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Taxonomic review of the *Chondrostoma* (Teleostei, Leuciscidae) species from inland waters of Turkey: an integrative approach

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Abstract

The genus *Chondrostoma* (Leuciscidae: Leuciscinae), composed of small to medium-sized fish with a scraper feeding characteristic, is distributed in the West and Middle East, Caucasus, Europe and Northern Mediterranean drainages. This genus spreads across Anatolia and Thrace, with the exception of Göksu and Eşen rivers in Turkey's Mediterranean basin. It is also difficult to understand the systematics of *Chondrostoma*, which is complicated morphologically. Therefore, in this study, an identification key was made by evaluating external morphology, osteology (some jaw bones and 5th ceratobrachial) and molecular features together. A total of 13 valid species have been so far recorded from Turkish inland waters, among which are *C. beysehirense*, *C. ceyhanensis*, *C. colchicum*, *C. cyri*, *C. holmwoodii*, *C. kinzelbachi*, *C. meandrense*, *C. nasus*, *C. regium*, *C. smyrnae*, *C. toros*, *C. turnai* and *C.vardarense.* Our molecular data showed that *C. angorense* (Kızılırmak and Sakarya rivers) is a synonym of *C. colchicum* (Çoruh and Yeşilırmak rivers). In addition, *C. angorense* was morphologically similar to *C. colchicum*. Therefore, we explored the systematic position of *C. vardarense* (from Meriç River) and *C. nasus* (from Simav River) in this study.

Key Words

Chondrostoma, freshwater fish, phylogeny, taxonomy

Introduction

The family Leuciscidae is one of the most diversified monophyletic families in the Holarctic Cypriniformes order and has a wide distribution area. Despite abundant research, there have still been different hypotheses concerning their speciation and intrafamilial diversification. They were classified into 7 subfamilies based on morphological characters, 6 subfamilies on osteology, and 6 subfamilies on molecular characters. These subfamilies are Pogonichthyinae, Leuciscinae, Plagopterinae, Lavininae, Phoxininae and Pseudaspininae. The family consists of 358 taxa, according to molecular phylogenetic classification (Schönhuth et al. 2018; Van der Laan et al. 2020). Many studies reported that *Chondrostoma* (Leuciscidae: Leuciscinae) shows a wide geographical distribution across Europe and Asia. It is evident throughout Europe: from the continent's eastern border to the Atlantic coast, from the southern Mediterranean to the Baltic Sea, the Danube River basin and the Thrace region, and throughout Asia: all of Anatolia, the Caucasus and the Middle East, the Tigris-Euphrates basins, the Caspian Sea basin of Iran, Esfahan and the Kor rivers basins (Esmaeili et al. 2014; Coad 2017; Eagderi et al. 2017; Küçük et al. 2017; Güçlü et al. 2018).

A recent molecular phylogenetic study showed that the genus consisted of two lineage groups, the first of which was the Mediterranean species and the other was the Danube and Mesopotamian species (Durand et al. 2003). Robalo et al. (2007) determined the phylogeny of

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Chondrostoma using molecular data (mitochondrial gene and nuclear actin gene). It has been reported, however, that rasping during feeding may change mouth structure, resulting in morphology-based misidentification. Also, some bones (jaw, ethmoid, basioccipital and pharyngeal etc.) were reported to be sinomorphic (Robalo et al. (2007). Küçük et al. (2017) which suggested that osteology and molecular data could be more suitable in the clarification of *Chondrostoma* systematics than classical morphological data.

Çiftçi et al. (2020) investigated the phylogeny of previously described *Chondrostoma* species in Turkish inland waters. Their study found that Regium and Nasus groups consisted of inland waters of Turkey. The first group represents the species in the Caucasus, Black Sea region and Marmara region and, in the Aegean and Central Anatolian basin, while the second group represents species dispersed in the eastern Mediterranean rivers and Tigris-Euphrates rivers basin. In addition, *C. nasus* was recorded from the Simav River (Susurluk basin). On the other hand, *Chondrostoma fahirae* was not included in all molecular studies (Çiftçi et al. 2020). In the latest study (Turan et al. 2021), *C. fahirae* was re-identified from the Dalaman River basin in south western Turkey as the species *Turcichondrostoma fahirae*.

