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Salmo emireae (Salmoniformes, Salmonidae), a new trout species from the Lake Balık, northeastern Turkey

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Abstract Trout species of the genus *Salmo* in Eastern Anatolia offer valuable insights into the evolution and diversity of freshwater fishes in the Caspian Sea basin. In the present study, we describe a new trout species, *Salmo emireae* sp. nov., from Lake Balık in northeastern Turkey, based on combined morphological and mitochondrial (cytochrome b) data. Phylogenetic analyses using maximum likelihood and Bayesian inference methods revealed that *S. emireae* belongs to the Danubian lineage and forms a well-supported monophyletic clade, closely related to *S. ischchan*, *S. aestivalis*, and *S. gegarkuni* from Lake Sevan. Moreover, unique morphological traits—by a shorter head, a slenderer caudal peduncle, a shorter maxilla, and a shorter mouth gape—distinguish *S. emireae* from its congeners. Given its limited distribution and pressure from overfishing, *S. emireae* may be considered endemic and potentially vulnerable. Our findings highlight the need for further taxonomic and conservation studies on endemic trout species in the region. The study provides a detailed diagnosis, morphometric description, distribution map for the new species, and an extensive phylogeny for genus *Salmo*, contributing to the growing biodiversity knowledge of Anatolian trout. The new species is endemic to a single, isolated lake that is subject to regular commercial fishing and recent drought-related habitat changes. These pressures, together with its restricted range, make *S. emireae* potentially vulnerable and of conservation concern.

Keywords Trout · Endemic · Aras River · Biodiversity · Cytochrome b

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Introduction

The brown trout (*Salmo trutta*, Linnaeus, 1758) is native to Atlantic, North, White, and Baltic Sea basins, with a natural range extending from Spain in the southwest to Chosha Bay in northern Russia. It is also present in Iceland, as well as in the northernmost rivers of Great Britain and Scandinavia (Freyhof et al. 2025). Such wide distribution across various climates and landscapes of the northern hemisphere makes it one of the most broadly distributed salmonid species. As a result, *S. trutta* exhibits remarkable levels of phenotypic, genotypic, behavioral, and ecologic variation (Bernatchez 2001; Ferguson 2004; Kottelat and Freyhof 2007). Phylogenetic studies based on the control region of mtDNA have revealed five major genetically distinct lineages: Adriatic (AD), Atlantic (AT), Danubian (DA), Marmoratus (MA), and Mediterranean (ME) (Bernatchez et al. 1992; Bernatchez 1995, 2001). Some of these lineages have further diversified due to shorter periods of geographic isolation, leading to the recognition of local haplogroups such as Duero (DU) (Vera et al. 2010) reported in Spain, Tigris (TI) reported in Turkey (Sušnik et al. 2005; Bardakçı et al. 2006), and Dades (Snoj et al. 2011) in Morocco. In recent years, the validity of mitochondrial markers has been widely confirmed for classifying and identifying *Salmo* populations (Sušnik et al. 2005; Turan et al. 2009, 2020, 2021 ; Ninua et al. 2018, 2023).

Genus *Salmo* is distributed in almost all rivers in Anatolia, except in the rivers in the Aegean basin (represented only in Karamenderes Stream in the Aegean). Anatolia has a high level of species richness and endemism and is considered part of the Mediterranean biodiversity "hot-spot" (Myers et al. 2000; Mittermeier et al. 2004; Şekercioğlu et al. 2011), which has also positively affected salmonids (Bardakçı et al. 2006). Such rich diversity of Anatolian trouts has been revealed by several morphological (Tortonese 1955; Behnke 1968; Turan et al. 2011, 2012, 2014a 2017, 2022; Turan and Aksu 2021) and genetically-assisted morphological (Turan et al. 2009, 2014b, 2020, 2021) studies. These efforts resulted in twenty native species in Anatolia (Küçük et al. 2024).

Three of these species (*Salmo ardahanensis* Turan, Kottelat & Kaya, 2022, *Salmo araxensis* Turan, Kottelat & Kaya, 2022 and *Salmo murathani* Turan, Kottelat & Kaya, 2022) are found in the upper Kura and Aras rivers. In addition to these species, *Salmo caspius* Kessler 1877 (lower basin of Kura River) and *Salmo ciscaucasicus* Dorofeeva, 1967 (Samur River) are valid species in the Caspian Sea basin (Kawraisky 1896; Levin et al. 2018; Turan et al. 2022). Furthermore, four valid species are known to occur in the natural lakes of the region. These include *Salmo ezenami* Berg 1948 from Lake Kezenoyam and *Salmo ischchan* Kessler 1877 (extinct), *Salmo gegarkuni* Kessler 1877, and *S. aestivalis* Fortunatov, 1926 from Lake Sevan. *Salmo danilewskii* Gulelmi, 1888, originally described from Lake Sevan, has been reported as a synonym of *S. ischchan* (Freyhof et al. 2025). Recent studies, including those by Levin et al. (2022) and Hashemzadeh Segherloo et al. (2022), have further explored the taxonomic status of these species, with Levin et al. (2022) recognizing the distinct ecological forms in Lake Sevan and Hashemzadeh Segherloo et al. (2022) highlighting genetic differences between populations in the Aralo-Caspian region.

Building on previous studies, our research focused on a new species described in Lake Balık, Aras River drainage (Caspian Sea basin), and named as *Salmo emireae* sp. nov. The new species was compared in detail with other species from the adjacent regions to provide a clearer understanding of its taxonomic position.

Materials and methods

Study area

The study was conducted in Balık Lake, which is located at the Aras Catchment, 22 km northeast of Taşlıçay. It has a surface area of 34 km^2 and is situated among high mountain ranges (Bozaoğlu 2020). Balık Lake is the highest natural lake in Turkey and includes irregular shorelines (Bozaoğlu 2020). The fish samples were collected from the lake in July 2024 with the assistance of local fishermen using gill nets.

