

The trouts of the Marmara and Aegean Sea drainages in Türkiye, with the description of a new species (Teleostei, Salmonidae)

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Abstract

The taxonomic status of native trout species of the Marmara and Aegean Sea drainages is evaluated and three species, *Salmo duhani*, *S. coruhensis* and *S. brunoi* **sp. nov.**, are recognized. *Salmo brunoi*, a new species, is described from the Nilüfer River, a tributary of the Susurluk River. It is distinguished by a general brownish body color in life; few black spots (fewer than 60) on the body, generally scattered on the back and the upper part of the flank, rarely in the median part; few (fewer than 40) and small (smaller than pupil) red spots on the body, scattered on the median part and lower half of the flank; a number of black and red spots not increasing with size in both sexes; a long adipose fin (adipose-fin height 8–9% SL); a short distance between adipose-fin and caudal-fin (12–14% SL); and a short anal fin (anal-fin height 12–15% SL). *Salmo brunoi* **sp. nov.** is separated from the rest of the Marmara and Aegean trouts of Anatolia based on genome-wide distributed 187,385 unlinked SNP markers. According to the best of the authors' knowledge, whole genome data is used for the first time here to characterize a new species of trout.

Key Words

Anatolia, biodiversity, freshwater fish, *Salmo*, taxonomy

Introduction

Salmo trutta Linnaeus, 1758 (brown trout) has long been considered a species widely distributed throughout Europe, reaching the Atlas Range southwards (Morocco, Algeria) and the upper Amu Darya drainage in Afghanistan eastwards (Kottelat and Freyhof 2007).

Anatolia has a high level of species richness and endemism and thus has been classified as a European biodiversity hotspot (Kosswig 1955; Durand et al. 2002; Şekercioğlu et al. 2011), and salmonids are no exception with a high level of endemism in the area (Bardakçı et al. 2006). As it is conveniently located at the intersection of three major biodiversity hotspots, namely: Caucasian, Mediterranean and Irano-Anatolia, Türkiye harbors a high genetic and morphological diversity across a wide range of taxa (Noroozi et al. 2019). So far, the rich diversity of Anatolian trouts has been mostly revealed by

the examination of morphological characters (Tortonese 1955; Behnke 1968; Turan et al. 2010, 2011, 2012, 2014a, 2014b, 2017, 2021, 2022; Turan and Bayçelebi 2020; Turan and Aksu 2021) and more recently based on the joint use of genetic and morphological characters (Turan et al. 2010, 2020, 2021; Kaya, 2020). With these comprehensive studies, there are seventeen *Salmo* species naturally distributed in Türkiye. These are: *Salmo abanticus* Tortonese, 1954 (Lake Abant), *Salmo araxensis* Turan, Kottelat & Kaya, 2022 (Aras River), *Salmo ardahanensis* Turan, Kottelat & Kaya, 2022 (upper drainages of Kura River), *Salmo baliki* Turan, Aksu, Oral, Kaya & Bayçelebi, 2021 (upper drainages of Murat River, Euphrates drainage), *Salmo chilo* Turan, Kottelat & Engin, 2012 (Ceyhan River), *Salmo coruhensis* Turan, Kottelat & Engin, 2010 (the streams and rivers from the Turkish Black Sea coast and Marmara drainages), *Salmo duhani* Turan & Aksu, 2021 (Gönen Stream, south western Marmara

drainage) *Salmo euphrataeus* Turan, Kottelat & Engin, 2014 (Karasu River, northern Euphrates drainage), *Salmo fahrettini* Turan, Kalayci, Bektaş, Kaya & Bayçelebi, 2020 (Karasu River, northern Euphrates drainage), *Salmo kottelati* Turan, Doğan, Kaya & Kanyılmaz, 2014 (Alakır Stream, Mediterranean drainage), *Salmo labecula* Turan, Kottelat & Engin, 2012 (lower drainages of Seyhan River), *Salmo munzuricus* Turan, Kottelat & Kaya, 2017 (Munzur Stream, northern Euphrates drainage), *Salmo murathani* Turan, Kottelat & Kaya, 2022 (Aras River), *Salmo okumusi* Turan, Kottelat & Engin, 2014 (Tohma and Göksu streams, western Euphrates drainage), *Salmo opimus* Turan, Kottelat & Engin, 2012 (Alara Stream, Mediterranean drainage), *Salmo platycephalus* Behnke, 1968 (upper drainages of Seyhan River), *Salmo rizeensis* Turan, Kottelat & Engin, 2010 (Turkish Black Sea coast) and *Salmo tigridis* Turan, Kottelat & Bektaş, 2011 (Tigris River) (Turan et al. 2022). Of these, nine species (*S. abanticus*, *S. araxensis*, *S. ardahanensis*, *S. coruhensis*, *S. duhani*, *S. euphrataeus*, *S. fahrettini*, *S. murathani*, *S. rizeensis*) belong to the Danubian lineage, while *S. tigridis* belongs to the Tigris lineage and the remaining species belong to the Adriatic lineage.

A previous phylogenetic study of the brown trout based on mitochondrial DNA sequences revealed five major brown trout evolutionary lineages including AD (Adriatic origin), AT (Atlantic), DA (Danubian), MA (Marmaratus), and ME (Mediterranean) (Bernatchez 2001). Subsequently, new lineages were described from Spain as Duero (DU; Suárez et al. 2001), from Türkiye as TI (Tigris; Sušnik et al. 2005; Bardakçı et al. 2006), from Morocco as Dades (Snoj et al. 2011), from Northern Africa (Tougard et al. 2018). A molecular study of the brown trout populations inhabiting the Marmara Sea drainages (*S. coruhensis* and *S. duhani*) placed them in the DA lineage (Bardakci et al. 2006).

Next-generation sequencing (NGS) technologies have revolutionized genomic research, enabling the identification of a massive number of genome-wide markers in a single reaction (Metzker 2010; Goodwin et al. 2016; McCombie et al. 2019). Advances have dramatically reduced the cost while providing high-quality sequence data. NGS has been extensively used in aquatic species, including population structure analysis (Segherloo et al. 2021), genetic linkage map construction (Leitwein et al. 2017), quantitative trait locus mapping for economically important traits (Palaikostas et al. 2013, 2015) and improving the quality of reference genome assemblies (Lien et al. 2016; Hansen et al. 2021).

Based on current knowledge, only two valid species, namely *Salmo coruhensis* and *S. duhani*, inhabit the rivers flowing to the Marmara Sea. *Salmo coruhensis* is distributed in the drainages of the Southern Black Sea and the northern part of the Marmara drainages [Elmalı Stream (İznik Lake drainage) and Kurtköy Stream]. *Salmo duhani* is restricted to the upper part of Gönen Stream, the southern drainage of the Marmara Sea. During the present study, additional populations of *Salmo* were discovered in the Nilüfer River (another drainage of

the Marmara Sea) and cannot be reliably assigned to one of the two known species from the area. To determine their taxonomic status, we compared their morphological characters and genome-wide molecular data to other known *Salmo* species in the area. In addition, the status of the *Salmo* populations from the Ayazma Stream is reexamined here. Our comparisons indicate that *Salmo* populations from the Nilüfer River correspond to a distinct and undescribed species belonging to the DA lineage.