As mentioned above, *Chondrostoma* species, which are distributed in the inland waters of Anatolia and Thrace, have not been fully addressed until recently due to their complex morphology and lack of distinctive taxonomical characters. In this study, we gathered the comprehensive external morphology, osteology (some jaw bones and 5th ceratobrachial) and molecular data on the genus. In addition to traditional morphology, we classified the genus *Chondrostoma* based on the osteology (premaxilla, maxilla, dentary and fifth brachial gill arc) and molecular data (16S rRNA, Cytb and COI). Furthermore, we addressed the external morphology and osteology of *C. nasus*, which is widely distributed throughout Europe and recorded from Simav River (Geiger et al. 2014) and of *C. vardarense*, which was already known from Meriç (Evros) River.

Material 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). Fish samples were caught with electroshock and fishing nets from all regions of Turkey (Fig. 1). After anaesthesia (anesthesia), fishes were fixed in 4% formaldehyde and stored in 70% ethanol or directly fixed in 99% ethanol. 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. 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 anterior-most rays are deeply embedded.

For osteological preparations, one specimen of a Regium group (*C. regium*, 182.5 mm from Tigris River), two specimens Nasus group (*C. nasus*, 181.2 mm SL from Simav River and *C. meandrense*, 183.8 mm SL from Işıklı Lake spring) were cleared and stained with alizarin red S, according to the protocol of Taylor and van Dyke (1985). Osteology of *C. meandrense* from Turan et al. (2021), *C. smyrnae* and *C. turnai* from Küçük et al. (2021). The specimens were studied using a stereomicroscope (Nikon SMZ1500), and photos taken with a digital machine with a glycerol bath. The nomenclature of the skeletal elements was followed Bogutskaya (1996). Vertebral counts were obtained from radiographs and counted as total, pre-dorsal, abdominal and caudal vertebrae following Naseka (1996).

Abbreviations used

SL, standard length; **HL**, head 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; **FSJF**, Fischsammlung J. Freyhof, Berlin.

Molecular analyses

Total DNA extraction was carried out from ethanol-preserved tissue samples of each specimen using Invitrogen PureLink ® Genomic DNA Mini Kit following the manufacturer's protocols, and stored at −20 °C prior to use. The mitochondrial cytochrome b (Cytb) 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 and sequenced with Forward (5'– AGG CGG CTT CTC AGT AGA CA -3') and Reverse (5'– AGA AAT TTT GTC GGC GTC TG -3') internal primers (Çiftçi et al. 2020). Partial sequence of mitochondrial 16S ribosomal RNA gene (577 bp) was amplified using the Forward (5' – AAG CCT CGC CTG TTT ACC AA - 3') and Reverse (5' – CTG AAC TCA GAT CAC GTA GG - 3') (Robalo et al. 2007) primer pairs. The PCR reaction was carried out in a final volume of 50 µl using 25 µl of PCR Master Mix (2X) (Promega Corporation, Madison, WI, USA), 5 µl of template DNA (25–50 ng/µL), 2 µl each of forward and reverse primers (10 pM of each primer), and

ddH2O to the total volume. PCR amplifications were performed using the following protocol: initial denaturation at 94 °C for 1 min, followed by denaturation at 94 °C (1 min), annealing at 60 °C (55 °C for 16S rRNA) (30 s) and primer elongation at 72 °C (1.5 min for Cytb and 1.0 min for 16S rRNA), repeated for 35 cycles, a final elongation cycle at 72 °C for 5 min, and cooling to 4 °C. Successfully amplified PCR products were purified prior to sequencing using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to protocol supplied by the manufacturer. The purified DNA fragments were sent to Macrogen (Macrogen Inc., Seoul, Korea; www.macrogen.com) for sequencing of both strands. Sequences were assembled and edited in BIOEDIT (Hall 1999) and aligned with CLUSTAL W (Thompson, Higgins and Gibson 1994) as implemented in BIOEDIT. All sequences of *Chondrostoma* species were deposited in GenBank under accession numbers given in Suppl. material 1: Table S1.