Fish sampling

All procedures were in accordance with the animal welfare laws of Turkey, permitted by RTE University Local Ethics Committee for Animal Experiments (License ID: 2014/72). Specimens were first anaesthetized by using MS222, then a small piece of fin tissue was taken and stored in %96 ethanol for genetic analysis. Later, fish samples collected for the faunal survey were preserved in 5% formaldehyde in a horizontal position, ensuring homogenous fixation. The material examined is deposited in Recep Tayyip Erdogan University Zoology Collection of the Faculty of Fisheries, Rize (FFR).

Morphological analyses

Measurements and counts were based on methods of Turan et al. (2017) and Freyhof et al. (2025). Measurements were conducted using a point-to-point approach, utilizing a dial caliper calibrated to an accuracy of 1 mm. In instances where the last two branched rays articulate on a single pterygiophore in either the anal or dorsal fins, these were treated as "1½". Thirty measurements and three meristic characters of the new species (*n* = 9), *S. murathani* (*n* = 20), and *S. araxensis* (*n* = 31) were analyzed using principal component analysis (PCA) with the software PAST version 1.8 (Hammer et al. 2001). All metric characters (raw data) were standardized to standard length (SL), and three meristic characters were used in the analysis. For comparison, this study incorporated materials reported in Turan et al. (2009, 2011, 2014a, 2017, 2020, 2021, 2022) and Freyhof et al. (2025).

DNA isolation, amplification, and sequencing

The genomic DNA was isolated from fin clips stored in ethanol using a commercial kit, the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), and processed using the Qiacube Automated DNA purification equipment, as recommended by the manufacturer. The vertebrate mitochondrial DNA cytochrome b (Cyt b) partial gene (993 bp) was amplified using the primer pairs StrCBF (5′-GCTAATGACGCA CTAGTCG-3′) and StrCBR (5′-GGGGGCGAGRAC TAGGAAGAT-3′; Turan et al. 2009). The PCR protocol was as follows: an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. After assessing amplification efficiency on an agarose gel, the amplicons were purified and sequenced bidirectionally at Macrogen Europe (Netherlands).

Data analysis

In this study, we analyzed a total of 82 sequences (9 new sequences from the present study and 73 sequences downloaded from GenBank) for 29 *Salmo* species to evaluate the newly described species based on molecular data (mtDNA Cyt b). The GenBank accession numbers of the sequences belonging to the newly described species are provided in the "Genetic Material" section. The accession numbers and locality information of the sequences downloaded from GenBank are provided in Fig. 1. The corresponding references are listed below: Crête-Lafrenière et al. 2012; Amini and Rezaei 2017; Levin et al. 2018; Ninua et al. 2018; Tougard et al. 2018; Turan et al. 2020, 2021; Turan et al., unpublished study; Küçük et al. 2024. Alignment of all Cyt b sequences was performed using the Clustal W algorithm (Thompson et al. 1994) in BioEdit v7.2.5 software (Hall 1999). Within the Cyt b dataset, sequence lengths range from 842 to 1141 base pairs. The alignment gaps were replaced with "N" nucleotides to account for missing data, and all analyses were performed. Inter-species genetic distance values of *Salmo* species were computed according to the uncorrected *p*-distance model in MEGA X (Kumar et al. 2018).

The phylogenetic relationships were assessed using the maximum likelihood (ML) approach in MEGA X and Bayesian Analysis (BI) in MrBayes v3.2.1 (Ronquist et al. 2012). The best-fit evolution models of nucleotide substitution were selected based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) in jModelTest 0.1.1 (Posada 2008). The ML tree was constructed with 1000 bootstrap replicates using the TrN + G + I model (Tamura and Nei 1993), and the BI tree was generated using the same model. For the BI analysis, 10 million generations were run with Metropolis-coupled Markov Chain Monte Carlo (MCMC) sampling every 1000 generations. A conservative 10% of the trees were removed as burn-in to ensure that the analysis reached stationarity. The convergence of the runs was confirmed using Tracer v1.7.1 (Rambaut et al. 2018). The iTOL (Interactive Tree of Life; https://itol.embl.de/) web-based program was used to visualize

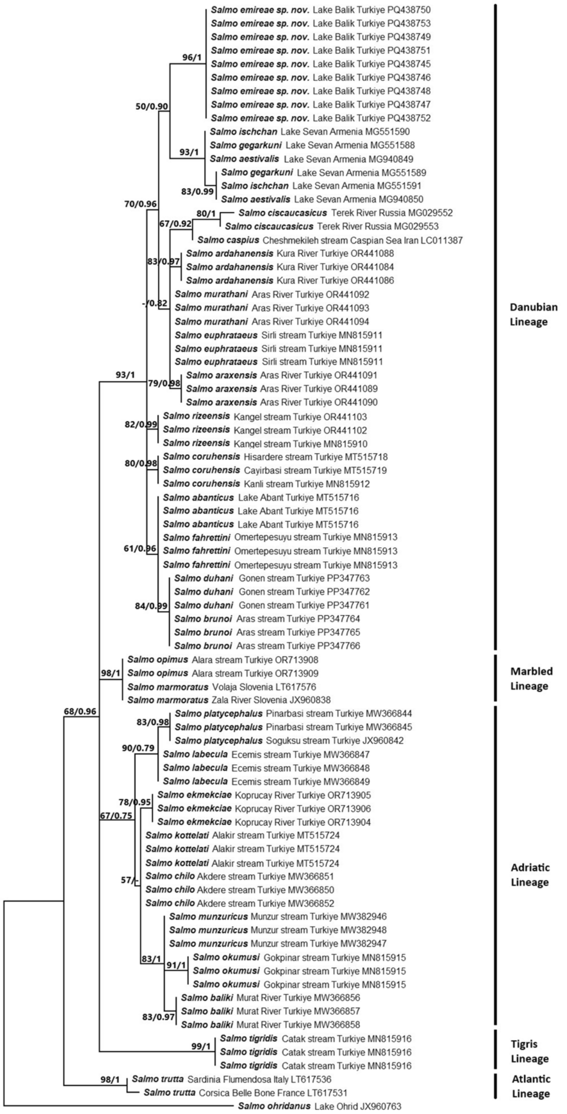

Fig. 1 The phylogenetic relationships of *Salmo* species were reconstructed using the maximum likelihood (ML) method based on the Cyt b data set. Since the topologies generated by the ML and Bayesian inference (BI) methods have complete similarity, only the ML phylogeny is presented. The nodes indicate bootstrap percentages and Bayesian posterior probabilities (ML/BI)

the BI tree. *Salmo ohridanus* (GenBank Accession no: JX960763) was selected as the outgroup species to root the phylogenetic tree.