Materials and methods

The fieldwork followed the guidelines of the Local Ethics Committee of RTE University related to the use of animals in scientific experiments with a permit reference number of 2014/72. Samples were collected from the streams Aras, Ericek and Deliçay, drainages of the Marmara Sea and western Türkiye (Fig. 1). These are known to be the uppermost tributaries of the Nilüfer River. Specimens were captured using an electrofishing device (Samus 1000) and euthanized using tricaine methanesulphonate solution (MS-222). Subsequently, fin clips were collected from one of the pelvic fins and placed into 96% ethanol for subsequent molecular work. Finally, specimens were fixed in a 4% formaldehyde solution in a vertical position. These specimens were deposited at the FFR, Zoology Museum of the Faculty of Fisheries, Recep Tayyip Erdogan University, Rize (Sabaj 2020) FSJF, Fischsammlung J. Freyhof, Berlin for detailed morphologic analysis.

Abbreviations: **SL**: Standard length; **HL**: Head length.

Morphological analyses

The study by Turan et al. (2010) was used as a guideline for morphometric analysis. All measurements were carried out in the form of a point-to-point approach (projections were not used) using a dial calliper calibrated to 0.01 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”.

Comparison material

All materials are from Türkiye except *Salmo labrax*.

Salmo abanticus: FFR 3163, 7,77–272 mm SL; Bolu prov.: outlet of Abant Lake, 40.5737°N, 31.2957°E.

Salmo ardahanensis: FFR 3164, 10, 154–217 mm SL; Ardahan prov.: stream Toros, Kura River drainage, 41.1000°N, 42.4333°E.—FFR 3107, 4, 156–192; FFR 3167, 2, 155–182 mm SL; Ardahan prov.: stream Alabalık, Kura River drainage, 41.0500°N, 42.3666°E.—FFR 3110, 4, 67–118 mm SL; Ardahan prov.: stream Karaman at Aşıkzülal, Kura River drainage, 41.4166°N, 42.6500°E.—FFR 3136, 16, 99–185 mm SL; Ardahan prov.: stream Kınavur at Çataldere, Kura River drainage, 41.1833°N, 42.6000°E.

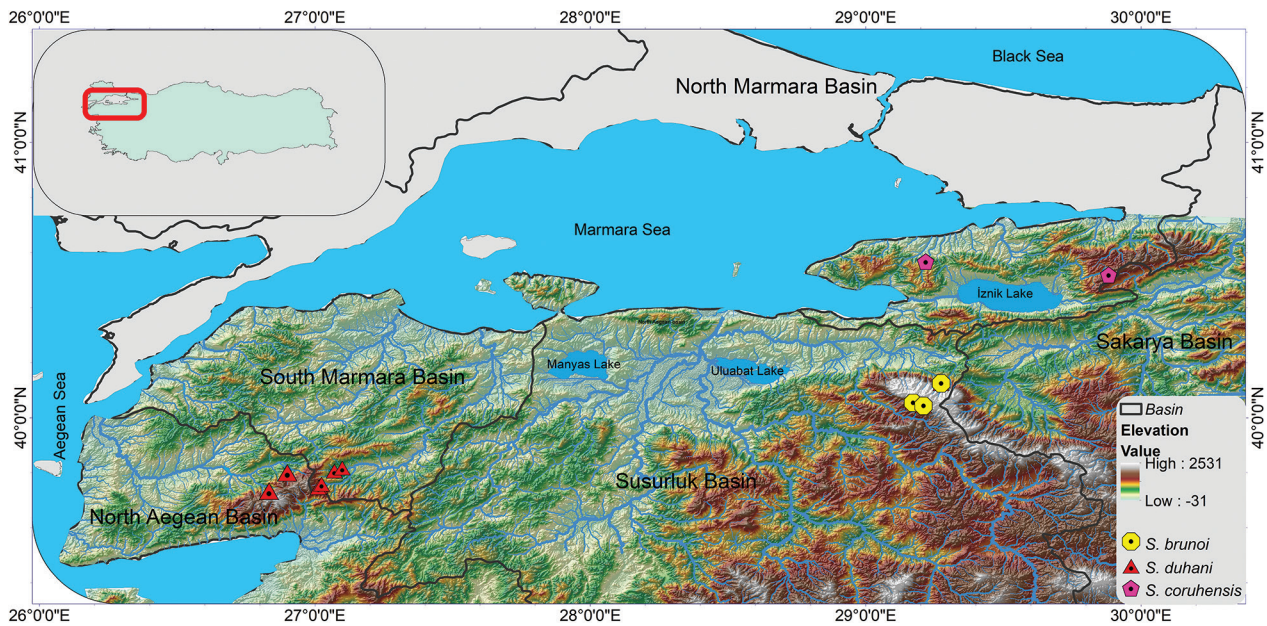


Figure 1. Distributions of *Salmo* in Marmara and Aegean Sea basins.

***Salmo araxensis*:** FFR 3114, 12, 116–201 mm SL; Kars prov.: Susuz district Kayalık stream, a tributary of Kars stream, Aras River drainage, 40.8166°N, 43.1166°E.—FFR 3115, 15, 93–237 mm SL; Kars prov.: Susuz district: Porsuklu (Akçalı) stream, a tributary of Kars stream, Aras River drainage, 40.8000°N, 43.1833°E.—FFR 3118, 6, 95–132 mm SL; Kars prov.: Sarıkamış district: Boyalı stream, a tributary of Kars stream, Aras River drainage, 40.4333°N, 42.5666°E.—FFR 3144, 16, 87–265 mm SL; Kars prov.: Susuz district: İncilipınar stream, a tributary of Kars stream, Aras River drainage, 40.8166°N, 43.0666°E.

***Salmo baliki*:** FFR 3234, 6, 132–276 mm SL; Ağrı prov.: stream Sinek a tributary of Murat River at Taşlıçay, 39.7587°N, 43.4644°E.—FFR 3205, 3, 175–267 mm SL; Ağrı prov.: a tributary of Murat River 39.7307°N, 43.4818°E.

***Salmo chilo*:** FFR 3055, 23, 65–235 mm SL; Sivas prov.: stream Akdere at Gürün, Ceyhan River drainage, 38.6088°N, 36.8962°E.

***Salmo coruhensis*:** FFR 3004, 16, 95–240 mm SL; Artvin prov.: stream Osmaniye at Karaosmaniye village, 41.4689°N, 41.5105°E.—FFR 3011, 11, 90–189 mm SL; Artvin prov.: stream Hopa at Çavuşlu village, 41.4509°N, 41.7001°E.—FFR 3021, 25, 90–520 mm SL; Rize prov.: stream Fırtına at Çat village 40.8653°N, 40.9311°E.—FFR 3022, 9, 95–228 mm SL; Rize prov.: stream Kendirli at Kalkandere District on road to Kendirli village, İyidere drainage 40.9373°N, 40.4320°E.—FFR 3023, 13, 120–450 mm SL; Rize prov.: stream İyidere (İkizdere) at Güneyce 40.8219°N, 40.4765°E.—FFR 3024, 13, 115–330 mm SL; Artvin prov.: stream Dörtkilise at Tekkale village, Çoruh River, 40.7877°N, 41.4946°E.—FFR 3025, 13, 80–550 mm SL; Erzurum prov.: stream Çayırbaşı (Kırık) at Kırık village, Çoruh River, 40.2904°N,

