

Chondrostoma smyrnae, a new nase from the Tahtalı reservoir drainage in the Aegean Sea basin (Teleostei, Leuciscidae)

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Abstract

Chondrostoma smyrnae, a new species, from the Tahtalı reservoir drainage is distinguished by having a slightly arched lower jaw with a well-developed keratinised edge, a deep and cylindric body, a complete lateral line with 47–52+1 total scales, 8–9 scale rows between the lateral line and the dorsal-fin origin, 4 scale rows between the lateral line and the pelvic fin-origin, and 19–23 gill rakers on the first gill arch. Moreover, molecular analyses using full *cyt b* (1141 bp) and partial *colI* (652 bp) sequences of the mitochondrial genome from specimens of the new species, *C. smyrnae* and specimens belonging to other *Chondrostoma* species from central and western Anatolia demonstrated that the *C. smyrnae* is easily differentiated by their high pairwise genetic distances of *cyt b* and *colI* data set (>2.20 and 1.03%, respectively) and by their position in the phylogenetic trees obtained through Maximum Likelihood (ML) methodology.

Key Words

Cytochrome b, Cytochrome oxidase I, freshwater fish, taxonomy, Western Anatolia

Introduction

Nases of the genus *Chondrostoma* Agassiz, 1832 are medium-to large-sized leuciscid fishes geographically widespread from France to the Volga River and Central Iran (Elvira 1997, Kottelat and Freyhof 2007, Küçük et al. 2017, Güçlü et al. 2018). Some species of *Chondrostoma* have a wide distribution (as *C. nasus*) and exhibit ecological and morphological diversity (Kottelat and Freyhof 2007, Durand et al. 2003), with some ambiguities relating to the taxonomy of some morphologically defined as *C. fahirae* (Elvira 1987, Durand et al. 2003, Freyhof and Özuluğ 2009). The major divergence times within *Chondrostoma* in Turkey coincided with the tectonic and climatic evolution of Anatolia in the Late Pliocene and Early Pleistocene which were seen as the uplift of the Anatolian plateau and an ice age that involved both the migration and isolation of species, respectively (Çiftçi et al. 2020).

Currently, 14 species of the genus lives in Turkey, of which eight are endemic to Anatolia (Güçlü et al. 2018, Çiftçi et al. 2020). In the Eastern Aegean Sea basin, 4 species are known: *C. fahirae* (Ladiges, 1960) from the Dalaman River drainage, *C. holmwoodii* (Boulenger, 1896) from the Gediz and Bakır River drainages, *C. meandrense* Elvira, 1987 from the Lake Işıklı basin, and *C. turnai* Güçlü, Küçük, Turan, Çiftçi & Mutlu, 2018 from the lower and middle Büyük Menderes River drainage. Additional species distributed adjacent to the Eastern Aegean basin are *C. angorense* Elvira, 1987, which is widespread in the Southern Marmara and Black Sea basins, and *C. beysehirense* Bogutskaya, 1997 that is endemic to tributaries of the Lake Beyşehir in Central Anatolia.

During a revision project of the genus *Chondrostoma* in Turkey, we already described three new *Chondrostoma* species suggested as evolutionary lineages by Geiger et al. (2014), including *C. turnai* from the Büyük Menderes River (Güçlü et al. 2018), *C. toros* Küçük, Turan,

Güçlü, Mutlu & Çiftci, 2017 from the Göksu River, and *C. ceyhanensis* Küçük, Turan, Güçlü, Mutlu & Çiftci, 2017 from the Seyhan and Ceyhan rivers (Küçük et al. 2017). Here, we describe an additional species from the Tahtalı River drainage in Turkey.

Materials and methods

The care of experimental animals was consistent with the Republic of Turkey animal welfare laws, guidelines and policies approved by Süleyman Demirel University Local Ethics Committee for Animal Experiments (permit reference number 2011/6/5). Samples were collected by electrocoker. After anaesthesia, samples of caudal fin tissue taken from each specimen for the molecular analysis were fixed and stored in 98% ethanol and fish were fixed in 4% formaldehyde. Measurements were made with a dial caliper and recorded to 1 mm. All measurements were made point-to-point, never by projections. Methods for counts and measurements follow Kottelat and Freyhof (2007). Standard length (SL) was measured from the tip of the snout to the posterior extremity of the hypural complex. The length of the caudal peduncle was measured from behind the base of the posterior anal-fin ray to the posterior extremity of the hypural complex, at mid-height of the caudal-fin base. The lateral line scales were counted from the first scale touching the shoulder girdle to the posterior-most scale at the end of the hypural complex. Scales on the caudal-fin were indicated by "+". The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins were counted as "1½". The simple dorsal-and anal-fin rays were not counted since the anteriormost rays are deeply embedded.

For osteological preparations, one specimen of a new species (168.8 mm SL) and one specimen of *C. turnai* (139.4 mm SL) were cleared and stained with alizarin red S, according to the protocol of Taylor and van Dyke (1985). The specimens were examined using a stereomicroscope (Nikon SMZ1500), photos taken with a digital machine with a glycerol bath. The nomenclature of the skeletal elements followed Bogutskaya (1996).

Abbreviations used

SL standard length;
BI Bayesian Inference;
ML Maximum Likelihood;
mt mitochondrial.

Collection codes:

IFC-ESUF Inland Fishes Collection, Eğirdir Fisheries Faculty of Isparta University of Applied Sciences;
FFR Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize;
FSJF Fischsammlung J. Freyhof, Berlin.

DNA extraction, PCR amplification and sequencing

Total DNA was isolated from ethanol-preserved tissue samples using the Invitrogen PureLink Genomic DNA Mini Kit according to the manufacturer's instructions and stored at -20 °C prior to use. The mitochondrial cytochrome b (*cyt b*) gene (1141 bp) was amplified using Forward (5'-AAT GAC TTG AAG AAC CAC CGT-3') and Reverse (5'-CAA CGA TCT CCG GTT TAC AAG AC-3') (Robalo et al. 2007) primers. Cytochrome c oxidase subunit 1 (*coI*) barcode region (652 bp) was amplified using the FishF1 (5'-TCAACCAACCACAAAG ACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGG-GTGGCCAAAGAATCA-3') (Ward et al. 2005) primer pairs. PCR reactions contained 5 µl of template DNA (25–50 ng/µl), 2 µl each of forward and reverse primers (10 pM of each primer), 25 µl of PCR Master Mix (2X) (Promega) and ddH₂O for 50 µl reaction mixture. PCR amplifications were performed using a Techne (TC-Plus) thermal cycler with the conditions as follows: after a preliminary denaturation at 94 °C for 4 min, each of the 35 cycles consisted of denaturation at 94 °C for 1 min, annealing (for *cyt b*, 30 s at 60 °C and for *coI*, 30 s at 54 °C), and primer extension at 72 °C for 1.5 min (1 min for *coI*) and a final extension at 72 °C for 10 min, followed by cooling to 4 °C. The PCR products were purified with the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, and bidirectional sequencing of purified PCR products was performed using the same PCR primers on an Applied Biosystems 3730 XL Genetic Analyser (Applied Biosystem, Foster City, CA, USA) using a BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystem) at the MacroGen Inc. (Amsterdam, Netherlands) (<https://dna.macrogen-europe.com/eng/>).

Phylogenetic reconstruction

For the phylogenetic analyses, two data sets were used, an 1140 bp fragment of *cyt b* and 652 bp fragment of *coI* sequences. *Cyt b* and *coI* sequences were aligned with the previous sequences from GenBank (Table 1) with the Clustal W algorithm (Thompson et al. 1994 available in Bioedit 7.2.5 (Hall 1999)) with default parameters (gap opening: 10.00 and gap extension: 0.10; Hall 2008) and all alignments were inspected and corrected visually. Sequences obtained in this study were deposited in GenBank (accession numbers: **MT387055–MT387058** for the *cyt b* and **MW719591–MW719611** and **MW722822–MW722824** for the *coI*). After translating the nucleotide sequence of protein coding genes to proteins using MEGA X (Kumar et al. 2018), we found no stop codons or indels present in this alignment. Haplotypes were detected using DnaSP v5 software (Librado and Rozas 2009). Duplicate sequences were not used for phylogenetic tree construction. Subsequently, phylogenetic analyses have been

Table 1. List of *Chondrostoma* species analysed, sampling locality, sources and GenBank accession numbers.

