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# Contribution to the trout of Euphrates River, with description of a new species, and range extension of *Salmo munzuricus* (Salmoniformes, Salmonidae)

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*http://zoobank.org/D06D2FA3-C8F9-4799-9E39-3E3ACA5A8337*

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## Abstract

In an effort to reveal the Euphrates trout taxonomy, the Karasu River, which is one of the eastern drainages of the river, was investigated and three independent populations were identified. Result revealed that two populations belonged to *Salmo munzuricus*, which was known only in Munzur River, while the other population belonged to an unnamed species. *Salmo baliki*, a new species, is described from the Murat River, a drainage of Euphrates River. It differs from *Salmo* species in adjacent water by the combination of the following characters: a grayish body; commonly one, rarely two pale black spots behind eye and on cheek; two to seven black spots on opercle; a few black spots on back and upper part of flank, missing on predorsal area; few to numerous large irregular-shaped red spots in median, upper and lower part of flank, surrounded by a large irregular-shaped white ring; the number of black and red spots not increasing in parallel with size; maxilla short and narrow; adipose-fin medium size, no or rarely one or two red spot its posterior edge; 107–118 lateral line scales; 24–28 scales rows between dorsal-in origin and lateral line; 18–22 scale rows between lateral line and anal–fin origin; maxilla length 7.7–9.1% SL in males, 8.2–9.6 in females. Finally, the genetic study of the Cyt *b* mitochondrial gene confirmed the morphological data, suggesting the separation of *S. baliki* from other *Salmo* species.

## Key Words

Anatolia, cytochrome b, freshwater fish, *Salmo*, taxonomy

## Introduction

Anatolia has a high level of species richness and endemism, thus it has been classified as a European biodiversity “*hot-spot*” (Kosswig 1955; Şekercioğlu et al. 2011), which has also positively reflected in salmonid biodiversity (Bardakçı et al. 2006). *Salmo trutta* L. 1758 is the most widely distributed freshwater fish native to the Palearctic region. Its natural habitat extends from Northeast Russia and Norway, southward to the Atlas Mountains, also, from the spring waters of the Aral Sea to Iceland (Bernatchez 2001; Lobón-Cerviá 2018 and references there in). Initially, all Anatolian trout had been grouped within the *S. trutta* or its subspecies (e.g. Kuru 1975; Geldiay and Balık 2007). Further studies based on morphology (Turan et al. 2012, 2014a, 2014b, 2017; Turan and Bayçelebi 2020) and genetic-aided morphology (Turan et al. 2010, 2011, 2020) of Anatolian trout have revealed a much more complex species structure. Overall, fourteen species have been identified in Anatolia within the last decade. Based on our current knowledge, the upper Euphrates River is one of the most species-rich areas for the genus *Salmo* genus including four well-described species: *Salmo euphrataeus* Turan, Kottelat & Engin, 2014 from the streams Şenyurt, Kuzgun, Rizekent, Ağırcık and Sırlı, northern Euphrates; *S. okumusi* Turan, Kottelat & Engin, 2014 from the streams Göksu, Gökpınar and Sürgü, western Euphrates; *S. munzuricus* Turan, Kottelat & Kaya,