40.8097°E.—FFR 3026, 6, 160–290 mm SL; Erzurum prov.: stream Büyük at Büyükköy village, Çoruh River, 40.4452°N, 40.8513°E.—FFR 3027, 6, 130–420 mm SL; Rize prov.: stream Veliköy at Veliköy village, 41.0332°N, 40.6145°E.—FFR 3029, 6, 130–220 mm SL; Rize prov.: stream Bozukkale at Bozukkale village, 41.0543°N, 40.6297°E.—FFR 3030, 6, 80–170 mm SL; Rize prov.: stream Çağlayan at Çağlayan district 40.9230°N, 40.4452°E.—FFR 3031, 6, 190–265 mm SL; Bayburt prov.: stream Ölçer at Ölçer village, Çoruh River, 40.5147°N, 40.5609°E.—FFR 3032, 16, 70–310 mm SL; Rize prov.: stream Söğütlü at Söğütlü village, about 5 km west of Çayeli, 41.0659°N, 40.6526°E.—FFR 3033, 16, 110–210 mm SL; Bayburt prov.: stream Kurtbogazı at Kurtbogazı village, Çoruh River, 40.1883°N, 40.5033°E.—FFR 3034, 16, 70–210 mm SL; Gümüşhane prov.: stream Harşit at Yağmurdere, 40.5746°N, 39.8645°E.—FFR 3035, 9, 160–450 mm SL; Sivas prov.: stream Gemin at Camili, Yeşirmak River drainage, 40.0619°E, 38.0536°N.—FFR 3037, 10, 90–380 mm SL; Erzurum prov.: stream Pehlivanlı at Pehlivanlı village, tributary of Tortum, Çoruh River, 40.5176°N, 41.4780°E.—FFR 3041, 10, 115–250 mm SL; Trabzon prov.: stream Solaklı at Taskıran village 40.6722°N, 40.2568°E.—FFR 3042, 6, 95–117 mm SL; Rize prov.: stream Sarayköy at Sarayköy village, 41.0190°N, 40.3807°E.—FFR 3043, 5, 130–229 mm SL; Artvin prov.: stream Barhal at Sarıgöl village, Çoruh River, 40.9744°N, 41.4184°E.—FFR 3043, 9, 110–223 mm SL; Rize prov.: stream Derapazarı at Derapazarı 41.0237°N, 40.4293°E.—FFR 3044, 6, 100–250 mm SL; Rize prov.: stream İyidere at İyidere 40.9676°N, 40.3778°E.—FFR 3045, 7, 150–450 mm SL; Rize prov.: stream Fırtına at Çamlıhemsin 41.0517°N, 41.0032°E.—FFR 3046, 5, 10–280 mm SL; Rize prov.: stream Limanköy at Limanköy village, 41.0714°N, 40.7121°E.

***Salmo duhani*:** FFR 3184, 15, 95–287 mm SL; Çanakkale prov.: stream Zeytinli about 9 km east of Kazdağı National Park, 39.750°N, 27.017°E, 28.11.2006. —FFR 3185, 14, 85–170 mm SL; Çanakkale prov.: stream Zeytinli, 39.749°N, 27.015°E.—FFR 3186, 12, 108–160 mm SL; Çanakkale prov.: stream Zeytinli 39.759°N, 27.021°E.—FFR 3194, 10, 62–122 mm SL; Çanakkale prov.: stream Kocaçayı, 12 km west of Kalkım, 39.804°N, 27.071°E.—FFR 3195, 15, 93–275 mm SL; Çanakkale prov.: stream Kocaçayı at Yenice, 39.817°N, 27.099°E.

***Salmo euphrataeus*:** FFR 1220, 24, 80–260 mm SL; Erzurum prov.: stream Kuzgun, a tributary of Karasu Stream, Euphrates River drainage, 40.2198°N, 41.1051°E.—FFR 1255, 25, 88–230 mm SL; Erzurum prov.: stream Şenyurt at Şenyurt, a tributary of Karasu Stream, Euphrates River, 40.1830°N, 41.5037°E.—FFR 1223, 5, 122–222 mm SL; Erzurum prov.: stream Sırlı, a tributary of Karasu Stream, Euphrates River, 40.2183°N, 41.1010°E.—FFR 1269, 8, 117–198 mm SL; Erzurum prov.: stream Kuzgun, Euphrates River, 40.2198°N, 41.1050°E.

***Salmo fahrettini*:** FFR 3232, 20, 134–227 mm SL; Erzurum prov.: stream Ömertepesuyu at Palandöken 39.7958°N, 40.9444°E.—FFR 3233, 5, 126–194 mm SL; Erzurum prov.: stream Tekke at Palandöken, 39.8197°N, 41.1516°E.

***Salmo kottelati*:** FFR 3181, 21, 98–210 mm SL; Antalya prov.: stream Alakır at Altınyaka, 36.5608°N, 30.3428°E.—FFR 3182, 16, 98–176 mm SL; Antalya prov.: stream Alakır at Altınyaka, 36.5608°N, 30.3428°E.

***Salmo labecula*:** FFR 3057, 4, 103–237 mm SL; Niğde prov.: stream Ecemiş at Çamardı, Seyhan River drainage, 37.8253°N, 34.9902°E.—FFR 3058, 5, 142–241 mm SL; Isparta prov.: stream Kartoz at Aşağıyaylabel, Köprüçay drainage, 37.5532°N, 31.3070°E.—FFR 3059, 5, 140–184 mm SL; Antalya prov.: stream Zindan at Aksu, Köprüçay drainage, 37.8064°N, 31.0734°E.

***Salmo labrax*:** FSJF 396, 6, 107–147 mm SL; Ukraine: Ula-Uzev River; N. Bogustkaya, A. Nesecka, J. Bohlen & J. Freyhof, 12 June 2002.—FSJF 10, 6, 102–160 mm SL; Russia: Crasnodar prov.: Khosta River; J. Freyhof, 19 July 2002.

***Salmo munzuricus*:** FFR 3162, 17, 127–270 mm SL; Tunceli prov.: stream Munzur at Koyungölü, 39.3472°N, 39.1341°E.—FFR 3147, 8, 146–320 mm SL; stream Munzur at Koyungölü, 39.3461°N, 39.1316°E.

***Salmo murathani*:** FFR 3121, 18, 60–233 mm SL; Kars prov.: Keklik stream [a tributary of Kars stream], Sarıkamış district, Aras River drainage, 40.2833°N, 42.6500°E.—FFR 3117, 22, 95–192 mm SL; FFR 3113, 17, 91–206; Kars prov.: Keklik stream [a tributary of Kars stream] Sarıkamış district, Aras River drainage, 40.2500°N, 42.6666°E.—FFR 3120, 10, 69–163 mm SL, Kars prov.: Maksutçuk stream [a tributary of Kars stream], Aras River drainage, 40.5333°N, 42.8666°E.—FFR 3108, 14, 90–186 mm SL; Ardahan prov.: Çıldır Lake, Aras River drainage 41.0500°N,

43.3166°E.—FFR 3228, 23, 95–241 mm SL; Kars prov.: Arpaçay stream [a tributary of Kars stream] Arpaçay district, Aras River drainage 40.9000°N, 43.1666°E.—FFR 3229, 8, 110–156 mm SL; Kars prov.: Keklik stream [a tributary of Kars stream] Sarıkamış District, Aras River drainage, 40.2833°N, 42.6500°E.

***Salmo okumusi*:** FFR 1254, 10, 75–202 mm SL; Malatya prov.: stream Sürgü, Euphrates River drainage, 37.9975°N, 37.9583°E.—FFR 125, 10, 129–169 mm SL; Sivas prov.: stream Gökpınar, a tributary of Tohma stream, Euphrates River, 38.6600°N, 37.3089°E.—FFR 1256, 10, 68–280 mm SL; Sivas prov.: stream Gökpınar, Euphrates River, 38.6600°N, 37.3089°E.—FFR 124, 2, 149–175 mm SL; Kahramanmaraş prov.: stream Göksu 4 km north of Düzbağ, Euphrates River, 37.8331°N, 37.4756°E.