Species	River/Drainage	Country	Sources	Accession numbers	
				Cyt b	Col
<i>C. holmwoodii</i>	Gölmarmara/ Akpınar	Turkey	Çiftci et al. (2020)	MT387078	
	Gediz river/ Derbent	Turkey	Çiftci et al. (2020)	MT387099	
	Bakırçay/ Bergama	Turkey	Durand et al. (2003)	AF533765	
	Gediz river/ Uşak	Turkey	Durand et al. (2003)	AF533762	
	Bakır stream	Turkey	Geiger et al. (2014)		KJ553066.1
	Gediz river /Uşak	Turkey	This study		MW719600
	Gediz river /Uşak	Turkey	This study		MW719598
	Gediz river /Uşak	Turkey	This study		MW719599
<i>C. smyrnae</i>	Bakır stream	Turkey	Geiger et al. (2014)		KJ552962.1
	Şaşal stream/ Tahtalı	Turkey	This study	MT387055	MW722822
	Şaşal stream/ Tahtalı	Turkey	This study	MT387056	MW722823
	Şaşal stream/ Tahtalı	Turkey	This study	MT387057	MW722824
<i>C. turnai</i>	Şaşal stream/ Tahtalı	Turkey	This study	MT387058	
	Çine stream	Turkey	Çiftci et al.(2020)	MT387091	
	Çine stream	Turkey	Çiftci et al. (2020)	MT387093	
	Çine stream	Turkey	Çiftci et al. (2020)	MT387092	
	Çine stream	Turkey	Çiftci et al. (2020)	MT387095	
	Çine stream	Turkey	Çiftci et al. (2020)	MT387094	
	Büyük Menderes	Turkey	Geiger et al. (2014)		KJ553058.1
	Yayla lake/Buldan	Turkey	This study		MW719601
	Yayla lake/Buldan	Turkey	This study		MW719602
	Yayla lake/Buldan	Turkey	This study		MW719603
	Büyük Menderes	Turkey	Geiger et al. (2014)		KJ553026.1
	Büyük Menderes	Turkey	Geiger et al. (2014)		KJ552989.1
<i>C. angorense</i>	Akin stream/ Sakarya	Turkey	Çiftci et al. (2020)	MT387066	
	Porsuk stream/ Sakarya	Turkey	Çiftci et al. (2020)	MT387114	
	Porsuk stream/ Sakarya	Turkey	Çiftci et al. (2020)	MT387112	
	Kızılırmak river Kırşehir	Turkey	Perea et al. (2010)	HM560078	
	Porsuk stream/ Sakarya	Turkey	Çiftci et al. (2020)	MT387113	
	Sakarya river/ Kızılcahamam	Turkey	This study		MW719604
	Sakarya river/ Kızılcahamam	Turkey	This study		MW719605
	Kızılırmak river/ Zara	Turkey	This study		MW719606
	Kızılırmak river/Zara	Turkey	This study		MW719607
	Delice stream/Yerköy	Turkey	This study		MW719608
	Delice stream /Yerköy	Turkey	This study		MW719609
	Kızılırmak river/ Boyabat	Turkey	This study		MW719610
	Kızılırmak river/ Boyabat	Turkey	This study		MW719611
<i>C. meandrense</i>	Işıklı spring/ Çivril	Turkey	Çiftci et al. (2020)	MT387085	
	Işıklı spring/ Çivril	Turkey	Çiftci et al. (2020)	MT387087	
	Işıklı spring/ Çivril	Turkey	Çiftci et al. (2020)	MT387088	
	Işıklı spring/ Çivril	Turkey	Çiftci et al. (2020)	MT387086	
	Büyük Menderes	Turkey	Geiger et al. (2014)		KJ553109
	Büyük Menderes /Çivril	Turkey	This study		MW719591
	Büyük Menderes/ Çivril	Turkey	This study		MW719592
	Büyük Menderes/ Dinar	Turkey	This study		MW719593
	Büyük Menderes/ Dinar	Turkey	This study		MW719594
	Büyük Menderes/ Dinar	Turkey	This study		MW719595
	Büyük Menderes/ Akçay	Turkey	This study		MW719596
	Büyük Menderes	Turkey	Geiger et al. (2014)		KJ553083
<i>C. nasus</i>	Diina River	Serbia	Schönhuth et al. (2014)		MG806833
	Soca drainage	Slovenia	Geiger et al. (2014)		KJ553248
	Simav drainage	Turkey	Geiger et al. (2014)		KJ552881
	Danube River	Romania	Org (2018)		MF135845
	Susurluk, Kocacay	Turkey	Durand et al. (2003)	AF533760	
	Danube River	Austria	Durand et al. (2002)	AY026402	
	Simav Stream, Yanıkburnu	Turkey	Çiftci et al. (2020)	MT387104	
	Simav Stream, Yanıkburnu	Turkey	Çiftci et al. (2020)	MT387105	
	Simav Stream, Yanıkburnu	Turkey	Çiftci et al. (2020)	MT387106	
	Simav Stream, Bigadiç	Turkey	Çiftci et al. (2020)	MT387109	
<i>C. beysehirense</i>	Simav Stream, Bigadiç	Turkey	Çiftci et al. (2020)	MT387110	
	Beyşehir lake	Turkey	Çiftci et al. (2020)	MT387079	
	Beyşehir lake	Turkey	Çiftci et al. (2020)	MT387080	
	Beyşehir drainage	Turkey	Geiger et al. (2014)		KJ552898
<i>C. fahirae</i>	Beyşehir lake	Turkey	This study		MW719597
	Tefenni Burdur	Turkey	Çiftci et al. (2020)	MT387128	
<i>Pseudochondrostoma willkommii</i>				KF529128	KJ554279
<i>Achondrostoma arcasii</i>				KF529113	KJ552518

performed over aligned nucleotides containing polymorphic sites for two different data sets (*cyt b* and *coI*). Computation of phylogenetic tree reconstructions of haplotypes was carried out using Maximum Likelihood (ML) analyses. ML analyses for these two data-sets were performed using PhyML version 3.0 (Guindon and Gascuel 2003), with 1000 bootstraps under the best-fit models (TIM3+G for *cyt b*, TPM1uf+G for *coI*) which were calculated by the Akaike and Bayesian Information Criteria (AIC and BIC) approaches in the programme jModelTest 0.1.1 (Posada 2008). All of the trees deduced from the *Cyt b* and *CoI* sequences were rooted with *Achondrostoma arcasii* (Steindachner, 1866) and *Pseudochondrostoma willkommii* as an outgroup taxa. Constrained trees were generated in TreeWiev (Page 1996). A calculation of pairwise genetic distance among different species with Kimura 2-parameter (K2P) distances model (Kimura 1980) was performed using MEGA X (Kumar et al. 2018).

Results

Molecular analysis

The nucleotide sequences of both strands resulted in the full length of mitochondrial *cyt b* (1141 bp) for the samples. Four haplotype sequences belonging to *Chondrostoma smyrnae* were compared with other *Chondrostoma* sequences from Western Anatolia available from GenBank (Table 1). A total of 1140 homologous sites for each of the 32 individual sequenced were aligned, with 215 (18.9%) variable and 119 (10.4%) variable characters parsimony-informative under the maximum parsimony optimality criterion. For *cyt b* gene, Intrageneric K2P distances among analysed *Chondrostoma* species from western Anatolia ranged from 1.26% between *C. angorense* and *C. meandrense* to 9.95% between *C. fahirae* and *C. turnai* (Table 2). The mean intraspecific divergence ranged between 0.13% for *C. smyrnae* and 0.53% for *C. holmwoodii* (Table 2). In addition, the pairwise genetic distances between *C. smyrnae* and the other species ranged from 2.20% to 9.88% (Table 2).

The dataset for cytochrome c oxidase subunit 1 (*coI*) included 24 individuals sequenced by this study and eight individuals from GenBank belonging to seven

Chondrostoma species (*Chondrostoma smyrnae*, *C. turnai*, *C. meandrense*, *C. holmwoodii*, *C. beysehirense*, *C. angorense* and *C. nasus*) in western Anatolia (Table 1). A total of 652 characters for each of the 36 individuals sequenced were aligned, 597 (91.6%) characters were constant, 27 (4.14%) variable characters were parsimony-uninformative and 28 (4.29%) characters were parsimony-informative under the maximum parsimony optimality criterion. For *coI* gene, Intrageneric K2P distances among analysed *Chondrostoma* species from western Anatolia ranged from 0.38% between *C. angorense* and *C. beysehirense* to 1.47% between *C. holmwoodii* and *C. turnai* (Table 2). The mean intraspecific divergence ranged between 0.00% for *C. angorense* and *C. nasus* and 0.21% for *C. smyrnae* (Table 2). In addition, the pairwise genetic distances between *C. smyrnae* and the other species ranged from 1.03% to 1.32% (Table 2).

Phylogenetic relationships among the sequences were reconstructed independently for *cyt b* and *coI* genes using the ML method. In the phylogenetic reconstructions for *cyt b*, *Chondrostoma* haplotypes formed three distinct monophyletic clades, hereafter referred to as clades I, II, and III as in Figure 1, which were consistently supported by high bootstrap values (100). The first clade consisted of *C. holmwoodii*, *C. smyrnae* and *C. turnai* while the other clade contains *C. angorense*, *C. meandrense* and *C. nasus*. *C. smyrnae* formed a well-supported clade sister to *C. turnai* and both taxa were clustered in a monophyletic group distinguished from *C. holmwoodii*. In addition, *C. fahirae* were positioned basal to all species of *Chondrostoma* (Figure 1).

For *coI*, the phylogenetic tree demonstrated that *Chondrostoma* species are divided into four clades (Figure 2). Clade I is formed by *C. meandrense* and *C. nasus* species while clade II is made up of the *C. turnai*, *C. smyrnae* and *C. holmwoodii*. Compared to *cyt b* results, the position of *C. angorense* was clearly separated from the clade including *C. meandrense* and *C. nasus* species, and formed a distinct and well-supported phylogenetic clade (93). *C. smyrnae* formed a well-supported lineage (85) and clustered together with *C. turnai*.