Results

Salmo emireae, new species

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Holotype

FFR 3247, 304 mm SL; Turkey: Ağrı Province: Lake Balık, 4 km north of Tanyolu, 39.7921, 43.5511.

Paratypes

FFR 3248, 8, 335–390 mm SL; same data as holotype.

Genetic material

FFR DNA ALAB 45, 46, 47, 48, 49, 50, 51, 52, 53; *N*=9; same data as holotype (GenBank accession numbers: between PQ438745—PQ438753).

Diagnosis

Salmo emireae is distinguished from the extant species of trouts distributed in adjacent water: The general body color is silvery in life; zero or one black spot behind the eye, approximately equal to the pupil; zero to three black spots on the opercle, smaller than the pupil; no black spot on the top of the head; black spots on the flank are few (less than 61), irregularly shaped, large (spots along the lateral line larger than half of the pupil or equal to), scattered on the upper and middle part of the flank; red spots are few (less than 10), large (larger than half of the pupil), ocellated, organized in one or two irregular longitudinal rows on the median part of the body, with no red spots in larger specimens than about 310 mm SL; the head is short (length 19.7–23.6% SL); the maxilla is short (length of maxilla 5.8–7.4% SL); the mouth is small (length of mouth gape 8.6–10.8% SL); the caudal peduncle is deep (depth 8.1–9.3% SL); the lateral line has 106–115 scales; there are 22–26 scale rows between dorsal-fin origin and lateral line; 17–20 scale rows between anal-fin origin and lateral line; 14–17 scale rows between the origin of the adipose fin and lateral line; and there are 18–20 gill rakers on the outer side of the first gill arch.

Fig. 2 *Salmo emireae* from Lake Balık: **a** FFR 3247, holotype, 304 mm SL, male; **b** FFR 3248, paratype, 345 mm SL, male; **c** 390 mm SL, female

Description

The general appearance of the holotype and two paratypes is shown in Fig. 2,; morphometric data are provided in Table 1. Body slender, slightly compressed laterally, its depth at dorsal-fin origin 20.4–24.3% SL. Dorsal profile straight or slightly arched, and ventral profile arched. Head short, length 19.7–23.6% SL. The upper profile of the head is straight or slightly convex in the interorbital area and convex on the snout. Mouth small, length of mouth gape 8.6–10.7%, slightly subterminal. Tip of the lower jaw pointed, slightly curved upwards, with a slightly developed process at the symphysis in males. Maxilla short, length 5.7–7.4% SL. The maxilla reaches slightly beyond the posterior margin of the eye. Snout short and pointed in males, slightly rounded in females. Adipose fin small in size, its height 4.0–5.5% SL. Largest observed specimen 390 mm SL.

Anal fin with 3 unbranched and 8½ (9½) branched rays, its distal margin convex in males and convex anteriorly concave posteriorly in females. Dorsal fin with (8½) 9½ branched and 3–4 unbranched rays,

Table 1 Morphometry of *Salmo emireae* (holotype, FFR 3247; paratypes FFR 3248, $n=8$). The calculations include the holotype

	Holotype	Paratypes
Standard length (mm)	304	323–390
In percentage of standard length		Range (mean)
Head length	23.6	19.7–23.6 (21.6)
Predorsal length	45.9	42.6–46.4 (44.5)
Prepelvic length	55.3	51.5–56.9 (54.2)
Preanal length	78.9	73.6–80.0 (77.2)
Body depth at dorsal-fin origin	21.6	20.4–24.3 (22.4)
Body depth at adipose-fin origin	12.8	11.2–13.3 (12.4)
Depth of caudal peduncle	9.2	8.1–9.3 (8.8)
Length of caudal peduncle	17.6	16.7–20.2 (18.3)
Distance between adipose- and caudal-fins	15.1	14.1–16.4 (15.4)
Body width at anal-fin origin	10.5	10.5–12.4 (11.4)
Length of dorsal-fin base	12.5	11.1–12.9 (12.1)
Depth of dorsal-fin	14.2	9.6–15.8 (13.3)
Length of pectoral-fin	16.7	12.8–16.7 (14.7)
Length of adipose-fin base	3.1	1.6–3.4 (2.7)
Depth of adipose-fin	5.2	4.0–5.5 (4.7)
Length of pelvic-fin	11.7	8.4–12.8 (10.9)
Depth of anal-fin	9.5	9.4–14.7 (12.5)
Length of anal-fin base	11.0	8.5–13.5 (10.3)
Length of upper caudal-fin lobe	13.7	11.5–16.3 (14.1)
Length of median caudal-fin rays	11.6	9.3–11.7 (10.6)
Length of lower caudal-fin lobe	13.5	12.4–16.2 (13.7)
Snout length	7.0	5.6–7.2 (6.4)
Distance between nasal openings	4.6	3.3–4.6 (3.9)
Eye diameter	3.2	3.0–4.1 (3.4)
Interorbital width	7.8	6.7–7.8 (7.2)
Head depth through eye	12.1	10.8–12.5 (11.6)
Head depth at nape	15.1	14.0–16.7 (15.2)
Length of maxilla	7.3	5.8–7.4 (6.4)
Width of mouth gape	7.8	6.8–9.0 (7.0)
Length of mouth gape	10.5	8.6–10.8 (9.6)

its distal margin concave or slightly convex. Pelvic fin with 1 unbranched and 8 branched rays, its external margin slightly convex. Pectoral fin with 1 unbranched and 10–12 branched rays, its external margin slightly convex. Caudal fin slightly forked. Ligne transversal with 22–26 scale rows between dorsal-fin origin and lateral line; 17–20 scale rows between anal-fin origin and lateral line; 14–17 scale rows between the origin of the adipose fin and lateral line. Lateral line with 106–115 scales. 18–20 gill rakers on the outer side of the first gill arch.