***Salmo opimus*:** FFR 3048, 12, 118–180 mm SL; Antalya prov.: stream Alara at Gündoğmuş, 36.7921°N, 31.9749°E.—FFR 3049, 20, 115–186; Kahramanmaraş prov.: stream Göçüksu at Kömürköy, Ceyhan River drainage, 38.1447°N, 36.5630°E.—FFR 3050, 4, 175–210 mm SL; Kahramanmaraş prov.: drainage of stream Tekir at Tekir, Ceyhan River drainage, 37.8767°N, 36.6058°E.—FFR 3051, 9, 90–300 mm SL; Kahramanmaraş prov.: stream Fırınz at Fırınz, Ceyhan River drainage, 37.7591°N, 36.6983°E.

***Salmo platycephalus*:** FFR 972, 7, 145–184 mm SL; Kayseri prov.: Pınarbası stream at Pınarbası district, Seyhan River drainage.—FFR 1260, 10, 137–237 mm SL; Kayseri prov.: Pınarbası Stream at Pınarbası district, Seyhan River drainage.

***Salmo rizeensis*:** FFR 3001, 15, 90–220 mm SL; Erzurum prov.: stream Ovit (2) [Kan] at Ovit mountain, Çoruh River, 40.5887°N, 40.8583°E.—FFR 3002, 10, 114–245 mm SL; Trabzon prov.: stream Degirmen at Çosandere village, 40.7512°N, 39.5908°E.—FFR 3003, 12, 112–230 mm SL; Trabzon prov.: stream Solaklı at Demirkapı village, 40.7586°N, 40.5913°E.—FFR 3005, 13, 111–220 mm SL; Rize prov.: stream Çağlayan at Gürcüdüzü plateau 41.1905°N, 41.3086°E.—FFR 3006, 18, 95–226 mm SL; Rize prov.: stream Sehlik at Sehlik village, 41.1407°N, 40.9828°E.—FFR 3007, 12, 90–118 mm SL; Rize prov.: stream Çayeli at Kaptanpasa village, 40.958°N, 40.7794°E.—FFR 3008, 18, 91–198 mm SL; Rize prov.: stream Fırtına at Tuncavilla village, 41.1259°N, 41.1310°E.—FFR 3009, 10, 110–240 mm SL; Rize prov.: stream Taşlıdere at Pasacı village, 40.8837°N, 40.5796°E.—FFR 3010, 9, 110–240 mm SL; Rize prov.: stream Taşlıdere at Kangel village, 40.9453°N, 40.6642°E.—FFR 3011, 7, 100–180 mm SL; Rize prov.: stream Erenler at Erenler village, 41.0914°N, 40.8298°E.—FFR 3012, 7, 88–237 mm SL; Artvin prov.: stream Dörtkilise at Tekkale Village, Çoruh River, 40.7800°N, 41.5098°E.—FFR 3013, 12, 75–167 mm SL; Artvin prov.: Çiftköprü stream at Cankurtaran mountain, Çoruh River, 41.3844°N, 41.5691°E.—FFR 3014, 7, 112–201 mm SL; Artvin prov.: stream Kapisre at Küçüköy village, 41.2753°N,

41.3755°E.—FFR 3015, 9, 113–228 mm SL; Bayburt prov.: stream Kop at Kop Mountain, Çoruh River, 40.0654°N, 40.4331°E.—FFR 3016, 9, 113–221 mm SL; Erzurum prov.: stream Yağlı at Yağlı village, Çoruh River, 40.3643°N, 41.0728°E.—FFR 3017, 12, 112–223 mm SL; Erzurum prov.: stream Büyük at Büyükdere plateau, Çoruh River drainage, 40.5698°N, 40.7140°E.—FFR 3018, 16, 145–224 mm SL; Gümüşhane prov.: stream Akbulak at Akbulak village, Yesilirmak River drainage, 40.281462°N, 39.0896°E.—FFR 3019, 10, 122–221 mm SL; Kütahya prov.: stream Sefaköy at Domaniç, Sakarya River drainage, 39.8426°N, 29.6706°E.—FFR 3020, 10, 111–119 mm SL; Kütahya prov.: Çatalalç Stream at Domaniç, Sakarya River, 39.8600°N, 29.6291°E.—FFR 3036, 10, 130–170 mm SL; Rize prov.: stream İkizdere at Anzer plateau, 40.5926°N, 40.5148°E.—FFR 3038b, 7, 130–170 mm SL; Rize prov.: stream Çiftekavak at Ortapazar village, 40.9959°N, 40.4851°E.—FFR 3039a, 14, 120–200 mm SL; Rize prov.: stream Fırtına at Elevit Plateau, 40.8471°N, 41.0151°E.—FFR 3038a, 1, 250 mm SL; Erzurum prov.: stream Ovit (2) [Kan] at Ovit mountain, Çoruh River, 40.5735°N, 40.8634°E.—FFR 3039b, 10, 90–238 mm SL; Rize prov.: stream Ovit at Ovit mountain, Iyidere drainage, 40.6361°N, 40.8214°E.—FFR 3040, 14, 90–190 mm SL; Erzurum prov.: stream Merekum at Merekum, Çoruh River, 40.5527°N, 41.4592°E.

Salmo tigris: FFR 1253, 9, 136–227 mm SL; Van prov.: stream Çatak, Tigris River, 38.0077°N, 43.0652°E.

Samples

In total, 71 samples fixed in formalin were investigated morphologically (see Paratypes section) and tissue samples were collected from two specimens of the new species, *S. brunoi*, originating from Bursa, Uludağ, Aras Stream, Türkiye. In total, 12 samples were examined for genetic analysis including 2 specimens of new species *S. brunoi* from Bursa, Uludağ, Aras Stream, 1 specimen of *Salmo coruhensis*, collected from Bursa, İznik, Sığırhisar village and 3 specimens of *Salmo coruhensis* from Sultaniye Stream, Kartepe, İzmit, 3 specimens of *Salmo pelagonicus* collected from Çanakkale, Bayramiç, Ayazma Stream and 3 specimens of *S. duhani* taken from the type locality, in Çanakkale, Yenice, Kalkım. In addition to Anatolian samples, globally recognized *Salmo* lineages were included as references in the genetic analyses. From these references 3 specimens of Danubian lineage samples included in the genetic analysis (1 specimen provided from the Kuban River, Russia, has been treated as *S. labrax* based on Turan et al. (2014b) who have previously reported the distribution of the species from the northwest Caucasus in Russia to the Danube River and 2 specimens from the Sevan River, Armenia were treated as *S. ischchan* (thus Danubian reference). The rest of the reference samples included 2 specimens from *S. marmoratus* from Svenica and Trebuscica in Slovenia; 3 specimens from Atlantic fish origin of Babeau hatchery

in France (unidentified species), 2 specimens of *S. obtusirostris* from Studenčica in Bosna and Herzegovina, 3 specimens of Adriatic lineage samples (unidentified species) from Alfios and Kalamos in Greece and 1 specimen from Ohrid-Drin-Skadar in Albania, respectively.