All phylogenetic analysis showed that these species are separated from one another. Also, *C. smyrnae* individuals from the Tahtalı reservoir drainage in the Aegean Sea basin are concluded to be distinct members of the *Chondrostoma* species studied in all phylogenetic trees.

Table 2. Pairwise distance of *cyt b* and *coI* data set within and among *Chondrostoma* species based on 1.000 bootstrap replications using the Kimura 2-parameter distance method; values in lower left cells = percent difference among taxa for *cyt b*, values in upper right cells = percent difference among taxa for *coI* and diagonal = percent difference within taxa for *cyt b/coI*.

	<i>C.holmwoodii</i>	<i>C.smyrnae</i>	<i>C.turnai</i>	<i>C.angorense</i>	<i>C.meandrense</i>	<i>C.nasus</i>	<i>C.beysehirense</i>
<i>C.holmwoodii</i>	0.53 /0.12	1.19	1.47	0.77	0.95	0.93	0.85
<i>C.smyrnae</i>	2.95	0.13 /0.21	1.32	1.03	1.21	1.19	1.11
<i>C.turnai</i>	2.91	2.20	0.33 /0.09	1.01	1.18	1.16	1.08
<i>C.angorense</i>	2.47	2.61	2.48	0.35 /0.00	0.48	0.46	0.38
<i>C.meandrense</i>	2.87	2.77	2.67	1.26	0.26 /0.04	0.64	0.56
<i>C.nasus</i>	2.96	2.64	2.45	1.47	1.53	0.50 /0.00	0.54
<i>C.beysehirense</i>	3.29	2.86	3.00	2.30	2.28	2.18	0.26 /0.15
<i>C.fahirae</i>	9.67	9.88	9.95	9.18	8.75	9.36	9.08

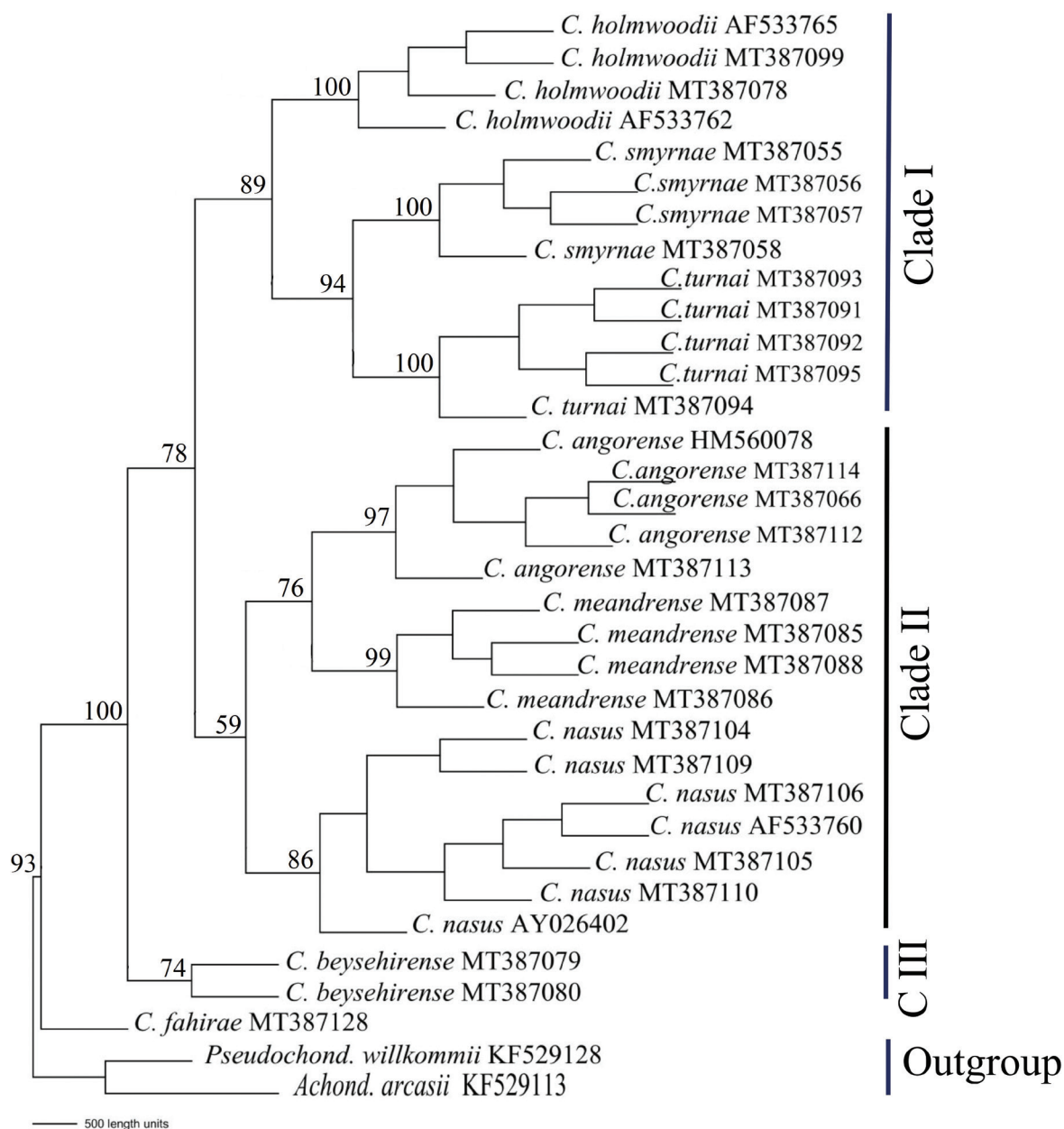


Figure 1. Maximum Likelihood (ML) estimation of the phylogenetic relationships of *Chondrostoma* species based on the mitochondrial *cyt b* sequence data. The tree was created with the TIM3+G substitution model. Branches are labelled with maximum likelihood bootstrap $\geq 50\%$.

Key to species of *Chondrostoma* in the Anatolian Aegean Sea basin

- 1 12–14 gill rakers on first gill arch; no keratinised cutting edge on lower jaw *C. fahirae*
- 19–28 gill rakers on first gill arch; a keratinised cutting edge on lower jaw 2
- 2 Lateral line with 56–68+2–3 scales 3
- Lateral line with 43–52+1–2 scales 5
- 3 Lower jaw markedly arched and keratin not well developed *C. meandrensis*
- Lower jaw slightly arched and keratin is well developed 4
- 4 5–7 scale rows between lateral line and pelvic-fin origin. In life, a longitudinal violet stripe (band) in middle of flank from caudal-fin base to dorsal-fin base *C. holmwoodii*
- 5 scale rows between lateral line and pelvic-fin origin. Not a longitudinal violet stripe in life (band) *C. angorensis*
- 5 Body width at dorsal-fin origin 13–15% SL; body width at mid-point of caudal peduncle 4–6% SL *C. turnai*
- Body width at dorsal-fin origin 16–19% SL; body width at mid-point of caudal peduncle 6–8% SL *C. smyrnae*