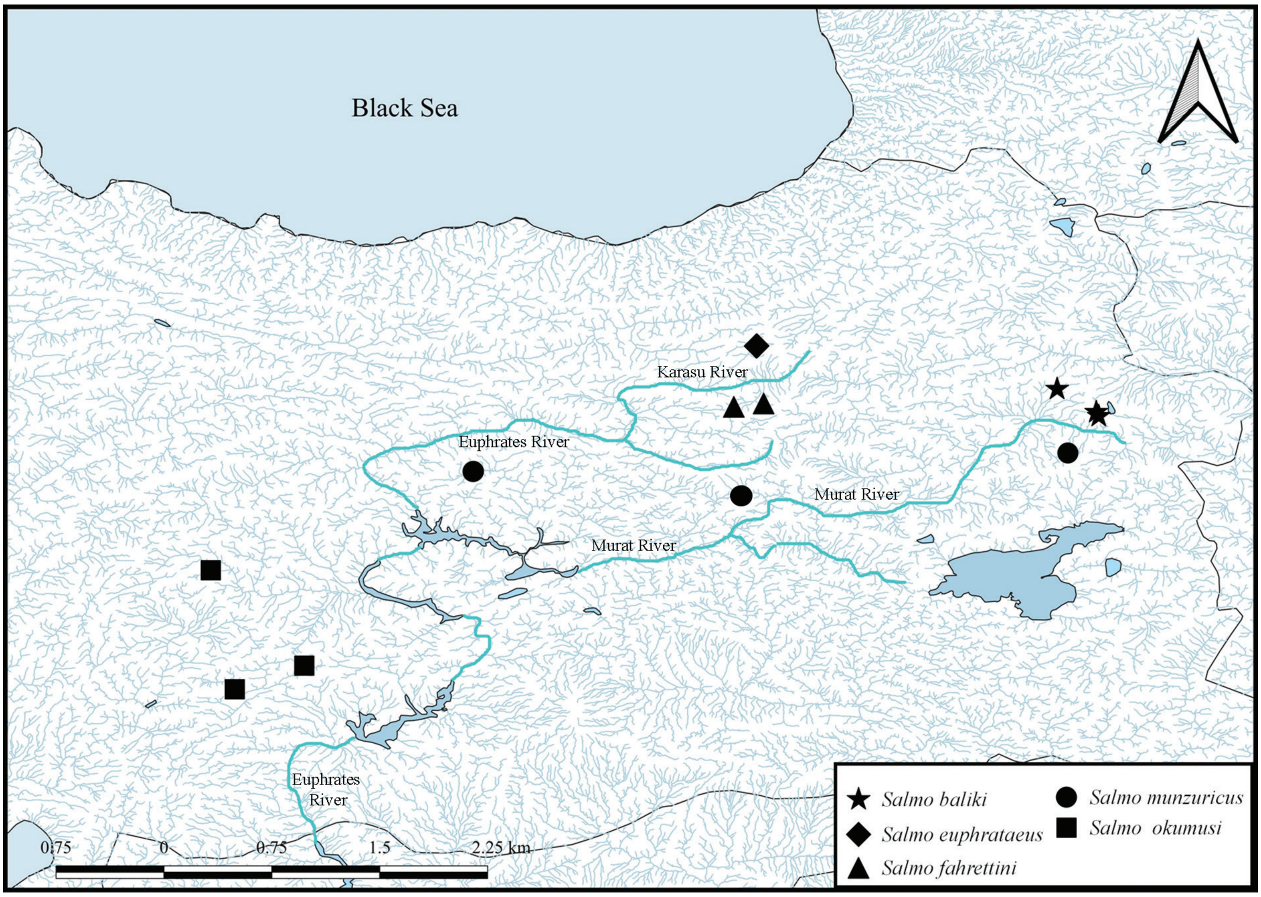

**Figure 1.** Distribution of *Salmo* species in the Euphrates River basin.

2017 from the stream Munzur, northwestern Euphrates; *S. fahrettini* Turan, Kalaycı, Bektaş, Kaya & Bayçelebi, 2020 from the streams Ömertepe suyu and Tekke, northern Euphrates.

Traditionally, five major evolutionary lineages of brown trout were described based on their origin, and phylogenetic; including the AD (Adriatic origin), AT (Atlantic), DA (Danubian), MA (Marmaratus) and ME (Mediterranean) (Bernatchez 2001). Further investigations identified new lineages as Duero from Spain (DU; Suárez et al. 2001), TI from Turkey (Tigris; Sušnik et al. 2005; Bardakçı et al. 2006), Dades from Morocco (Snoj et al. 2011) and from Northern Africa (Tougard et al. 2018). Additional molecular studies have placed the trout species from the Euphrates River drainages in the Danubian (*S. euphrataeus* and *S. fahrettini*), and the Adriatic (*S. okumusi* and *S. munzuricus*) lineages providing the significant species diversity in the Euphrates.

In the scope of this study, three additional trout populations in the Murat River were determined. To reveal the taxonomic status of these novel populations, morphologic and molecular studies were carried out to compare them with the previously identified species in the adjacent waters. Our studies demonstrated that two of these populations belonged to the *S. munzuricus*, which was previously known from a single locality, while the other population belongs to an unnamed species within the Adriatic lineage.

## Material and methods

The field work was carried out by following the guidelines of the Local Ethics Committee of RTE University for the use of animals in scientific experiments with a permit reference number of 2014/72. Samples were collected from the stream Sinek, drainage of the Murat River, Ağrı, and eastern Turkey (Figure 1). This water is known to be one of the uppermost tributaries of the Euphrates River. Samples were caught using an electrofishing device (Samus, 1000). First, live photographs were taken in an aquarium, filled with the water of the sampling reservoir so as to capture the natural coloration and patterns of the specimens. Then, anesthesia was performed using tricaine methane sulphonate solution (MS222). Subsequently, fin clips were collected from one of the pelvic fins, placed into 96% ethanol, for molecular work. Following a surgical procedure, samples were fixed in 4% formaldehyde in the vertical position. These specimens were taken to FFR, Zoology Museum of the Faculty of Fisheries, Recep Tayyip Erdogan University, Rize for detailed morphologic analysis.

## Morphological analyses

Turan et al. (2010) was used as a guideline for morphometric analysis. All measurements were carried out in the form of point to point approach (projections were refused) using a dial caliper calibrated to 1 mm. Specific to the present study, the last two branched rays articulating on a single pterygiophore in the anal and dorsal fins were counted as "1½". Comparative materials used in this study were listed in Turan et al. (2010, 2011, 2012, 2014a, 2017, 2020).

## DNA extraction, PCR and sequencing

Total genomic DNA was extracted from the ethanol-fixed fin clips using DNeasy Blood & Tissue Kit (Qiagen, USA) following the manufacturer's protocol carried out in the Qiacube Automated DNA purification system. The DNA concentration and purity of each sample were assessed by spectrophotometry (Nanodrop, 2000/c, Thermo Scientific, USA), while the integrity was assessed by 0.8% agarose gel electrophoresis. Mitochondrial cytochrome b (Cyt *b*) gene was amplified using SsaL14437 (Warheit and Bowman 2008) and StrCBR (Turan et al. 2010) primer pairs following the PCR conditions specified in Turan et al. (2020). The amplicons were visualized on UV Quantum–Capt ST4 system (Vilber Lourmat, France) and sequenced in both directions by Macrogen Inc. (Amsterdam, Netherlands).

## Phylogenetic analysis

A total of 65 Cyt *b* sequences were assessed from the *Salmo* species (Table 2) inhabited in the Tigris, Euphrates and Kura River drainages as well as the Black Sea and eastern Mediterranean Sea basins. Generated sequences were aligned using BioEdit 7.2.5 (Hall 1999) with Clustal W (Thompson et al. 1994). Trimming was essential thus, applied to both ends of the fragments to set the equal lengths of 993 bp for each and every fragment. The phylogenetic relationships among *Salmo* species were assessed by maximum likelihood (ML) approach in MEGA X software (Kumar et al. 2018) and by Bayesian analysis (BI) in MrBayes v3.2.1 (Ronquist et al. 2012).

The most appropriate evolution model of nucleotide substitution was selected by the Akaike Information Criteria (AIC) and Bayesian Information Criteria (BIC) approaches in jmodelTest 0.1.1 (Posada 2008). ML tree was generated by selecting TrN+I+G (Tamura and Nei 1993) model according to AIC and 1000 bootstrap replicates applied. The BI was generated according to the TrN+I+G (Tamura and Nei 1993) model that the evolution model was selected by the lowest BIC score. For BI, analyses were run for 1×$10^6$ generations with Metropolis coupled Monte Carlo Markov Chains (MCMC) sampled every 1000 generations.

