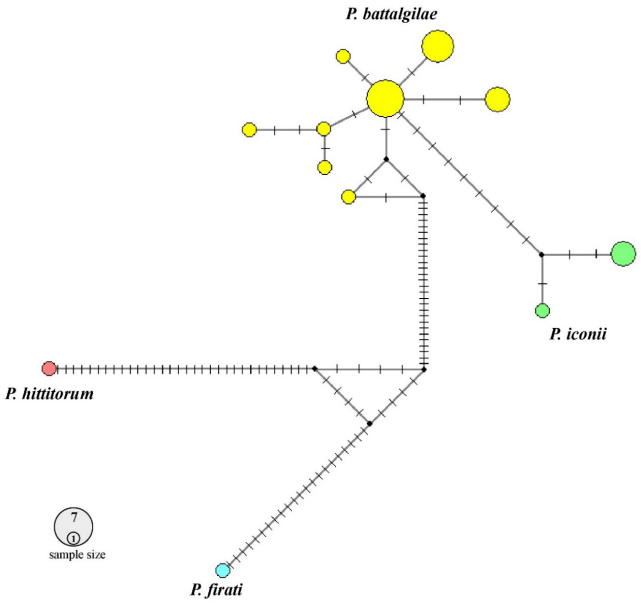

Figure 7. Haplotype network analysis for the *Pseudophoxinus* species.

Table 4. Genetic distance between *Pseudophoxinus* species/populations based on COI sequence data

		P. battalgilae								*P. iconii*	*P. firati*
		GS	**BC**	**GR**	**ID**	**TD**	**MR**	**S**	**AC**		
P. battalgilae	**GS**										
	BC	0.0015									
	GR	0.0015	0.0000								
	ID	0.0015	0.0000	0.0000							
	TD	0.0031	0.0015	0.0015	0.0015						
	MR	0.0034	0.0018	0.0018	0.0018	0.0034					
	S	0.0046	0.0031	0.0031	0.0031	0.0046	0.0043				
	AC	0.0046	0.0031	0.0031	0.0031	0.0046	0.0049	0.0061			
P. iconii		0.0180	0.0165	0.0165	0.0165	0.0181	0.0183	0.0196	0.0196		
P. firati		0.0874	0.0859	0.0859	0.0859	0.0864	0.0877	0.0890	0.0844	0.0901	
P. hittitorum		0.0997	0.0982	0.0982	0.0982	0.0988	0.0982	0.0982	0.0982	0.0985	0.0890

GS = Gödet Stream; BC = Bulasın Creek; GR = Gümüşler Reservoir; ID = Ilgın Drainage; TD = Tuz Lake Drainage; MR = Manavgat River; S = Seydişehir's surroundings; AC = Aşıklar Canal

Ereğli Marshes basin in Konya, which are geographically very close (Table 4). The minimum genetic distance between *P. battalgilae* and the most related species (*P. iconii*; see Fig. 6) was determined as 0.0165 (Table 4). Furthermore, a maximum of five nucleotide differences are found among the *P. battalgilae* populations, with the populations showing a star-like structure in the haplotype network analysis (Fig. 7). As a result, the genetic analysis has confirmed the *Pseudophoxinus* specimens sampled from Gödet Stream to be *P. battalgilae.* The NCBI BLAST analysis has also confirmed the Gödet Stream population to belong to the *P. battalgilae* species (> 99% identity).

Conclusion

As a result of the recent taxonomic studies on and revisions to the *Pseudophoxinus* genus, a total of 22 valid species (*P. handlirschi* EX) has been recorded in Anatolia (Güçlü & Küçük, 2017). While the molecular phylogeny of the genus has been partially determined (Central Anatolia, Southeastern Anatolia and Eastern Mediterranean phylogenies), its morphology exhibits a rather complex structure (Hrbek *et al*., 2004; Perea *et al*., 2010). A v ariety of species have been noted to have diversified regarding a morphology-based classification, including those with a complete or nearly complete lateral line, relatively large body size, and small scales (e.g., *P. anatolicus*, *P. handlirschi*, *P. fahrettini*), as well as species with an underdeveloped lateral organ, relatively small body size, and large scales (e.g., *P. alii*, *P. maeandri*, *P. libani*). *P. battalgilae*, which is distributed in Anatolia and has the largest population after *P. firati*, has a complete lateral line and very small scales. The most distinctive feature of the species is the presence of a partially developed keel between the ventral and anal fins and of seven or more branched rays in the anal fin. Regarding the sequence analysis based on the COI barcoding region, no significant genetic difference is understood to be present among the *P. battalgilae* populations.

As a result based on what can be understood from the evaluations in this study, a total of eight populations are present, five in the Central Anatolian basin (two in the Gümüşler and Akkaya reservoirs in Niğde, one in the soil canals that supply the Akgöl and Ereğli marshes, one in the Gödet Stream in Karaman, and one in the small springs around Seydişehir) and two in the Manavgat River basin (one in the Oymapınar and Manavgat reservoirs and one in the area surrounding Ilgın, namely the two rivers flowing into Çavuşçu Lake). These populations, especially those around Akgöl, Niğde, and Ilgın, are expected to disappear in a short time due to drought resulting mainly from groundwater withdrawal for agricultural irrigation and the decreased rainfall in recent years, as well as due to domestic and agricultural pollution.

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