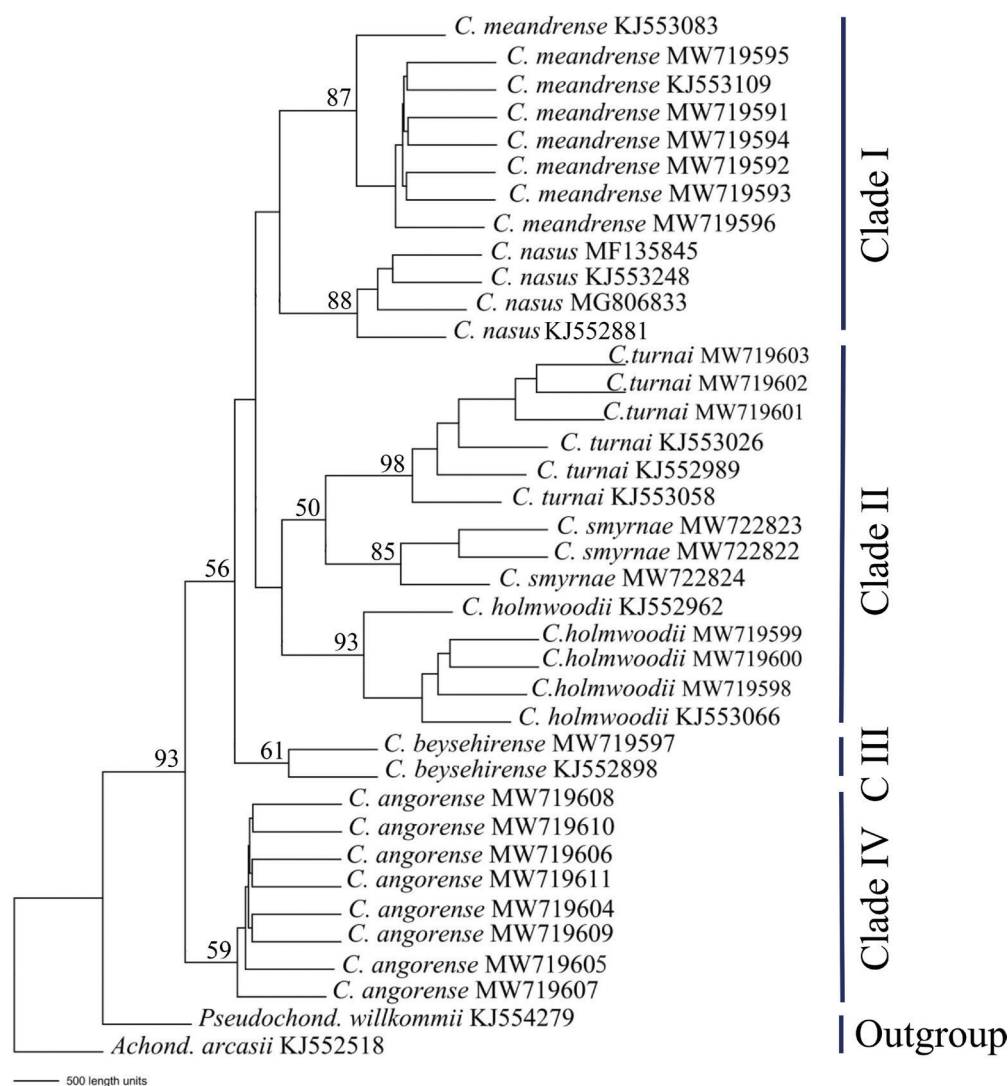


Figure 2. Maximum Likelihood (ML) estimation of the phylogenetic relationships of *Chondrostoma* species based on the mitochondrial *coI* barcode region. The tree was created with the TPM1uf+G substitution model. Branches are labelled with maximum likelihood bootstrap $\geq 50\%$.

Chondrostoma smyrnae, sp. nov.

<http://zoobank.org/44365C7F-0D20-4983-9E3B-7205046ED1FA>

Fig. 3a–c

Holotype. IFC-ESUF 03-1566, 191 mm SL; Turkey: İzmir prov.: Tahtalı reservoir about 2 km north of Değir-mendere, 38°08'19"N, 27°07'10"E.

Paratypes. IFC-ESUF 03-1567, 22, 152–205 mm SL; IFC-ESUF 03-1568, 22, 181–272 mm SL; same data as holotype. – IFC-ESUF 03-1550, 2, 92.68–109.02 mm SL; Turkey: İzmir prov.: stream Şaşal about 1 km south of Küner, 38°11'57"N, 27°08'09"E. – FFR 2079, 1, 92 mm SL; Turkey: İzmir prov.: stream Balaban at Küner, 38.213950 27.101505.

Material used in molecular genetic analysis. IFC-ESUF DNA-03-1550, 7, Turkey: İzmir prov.: stream Şaşal about 1 km south of Küner, 38°11'57"N, 27°08'09"E (GenBank accession number: MT387055–MT387058; MW719591–MW719611; MW722822–MW722824).

Diagnosis. *Chondrostoma smyrnae* is distinguished from other species occur to adjacent basin by a cylindrical body (body width at dorsal-fin origin 16.8–19.3% SL, vs. 13.3–15.4 in *C. turnai* (Fig. 3d), 14.1–16.6 in *C. meandrense*, 12.0–16.3 in *C. holmwoodii*, 12.4–15.7 in *C. fahirae*, except *C. beysehirense*), a wider head (head width at anterior margin of eye 55–65% HL, vs. 42–54), by having less lateral line scales (48–53 vs. 60–67 in *C. beysehirense*, 60–66 in *C. holmwoodii* and 56–60 in *meandrense*, except *C. turnai* and *C. fahirae*). *Chondrostoma smyrnae* is further distinguished from *C. turnai* by the absence keel between pelvic fin-origin and anus (vs. present in specimens larger than 160 mm SL), a straight or slightly arched lower jaw (vs. arched), more total lateral line scales (48–53, vs. 44–51), and fewer gill rakers on first gill arch (19–23, vs. 22–27). Also, *C. smyrnae* further differs from *C. turnai* by the shape of jaws, hyomandibular, quadrate and the fifth brachial gill arc. In *C. smyrnae*, the dentary thick and coronoid process inclined forward (vs. thin and

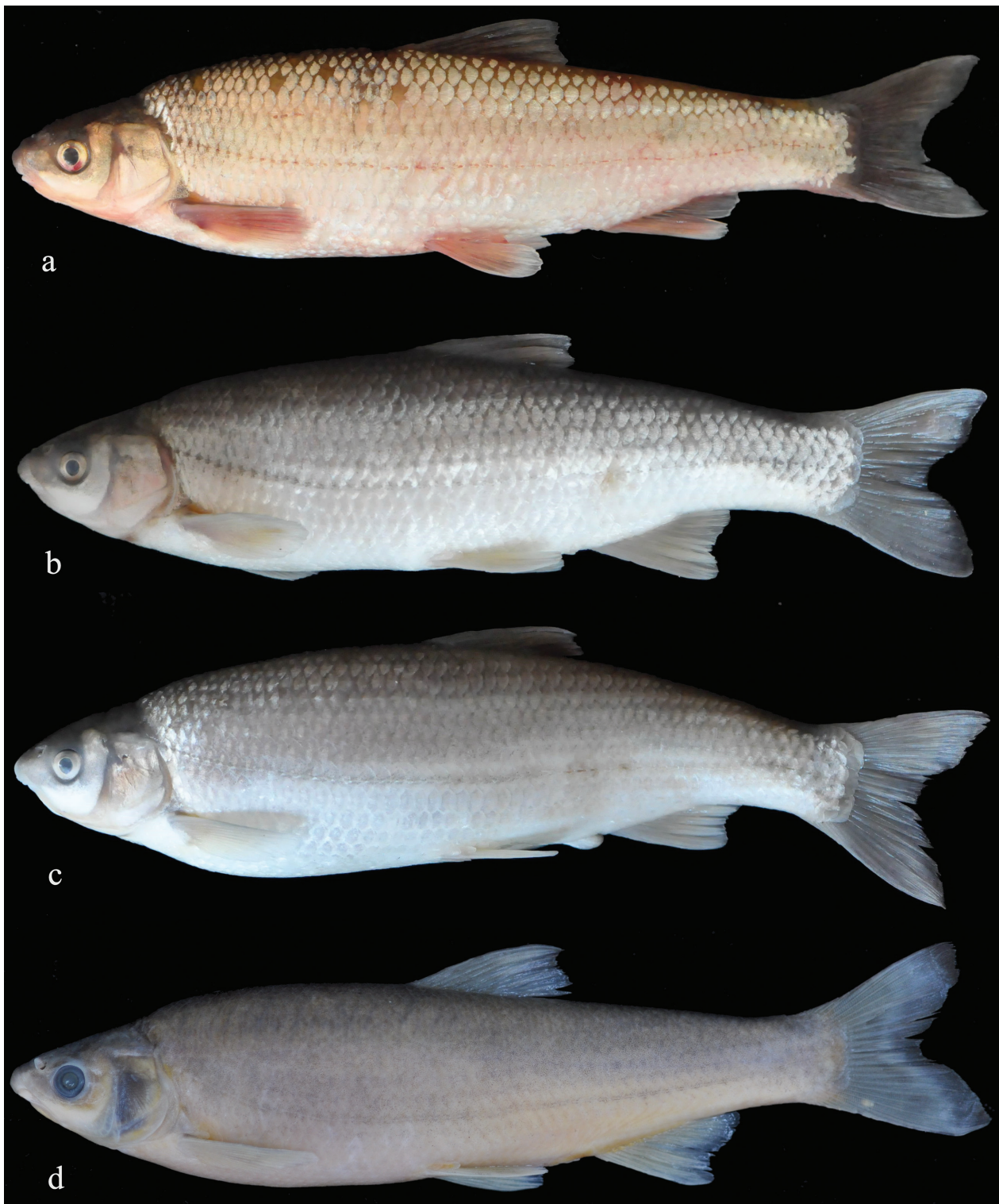


Figure 3. **a.** *Chondrostoma smyrnae*, IFC-ESUF 03–1567, just after fixation form, 167 mm SL, Turkey: Tahtalı reservoir; **b.** IFC-ESUF 03–1566, holotype, 191 mm SL, Turkey: Tahtalı reservoir; **c.** IFC-ESUF 03–1567, paratype 205 mm SL, Turkey: Tahtalı reservoir; **d.** *Chondrostoma turnai*, IFC-ESUF 03–1557, 197 mm SL, Turkey: Çine stream.

coronoid process nearly vertical); premaxilla very deep and posterior edge short (vs. slender and posterior edge long); hyomandibular long and narrow (vs. short and wide), the fifth brachial gill arc wide angle (vs. narrow angle) and pharyngeal teeth wide (vs. thin); outer margin of quadrate slightly pointed (vs. rounded) (Figs 4, 5).