*Salmo salar* Linnaeus, 1758 (JX960834) was used as an outgroup so as to root the phylogenetic tree. The new sequences generated in the present study were deposited

**Figure 2.** *Salmo baliki*, FFR 3242, holotype, 212 mm SL, male; Turkey: stream Sinek, a tributary of Murat River.

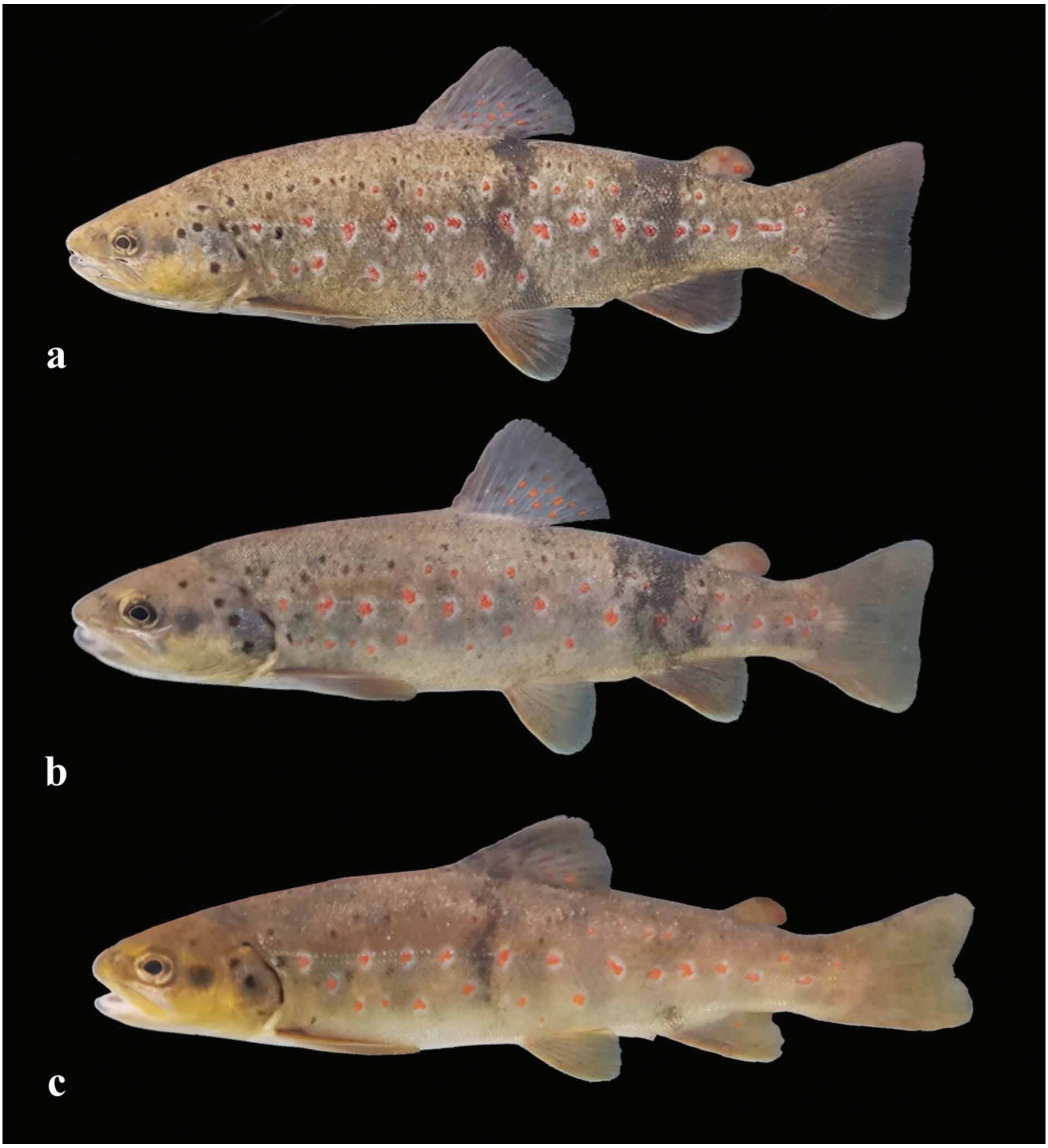

**Figure 3.** *Salmo baliki*, FFR 3234, paratypes, **a.** 216 mm SL, male; **b.** 170 mm SL, male; **c.** 164 mm SL, female; Turkey: stream Sinek, a tributary of Murat River.

to GenBank under the accession numbers: MW366844–MW366860 and MW382946–MW382950 (Table 2).

## Results

### *Salmo baliki* sp. nov.

http://zoobank.org/69483E41-85FA-42AF-AACF-2E69BBE83B0A

Figures 2–4

**Holotype.** FFR 3242, 212 mm SL; Turkey: Ağrı Province: stream Sinek a tributary of Murat River at Taşlıçay, 39.758749°N, 43.464480°E.

**Paratypes.** FFR 3234, 6, 132–276 mm SL; same data as holotype. —FFR 3205, 3, 175–267 mm SL; Turkey: Ağrı Province: a tributary of Murat River 39.730705°N, 43.481869°E.