For the phylogenetic analyses, three data sets were used, 577 bp fragment of 16S rRNA gene, 1140 bp of Cytb and 652 bp fragment of COI sequences. 16S rRNA, Cytb and COI sequences were aligned with the previous sequences from GenBank (Suppl. material 1: Table S1) 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. To determine whether the DNA sequence datasets (16S rRNA, Cytb and COI) were congruent, an incongruence length difference (ILD) test (Farris et al. 1995) implemented as partition homogeneity test in PAUP 4.0b10 (Swofford 1998) was performed using 1000 partition replicates, each comprising 100 random sequence addition replicates, and TBR branch swapping. Invariant characters were removed from the data sets prior to performing the ILD test (Cunningham 1997). The partition homogeneity test revealed no significant difference (P value = 1 - (599/1000) = 0.401) between three data partitions (16S rRNA, Cytb and COI), indicating that the three data sets could be combined for analysis. 16S rRNA, Cytb and COI sequences of *Turcichondrostoma fahirae* and *Telestes souffia* were also included in the phylogenetic analysis as outgroup (Suppl. material 1: Table S1).

To assess the best fitting nucleotide substitution model for combined data set of *Chondrostoma* species, we used the jModelTest v.0.1 (Guindon and Gascuel 2003; Posada 2008), based on hierarchical series of likelihood ratio tests. GTR+I+G substitution model (gamma shape = 0.7890; p-inv = 0.6520) was selected as the best fit to the genus *Chondrostoma* data set by the lowest AIC score in jModeltest 0.1.1 (Posada 2008) and therefore, we used this model for the subsequent phylogenetic analysis. Phylogenetic trees were generated using Bayesian inference (BI), and maximum likelihood (ML) to determine the evolutionary relationships among haplotypes. PhyML 3.0 (Guindon and Gascuel 2003) software was used for ML analyses. Bootstrap tests (Felsenstein 1985) were performed with 1000 pseudo replicates for ML analyses with the same software used for phylogenies. Bayesian inferences of *Chondrostoma* phylogeny were performed as implemented in MrBayes 3.2 (Ronquist et al. 2012) software. Four independent Markov Monte-Carlo coupled chains were run with 10^6 generations and sampled every 100