Coloration

General coloration of freshly preserved specimens is silvery on the back and flank, yellowish on the belly. There is zero or one spot in the postorbital and suborbital areas, smaller than the pupil; there are zero to three black spots on the opercle, smaller than the pupil. Black spots on the body are few (31–60), large (larger than the pupil), ocellated, scattered on the upper part of the flank and the middle part of the flank. There is no black spot on the top of the head and back. Red spots are few (less than 10), large (larger than half of the pupil), ocellated, organized in two irregular longitudinal rows on the median part of the body. Black and red spots are irregularly shaped. The number of black and red spots on the flanks does not increase with increasing size. The dorsal fin is grey, with two to four rows of black spots (smaller than half of the pupil). The caudal fin is dark grey; the pectoral, anal, and pelvic fins are grayish. The adipose fin is plain greyish, with its posterior edge convex.

Distribution

Salmo emireae is distributed in Lake Balık (Fig. 3).

Sexual dimorphism

Distal margin of anal fin convex in males, and convex anteriorly, concave posteriorly in females. The snout of males is more pointed than that of females. Tip of lower jaw pointed, slightly curved upwards, with a slightly developed process at symphysis in males, absent in females.

Etymology

The new species is named in honour of Emire Aksu, wife of the last author, in appreciation of her continuous support throughout his scientific work. A noun in the genitive case, indeclinable.

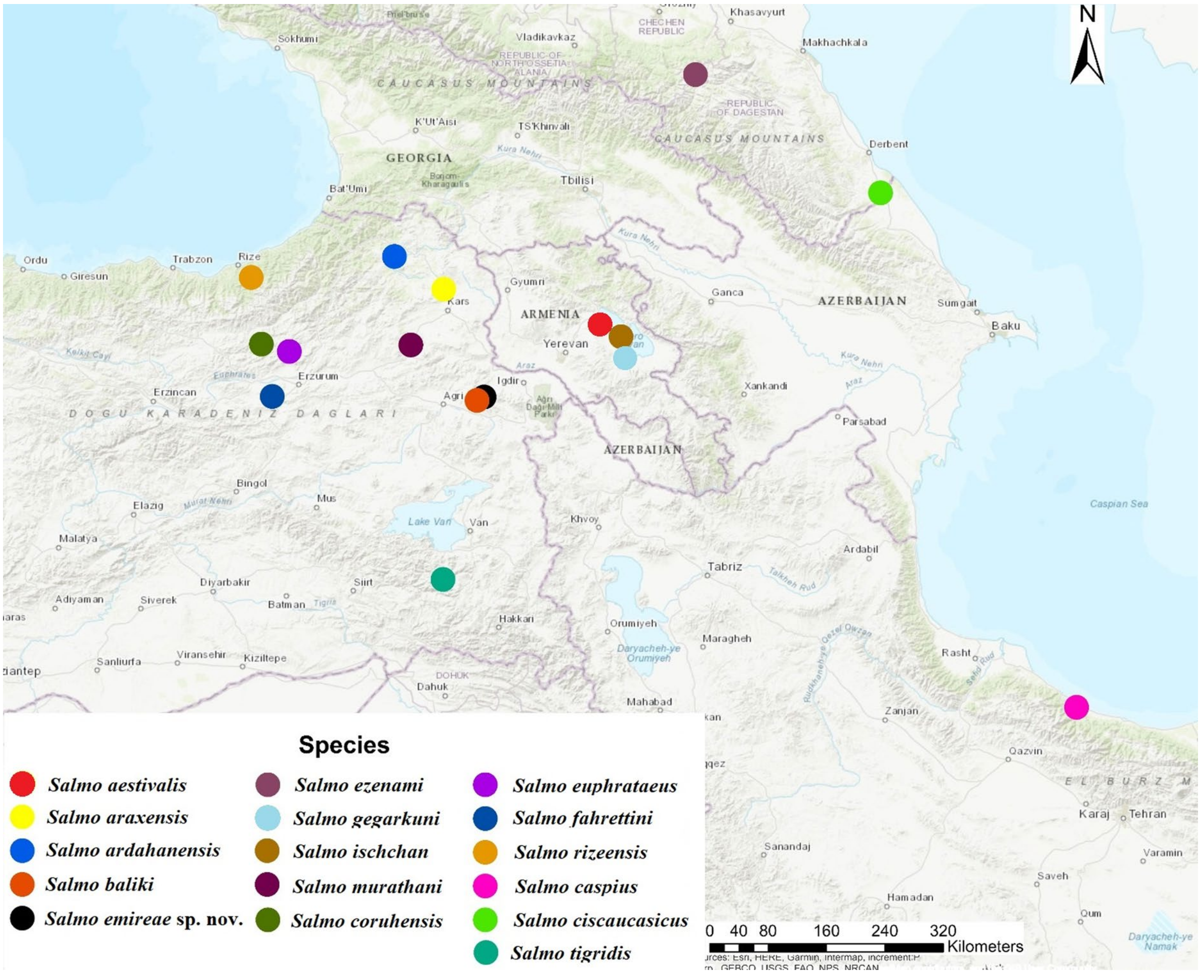

Fig. 3 The map shows the distribution of *Salmo* species in the adjacent area

Conservation status

The new species is endemic to a single, isolated lake that is subject to regular commercial fishing and recent drought-related habitat changes. These pressures, together with its restricted range, make *S. emireae* potentially vulnerable and of conservation concern.