**Additional record.** Turkey: Ağrı Province: stream Cuma at Cumaçay, 39.919118°N, 43.192272°E.

**Diagnosis.** *Salmo baliki* differs from the other species of trout recorded from the Euphrates and Tigris River drainages (*S. euphrataeus*, *S. okumusi*, *S. munzuricus*, *S. fahrettini* and *S. tigridis*) by having large and irregular-shaped red spots on its body (red spots larger than pupil, vs. smaller than pupil). *Salmo baliki* further differs from *S. euphrataeus* by the general body color silvery in live (vs. brownish), a shorter head in the male (length 24–27% SL, vs. 27–31), a shorter maxilla in the male (length 8–9% SL, vs. 10–11), and a shorter mouth gape (12–14% SL in male, 11–12 in female, vs.14–17 in male, 12–15 in female). *Salmo baliki* further differs from *S. munzuricus* by having fewer black spots in postorbital and suborbital areas (commonly 1, rarely 2, vs. 3–24); fewer black spots on the body (less than 30, vs. more than 80 in adult specimens), whose number does not increase with size (vs. number increasing with size); the black spots circular (vs. irregularly shaped); commonly plain or rarely two roundish red spots on posterior edge of the adipose-fin in male and female (vs. plain or the adipose-fin with a very narrow white margin, then a red submarginal band, then a white band or spots, then a red band again in males, Figures 3, 5), a smaller adipose-fin (8–9% SL in male, 7–8 in female, vs. 9–12 in male, 8–10 in female) a deeper anal-fin in females (16–18% SL, vs. 13–16), a greater anal-fin base (10–12% SL, vs. 8–10), a longer median caudal-fin rays (14–16% SL, vs. 11–14), a greater mouth gape in females (12–13% SL, vs. 10–12), a deeper maxilla in females (maximum maxilla depth 3–4% SL, vs. 2–3), a smaller distance between the adipose and caudal fins in males (15–16% SL, vs. 16–18), and fewer lateral line scales (107–118, vs. 116–123). *Salmo baliki* further differs from *S. okumusi* by having one or two pale black spot in postorbital and suborbital area (vs. 1–22), fewer black spots on opercle (3–7, vs. 8–17), fewer black spots on the body in specimens smaller than 210 mm SL (less than 30, vs. more than 90), presence black spot on body in all size (vs. the body with black dots in specimens larger than about 230 mm SL), the shape of the black spots ocellated (vs. irregularly shaped), the black spots scatter on back and upper part of flank (vs. whole flank covered black spots or dots), no black spots on top head (vs. 5–18), one or two dark bands on posterior part of the flank (vs. four dark bands in specimens larger than about 230 mm SL), the parr marks vertically oblong (vs. vertically elongate), the shape of the black spots ocellated (vs. irregularly shaped), a more slender dorsal-fin in males (16–17% SL, vs. 17–19), a shorter pectoral-fin in males (17–19% SL, vs. 19–21), a smaller eye diameter (4–5% SL in males, 4–6 in females; vs. 5–7 in males, 6–8 in females), a shorter maxilla in males (8–9% SL, vs. 9–11) (see Figures 3, 6). *Salmo baliki* further differs from *S. tigridis* by having fewer scale rows between the dorsal-fin origin and the lateral line (24–28, vs. 32–35); fewer scale rows between the end of the adipose-fin base and the lateral line (13–15, vs. 19–20), a slenderer caudal peduncle depth (11–12% SL, vs. 12–13). *Salmo baliki* further differs from *S. fahrettini* by having fewer black spots on its body (less than 30, vs. more than 80); the black spots scatter on back and upper part of flank (vs. scattered on back, middle and upper part of flank, and anterior part of lower half of flank), their number not increasing with size (vs. increasing with size), fewer red spots on body (fewer than 42 in adult specimens, vs. more than 70 in adult specimens), their number not increasing with size (vs. increasing with size). See Discussion for comparison with other trout in adjacent waters of Anatolia.

**Table 1.** Morphometry of *Salmo baliki* (holotype, FFR 3242; paratypes FFR 3205, n = 3 and FFR 3234, n = 6). The calculations include the holotype.

| | **Holotype** | **Paratypes** | | | |
|---|---|---|---|---|---|
| **Sex** | **male** | **male** | | **Female** | |
| Number of specimens | | n = 4 | | n = 5 | |
| Standard length (mm) | 212 | 164–250 | | 132–267 | |
| In percentage of standard length | | Range (mean) | SD | Range (mean) | SD |
| Head length | 26.9 | 24.1–26.9 (25.7) | 1.1 | 24.2–26.0 (25.2) | 0.7 |
| Predorsal length | 47.4 | 45.0–49.7 (47.7) | 1.8 | 46.2–48.2 (47.4) | 0.8 |
| Prepelvic length | 52.7 | 52.7–55.6 (54.3) | 1.1 | 52.9–55.3 (54.1) | 1.0 |
| Preanal length | 72.7 | 72.7–76.0 (74.8) | 1.3 | 72.6–77.7 (75.2) | 2.0 |
| Body depth at dorsal-fin origin | 23.9 | 23.6–26.4 (24.7) | 1.2 | 22.2–25.8 (24.3) | 1.4 |
| Body depth at anal-fin origin | 19.1 | 18.5–20.8 (19.5) | 1.2 | 17.4–20.1 (18.9) | 1.2 |
| Depth of caudal peduncle | 10.6 | 10.6–11.7 (11.0) | 0.5 | 10.6–11.9 (11.2) | 0.5 |
| Length of caudal peduncle | 17.3 | 16.1–17.9 (17.2) | 0.7 | 15.8–17.9 (17.0) | 0.9 |
| Distance between adipose- and caudal-fins | 16.0 | 15.4–16.4 (15.8) | 0.4 | 16.1–17.9 (16.6) | 0.7 |
| Body width at anal-fin origin | 11.9 | 9.6–11.9 (10.6) | 0.9 | 8.9–11.7 (10.5) | 1.1 |
| Length of dorsal-fin base | 12.2 | 12.2–14.8 (13.5) | 1.0 | 12.9–14.0 (13.5) | 0.4 |
| Depth of dorsal-fin | 16.7 | 16.0–17.2 (16.6) | 0.5 | 12.8–18.2 (15.9) | 2.0 |
| Length of pectoral-fin | 17.8 | 17.0–19.0 (17.9) | 0.8 | 17.2–20.3 (19.1) | 1.2 |
| Length of adipose-fin base | 4.2 | 4.0–5.3 (4.7) | 0.6 | 3.9–5.0 (4.4) | 0.4 |
| Depth of adipose-fin | 8.4 | 7.5–8.7 (8.2) | 0.5 | 7.1–8.3 (7.7) | 0.6 |
| Length of pelvic-fin | 14.5 | 13.8–15.8 (14.8) | 0.8 | 14.0–15.2 (14.7) | 0.5 |
| Depth of anal-fin | 16.9 | 15.8–18.0 (16.9) | 0.8 | 16.3–18.3 (17.4) | 0.8 |
| Length of anal-fin base | 10.3 | 10.3–11.3 (10.9) | 0.4 | 10.2–11.8 (10.9) | 0.6 |
| Length of upper caudal-fin lobe | 17.0 | 15.3–17.2 (16.3) | 0.8 | 15.2–18.3 (16.7) | 1.1 |
| Length of median caudal-fin rays | 14.3 | 13.5–14.7 (14.3) | 0.4 | 13.5–15.7 (14.2) | 0.9 |
| Length of lower caudal-fin lobe | 17.5 | 15.6–18.2 (17.0) | 1.2 | 14.4–19.2 (16.9) | 1.9 |
| Snout length | 8.1 | 6.3–8.3 (7.2) | 0.9 | 6.6–7.6 (7.1) | 0.5 |
| Distance between nasal openings | 4.5 | 3.7–5.0 (4.4) | 0.5 | 4.0–4.7 (4.3) | 0.2 |
| Eye diameter | 4.0 | 3.6–4.9 (4.4) | 0.5 | 3.6–5.8 (5.1) | 0.9 |
| Interorbital width | 7.9 | 7.1–9.3 (8.3) | 0.8 | 6.9–8.1 (7.5) | 0.5 |
| Head depth through eye | 12.3 | 11.2–13.4 (12.5) | 0.9 | 11.4–13.5 (12.5) | 0.9 |
| Head depth at nape | 16.6 | 15.0–17.7 (16.3) | 1.0 | 16.0–18.6 (16.8) | 1.0 |
| Length of maxilla | 7.7 | 7.7–9.1 (8.4) | 0.5 | 8.2–9.6 (8.7) | 0.6 |
| Maximum height of maxilla | 3.1 | 2.5–3.1 (2.9) | 0.3 | 2.6–3.9 (3.2) | 0.5 |
| Width of mouth gape | 9.4 | 8.6–10.5 (9.6) | 0.7 | 8.7–10.1 (9.1) | 0.6 |
| Length of mouth gape | 13.9 | 11.7–13.9 (12.8) | 0.9 | 11.6–12.6 (11.8) | 0.3 |