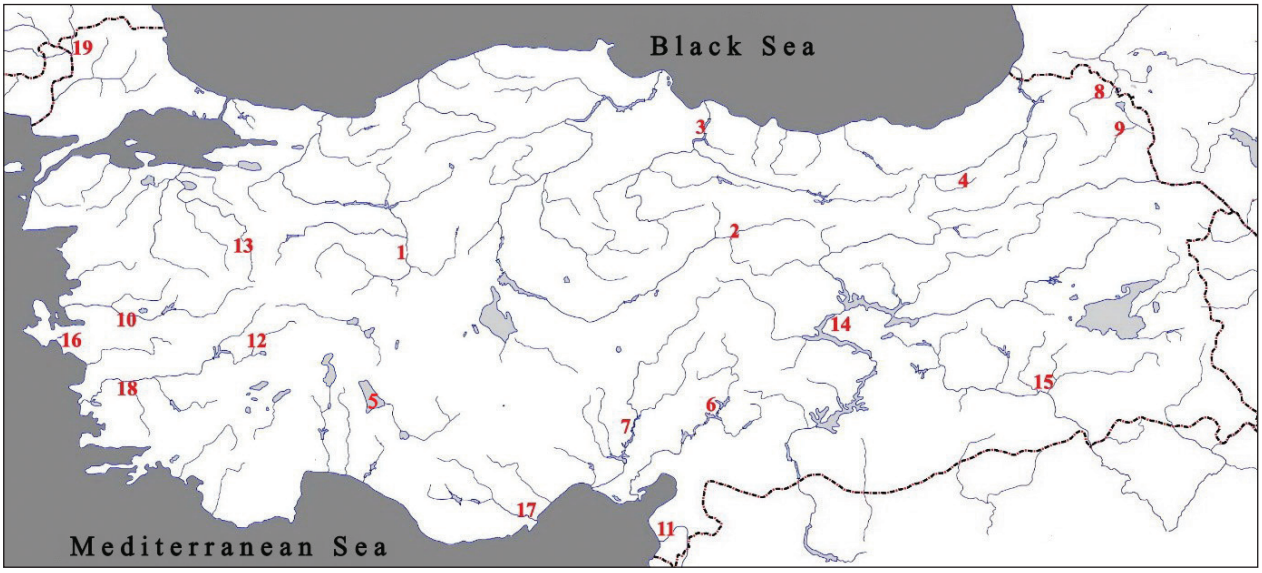

Figure 1. Distribution of *Chondrostoma* species in inland waters of Turkey (1. *C. colchicum* – Akin Stream, Sakarya River; 2. *C. colchicum* – Kızılırmak River; 3. *C. colchicum* – SuatUğurlu reservoir, Yeşilırmak River; 4. *C. colchicum* – Çoruh River; 5. *C. beysehirense* – Beyşehir Lake; 6. *C. ceyhanensis* – Sır reservoir, Ceyhan River; 7. *C. ceyhanensis* – Seyhan reservior, Seyhan River; 8. *C. cyri* – Kura River; 9. *C. cyri* – Aras River; 10. *C. holmwoodii* – Gediz River; 11. *C. kinzelbachi* – Gölbaşı Lake, Asi River; 12. *C. meandrense* – Işıklı Spring, Büyük Menderes River; 13. *C. nasus* – Simav Stream; 14. *C. regium* – Karakaya reservoir, Euphrates River; 15. *C. regium* – Ongözlü Bridge, Tigris River; 16. *C. smyrnae* – Tahtalı reservoir; 17. *C. toros* – Göksu River; 18. *C. turnai* – Çine Stream, Büyük Menderes River; 19. *C. vardarense* – Meriç River).

generations to yield tree topologies. The first 25% generations were discarded as burn-in. FigTree v.1.4.4 (Rambaut 2018) was used to build constrained trees. A calculation of pairwise genetic distance among different species with Kimura 2-parameter (K2P; Kimura 1980) model was performed using MEGA X (Kumar et al. 2018).

Results

Morphology of *Chondrostoma*

The body is typically fusiform, deep and somewhat cylindrical. The mouth position inferior, mouth usually straight or slightly arched, but markedly arched in *C. meandrense* and *C. cyri* (Fig. 2a–d), the lower lip is covered with a horny layer. The pre-dorsal distance is between 47.1–55.0/SL, pelvic-fin origin, usually in front of the dorsal fin origin; pre-pelvic distance /SL ratio is 48.4–55.0. Body depth markedly high, its depth 19.3–28.4/SL, the caudal peduncle length/its depth ratio is between 1.73–1.92. The caudal-fin deeply forked and the lobes are pointed (some species have a black band on the outer margin of the caudal fin); eye diameter relatively large, the eye diameter/HL ratio is 17.5–25.2 (Table 1). A complete lateral line with 44–74 total scales, 8–11 scale rows between the lateral line and the dorsal-fin origin, 4–7 scale rows between the lateral line and the pelvic fin-origin (Table 2). The scales are partially angular and arranged properly on the body, and rays are positioned anteriorly and posteriorly. The focus closer to the anterior edge of the scale. The angle between the focus and anterior margin varies by species.