Molecular data analysis

The maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses produced largely congruent topologies for 29 *Salmo* species. Both approaches consistently identified five major clades, each supported by high bootstrap and posterior probability values (Fig. 1), with the exception of the Adriatic lineage, which exhibited only moderate support. The identified clades are as follows:

> Clade I (Danubian lineage): *Salmo emireae* sp. nov., *S. ischchan*, *S. gegarkuni*, *S. aestivalis*, *S. ciscaucasicus*, *S. caspius*, *S. ardahanensis*, *S. murathani*, *S. euphrataeus*, *S. araxensis*, *S. coruhensis*, *S. rizeensis*, *S. abanticus*, *S. fahrettini*, *S. brunoi*, and *S. duhani*.
> Clade II (Marbled lineage): *S. marmoratus* and *S. opimus*.
> Clade III (Adriatic lineage): *S. labecula*, *S. platycephalus*, *S. ekmekciae*, *S. kottelati*, *S. chilo*, *S. okumusi*, *S. baliki*, and *S. munzuricus*.
> Clade IV (Tigris lineage): *S. tigridis*.
> Clade V (Atlantic lineage): *S. trutta*.

The *Salmo* population in Lake Balık, *Salmo emireae*, forms a monophyletic group with strong branch support (ML: 96%, BI: 1.0) and is nested within the Danubian lineage. According to both tree topologies, the sister species of the new species is a clade comprising *S. ischchan*, *S. aestivalis*, and *S. gegarkuni*, which is resolved with moderate support (ML: 50%) in the ML analysis and with high support (BI: 0.90) in the BI analysis. However, it should be noted that *S. ischchan* is considered extinct for a long time (Freyhof et al. 2025), and the *S. ischchan* sequences used in our analysis were retrieved from GenBank (originally from Levin et al. 2018). Therefore, these sequences might in fact belong to *S. gegarkuni*, and this possibility should not be overlooked when interpreting the tree topology.

The interspecific sequence divergence values ranging from 0.00% (*S. kottelati* and *S. chilo*; *S. opimus* and *S. marmoratus*; *S. duhani* and *S. brunoi*; *S. euphrataeus* and *S. murathani*; *S. abanticus* and *S. fahrettini*) to 3.22% (between *S. emireae* and *S. ohridanus*) were determined among the 29 *Salmo* species computed using the *p*-distance model. The interspecific sequence divergence between the new species and other congeneric species ranged from 0.50% (*S. euphrataeus* and *S. murathani*) to 3.22% (*S. ohridanus*). All interspecific sequence divergence results are provided in Table S1.

PCA data analysis

Two species, *Salmo murathani* and *S. araxensis*, from the Aras River were compared with *S. emireae* sp. nov. using principal component analysis (PCA). In total, 60 individuals were examined, including *S. murathani* ($n=20$), *S. araxensis* ($n=31$), and *S. emireae* ($n=9$). Thirty morphometric characters standardized to standard length and three meristic characters were used in the analysis.

The PCA results revealed that *S. emireae* is clearly differentiated from both *S. murathani* and *S. araxensis*. The analysis demonstrates strong morphological differentiation among the examined *Salmo* species. The new species, *S. emireae* sp. nov., shows a distinct separation from *S. murathani* and *S. araxensis*, supporting its recognition as a separate taxon. In contrast, *S. murathani* and *S. araxensis* exhibit closer morphological affinities to each other, although they remain distinguishable along the first two principal components (Table 2; Fig. 4).

The first two principal components explained 97.4% of the total variation among the examined specimens. The first three components, which had the highest eigenvalues, accounted for 84.11%, 13.31%, and 2.57% of the total variance, respectively. The loadings on the first principal component (PC1) were mainly associated with three meristic characters: number of scales on the lateral line, number of scale rows between the lateral line and the dorsal-fin origin, and number of scale rows between the lateral line and the anal-fin origin (Table 2).

Table 2 Character loadings on principal components I and II (PC I and PC II) for 30 measurements and 3 meristic characters taken on 60 specimens of *Salmo emireae* sp. nov., *S. murathani* and *S. araxensis*

Metrics features	PC I	PC II
Head length	−0.0011	0.0068
Predorsal length	−0.0008	0.0048
Prepelvic length	−0.0005	0.0004
Preanal length	0.0007	−0.0019
Body depth at dorsal-fin origin	0.0014	0.0029
Body depth at adipose-fin origin	0.0004	0.0028
Depth of caudal peduncle	−0.0001	0.0032
Length of caudal peduncle	0.0011	−0.0009
Dist. bet. adipose- and caudal-fin	0.0002	−0.0001
Body width at anal-fin origin	0.0005	−0.0015
Length of dorsal-fin base	0.0003	0.0035
Depth of dorsal-fin	−0.0013	0.0047
Length of pectoral-fin	−0.0020	0.0043
Length of adipose-fin base	0.0004	0.0027
Depth of adipose-fin	−0.0005	0.0045
Length of pelvic-fin	−0.0015	0.0038
Depth of anal-fin	−0.0021	0.0048
Length of anal-fin base	−0.0000	−0.0013
Length of upper caudal-fin lobe	−0.0006	0.0025
Length of median caudal-fin rays	−0.0013	0.0037
Length of lower caudal-fin lobe	−0.0013	0.0031
Snout length	−0.0004	0.0002
Distance between nasal openings	0.0002	0.0012
Eye diameter	−0.0003	0.0041
Interorbital width	−0.0001	0.0012
Head depth through eye	0.0001	0.0013
Head depth at nape	0.0001	0.0033
Length of maxilla	−0.0007	0.0037
Width of mouth gape	−0.0013	0.0015
Length of mouth gape	−0.0013	0.0045
Scales on the lateral line	−0.9804	−0.1761
Scale rows bet. the lateral line and the dorsal-fin origin	−0.1493	0.9564
Scale rows bet. the lateral line and the dorsal-fin origin	−0.1281	0.2323