DNA extraction, ddRADseq library preparation and NGS sequencing

Total genomic DNA extraction was carried out on a King-Fisher Flex DNA extraction robot (Thermo Fisher Scientific, France) following the manufacturer's instructions. DNA quality was assessed on 0.8% agarose gels and DNA quantity was estimated using a NanoDrop 2000 (Thermo Fisher Scientific, France). High molecular weight genomic DNA samples were further assessed using Qubit (Thermo Fisher Scientific, France) BR assay for the final quantification of double-stranded DNA prior to ddRADseq library construction. The library construction was performed following the original ddRADseq protocol by Peterson et al. (2012) with slight modifications detailed by Leitwein et al. (2016) and Oral (2023). Genomic DNA was double-digested using *EcoRI* and *MspI* enzymes. Fragmented DNA was then individually barcoded using adaptors. Samples were pooled and processed into single tubes following adaptor ligation. Purified and size-selected fragments (c. 300–700 bp) were then enriched for 15 PCR cycles. The amplified library was quantified using NanoDrop spectrometry and Qubit fluorimetry and the size distribution of the library was further assessed on a Fragment Analyzer (Advanced Analytical Technologies, France). The ddRADseq library was sequenced on an Illumina NovaSeq platform with paired-end reads of 150 base pairs.

Bioinformatic data analysis

The initial quality control of the raw data files was carried out using FastQC (Andrews 2010; Babraham Bioinformatics). Reads of low quality (Phred score < 30), missing restriction sites and/or involving ambiguous barcodes were removed. Retained reads were then processed using Stacks v2.55 (Catchen et al. 2013) for demultiplexing based on their barcodes, restriction enzymes and cleaned with ProcessRadtags (-c -r -q --renz_1 ecoRI --renz_2 mspI). Cleaned reads were mapped against the *Salmo trutta* reference genome (accession number: GCA_901001165.2; Hansen et al. 2021) with BWA-mem2 v2.1 (-k 19 -c 500 -O 0,0 -E 2,2 -T 0 -R) (Li and Durbin 2010) and samtools v1.11 (-Sb -q 1 -F 4 -F 256 -F 2048). Then gstacks (--max-clipped 0.01) was run with a minimum number of 2 populations where a locus must be present (-p 2), a minimum 20% of individuals in a population (-r 0.2), a maximum observed heterozygosity of 60% (--max-obs-het 0.6), a minimum allele frequency of 1% (--min-maf 0.01) and a single representative of each overlapping site (--ordered-export).

Once variants were collected following the steps above mentioned, they were filtered with *vcftools* v0.1.16 (Danecek et al. 2011). First, we focused on individuals, removing those with more than 20% of missing data. Second, we filtered SNPs according to the sequencing depth, missing data, frequency and number of alleles per site (`--minDP 4 --minGQ 30 --max-missing 0.4 --min-alleles 2 --max-alleles 2 --maf 0.01`). Finally, we removed SNPs that were in high linkage disequilibrium using *11_extract_unlinked_snps_genome.py* (`diff_threshold=0.5` and `max_distance=50`) from *stacks_workflow* v2.62 (https://github.com/enormandeau/stacks_workflow). Bioinformatics analyses were performed with the support of LDgenX (www.ldgenx.com) and only a subset of these data was used in the present study.

Population structure analysis

We performed ADMIXTURE and Principal Component Analysis (PCA) on filtered and unlinked SNPs. ADMIXTURE v.1.3.0 (Alexander et al. 2009) was used to estimate individual cluster memberships. ADMIXTURE provides an estimation of individual ancestry proportion for K groups and the number of different groups was explored from 1 to 12. Based on the cross-validation procedure, the best K with the lowest cross-validation error was detected as 9. Q-values estimated by ADMIXTURE were used to produce bar plots with R v 4.2.1 (R Core Team 2015).

Alongside the ADMIXTURE analysis, the unlinked SNPs of 12 individuals from the Marmara Aegean basin were further investigated using PCA calculated with PLINK 1.9 (Chang et al. 2015). PCA was conducted to determine the population structure and the first two components of the PCA were plotted using R v 4.2.1.

Results

Salmo brunoi sp. nov.

<https://zoobank.org/6AB6FDA0-37BF-49D8-8A74-BE2FCED9212F>
Figs 2–4

Type material. Holotype: FFR 3243, 175 mm SL; Türkiye, Bursa prov.: stream Aras, a tributary of Nilüfer River, 40.0536°N, 29.1722°E.

Paratypes: FFR 3216, 188–153 mm SL; same data as holotype.—FFR 3213, 7, 142–195 mm SL;—FFR 3215, 7, 142–195 mm SL; Türkiye, Bursa prov.: stream Deliçay at Kestel, 40.1241°N, 29.2737°E.—FFR 3211, 18, 93–180 mm SL; —FFR 3217, 12, 85–153 mm SL; Türkiye, Bursa prov.: stream Ericek at Osmangazi, 40.0426°N, 29.2098°E.

Diagnosis. *Salmo brunoi* is distinguished from all the species of *Salmo* in Türkiye and adjacent areas by the combination of the following characters: a small size (known maximum size 187 mm SL); body brownish

in life; one black spot in postorbital and suborbital areas, greater than the pupil; two to four black spots on the opercle, approximately smaller than the pupil; black spots on the body few (fewer than 60), approximately equal to the pupil, ocellated, scattered on the back and the upper part of the flank (missing in the predorsal area); red spots few (fewer than 40), smaller than the pupil, irregularly shaped, surrounded by an irregularly shaped narrow ring, organized in two to four irregular longitudinal rows; number of black and red spots not increasing with size; anal fin short (12–15% SL in males, 12–14 in females), adipose fin large (adipose fin height 8–9% SL in males and females), short distance between adipose fin and caudal fin bases (13–14% SL in males, 12–14% in females).

Description. The general appearance is shown in Figs 2, 3, live images are in Fig. 4, morphometric data are in Table 1. Body moderately deep, compressed laterally, its depth smaller than head length. The dorsal profile is slightly arched, and the head is short, upper profile slightly convex on the interorbital area and the snout in males and markedly convex on both interorbital areas and the snout in females. Mouth large in males, small in females, terminal or slightly subterminal in males, subterminal in females. Tip of lower jaw slightly curved upwards, pointed, with a slightly developed process at symphysis in males larger than 160 mm SL. Maxilla somewhat long, with a length of 10–12% SL, reaching beyond the posterior margin of the eye in males larger than 140 mm SL and only reaching the posterior margin of the eye in females. Snout somewhat short, with a pointed tip in males, rounded in females. Adipose fin long, height about 8–9% SL in males and in females. Known maximum size 195 mm SL.

Dorsal fin with 3–4 unbranched and 8–10 branched rays, its distal margin convex. Pectoral fin with 1 unbranched and 11–13 branched rays, its external margin slightly convex. Pelvic fin with 1 unbranched and 7–8 branched rays, its external margin convex. Anal fin with 3 unbranched and 7–9 branched rays, its distal margin convex anteriorly and concave posteriorly. The caudal fin deeply emarginated in specimens less than 120 mm SL, slightly emarginated or truncated in specimens larger than 140 mm SL, lobes slightly pointed. Lateral line with 108–122 scales; 23–32 scale rows between dorsal fin origin and lateral line; 16–23 scale rows between anal fin origin and lateral line; 14–18 scale rows between origin of the adipose fin and lateral line. Gill rakers 15–18 on the first gill arch.