The new species is also distinguished from *C. meandrense* by the body colour silvery in life (silvery, vs. brownish). It is further distinguished from *C. holmwoodii* by having 8–9 scale rows between the lateral line and dorsal-fin origin (vs. 9–11), four scale rows between the lateral line and pelvic-fin origin (vs. 6–7). *Chondrostoma smyrnae*

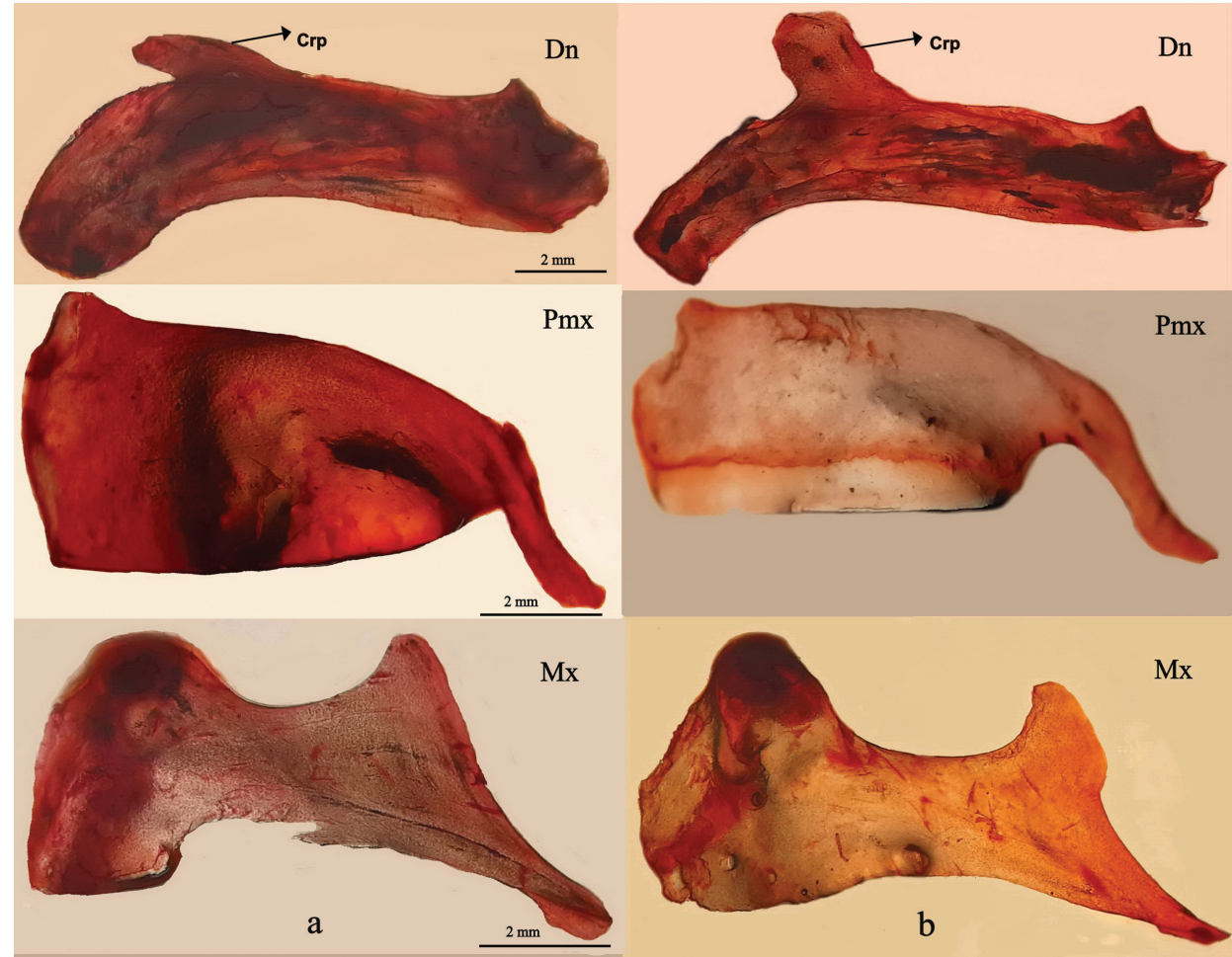


Figure 4. Jaws bones of *C. smyrnae* (a.) and *C. turnai* (b.). (Dn: dentary, Pmx: premaxilla, Mx: maxilla, Crp: Coronoid process).

is further distinguished from *C. fahirae* by having of 19–23 gill rakers on the first gill arch (vs. 12–14), well keratinised cutting edge present on the lower jaw (vs. slightly) (Fig. 6).

Description. See Figures (3) for general appearance, Table 3, 4 for morphometric and Table 5, 6 for meristic data. A small-sized individual of *Chondrostoma symrnae* with a cylindrical body shape is shown on Figure 7. Dorsal and ventral body profiles markedly convex. Interorbital area slightly convex. Mouth inferior, arched, with a keratinized cutting edge on lower jaw. The lower jaw slightly arched. Snout long, length 31–35% HL, with slightly rounded tip. Rostral cap almost covers upper jaw. Lower jaw relatively long, lower jaw-quadrate junction on vertical through eye pupil margin. Eye diameter about equal to interorbital distance. Caudal peduncle depth fits 10–12 times in its length. Dorsal-fin outer margin markedly concave. Anal-fin outer margin concave. Caudal-fin deeply forked, lobes with pointed tips. Outer margins of pectoral and pelvic-fins slightly convex. Pharyngeal teeth in two rows, 5-6, 5-5, sharp, serrated, hooked at tip (Fig. 5). Dentary thick and coronoid process inclined forward. Premaxilla very deep and its posterior edge short. Hyomandibular long and narrow. Fifth branchial gill arc wide angle and pharyngeal teeth wide. Outer margin of quadrate slightly pointed.

Table 3. Morphometric data of *Chondrostoma smyrnae* (holotype, IFC-ESUF 03-1566 and paratypes, IFC-ESUF 03-1567; n = 22).

	Holotype	Holotype & paratypes			
		mean	Min	Max	SD
Standard length (mm)	190.5	174.7	152.3	205.0	
In percent of standard length					
Head length	21.9	21.1	19.9	22.7	0.7
Body depth	26.6	25.9	24.2	28.4	1.4
Body width at dorsal-fin	17.5	17.4	16.0	19.4	1.2
Body width at caudal peduncle	6.8	6.8	5.7	8.0	0.9
Predorsal distance	51.2	50.8	49.0	52.2	1.2
Prepelvic distance	52.6	53.1	51.1	54.6	1.8
Preanal distance	71.4	73.2	70.4	75.7	2.6
Pectoral-fin origin to anal-fin	49.6	52.8	49.7	58.7	1.7
Pectoral-fin origin to pelvic-fin	29.8	32.0	29.8	36.2	1.5
Pelvic-fin origin to anal-fin	19.6	22.1	19.8	24.6	0.9
Dorsal-fin depth	18.4	18.4	17.1	20.6	0.8
Anal-fin depth	16.2	15.4	14.4	16.9	0.9
Pectoral-fin length	16.9	17.3	16.4	19.9	1.0
Pelvic-fin length	15.9	14.9	11.3	17.3	0.9
Caudal peduncle length	17.9	18.5	16.5	20.4	1.4
Caudal peduncle depth	11.8	11.0	10.2	11.8	0.9
In percent of head length					
Snout length	31.6	33.1	31.1	35.3	1.2
Eye diameter	18.5	20.7	17.5	22.8	1.4
Interorbital distance	41.8	41.7	38.7	46.4	2.1
Head width ₁	56.9	59.7	54.9	65.3	2.3
Head depth ₁	54.7	54.6	50.9	57.8	2.4
Head depth ₂	81.1	79.9	75.0	85.2	2.6
Mouth width	27.2	27.2	24.1	27.9	1.0