Morphological differences and comparisons

Kessler (1877) described *Salmo caspius* from the Kura River near Bozhii in Azerbaijan. However, there is still a dearth of knowledge regarding the trout species found in the Caspian Sea basin. Prior to Dorofeeva's (1967) classification of *Salmo caspius* as limited to the drainages south of the Great Caucasus and *S. ciscaucasicus* as a separate species for drainages north of it, Berg (1948) claimed that *Salmo caspius* was a large anadromous species present in all drainages of the basin. Additionally, several lacustrine populations or species were documented: *S. ischchan* in Lake Sevan, Armenia, and *S. ezenami* in Lake Kezenoiyam, Dagestan (Kessler 1874; Berg 1948). Previous researchers (e.g., Schöffmann 2002; Kuljanishvili et al. 2020) had identified several forms, variations, or subspecies of *S. ischchan* (*S. ischchan*, *S. gegarkuni*, *S. danilewskii*, and *S. aestivalis*). Levin et al. (2018) reported all these as *S. ischchan*. Kuljanishvili et al. (2020) provisionally considered the ecomorphs of Sevan trout as separate species. By following Kuljanishvili et al. (2020), Levin et al. (2022) adopted the existing species names while also using

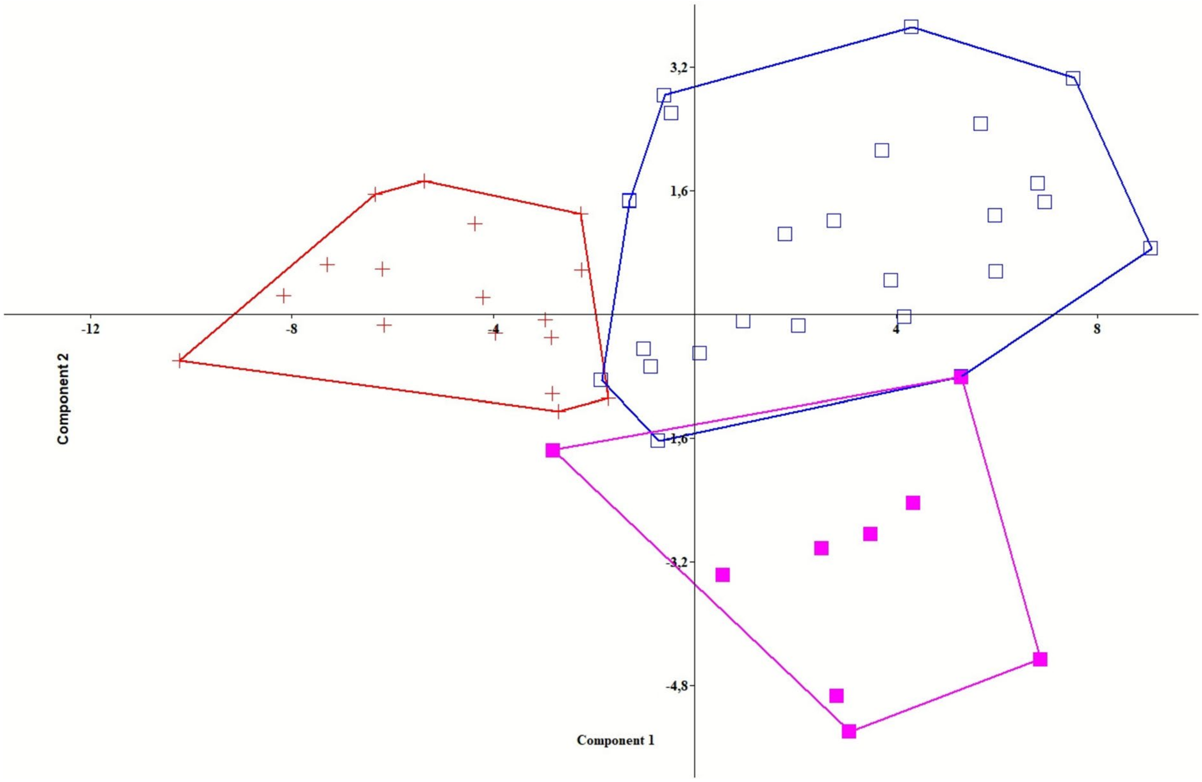

Fig. 4 Principal component analysis (PCA) scatter plot based on 30 morphometric and three meristic characters of *Salmo murathani* (red crosses; +), *S. araxensis* (blue open squares; □), and *S. emireae* sp. nov. (purple filled squares; ■) from the Aras River drainage

the taxonomically neutral term “ectomorph” due to the clear ecological differences between the discussed forms. More recently, Hashemzadeh Segherloo et al. (2022) examined the genetic structure of *Salmo* trouts from the Aralo-Caspian region and suggested that trouts from Lake Sevan, which were not included in their genomic analyses, are distinct from *S. caspius* and other Caspian and Aral basin populations. Their findings emphasized the need for further research to clarify the taxonomic boundaries of these trout populations. However, very recently, Freyhof et al. (2025) re-evaluated the taxonomic status of Lake Sevan trout and recognized *S. ischchan*, *S. gegarkuni*, and *S. aestivalis* as valid species, while treating *S. danilewskii* as a synonym of *S. ischchan*. According to Freyhof et al. (2025), *S. danilewskii* has been used to describe small individuals that primarily feed on gammarids and plankton, but these fish were most likely juveniles of *S. ischchan*.

Salmo caspius is a gigantic silvery fish, up to around 50 kg, or above 1.2 m, with many small black markings shaped like ‘X’s and no red spots. Males have a massive hook at the point of their lower jaw (Kawraisky 1896; Turan et al. 2022).