Coloration. In life: General body color brownish or light brownish. Back and flank brownish and belly yellowish. Red spots conspicuously organized in two to four irregular longitudinal rows on the median part of the body and half of the lower part of the flank. Conspicuously black spots in postorbital and suborbital areas. Black spots roundish, scattered on back and upper part of flank. Pectoral, pelvic and anal fins yellowish, dorsal and anal fins yellowish or light brownish. Adipose fin with reddish margin (see Fig. 4).



Figure 2. *Salmo brunoi*, FFR 3243, holotype, 175 mm SL, male; Türkiye: stream Aras, a tributary of Nilüfer River.



Figure 3. *Salmo brunoi*, from top: FFR 3216, paratypes, 137 mm SL, male; 105 mm SL, female; Türkiye: stream Aras, a tributary of Nilüfer River.

In formalin: The general coloration of freshly preserved specimens dark brown on the back and upper part of the flank, brownish on the lower part of the flank and yellowish on the belly. One black spot in postorbital and suborbital areas, greater than the pupil; two to four black spots on the opercle, approximately smaller than the pupil. Black spots on the body few (fewer than 60), approximately equal to the pupil, ocellated, commonly scattered on the back and the upper part of the flank (missing in the predorsal area) and rarely median part of the flank; no black spot on top of the head. Red spots few (fewer than 40), small (smaller than the pupil), irregularly shaped, surrounded by an irregularly shaped narrow ring, organized in two to four irregular longitudinal rows on the median part of the body and half of the lower part of the flank. The number of black and red spots on the flanks do not increase with size. Dorsal fin gray, with two or three

rows of black spots (smaller than pupil) and one or two rows of red spots (smaller than pupil). Caudal fin dark gray; pectoral, anal and pelvic fins grayish. Adipose fin plain grayish, rarely one or two red spots on its posterior edge (Figs 2,3). Eleven to thirteen parr marks on the body, distinct in specimens up to about 195 mm SL.

Distribution and habitat. *Salmo brunoi* sp. nov. inhabits clear and swift-flowing water, with a substrate consisting of gravel and pebbles. The observed material for this species has been collected from streams Aras, Deliçay and Ericek, drainages of Nilüfer River (Fig. 1).

Conservation status. According to the First Author's (DT) observations, *Salmo brunoi* sp. nov. is under the influence of overfishing. Besides fresh consumption, trout oil is a widely preferred natural remedy, particularly for the treatment of rheumatism, muscle, and joint pains among local people (Turan et al. 2006). Therefore, the

Table 1. Morphometry of *Salmo brunoi* (holotype, FFR 3243; paratypes FFR 3215, n=6, and FFR 3216, n=8). The calculations include the holotype.

Sex	Holotype		SD	Paratypes	
	male	male		female	SD
	Number of specimens	n=6		n=8	
	Standard length (mm)	112–179		110–153	
In percentage of standard length	175	Range (mean)		Range (mean)	
Head length	29.6	26.1–29.6 (27.7)	1.3	24.8–26.9 (26.0)	0.7
Predorsal length	49.6	47.1–49.6 (48.7)	0.8	44.2–48.4 (47.1)	1.4
Prepelvic length	55.9	53.8–55.9 (54.8)	0.8	52.7–55.5 (53.8)	1.0
Preanal length	73.6	73.2–75.0 (74.3)	0.7	73.3–75.7 (74.2)	1.0
Body depth at dorsal-fin origin	24.8	22.1–25.3 (23.8)	0.4	19.9–24.3 (21.6)	1.3
Body depth at anal-fin origin	19.2	16.2–19.3 (17.7)	1.1	16.1–17.8 (16.8)	0.6
Depth of caudal peduncle	10.9	9.9–10.9 (10.1)	0.3	8.8–10.0 (9.4)	0.4
Length of caudal peduncle	17.0	15.5–18.0 (16.6)	0.9	15.3–17.8 (17.0)	0.8
Distance between adipose- and caudal-fins	14.0	12.6–14.4 (13.5)	0.6	11.5–13.6 (12.6)	0.7
Body width at anal-fin origin	9.0	7.1–10.4 (8.9)	1.3	7.0–9.9 (9.0)	1.0
Length of dorsal-fin base	9.0	12.2–14.5 (13.3)	0.9	12.7–13.8 (13.1)	0.5
Height of dorsal fin	19.4	16.3–19.5 (17.6)	1.2	15.2–17.1 (16.2)	0.7
Length of pectoral fin	14.1	15.9–20.1 (18.0)	1.6	16.3–18.5 (17.3)	0.8
Length of adipose-fin base	3.7	2.9–4.1 (3.6)	0.5	2.8–4.8 (3.8)	0.4
Height of adipose fin	8.6	8.0–9.2 (8.4)	0.5	7.8–8.5 (8.1)	0.2
Length of pelvic fin	19.4	12.1–15.3 (13.4)	1.6	11.9–14.4 (13.1)	0.9
Height of anal fin	13.4	12.1–14.7 (13.4)	1.2	11.9–14.4 (13.1)	0.9
Length of anal-fin base	10.7	9.3–12.2 (10.4)	0.9	8.3–11.6 (10.3)	1.2
Length of upper caudal-fin lobe	19.9	12.9–17.6 (15.6)	1.6	15.4–17.7 (16.3)	0.9
Length of median caudal-fin rays	14.3	10.9–14.7 (13.0)	1.4	10.8–14.0 (12.4)	1.1
Length of lower caudal-fin lobe	14.7	14.0–18.0 (16.2)	1.6	15.2–18.5 (16.6)	1.1
Snout length	8.8	6.7–8.7 (7.7)	0.8	6.6–7.4 (7.0)	0.3
Distance between nasal openings	4.8	4.0–5.6 (4.9)	0.5	4.0–4.8 (4.4)	0.2
Eye diameter	6.1	5.2–7.0 (6.0)	0.6	5.4–6.6 (5.8)	0.4
Interorbital width	8.4	7.3–8.4 (7.8)	0.4	7.0–8.0 (7.5)	0.3
Head depth through eye	13.4	11.2–13.4 (12.5)	0.8	11.5–13.4 (12.8)	0.6
Head depth at nape	17.5	15.0–17.7 (16.3)	0.4	16.1–17.9 (16.9)	0.7
Length of maxilla	12.0	9.5–12.0 (10.5)	0.9	8.5–9.7 (9.2)	0.5
Maximum height of maxilla	2.5	2.6–3.7 (3.1)	0.3	2.6–3.8 (3.1)	0.4
Width of mouth gape	9.7	7.9–11.0 (9.2)	1.0	8.0–9.3 (8.7)	0.4
Length of mouth gape	16.6	12.2–16.7 (14.0)	1.6	12.0–13.2 (12.6)	0.5

species is in high demand. Given the highly restricted distribution of *S. brunoi* sp. nov. to a very limited area (only three streams), and considering the above mentioned socio-economic interest, this species is likely to be under a serious threat. Thus, there is a need for the species to be conserved under international legislation.