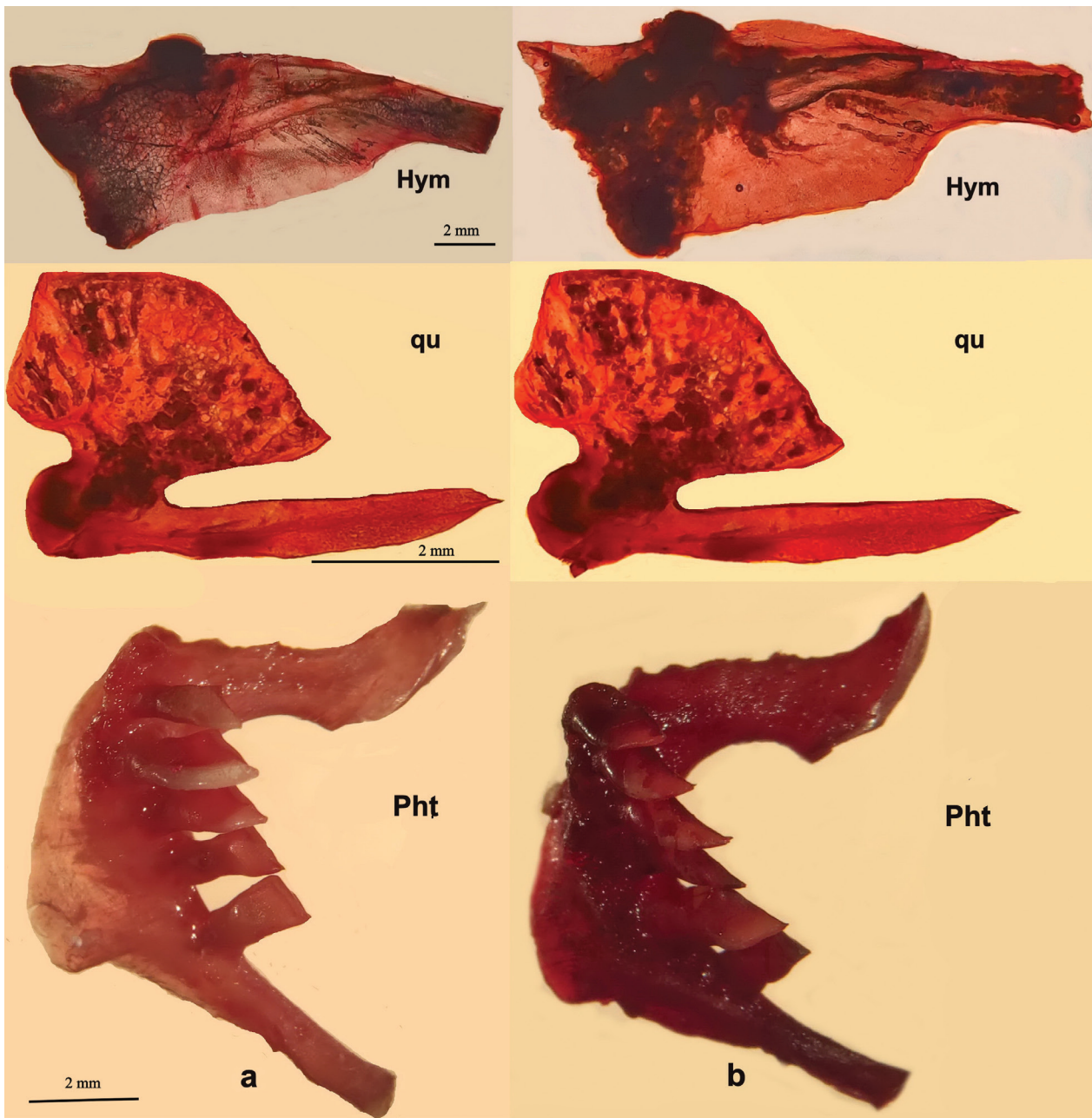


Figure 5. Hyomandibular, quadrate and pharyngeal teeth of *C. smyrnae* (a.) and *C. turnai* (b.) (Hym: hyomandibulare, qu: quadrate, Pht: Pharyngeal teeth).

The number of lateral line scales, scale rows between the lateral line and dorsal-fin origin, scale rows between the lateral line and pelvic-fin origin, branched dorsal-fin rays, branched anal-fin rays and rakers on the outer side of the first gill arch are shown in Tables 5–6.

Colouration. In life: fins pinkish with hyaline margins; back brown; flank silvery with pinkish hue. After fixation: back and upper portion of flank dark greyish; mid-lateral portion of flank and belly yellowish. Dorsal and caudal fins dark grey, pelvic and anal fins yellowish. Peritoneal membrane black.

Distribution. *Chondrostoma smyrnae* is known from the Tahtalı reservoir basin (Fig. 8). It is also expected to be native to the Küçük Menderes River drainage but at-

tempts to find it there have thus far proven unsuccessful and it may have been extirpated.

Etymology. The species is named for Smyrna, the historic name of the city known today as Izmir. A noun in genitive, indeclinable.

Discussion

The identification of *Chondrostoma smyrnae* is a significant contribution to the genus *Chondrostoma*. There are six species of *Chondrostoma* (*C. angorense*, *C. beysehirense*, *C. fahirae*, *C. holmwoodii*, *C. meandrense* and *C. turnai*) in western Anatolia. The differences between *Chondrostoma smyrnae*,

Table 4. Morphometry of *C. turnai* (IFC-ESUF 03-1558. n = 43), *C. meandrense* (IFC-ESUF 03-1519. n = 24), *C. holmwoodii* (IFC-ESUF 03-1513. n = 19), and *C. fahirae* (IFCESUF 03-1512. n = 36).

SL (mm)	<i>C. turnai</i>				<i>C. meandrense</i>				<i>C. holmwoodii</i>				<i>C. fahirae</i>			
	132.0–191.8				120.6–200.9				88.8–161.3				82.8–96.8			
	Mean	min	max	SD	mean	min	max	SD	mean	Min	max	SD	mean	min	max	SD
In percent of standard length																
Head length	21.9	20.7	23.4	0.8	22.6	21.9	23.6	0.5	23.1	21.5	24.0	0.4	24.1	23.3	25.1	0.6
Body depth	24.6	22.9	27.0	1.0	23.9	21.8	25.5	1.0	23.6	22.0	24.8	0.7	24.0	21.9	25.3	1.1
Body width at dorsal-fin	14.3	12.8	15.1	0.8	14.4	12.5	16.7	0.8	13.0	12.3	14.6	0.5	12.6	10.7	14.6	0.9
Body width at caudal peduncle	5.3	4.0	5.8	1.4	5.00	3.5	5.9	0.9	5.6	4.7	6.6	0.7	5.6	4.1	6.9	1.2
Predorsal distance	51.5	50.0	54.5	1.2	51.3	49.6	52.6	1.5	52.2	50.7	54.0	1.1	53.5	51.8	55.0	1.6
Prepelvic distance	52.7	50.1	53.8	0.9	52.4	50.7	54.0	1.6	52.7	50.6	54.2	1.4	52.5	49.8	53.8	1.2
Preanal distance	73.4	71.8	75.1	1.4	72.1	69.4	74.8	1.3	72.2	70.4	73.4	1.5	70.3	67.3	72.9	1.4
Pectoral-fin origin to anal-fin	52.9	48.7	54.3	1.4	49.9	46.1	52.4	0.9	49.9	48.2	51.1	0.6	47.1	45.7	49.9	0.6
Pectoral-fin origin to pelvic-fin	31.4	29.0	34.3	1.0	29.2	26.5	31.5	1.2	28.9	28.2	29.4	0.7	28.0	25.4	30.3	0.6
Pelvic-fin origin to anal-fin	22.0	20.5	23.0	1.2	20.9	19.5	23.5	0.5	20.9	19.8	21.6	0.4	28.0	25.4	30.3	0.6
Dorsal-fin depth	19.2	17.5	21.4	0.8	18.3	16.2	20.2	1.0	19.5	17.2	20.9	0.7	21.0	20.0	22.4	1.1
Anal-fin depth	15.3	13.5	16.4	0.9	14.9	13.4	16.8	0.8	16.2	14.8	17.1	0.6	16.6	15.0	18.8	0.9
Pectoral-fin length	18.0	16.0	20.1	0.8	17.5	15.4	19.1	0.9	18.4	18.0	19.4	0.9	20.5	18.7	21.9	0.9
Pelvic-fin length	15.6	14.1	17.5	1.0	15.4	13.8	17.5	0.9	15.4	14.6	16.2	0.8	17.3	14.1	19.0	1.2
Caudal peduncle length	17.4	14.5	18.5	0.5	18.2	16.7	20.3	0.9	17.8	16.2	19.4	0.6	19.4	18.2	20.9	0.9
Caudal peduncle depth	10.5	9.70	11.7	0.8	10.5	9.9	11.2	0.4	10.2	9.4	10.9	0.2	10.8	9.9	11.9	0.5
In percent of head length																
Snout length	30.6	27.2	33.8	1.6	32.5	29.1	34.8	1.5	31.4	26.9	33.3	1.0	31.2	28.0	32.6	1.2
Eye diameter	22.2	20.1	24.7	1.3	21.9	19.7	26.3	1.7	20.6	17.6	22.4	0.8	25.7	23.2	29.1	1.7
Interorbital distance	38.2	33.1	41.9	2.1	36.4	33.7	38.7	1.4	36.1	32.7	39.0	1.1	37.8	35.1	39.6	1.6
Head width ₁	54.4	49.8	57.0	2.4	43.4	37.6	48.2	2.2	44.0	40.5	47.7	1.7	42.6	38.9	48.1	1.6
Head depth ₁	50.6	47.1	56.1	1.9	50.1	47.6	54.8	1.7	50.1	47.3	52.5	0.8	56.8	51.1	62.4	3.0
Head depth ₂	74.3	70.6	78.9	1.9	73.6	69.8	79.4	2.2	72.7	71.2	75.4	0.8	75.0	72.2	76.8	1.7
Mouth width	26.1	23.1	27.7	1.3	25.8	23.8	28.3	1.1	27.4	25.3	29.3	1.4	25.4	24.4	27.8	1.1

Table 5. Frequency of lateral line scales, scale rows between the lateral line and dorsal-fin origin, and the lateral line and pelvic-fin origin in Eastern Aegean *Chondrostoma* species.

	lateral line scales																									
	N	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	Mean	
<i>C. smyrnae</i>	29					1	6	4	7	6	5														50.9	
<i>C. turnai</i>	17	1	4	1	3	3	2	2	1																47.4	
<i>C. meandrense</i>	14													2	2	2	6	2							58.2	
<i>C. holmwoodii</i>	14																	1	1	4	1	2	2	3	63.4	
<i>C. fahirae</i>	24	1	1	5	6	5	3	1	1		1														47.6	
	scale rows between lateral line and dorsal-fin origin										scale rows between lateral line and pelvic-fin origin															
	N	8	8 _{1/2}	9	9 _{1/2}	10	11	Mean	3 _{1/2}	4	4 _{1/2}	5	5 _{1/2}	6	7	Mean										
<i>C. smyrnae</i>	28	19		9				8.3		28						4.0										
<i>C. turnai</i>	15	8	2	4	1			8.4	1	10	1	3				4.2										
<i>C. meandrense</i>	14	1		11		2		9.0				14				5.0										
<i>C. holmwoodii</i>	20			2		13	5	10.2				5		13	2	5.8										
<i>C. fahirae</i>	10	5		5				8.5		7		3				4.3										

Table 6. Frequency of gill rakers, branched dorsal-fin rays, and branched anal-fin rays in Eastern Aegean *Chondrostoma* species.