**Description.** The general appearance is shown in Figures 2–4, morphometric data are in Table 1. Body deep, compressed laterally, its depth approximately equal to head length. Dorsal profile markedly arched and ventral profile less arched than the dorsal profile. Head short, upper profile

**Table 2.** Materials used in genetic analysis.

| Species | Sample | Locality | Accession number | Reference |
|---|---|---|---|---|
| *S. baliki* | 5 | Turkey: Agri, Sinek stream, Murat River, Euphrates River | MW366856–MW366860 | This study |
| *S. munzuricus* | 3 | Turkey: Tunceli, Munzur Stream, Euphrates | MN815914 | Turan et al. 2020 |
| | 5 | Turkey: Agri, Murat River, Euphrates River | MW382946–MW382950 | This study |
| *S. okumusi* | 3 | Turkey: Sivas, Gökpinar Stream, Euphrates | MN815915 | Turan et al. 2020 |
| *S. opimus* | 3 | Turkey: K.Maras, Göksun, Ceyhan River drainage | MW366853–MW366855 | This study |
| *S. chilo* | 3 | Turkey: Sivas, Akdere stream, Ceyhan River drainage | MW366850–MW366852 | This study |
| *S. labecula* | 3 | Turkey: Nigde, Ecemis stream, Seyhan River drainage | MW366847–MW366849 | This study |
| *S. platycephalus* | 3 | Turkey: Kayseri, Pinarbasi stream, Seyhan River drainage | MW366844–MW366846 | This study |
| *S. fahrettini* | 3 | Turkey: Erzurum, Omertepesuyu Stream Euphrates | MN815913 | Turan et al. 2020 |
| *S. coruhensis* | 3 | Turkey: Rize, Cayeli Kanlidere Stream | MN815912 | Turan et al. 2020 |
| *S. rizeensis* | 3 | Turkey: Rize, Kangel stream | MN815910 | Turan et al. 2020 |
| | 3 | Turkey: Rize, Alakoz stream | MN815910 | Turan et al. 2020 |
| *S. euphrataeus* | 3 | Turkey: Erzurum, Sirli Stream, Euphrates | MN815911 | Turan et al. 2020 |
| *S. caspius* | 2 | Turkey: Ardahan, Toros Stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| | 2 | Turkey: Ardahan, Derindere stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| | 2 | Turkey: Ardahan, Karaman stream, Kura River drainage | MN815909 | Turan et al. 2020 |
| *S. tigridis* | 3 | Turkey: Van, Catak Stream | MN815916 | Turan et al. 2020 |
| *S. trutta* | 1 | Italy: Flumendosa | LT617538 | Tougard et al. 2018 |
| | 1 | France: Vidourle | LT617535 | Tougard et al. 2018 |
| | 1 | Slovenia: Volaja | LT617539 | Tougard et al. 2018 |
| | 1 | United Kingdom: Camel | LT617540 | Tougard et al. 2018 |
| | 1 | Austria: Kleiner Kamp | KF985687 | Schenekar et al. 2014 |
| | 1 | Norway: Leksa | JX960836 | Crête–Lafrenière et al. 2012 |
| | 2 | Turkey: Van, Arpet Stream, Tigris | MT981164–MT981165 | Kaya 2020 |
| | 2 | Turkey: Bitlis, Sapur Stream, Lake Van | MT981168–MT981169 | Kaya 2020 |
| *S. obtusirostris* | 1 | Bosnia and Herzegovina: Neretva | JX960841 | Crête–Lafrenière et al. 2012 |
| *S. ohridanus* | 1 | Macedonia: Lake Ohrid | AF053590 | Sušnik et al. 2006 |
| *S. salar* | 1 | Norway: Ims | JX960834 | Crête–Lafrenière et al. 2012 |

**Figure 4.** *Salmo baliki*, FFR 3205, paratypes, **a.** 250 mm SL, male; **b.** 267 mm SL, female; Turkey: stream Sinek, a tributary of Murat River.

straight both on the snout and above the eye in male, straight above the eye and convex on snout in female. Mouth small, terminal or slightly subterminal in male, subterminal in female. Tip of lower jaw slightly curved upwards, pointed, with a slightly-developed process at symphysis in male larger than 200 mm SL. Maxilla short, reaching slightly beyond posterior margin of the eye in males and female larger than about 200 mm SL. Snout somewhat long, with

**Figure 5.** *Salmo munzuricus*: **a.** FFR 3226, 211 mm SL, male; Turkey: Tunceli Prov., stream Kalan; **b.** FFR 3241, 205, male; Turkey: Muş Prov., stream Mengel; **c.** FFR 3226, 240, male; Turkey: Ağrı Prov., stream Alakoçlu.

pointed tip in male, rounded in female. Adipose fin somewhat large, its height 7.5–8.7% SL in males and 7.1–8.3 in females. Largest observed specimen 250 mm SL.