Comparison with other *Salmo* species. *Salmo brunoi* sp. nov. differs from the other species of trout recorded from Marmara, Aegean and Black Sea basins (*S. duhani*, *S. coruhensis*, *S. abanticus*, *S. rizeensis* and *S. labrax*) by having a shorter anal fin in females (12–14% SL, vs. 14–20), a longer adipose fin in females (adipose fin height 8–9% SL, vs. 4–8) and males (8–9% SL, vs. 4–8, except *S. coruhensis*), a shorter distance between adipose fin and caudal fin bases in females (12–14% SL, vs. 14–17, except *S. duhani*) and males (13–14% SL, vs. 15–17 in *S. labrax*, 14–16 in *S. rizeensis*, 14–16 in *S. duhani*, except *S. abanticus* and *S. coruhensis*). *Salmo brunoi* further differs from *S. abanticus*, *S. coruhensis* and *S. labrax* by the brownish body color in life (vs. silvery). *Salmo*

brunoi further differs from *S. abanticus* and *S. coruhensis* by fewer black spots on the body in adult males (fewer than 60, vs. more than 80), whose number does not increase with size (vs. number increasing with size). *Salmo brunoi* further differs from *S. duhani* by having fewer black spots on the back and flank in females (fewer than 60, vs. more than 80). *Salmo brunoi* is further distinguished from *S. abanticus* by the presence of red spots on the body in all sizes (vs. absent in specimens larger than about 200 mm SL) and black spots circular (vs. polygonal). *Salmo brunoi* is further distinguished from *S. labrax* by having a shorter predorsal distance in males (47–50% SL, vs. 46–47), a slenderer body in males (body depth at anal fin origin 16–19% SL, vs. 19–21) and a slenderer caudal peduncle in females (9–10% SL, vs. 10–11). *Salmo brunoi* is further distinguished from *S. rizeensis* by having a slenderer caudal peduncle in females (9–10% SL, vs. 10–11).

The new species, *Salmo brunoi*, is also distinguished from *S. ardahanensis* by having fewer gill rakers on



Figure 4. *Salmo brunoi*, from top: not preserved, ~145 mm SL, male; not preserved, ~150 mm SL, female; Türkiye: stream Aras, a tributary of Nilüfer River.

the outer side of the first gill arch (15–18, vs. 19–21), no black spots on the top of the head (vs. small black spots). It further differs from *S. ardahanensis* by having a smaller distance between adipose and caudal fins in females (12–14% SL, vs. 14–17) and a shorter anal fin in males (12–15% SL, vs. 15–18). In males of *Salmo brunoi*, anal and adipose fins do not reach the caudal fin base (vs. reaching in specimens larger than 200 mm SL) and the interorbital area is convex (vs. flat straight).

Salmo brunoi is further distinguished from *Salmo murathani* by having fewer black spots on flank and back in adult specimens (fewer than 60, vs. more than 66); one black spot behind eye (larger than pupil); 2–4 spots on preopercle and opercle (vs. 4–15); black spots scattered on back (missing in predorsal area), the upper part of flank, sometimes a few black spots below lateral line behind head (vs. black spots scattered on back, the middle and upper part of the flank and the anterior part of the lower flank in males) black spots few (34–47), restricted to the back and upper part of flank in females smaller than about 210 mm SL). It further differs from *S. murathani* by having a smaller distance between adipose and caudal fins in females (12–14% SL, vs. 15–17) and a shorter anal fin in females (12–14% SL, vs. 14–18) and a slenderer caudal peduncle depth in females (9–10% SL, vs. 10–12).

Salmo brunoi is distinguished from *Salmo araxensis* by having a longer maxilla in males (10–12% SL, vs. 9–10), a shorter anal fin (12–15% SL in males, 12–14 in females, vs. 15–18 in males, 14–18 in females), a slenderer caudal

peduncle in females (9–10% SL, vs. 10–12) and a smaller distance between adipose and caudal fins in females (12–14% SL, vs. 14–17).

Salmo brunoi is distinguished from *S. fahrettini* by having the general body color brownish in life (vs. silvery); fewer black spots on the body (fewer than 60, vs. more than 80); black spots on the back (missing on the predorsal area) and upper part of flank, sometimes a few below lateral line behind the head (vs. black spots scattered on back, middle and upper part of flank and anterior part of lower half of flank); their number not increasing with size (vs. their number increasing with size); fewer red spots on body (fewer than 40, vs. more than 70 in adult specimens), their number not increasing with size (vs. increasing with size); a longer maxilla in males (length 10–12% SL, vs. 9–10); a longer adipose fin in males (8–9% SL vs. 3–8); a smaller distance between adipose and caudal fins in females (12–14% SL, vs. 15–18) and a shorter anal fin in females (12–14% SL, vs. 15–17).

Salmo brunoi is distinguished from *S. euphrataeus* by having a smaller distance between adipose and caudal fins in males (13–14% SL, vs. 14–16), a slenderer caudal peduncle in females (9–10% SL, vs. 10–12), a shorter anal fin in females (12–14% SL, vs. 16–19), and the adipose fins do not reach the caudal fin base (vs. reaching in specimens larger than 200 mm SL).

Salmo brunoi is distinguished from *S. platycephalus*, *S. chilo*, *S. labecula*, *S. kottelati*, *S. opimus*, all from streams draining to the Mediterranean and *S. okumusi*,

S. munzuricus and *S. baliki* from Euphrates River, by having a smaller distance between adipose and caudal fins in males (12–14% SL, vs. 14–19), a slenderer caudal peduncle in females (9–10% SL, vs. 10–13), a shorter anal fin (12–15% SL, vs. 15–21, except *S. labecula* and *S. munzuricus*) and fewer gill rakers on first gill arch (15–18, vs. 18–25, except *S. munzuricus* and *S. baliki*). *Salmo brunoi* is further differs from *S. platycephalus*, *S. chilo*, *S. labecula*, *S. kottelati* and *S. opimus*, by the absence of four dark bands on the flank (vs. presence). It further differs from *S. munzuricus* by having a smaller adipose in males (8–9% SL, vs. 9–12) and a longer maxilla in males (10–12% SL, vs. 8–10). It further differs from *S. platycephalus* by the presence of red spots on flank (vs. absent in specimens larger than about 70 mm SL) also differs from *S. labecula* by the presence of red spots on flank in all size (vs. absent in specimens larger than about 70 mm SL).

Salmo brunoi differs from *S. tigridis* by having fewer scale rows between the dorsal fin origin and the lateral line (23–32, vs. 32–35); fewer scale rows between the end of the adipose fin base and the lateral line (14–18, vs. 19–20), a slenderer caudal peduncle depth 9–11% SL, vs. 12–13).

Sexual dimorphism. The maxillary length in males is longer than that of females (10–12% SL in males, 9–10 in females). The length of mouth gape in males is longer than that of females (12–17% SL, 12–13). The snout of the male is more pointed than that of the female.

Etymology. The species is named after Dr. Bruno Guinand (University of Montpellier, ISEM, France) for his valuable contribution to *Salmo* population genomics research.

ddRAD loci and SNP calling

In total, an average of thirteen million raw reads were generated per individual with a mean sequence depth of 30. Sequences with a missingness index higher than 20% were removed from the dataset. Once filtered according to sequencing depth, missing data, frequency and number of alleles, a total of 215k SNPs were retrieved. More than 187k unlinked SNPs within the 50 bp window were used for downstream population analysis.

Interference of ADMIXTURE and PCA analysis

The ADMIXTURE program identified 9 separate clusters. In the reference lineages, the Danubian (DA) cluster was placed in two groups of which DA-1 (*S. labrax*) separated from DA-2 and DA-3 (*S. ischchan*) corresponding to the origin and the geographic basin. The rest of the reference *Salmo* species including *S. obtusirostris* and *S. marmaratus* clustered separately, as expected. Similarly, *S. brunoi* sp. nov., generated a separate cluster from the rest of the Marmara and Aegean trout of Anatolia. The

only exception was observed in *Salmo duhani*, which individuals clustered together (K=9; Fig. 5; see Discussion for detailed explanation).