The Lake Balık population has fewer black spots on the body (less than 61), the presence of red spots in specimens about 300 mm SL, the presence of roundish black spots on the body, and a very small hook at the tip of the lower jaw in males.

Salmo emireae sp. nov. is distinguished from *S. ezenami* by having fewer branched dorsal-fin rays (8–9½, vs. 10–12½ [from Berg 1948:267]), fewer branched anal-fin rays (8–9, vs. 10–12 [from Berg 1948:267]), and the absence of red spots on the body in specimens larger than about 310 mm SL (vs. present in all sizes [data from Berg 1948:267]).

Salmo emireae sp. nov. is distinguished from *S. ischchan* by having red (or orange) spots in individuals up to 310 mm SL (vs. absent, only in juveniles), fewer scale rows between the lateral line and anal-fin origin (17–20 vs. 24–26 [data from Berg 1948:274]), and having fewer scale rows between the lateral line and dorsal-fin origin (22–26 vs. 25–29 [data from Berg 1948:274]). However, although we are not completely certain whether Berg's (1948) method of counting scale rows is identical to ours, the fact that this character is well-defined and well-understood by taxonomists, and that the differences between our findings and Berg's (1948) data are substantial, suggests that using these characters is appropriate.

Salmo emireae sp. nov. is distinguished from *S. aestivalis* by having an irregular black spot and a ring around it (vs. a black spot and ring that are both rounded); the maxilla reaching slightly behind the posterior margin of the eye (vs. not reaching [from Berg 1948:267]) and no black spots in specimens larger than about 310 mm SL (vs. mostly present in all sizes [from Berg 1948:267]).

Salmo emireae sp. nov. is distinguished from *S. gegarkuni* by having red (or orange) spots in individuals up to 310 mm SL (vs. absent, only in juveniles), spots along the lateral line larger than half of the pupil or equal to in the individual larger than 300 mm SL (vs. larger than the pupil, even sometimes larger than the eye). We examined trouts from Tyurkidagunchay in Dagestan. The Tyurkidagunchay is a tributary of the Mukhun stream, itself a tributary of the Chiragchay, itself a tributary of the Samur. The type locality of *S. ciscaucasicus* is in the Samur delta, about 60 km away from the sampling site of the specimens we examined (N. Bogutskaya, pers. comm.). However, the type series is made up of anadromous trouts (Dorofeyeva 1967), while the Samur material we examined is comprised of resident trouts. We identified Samur materials as *S.* cf. *ciscaucasicus*.

Salmo emireae sp. nov. is distinguished from other species occurring in adjacent waters, including *S. cf. ciscaucasicus*, *S. araxensis* (upper Aras River), *S. murathani* (upper Aras River), *S. ardahanensis* (upper Kura River), *S. rizeensis* (upper streams draining to the south and southeastern Black Sea), *S. coruhensis* (lower and middle streams of the south and southeastern Black Sea), *S. abanticus* (Lake Abant basin), *S. duhani* (Gönen and Ayazma Streams, Marmara and Northern Aegean basins), *S. brunoi* (Nilüfer River, Marmara Sea drainage), *S. baliki* (northeastern tributaries of the Euphrates River), *S. okumusi* (northwestern tributaries of the Euphrates River), *S. euphrataeus* and *S. fahrettini* (northern tributaries of the Euphrates River), *S. munzuricus* (Munzur River, northern tributaries of the Euphrates River), *S. platycephalus* (upper Seyhan River, Mediterranean drainage), *S. labecula* (lower Seyhan River), *S. chilo* (Ceyhan River, Mediterranean drainage), *S. opimus* (Alara Stream, Mediterranean drainage), *S. kottelati* (Alakır Stream, Mediterranean drainage), *S. ekmekciae* (Köprüçay River, Mediterranean drainage), and *S. tigridis* (Tigris River). It can be differentiated by a combination of morphological characters, including a shorter head (19.7–23.6% SL, vs. 23.7–32.7, except *S. munzuricus*), a slender caudal peduncle (8.1–9.3% SL, vs. 9.3–13.3, except *S. abanticus, S. brunoi*, and *S. duhani*), a shorter maxilla (5.8–7.4% SL, vs. 7.5–13.4), a smaller mouth gape (8.6–10.8% SL, vs. 10.8–18.9, except *S. coruhensis*, *S. munzuricus*, and *S. opimus*), and a reduced adipose fin (4.0–5.5% SL, vs. 5.6–11.5, except *S. coruhensis*, *S. rizeensis*, *S. duhani*, and *S. brunoi*).

Salmo emireae sp. nov. is further distinguished from *S. platycephalus, S. opimus, S. chilo, S. labecula,* and *S. ekmekciae* by the absence of four dark bands on the flank (vs. present in these species). In addition, it differs from *S. platycephalus* by the presence of black spots in individuals of all sizes (vs. absent in specimens larger than ~ 170 mm SL). *S. emireae* also differs from *S. chilo* by having a slightly convex dorsal profile of the head (vs. strongly convex), a slightly subterminal mouth (vs. conspicuously subterminal), and non-fleshy maxilla and lower lip (vs. fleshy). Compared to *S. labecula*, it shows red spots on the flank up to ~ 300 mm SL (vs. absent in specimens larger than ~ 70 mm SL). Furthermore, *S. emireae* differs from *S. tigridis* by having fewer scale rows between the lateral line and the dorsal-fin origin (22–26 vs. 32–35), fewer scale rows between the lateral line and the anal-fin origin (17–20 vs. 22–26), and fewer scale rows between the adipose fin insertion and the lateral line (14–17 vs. 19–20).