Dorsal fin with 3–4 unbranched and 8–10 branched rays, its distal margin slightly convex. Pectoral fin with 1 unbranched and 10–11 branched rays, its external margin slightly convex. Pelvic fin with 1 unbranched and 7–8 branched rays, its external margin slightly convex. Anal fin with 3 unbranched and 7–9 branched rays, its distal margin convex anteriorly and straight or concave posteriorly. Caudal fin deeply emarginated in specimens less than 160 mm SL, slightly emarginated or truncate in specimens larger than about 200 mm SL, lobes slightly pointed. Lateral line with 107–118 scales; 24–28 scale rows between dorsal-fin origin and lateral line; 18–22 scale rows between anal-fin origin and lateral line; 13–15 scale rows between origin of the adipose fin and lateral line. Gill rakers 16–18 on first gill arch.

**Coloration.** In formalin: General coloration of freshly preserved specimens silvery on back and flank, yellowish on the belly. One pale black spot in postorbital and suborbital areas, greater than pupil; three to seven black spots on opercle, approximately smaller than pupil. Black spots on body few (fewer than 30), smaller than the pupil, ocellated, scattered on the upper part of flank (missing in back). No black spot on top of the head. Red spots few (fewer than 30), large (greater than pupil), irregularly-shaped, surrounded by an irregularly shaped narrow ring, organized in two to four irregular longitudinal rows on median part of the body, and half

**Figure 6.** *Salmo okumusi*, FFR 3157, 260 mm SL, male; Turkey: stream Gökpınar, a tributary of Tohma River.

**Figure 7. a.** Stream Sinek, Murat River, Turkey; type locality of *Salmo baliki*: **b.** Stream Alakoçlu; **c.** Stream Mengel, Murat River Turkey, two new localities of *S. munzuricus*.

of lower part of the flank. The number of black and red spots on flanks does not increase with increasing size. Dorsal fin grey, with three or four rows of black spots (smaller than pupil) and one or two rows of red spots (smaller than pupil). Caudal fin dark gray; pectoral, anal pelvic fins greyish. Adipose-fin plain grayish, rarely one or two red spots on its posterior edge. Eight to nine oblong parr marks on the body, distinct in specimens up to about 190 mm SL. 1–2 vertical bands on posterior half of flank in most specimens.

**Distribution and habitat.** *Salmo baliki* inhabits clear and moderately swift-flowing water, with a substrate of stones and pebbles. The observed material for this species has only been collected from stream Sinek, drainage of Murat River (Figures 1, 7a). The species has not been found in adjacent waters except stream Cumaçay (39.919118°N, 43.192272°E) that is located approximately 32 km northwest of Sinek, another drainage of Murat River. However, there was no opportunity to carry out survey in the stream Cumaçay location. Following solid evidences of shape and size of the spots from the video records shown by local people, this location will further be investigated in the near future.

**Conservation status.** There is serious pressure on the populations of *Salmo baliki* due to overfishing. The species is taken for curative purposes, hence demand is very