The 187,385 unrelated SNPs for each of the 12 individuals from the Marmara Aegean basin were used for PCA. The analysis results indicated 3 clusters of which the first cluster included DA reference samples originated from Armenia as *S. ischchan*, the second cluster included *S. brunoi* and *S. coruhensis* clustered with *S. labrax* from Russia and the third cluster included *S. duhani* and that of *S. pelagonicus* (see discussion). The first two components of PCA represented 29.31% and 21.39% of the variance among individuals.

Discussion

Up until the present study, three species of trout have been reported from the Marmara and Aegean Sea drainages: *S. duhani* (Gönen Stream-Marmara Sea drainage), *S. coruhensis* (Elmalı and Kurtköy streams, İznik and Sapanca Lake drainages) and *S. pelagonicus* (Ayazma Stream; Karamenderes drainage, Aegean Sea drainage). In the present study, our molecular data (Q values, 0.99992, 0.9992 respectively for *S. pelagonicus* and *S. duhani*, Fig. 5) showed that trout samples from Gönen Stream (Marmara Sea drainage) largely overlapped in genetic diversity of 187,385 genome-wide SNP markers with those of Ayazma Stream samples (Aegean Sea drainage). Turan and Bayçelebi, (2020) reported Ayazma samples as *Salmo pelagonicus*. Indeed, *Salmo pelagonicus* was originally described from Mountain Brooks in Macedonia (Karaman, 1938). Although Turan and Bayçelebi, (2020) compared specimens from the Ayazma stream with 3 photographs of *S. pelagonicus* from the Aliakmon River in Greece, these authors did not compare the Ayazma population with that of the type locality for *S. pelagonicus* from Macedonia. Later, Turan and Aksu (2021) described *Salmo duhani* from Gönen Stream and gave a few morphological differences between *S. duhani* and *S. pelagonicus*. For example, *Salmo duhani* is distinguished from *S. pelagonicus* by having fewer lateral-line scales (115–121, vs. 109–115), a shorter maxilla in males (8–10% SL, vs. 10–11) and a slenderer body in males (body depth at dorsal-fin origin 20–23% SL, vs. 23–27). Taking all into account; the distance, geographic barriers between Macedonia and Ayazma stream, Türkiye and our molecular data; here, we treated this species as *S. duhani*. Furthermore, bases on our present results, *Salmo duhani* needs to be rediagnosed by considering all samples (Gönen ve Ayazma streams) in future studies.

In the present study, 187,385 unlinked SNP loci shared among the populations were analysed to provide support our recognition of a distinct species. Results provided evidence that *S. brunoi* sp. nov. separates from other *Salmo* species that inhabit adjacent basins (Figs 5, 6).

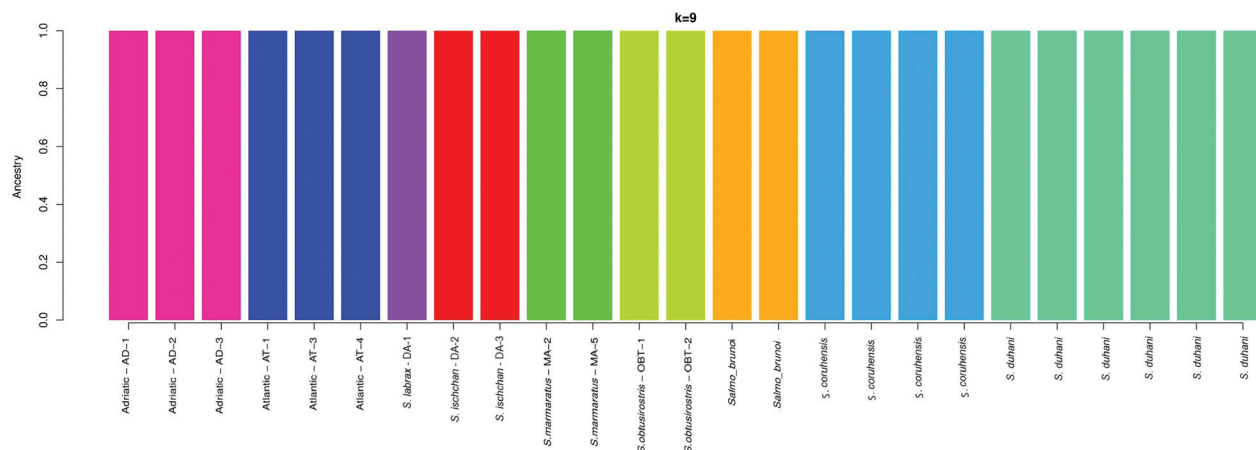


Figure 5. Bar plots of the individuals ancestry generated by ADMIXTURE v.1.3.0 using 187,385 unlinked SNPs. Vertical lines represent each individual and color-code defines the ancestry origin with $k=9$ groups. Reference trout specimens are: Adriatic (AD), Atlantic (AT) and Danubian (DA) lineage (originates from two different locations), *Salmo marmoratus* and *S. obtusirostris*.

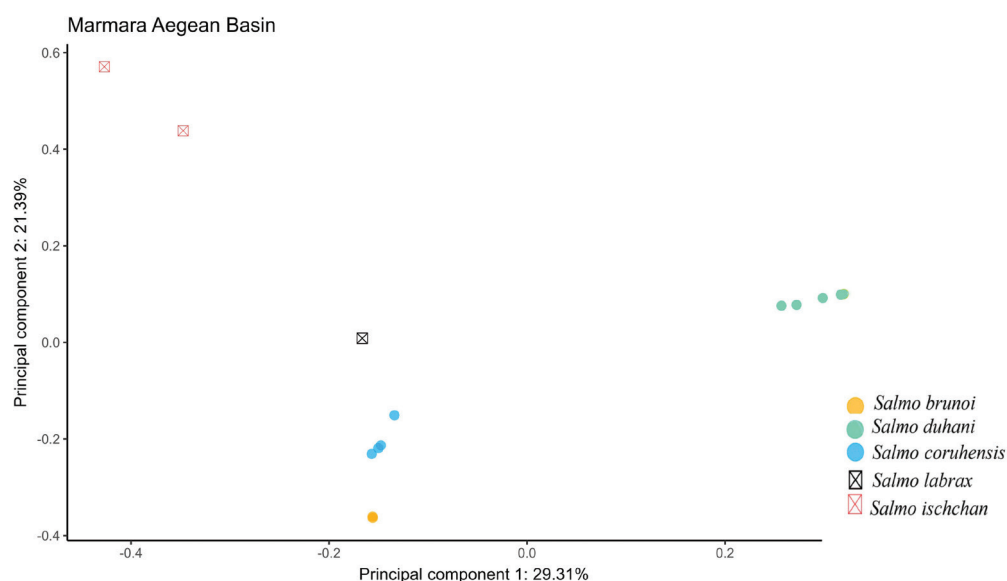


Figure 6. Principal Component Analysis (PCA) plot using 187,385 unlinked SNPs. Rectangular symbols represent the Danubian (DA) lineage reference, whereas circles indicate trout specimens used in the study. Colors represent the *Salmo coruhensis* and *S. duhani* specimens.

Author Contributions

DT conceptualized and conceived the idea. EB carried out morphometric measurements under the guidance of DT. SA carried out the fieldwork. MO performed genetic wet-lab work and data analysis and provided funding acquisition. The draft was written primarily by DT and all authors have read, edited and agreed with the final version.

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