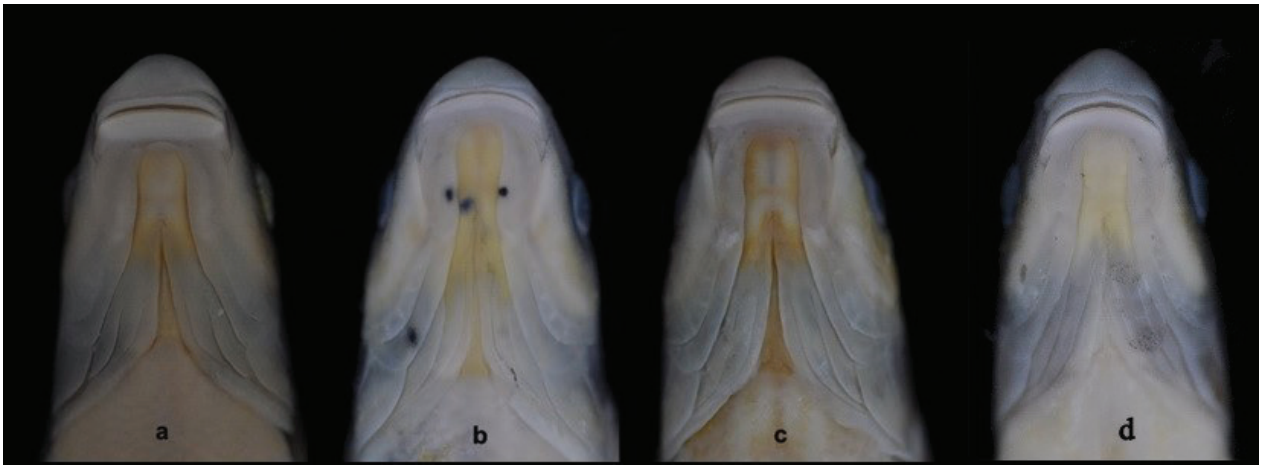

Figure 2. Mouth shape of *Chondrostoma* species (**a.** *C. nasus*; **b.** *C. meandrense*; **c.** *C. regium*; **d.** *C.cyri*).

Table 1. Some Morphometric data of *Chondrostoma* species of Turkey.