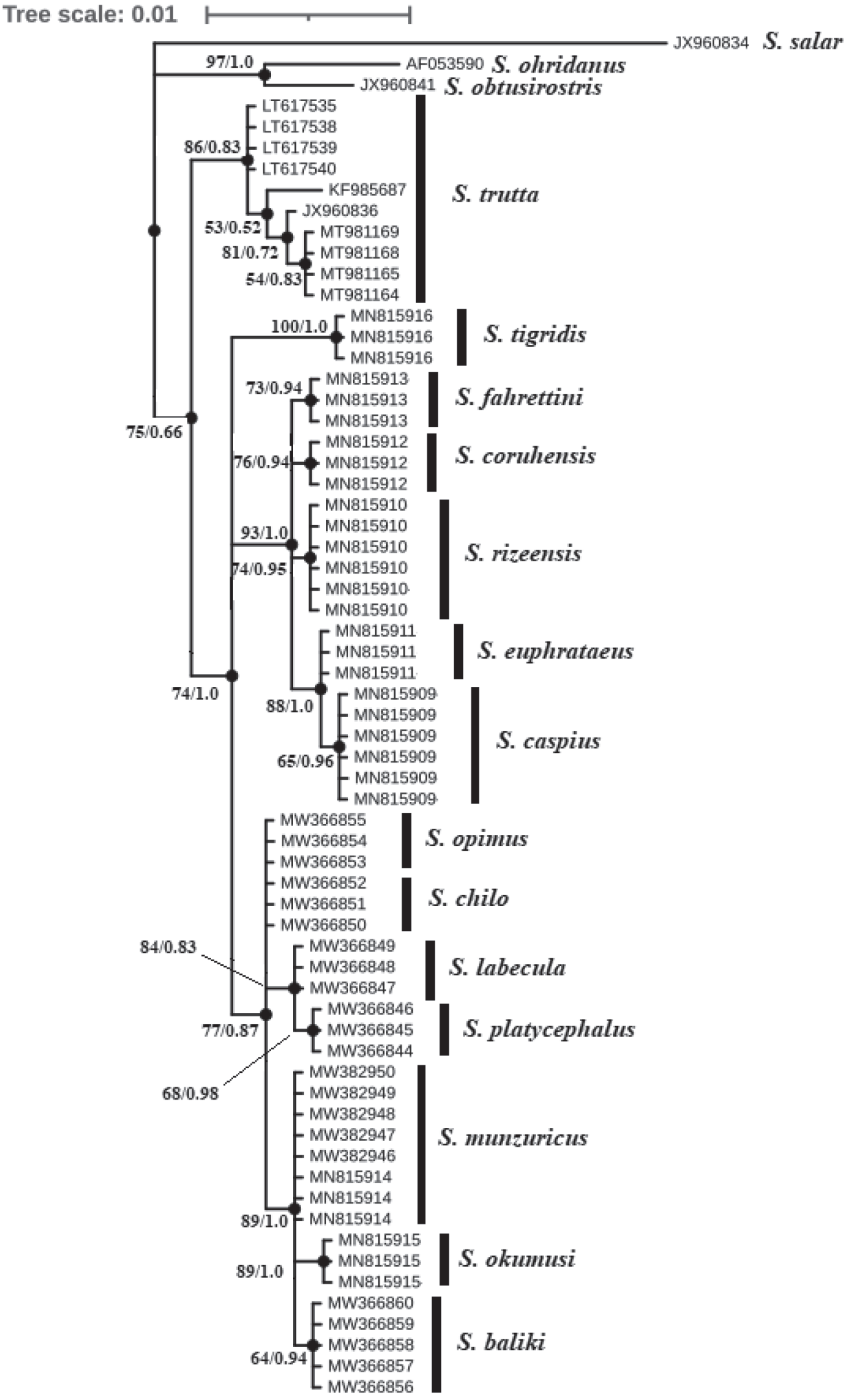

**Figure 8.** Bayesian inference (BI) phylogenetic tree based on Cyt *b* sequences of *Salmo* species. ML and BI methods generated the similar topologies and therefore only the BI tree is shown. The bootstrap values of ML and posterior probability values of BI are indicated on nodes (ML/BI).

high. Within the first fieldwork in the area which was carried out in 2006, in total 3 specimens were found in the middle of the stream (39.730705°N, 43.481869°E), however no specimens were detected in the same location during the recent survey, only a small population observed about 4 km upstream (a restricted area, far from the villages, and the only transportation is provided through a rough and muddy road). Taking all these factors into account, endemic *S. baliki* is stuck in a very limited area, thought to be under a serious threat. Therefore, there is a need for the species to be conserved under international legislation.

**Sexual dimorphism.** The snout of the male is more pointed than that of the female. The depth of the adipose-fin and the length of the mouth gape in male are greater than those of the female.

**Etymology.** The species is named after Dr. Süleyman Balık (Turkey), taxonomist, in appreciation of his contributions to the freshwater fish fauna of Turkey.

**Phylogenetic placement of *Salmo baliki*.** We analyzed a total of 65 sequences (22 new sequences in this study and 43 sequences from GenBank; Table 2) to assess if the phylogenetic relationship among *Salmo* sp. *Salmo baliki* new species is genetically different from the other *Salmo* species. The nodes separating the species in the phylogenetic tree topology of the Cyt *b* gene were supported by high posterior probability and bootstrap values. (Figure 8). The bootstrap values in ML analysis are relatively low compared to the posterior probability values in BI analysis. However, the two tree topologies do not contradict each other. According to the result of phylogenetic analysis, *Salmo baliki* is a sister taxon to *S. munzuricus* (Figure 8). For *Salmo* species, phylogenetic tree topology corresponds exactly with the fiction formed as a result of morphological data.

**Range extension of *Salmo munzuricus*.** Within the aim of the present study a geographic range extension for *Salmo munzuricus* was also recorded. This species was previously only described from Munzur River, north western Euphrates (Turan et al. 2017). Here, two new localities have been identified for *S. munzuricus* (Figure 7b, c). These new localities in the Murat River, located 140 and 340 km east of the previously known distribution range of the species, reveal the scarce bio-geographic knowledge of the species in the Euphrates basin.

## Discussion

The population of *Salmo baliki* has been experiencing a serious pressure caused by local people and fisherman. The trout inhabiting stream Sinek is thought to be a ‘healer fish’, as appeared in local and national press, thus sold for higher prices throughout the country. This is the main reason behind the relatively low number of specimens investigated in the present study as opposed to standard morphometric studies (10 fish versus 25 fish). However, evidence, discussions with local people and video recordings suggest an additional locality for *S. baliki*, soon to be confirmed.

Trout inhabit cold, well-oxygenated waters where the flow is relatively high and species get restricted to such locations. This leads to ecological isolation from the other populations inhabiting same water bodies. Hence, this, in turn, has a significant effect on speciation. Although there are significant morphological differences among the trout species, relatively lower genetic distances in mtDNA sequences indicate an early stage of speciation taking place in *Salmo* genus within the course of evolution. In total, fifteen native trout species have been identified in Turkey, six of which are known from the Euphrates and Tigris drainages, namely; *Salmo tigridis*, *S. okumusi*, *S. euphrataeus*, *S. munzuricus*, *S. fahrettini* and *S. baliki* (in the present study). Those of *Salmo baliki*, *S. munzuricus* and *S. okumusi* belong to Adriatic linage. Molecular distance among these species is not very distinct, however, the remarkable morphological differences easily separate these species which are presented above in the diagnosis section (see also Figure 2–4, 5 and 6).

*Salmo baliki* is easily distinguished from *S. platycephalus*, *S. chilo*, *S. labecula* and *S. opimus*, all from streams draining to the Mediterranean, by zero to two dark bands on the posterior part of the flank (vs. four dark bands on flank), a smaller eye in males (eye diameter 4–5% SL, vs. 6–7), in having more scale rows between the anal-fin origin and the lateral line (18–22, vs. 15–18).