	N	12	13	14	15	16	17	18	19	20	21	22	23	24	Gill Rakers	26	27	28	Mean
															25				
<i>C. smyrnae</i>	16								3	5	3	3	2						20.8
<i>C. turnai</i>	11											1	2	4	3		1		24.2
<i>C. meandrense</i>	20												2	2	3	6	6	1	25.8
<i>C. holmwoodii</i>	14										2	8	2	2					22.3
<i>C. fahirae</i>	7	1	3	3															13.3
	Branched dorsal-fin rays							Branched anal-fin rays											
	N	7	8	9	10	Mean	8	9	10	11	12	Mean							
<i>C. smyrnae</i>	36	2	34			7.9	2	5	29			9.8							
<i>C. turnai</i>	18		18			8.0		11	7			9.4							
<i>C. meandrense</i>	14		12	2		8.1		8	6			9.4							
<i>C. holmwoodii</i>	16		16			8.0		14	2			9.1							
<i>C. fahirae</i>	10	10				7.0	3	7				8.7							

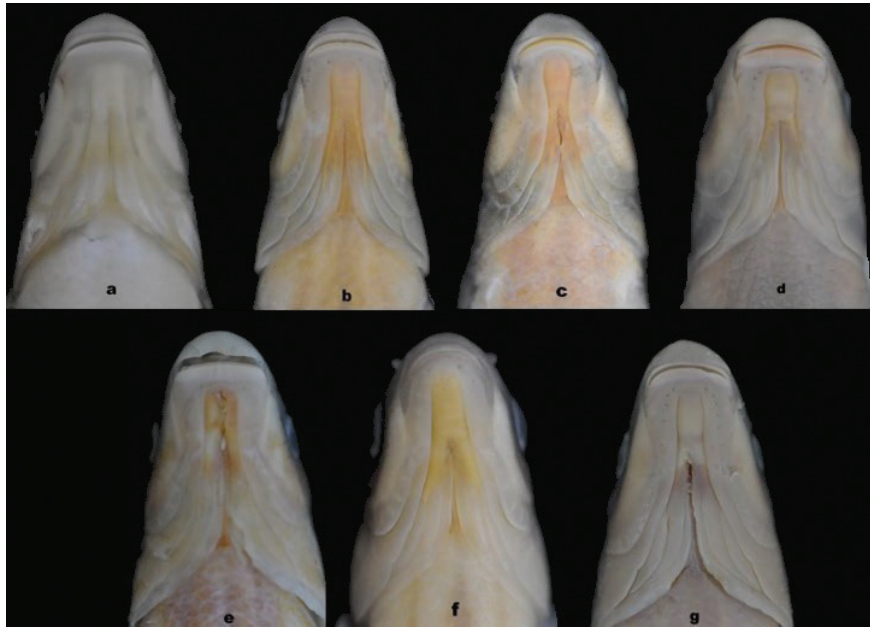


Figure 6. Ventral view of head: **a.** *C. smyrnae*, IFC-ESUF 03–1567, paratype, 189 mm SL, Turkey: Tahtalı reservoir; **b.** *C. turnai*, IFC-ESUF 03–1524, 174 mm SL, Turkey: Çine Stream; **c.** *C. meandrense*, IFC-ESUF 03–1519, 183 mm SL, Turkey: Işıklı Spring; **d.** *C. holmwoodii*, IFC-ESUF 03–1513, 156 mm SL, Turkey: Gediz River; **e.** *C. angorense*, IFC-ESUF 03–1502, 152 mm SL, Turkey: Sakarya River; **f.** *C. fahirae*, IFC-ESUF 03–1512, 109 mm SL, Turkey: Dalaman River; **g.** *C. beysehirense*, IFC-ESUF 03–1505, 288 mm SL, Turkey: Beyşehir Lake.



Figure 7. Top view of *Chondrostoma smyrnae* (**a.**) IFC-ESUF 03-1567, 194.2 mm SL; (**b.**) 190.0 mm SL, Tahtalı Reservoir; and *C. turnai* (**c.**) IFC-ESUF 03-1524, 195.1 mm SL; (**d.**) 181.3 mm SL, Stream Çine.

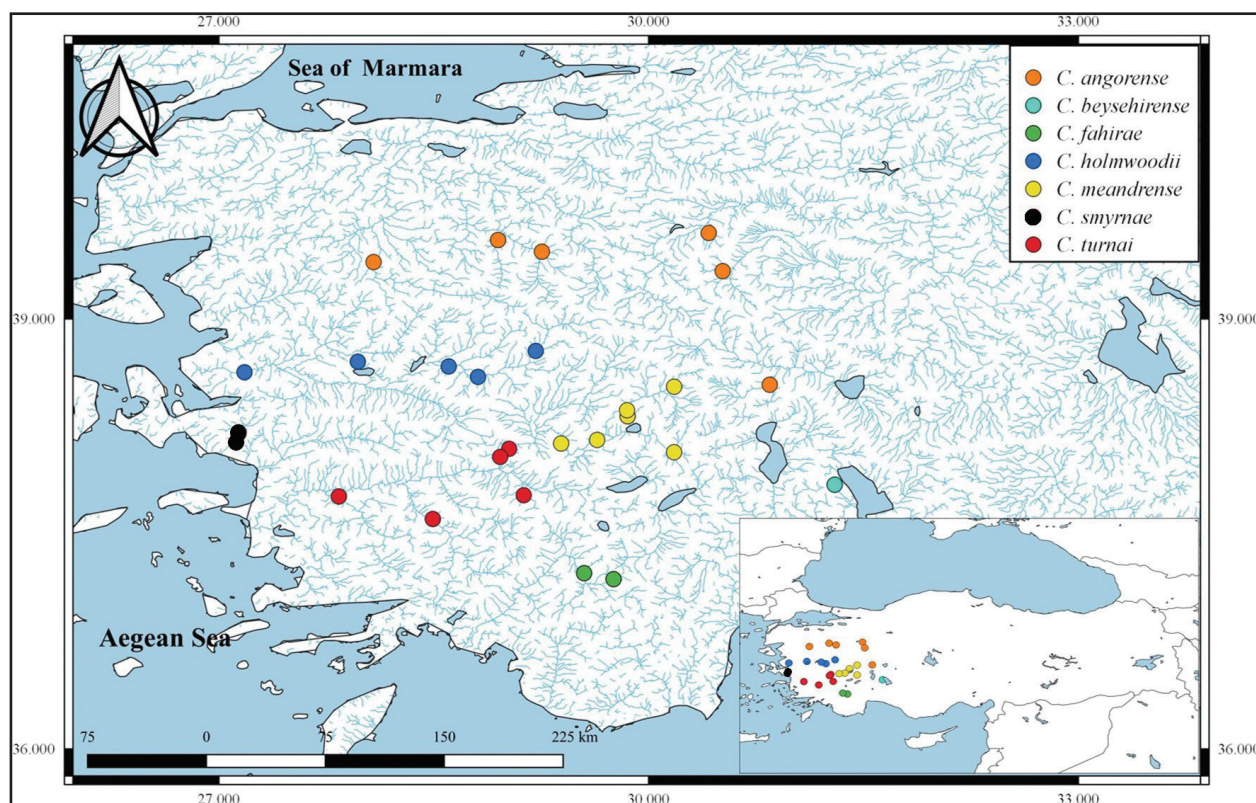


Figure 8. Distribution of *Chondrostoma* species in western Anatolia.

C. fahirae, *C. holmwoodii*, *C. meandrense* and *C. turnai*, which occur in the Aegean Sea basin of Turkey, are given in detail in the diagnosis section.

Chondrostoma smyrnae is distinguished from *C. angorense* and *C. beysehirense*, which occur adjacent to the Aegean Sea basin of Turkey, by having 48–53 lateral line scales (vs. 59–68 in *C. angorense*, 60–67 in *C. beysehirense*), 8–9 scale rows between the lateral line and the dorsal-fin origin (vs. 9–10 in *C. angorense*, vs. 10–11 in *C. beysehirense*), four scale rows between lateral line anal-fin origin (vs. 5 in *C. angorense*, 5–6 in *C. beysehirense*) and 19–23 gill rakers on first gill arch (vs. 33–39 in *C. beysehirense*).