Discussion

Six valid native trout species are found in the Caspian Sea basin, and four in the lakes in the region (Kessler 1874; Kawraisky 1896; Berg 1948; Dorofeeva 1967; Schöffmann 2002; Levin et al. 2018; Kuljanishvili et al. 2020; Turan et al. 2022; Freyhof et al. 2025). These are *S. aestivalis*, *S. araxensis*, *S. ardahanensis*, *S. caspius*, *S. ciscaucasicus*, *S. emireae*, *S. ezenami*, *S. gegarkuni*, *S. ischchan*, and *S. murathani.* We were unable to examine the samples of *S. aestivalis*, *S. caspius*, *S. ezenami*, *S. gegarkuni*, and *S. ischchan*. However, we compared our molecular data with those of previous studies (see above) and previous detailed molecular research conducted in the region. This comparison allowed us to support the hypothesis that our samples represent a new species. Additionally, we evaluated the evolutionary position of the new species and compared it with that of those belonging to the same lineage (*S. euphrataeus*, *S. fahrettini*, *S. rizeensis*, *S. coruhensis*) in adjacent areas.

The phylogenies constructed using ML and BI techniques based on molecular data (mtDNA Cyt b) placed the new species within the Danubian lineage. According to phylogenetic results, the closest relative of the new species is *S. aestivalis, S. gegarkuni,* and *S. ischchan* from Lake Sevan. In the study published by Levin et al. (2018), the taxa *Salmo aestivalis* and *Salmo gegarkuni* are treated not as distinct species, but as subordinate forms (or synonyms) of *Salmo ischchan*. This taxonomic interpretation is clarified through the use of form names such as "*Salmo ischchan aestivalis*" and "*Salmo ischchan gegarkuni*" within the study. Accordingly, *S. aestivalis* and *S. gegarkuni* are not considered separate species but are classified as variants associated with the morphologically and ecologically diverse species *S. ischchan*. The new species could not be clearly separated from its closest relative with moderate support (50%) in the ML phylogeny, whereas it was well resolved with strong support (0.90) in the BI phylogeny. In contrast, calculations based on *p*-distance sequence divergence indicate that the nearest taxa by 0.50% are *S. murathani* from the upper drainages of the Aras River, which is in the same basin, and *S. euphrataeus* from the northeastern tributaries of the Euphrates River, which are from very close basins.

The close phylogenetic affinity between the new species from Lake Balık and the Sevan trout (*S. ischchan*, *S. gegarkuni*, and *S. aestivalis*) indicates a common origin shaped by historical connectivity between Lake Sevan and the Aras-Kura rivers (Levin et al. 2018; Kuljanishvili et al. 2020; Turan et al. 2022). Climatic oscillations throughout the Pleistocene glacial-interglacial cycles strongly affected the distribution of freshwater fauna through the Caucasus and Anatolia. It is well known that such temperature and hydrological oscillations have repeatedly favored population isolation in glacial refugia, followed by secondary contact during warmer periods (Hewitt 2000; Bernatchez 2001). In trout, these often result in the persistence of distinct genetic lineages in separate drainage systems (Marić et al. 2006; Pustovrh et al. 2014). Repeated glaciation events most probably led to the isolation of ancestral trout of the Aras-Kura system in local refugial habitats where they diverged under site-specific ecological conditions. When glaciers retreated, meltwater and a change in drainage patterns might have periodically reconnected some basins, allowing limited gene flow among previously separated populations (Simonović et al. 2017). This dynamic history may explain the relatively low, yet constant, genetic differentiation between the Lake Balık trout and their closest relatives from Lake Sevan. Therefore, the present genetic and morphological distinctiveness of *S. emireae* most likely reflects ancient isolation and regionally constrained dispersal events in the upper Aras system.

Mouth size is recognized as an important diagnostic characteristic among trout species distributed across Anatolia (e.g., Turan et al. 2009, 2012). Although habitat type, such as streams or lakes, can influence certain morphological traits, mouth size appears to be species-specific rather than environmentally induced. Some Anatolian trout species inhabiting stream environments possess relatively small mouths, suggesting that this trait has evolved independently of habitat adaptation. For example, *Salmo opimus* and *S. munzuricus* are characterized by small mouth sizes, and the examined specimens do not differ significantly from these species in this respect. This finding supports the view that mouth size represents a stable taxonomic character within Anatolian *Salmo* species and may serve as a reliable criterion for distinguishing closely related taxa. As a result, *Salmo* specimens from Lake Balık are distinguished from other relative *Salmo* species by using genetic and morphological data.

In general, members of the *Adriatic*, *Marmaratus*, and *Tigris* lineages, including *Salmo opimus*, *S. platycephalus*, *S. chilo*, *S. ekmekciae*, *S. okumusi*, *S. tigridis*, and *S. munzuricus*, possess relatively smaller heads, maxillae, and mouths (Turan et al. 2011, 2012, 2014a, 2014b; Küçük et al. 2024). In contrast, most members of the *Danubian* lineage (*S. rizeensis*, *S. ardahanensis*, *S. abanticus*, *S. euphrataeus*, and *S. araxensis*) exhibit longer heads, longer maxillae, and longer mouth gapes (Turan et al. 2009, 2014a, 2022).

These morphological trends indicate that head length, maxilla length, and mouth size constitute key diagnostic features in distinguishing *Salmo* species across different evolutionary lineages.

Based on the results of both morphological and molecular differences, we described the *Salmo* specimens from Lake Balık as a new species, *Salmo emireae*.

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Author contribution Davut Turan: data analysis, methodology, investigation, visualization, writing—review and editing, supervision. Mehmet Kocabaş: data analysis, methodology, investigation, visualization. Cüneyt Kaya: data analysis, methodology, investigation, visualization, writing—review and editing. Münevver Oral Kaba: data analysis, methodology, investigation, visualization, writing—review and editing. İsmail Aksu: data analysis, methodology, investigation, visualization, writing—review and editing.

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Data availability The information, figures, and tables produced in the current study can be used with proper citation of this study. In addition, the sequences generated for this study are publicly available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Declarations

Conflict of interest The authors declare no competing interests.

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