*Salmo baliki* further differs from *S. platycephalus* and *S. labecula* by having fewer gill rakers on the first gill arch (16–18, vs. 21–25), a shorter head in males (head length 24–27% SL, vs. 27–29) and the presence of red spots in specimens larger than about 70 mm SL (vs. absence). In *S. baliki*, the top of the head is not flattened, while the top of the head is flattened in *S. platycephalus*,

*Salmo baliki* further differs from *S. chilo* by the dorsal profile of the head being straight in the interorbital area and at the level of the nostrils (vs. strongly convex), the snout slightly pointed in the male (vs. blunt), the maxilla and lower lip are not fleshy (vs. flesh), fewer black spots behind the eye (always one, vs.up to 12) and fewer black spots on the opercle (3–7, vs. 7–13). *Salmo baliki* also differs from *S. chilo* by the number and position of the black spots on the body in males. In *S. baliki*, black spots are fewer (less than 30) and located on the upper part of the flank. In *S. chilo*, there are numerous (more than 40) black spots which are scattered on the middle part of the body, mostly on the anterior part; however, these are missing on the back in specimens larger than 140 mm SL.

*Salmo baliki* further differs from *S. opimus* by having a slenderer body in male (23–26% SL, vs. 26–29), a shorter maxilla in male (maxilla length 8–9% SL, vs. 9–10) with the black circular (vs. irregularly shaped) spots. Additionally, the top of the head is straight in male (vs. convex) and the mouth is located terminally or slightly subterminal in male (vs. conspicuously subterminal).

*Salmo baliki* is most notably distinguished from *S. caspius* (from Kura River drainage) by having fewer gill rakers on the outer side of the first gill arch (16–18, vs. 19–21) and no black spots on the top of the head (vs. small black spot on top of head). It further differs from *S. caspius* by having a greater distance between adipose and caudal fins in male (15–18% SL, vs. 14–15), a shorter head in male (24–27% SL, vs. 27–31), a shorter and narrower maxilla in male (maxilla length 8–9% SL, vs. 9–11; maxilla width 2–3% SL, vs. 3–4). In the male specimen of *Salmo baliki*, the anal- and adipose-fins do not reach the caudal-fin base (vs. reaching in specimens larger than 200 mm SL) and the general body color is silvery in live (vs. brownish).

*Salmo baliki* differs from *S. rizeensis* by the general body color being silvery in live (vs. brownish) and the absence of black spots on the back (vs. presence). *Salmo baliki* also differs from *S. rizeensis* by having more scale rows between anal-fin origin and lateral line (24–28, vs. 18–22), less branched dorsal-fin rays (7–9, mode 9, vs. 9–12, mode 10), a shorter head in male (24–27% SL, vs. 29–31), a deeper caudal peduncle (11–12% SL, vs. 10–11), a greater adipose fin (length of base of adipose-fin 4–5% SL, vs. 3–4), a smaller maxilla in male (length of maxilla 8–9% SL, vs. 10–12), and a smaller mouth gape in male (length of mouth 12–14% SL, mean 12.8, vs. 13–18, mean 15.5).

*Salmo baliki* is immediately distinguished from *S. coruhensis* by the number and distribution of the black and red spots on the body and the way they vary with increasing size. In *S. baliki*, the black spots are few, small, ocellated and restricted to the upper part of the flank and missing on the back. The red spots are few, large, irregularly shaped and scatter on the half of the lower and the upper, and median part of the flank. The number of black spots does not increase with increasing size. In *S. coruhensis*, the black spots are numerous, from medium to large, ocellated, and present on the whole upper half of the flank and on the anterior part of the lower half. The red spots are irregularly shaped and ocellated, and do not increase with increasing size. The number of both kinds of spots increase with increasing size and age. *Salmo baliki* usually has a single pale black spot behind the eye (on cheek and preopercle) at all sizes in both sex; 3 to 7 spots on the opercle. *Salmo coruhensis* has two or three spots on the cheek and the preopercle in most specimens, rarely a single one spot found and this number increases to 4–17 in large adult male; 5–14 spots on the opercle.

*Salmo baliki* is also distinguished from *S. trutta* by having fewer lateral line scales (107–118, vs. 117–128), fewer scale rows between lateral line and dorsal–fin origin (27–30, vs. 30–34), a shorter head in males (24–27% SL, vs. 28–31), a smaller maxilla (length of maxilla 8–10% SL, vs. 10–12), a smaller mouth gape in males (length of mouth gape 11–14% SL, vs. 14–16), a deeper caudal peduncle (11–12, vs. 10–11) and greater adipose-fin in males (8–9% SL, vs. 7–8). *Salmo baliki* further differs by body color and pattern. In *S. baliki*, red spots are large (greater than eye diameter, vs. smaller than eye diameter) and irregular-shaped (vs. roundish); black spots are few (less than 30, vs. more than 70) and scatter on upper part of flank (vs. numerous and scatter on back, upper part and middle part of flank, sometimes lower part of flank).

Results of genetic work was in correspondence with the morphological observations. Although a single mtDNA region (Cyt *b*) was used to assess phylogenetic relationship among *Salmo* sp. in comparison with *S. baliki*, support of high bootstrap and posterior probability values indicated separation among *Salmo* species inhabiting Anatolia. *Salmo* sp. are known to be recently diverged (Lobón-Cerviá 2018 and references therein) within the course of evolution, thus diversification of species is still an ongoing process, and mostly supported by lower genetic distances among species. However, phylogenetic analysis of the present study indicated a separate branch for *Salmo baliki*, when compared with the *S. euphrataeus*, *S. fahrettini*, *S. munzuricus* inhabits the same basin. Additionally, each of these species formed unique haplotypes, supporting differences among closely related species of *S. munzuricus* and *S. okumusi*.

## Comparative material

See Turan et al. (2010, 2011, 2012, 2014a, 2017, 2020) for additional comparative materials examined.

***Salmo munzuricus*:** FFR 3235, 13, 170–253 mm SL; Turkey: Ağrı province: stream Alakoçlu, a tributary of Euphrates River at Taşlıçay, 39.475000°N, 43.267000°E. —FFR 3241, 4, 108–205 mm SL; Turkey: Muş province: stream Mengel at Alabalık village, a tributary of Murat River, 39.313686°N, 41.162689°E. —FFR 3226, 11, 123–211 mm SL; Turkey: Tunceli province: stream Kalan at Sarıtaş village, a tributary of Munzur River, 39.249975°N, 39.489062°E.

***Salmo trutta*:** Germany, 7, 111–156 mm SL; Rhine River, Plesibach Stream at Niederpleiss.

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