In other words, their high genetic distance and position in the phylogenetic trees with high bootstrap values easily differentiated *C. smyrnae* from other species. The results on comparative morphological and genetic studies based on *cyt b* and *col* genes showed that the new species differ from formerly described *Chondrostoma* species.

Comparative Material

Material used for morphometric and meristic comparison

***Chondrostoma angorense*:** IFC-ESUF 03-1501, 32, 80–174 mm SL; Turkey: Eskişehir prov.: Porsuk River about 2 km west of Yörükkırka, 39°36'00"N, 30°25'09"E. – IFC-ESUF 03-1502, 11, 35–162 mm SL; Turkey: Eskişehir prov.: stream Akin 0.5 km south of Akin, 39°20'02"N,

30°30'59"E. – IFC-ESUF 03-1503, 2, 245–300 mm SL; Turkey: Kütahya prov.: stream Emet about 10 km north of Eğriöz, 39°28'10"N, 29°15'17"E. – IFC-ESUF 03-1537, 23, 151–216 mm SL; Turkey: Kütahya prov.: stream Yanıkburnu about 25 km east of Dursunbey, 39°33'04"N, 28°56'55"E. – IFC-ESUF 03-1538, 3, 137–185 mm SL; Turkey: Balıkesir prov.: stream Bigadiç west of Bigadiç, 39°23'48"N, 28°04'50"E. – IFC-ESUF 03-1549, 1, 264 mm SL; Turkey: Afyonkarahisar prov.: stream Kali about 15 km west of Çay, 38°32'28"N, 30°50'41"E.

***Chondrostoma beysehirense*:** IFC-ESUF 03-1505, 16, 156–251 mm SL; Turkey: Konya prov.: Beyşehir Lake about 20 km south of Şarkikaraağaç, 37°52'42"N, 31°20'46"E.

***Chondrostoma fahirae*:** IFC-ESUF 03-1512, 36, 60–127 mm SL, Turkey: Burdur prov.: Başpınar Spring about 13 km south of Tefenni, 37°11'08"N, 29°45'16"E. – IFC-ESUF 03-1551, 1, 92 mm SL, Turkey: Burdur prov.: Dalaman River about 4 km north of Yusufça, 37°13'37"N, 29°32'57"E.

***Chondrostoma holmwoodii*:** IFC-ESUF 03-1513, 19, 68–160 mm SL; Turkey: Manisa prov.: Gediz River at Derbent, 38°46'37"N, 29°12'41"E. – IFC-ESUF 03-1514, 7, 58–118 mm SL; Turkey: Manisa prov.: Gediz River about 16 km east of Kula, 38°35'46"N, 28°48'30"E. – IFC-ESUF 03-1515, 1, 112 mm SL; Turkey: Manisa prov.: Gediz River about 15 km north of Kula, 38°40'08"N, 28°36'14"E. – IFC-ESUF 03-1516, 1, 145 mm SL; Turkey: Manisa prov.: Gediz River about 5 km east of Gölmar-mara, 38°42'08"N, 27°58'10"E. – IFC-ESUF 03-1517, 3,

85–102 mm SL; Turkey: İzmir prov.: Gediz River about 8 km east of Menemen, 38°37'42"N, 27°10'41"E.

***Chondrostoma meandrense*:** IFC-ESUF 03-1519, 45, 120–209 mm SL, Turkey: Denizli prov.: Işıklı Spring, 38°19'19"N, 29°51'10"E. – IFC-ESUF 03-1522, 19, 96–151 mm SL, Turkey: Denizli prov.: stream Küfi about 4 km north of Işıklı, 38°21'48"N, 29°50'56"E. – IFC-ESUF 03-1523, 20, 110–219 mm SL, Turkey: Afyonkarahisar prov.: Stream Karasandıklı 0.5 km east of Karasandıklı, 38°31'40"N, 30°10'39"E. – IFC-ESUF 03-1525, 4, 65–138 mm SL, Turkey: Afyonkarahisar prov.: Suçikan Spring 0.5 km east of Dinar, 38°04'14"N, 30°10'38"E. – IFC-ESUF 03-1561, 21, 50–158 mm SL, Turkey: Denizli prov.: Büyük Menderes River about 2 km west of Çıtak, 38°09'23"N, 29°38'24"E. – IFC-ESUF 03-1562, 3, 125–154 mm SL, Turkey: Denizli prov.: Büyük Menderes River about 1 km north of Hançalar, 38°07'54"N, 29°23'19"E.

***Chondrostoma turnai*:** IFC-ESUF 03-1524, 44, 75–210 mm SL; Turkey: Aydın prov.: stream Çine about 8 km south of Aydın, 37°45'43"N, 27°50'12"E. – IFC-ESUF 03-1563, 1, 145 mm SL; Turkey: Denizli prov.: Cindere reservoir about 8 km south of Güney, 38°05'40"N, 29°01'32"E. – IFC-ESUF 03-1564, 3, 92–99 mm SL; Turkey: Denizli prov.: Yenicekent DSI Pomp about 3 km east of Yenicekent, 38°02'16"N, 28°57'47"E. – IFC-ESUF 03-1565, 15, 113–175 mm SL; Turkey: Aydın prov.: Akçay Stream about 3 km east of Sırma, 37°36'18"N, 28°29'34"E. – IFC-ESUF 03-1569, 1, 239 mm SL; Turkey: Denizli prov.: Vali Recep Yazıcıoğlu reservoir about 3 km east of Denizli, 37°46'14"N, 29°07'39"E.

Material used in molecular genetic analysis

***Chondrostoma angorense*:** IFC-ESUF DNA-03-1502, 1; Turkey: Eskişehir prov.: stream Akin 0.5 km south of Akin, 39°20'02"N, 30°30'59"E (GenBank accession number: **MT387066**). – IFC-ESUF DNA-03-1501, 3; Turkey: Eskişehir prov.: Porsuk River about 2 km west of Yörükçürka, 39°36'00"N, 30°25'09"E (GenBank accession number: **MT387112–MT387114**). FFR DNA CH5-6, 2; Turkey: Kızılcahamam, Sakarya River drainage, (GenBank accession number: **MW719604, MW719605**). IFC-ESUF DNA-03-1500, 2; Turkey: Sivas prov.: Kızılırmak River about 3 km east of Zara, 39°54'27"N, 37°48'25"E (GenBank accession number: **MW719606, MW719607**). FFR DNA CH18-19, 2; Turkey: Yerköy, Delice stream, (GenBank accession number: **MW719608, MW719609**).

***Chondrostoma beyşehirense*:** IFC-ESUF DNA-03-1505, 2; Turkey: Konya prov.: Beyşehir Lake about 20 km south of Şarkikaraağaç, 37°52'42"N, 31°20'46"E (GenBank accession number: **MT387079–MT387080**). IFC-ESUF DNA-03-1505, 1; Turkey: Konya Prov.: Beyşehir, Beyşehir Lake, 37°55'43"N, 31°21'24"E (GenBank accession number: **MW719597**).

***Chondrostoma fahirae*:** IFC-ESUF DNA-03-1512, 1; Turkey: Burdur prov.: Başpınar Spring about 13 km south

of Tefenni, 37°11'08"N, 29°45'16"E (GenBank accession number: **MT387128**).

***Chondrostoma holmwoodii*:** IFC-ESUF DNA-03-1513, 1; Turkey: Manisa prov.: Gediz River at Derbent, 38°46'37"N, 29°12'41"E (GenBank accession number: **MT387099**). – IFC-ESUF DNA-03-1516, 1; Turkey: Manisa prov.: Gediz River about 5 km east of Gölmarmara, 38°42'08"N, 27°58'10"E (GenBank accession number: **MT387078**). IFC-ESUF DNA-03-1513, 3; Turkey: Derbent Bridge, Manisa Prov.: Gediz River drainage, 38°46'40"N, 29°12'45"E (GenBank accession number: **MW719598–MW719600**).

***Chondrostoma meandrense*:** IFC-ESUF DNA-03-1519, 4; Turkey: Denizli prov.: Işıklı Spring, 38°19'19"N, 29°51'10"E (GenBank accession number: **MT387085–MT387088**). IFC-ESUF DNA-03-1519, 2; Turkey: Işıklı Village, Denizli Prov.: Işıklı Spring, Büyük Menderes River drainage, 38°19'21"N, 29°51'07"E (GenBank accession number: **MW719591–MW719592**). IFC-ESUF DNA-03-1525, 3; Turkey: Afyonkarahisar prov.: Suçikan Spring, Büyük Menderes River east of Dinar, 38°04'14"N, 30°10'38"E (GenBank accession number: **MW719593–MW719595**). FFR DNA CH13, 1; Turkey: Akçay, Büyük Menderes River drainage (GenBank accession number: **MW719596**).

***Chondrostoma turnai*:** IFC-ESUF 03-1557, 5; Turkey: Aydın prov.: Stream Çine about 8 km south of Aydın, 37°45'43"N, 27°50'12"E. IFC-ESUF DNA-03-1524, 3; Turkey: Çiftlikburnu Village, Aydın Prov.: Çine Stream, Büyük Menderes River, 37°42'52"N, 27°50'04"E (GenBank accession number: **MW719601–MW719602**).

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