Species	Body depth / SL (%)			Pre-dorsal / SL (%)			Pre-pelvic / SL (%)			Eye diameter / HL (%)			Caudal peduncle length / depth (%)		
	min	max	x̄	min.	max	x̄	min	max	x̄	min	max	x̄	min	max	x̄
C. colchicum (Sakarya)	22.5	24.5	23.8	49.2	55.0	52.1	51.8	54.3	53.2	22.1	25.2	23.3	1.69	1.92	1.79
C. colchicum (Kızılırmak)	21.8	23.2	22.7	49.4	53.8	51.7	51.9	55.2	53.5	18.7	22.3	20.4	1.74	2.11	1.91
C. colchicum (Yeşilırmak)	21.1	24.1	21.9	48.5	52.5	50.7	50.9	53.2	52.1	19.5	23.0	21.8	1.70	2.18	1.91
C. colchicum (Çoruh)	22.6	24.9	23.8	49.6	50.8	49.9	49.6	51.4	50.9	15.8	18.3	17.3	1.67	1.88	1.75
C. beysehirense (Beyşehir)	21.7	25.2	23.1	48.6	50.9	49.7	48.9	51.0	50.0	18.3	20.5	19.3	1.71	1.95	1.86
C. ceyhanensis (Ceyhan)	20.9	24.5	23.2	48.5	52.9	50.9	50.5	53.1	51.4	18.9	23.8	22.3	1.61	1.94	1.80
C. ceyhanensis (Seyhan)	20.1	24.1	22.8	48.1	53.2	51.2	50.2	53.4	51.0	19.2	23.4	22.7	1.57	1.90	1.84
C. cyri (Aras)	19.3	21.8	20.9	47.1	50.5	49.4	50.1	52.3	51.5	20.0	23.1	22.1	1.73	2.02	1.91
C. cyri (Kura)	19.0	21.2	20.0	46.8	50.0	49.9	50.7	51.8	51.4	19.6	23.0	22.7	1.68	2.03	1.94
C. holmwoodii (Gediz)	22.0	24.8	23.6	50.7	54.0	52.2	50.6	54.2	52.7	17.6	22.4	20.6	1.62	1.94	1.78
C. kinzelbachi (Asi)	19.7	22.2	20.7	48.7	53.3	50.0	48.4	51.2	49.7	18.4	22.2	20.0	1.80	2.03	1.92
C. meandrense (B.Menderes)	23.0	25.5	24.1	49.6	52.1	51.1	50.7	53.4	52.3	20.1	23.6	21.9	1.70	1.92	1.80
C. nasus (Simav)	23.1	25.9	24.1	50.2	52.7	51.6	50.9	53.3	52.0	18.2	21.0	19.9	1.64	1.81	1.73
C. nasus (Danube)	23.1	25.9	24.1	50.2	52.7	51.6	50.9	53.3	52.0	18.2	21.0	19.9	1.64	1.81	1.73
C. nasus (Rhine)	23.5	26.2	24.3	50.5	52.5	51.2	50.1	53.3	51.8	18.0	21.1	19.6	1.52	1.82	1.70
C. regium (Tigris)	19.8	22.5	21.3	47.1	50.7	49.0	49.0	52.1	50.5	19.0	23.8	21.4	1.77	2.00	1.85
C. regium (Euphrates)	19.6	21.9	21.6	47.0	50.1	48.4	49.2	52.1	50.0	19.5	23.2	19.8	1.70	2.05	1.81
C. smyrnae (Tahtalı)	24.2	28.4	25.9	49.0	52.2	50.8	51.1	54.6	53.1	17.5	22.8	20.7	1.65	2.04	1.85
C. toros (Göksu)	23.3	26.7	24.5	49.9	53.1	51.8	52.3	55.0	53.5	19.5	24.9	22.7	1.64	1.88	1.74
C. turnai (Çine)	22.9	27.0	24.6	50.0	54.5	51.5	50.1	53.8	52.7	20.1	24.7	22.2	1.45	1.85	1.74
C.vardarense (Meriç)	22.7	25.0	24.1	47.3	52.9	50.1	49.9	53.9	51.8	17.5	22.5	19.6	1.59	1.94	1.77

Osteology of *Chondrostoma*

In the classification of *Chondrostoma* species, some jaw bones (premaxilla, maxilla and dentary), hyomandibular bone, the 5th ceratobrachial arc and pharyngeal teeth, and the numbers of vertebrae were examined.

The anterior part of the dentary medially curved at an angle of about 90 degrees or slightly contorted. There is a cavity on its outer surface where the keratin layer is found. This cavity is larger and deeper in species, a well-developed keratinized layer. The coronoid process is usually anteriorly inclined or slightly vertical unlike in *C. meandrense*.

Premaxilla deep and without ascendant process, slightly concave for scraping, bottom edge sharp, posterior edge is thin and long; maxilla is deep and ascending process is vertical (Fig. 3a–c). Hyomandibular bone is long-narrow or short-wide, it varies across species. Upper edge of 5th ceratobranchial enlarged and axe-shaped. Pharyngeal teeth knife-like in one row; 5-5, 5-6, 6-6, 6-7, 7-7; some species slightly serrated and slightly hooked at tip (Fig. 4a–c); in *C. colchicum*, *C. cyri* and *C. meandrense* usually 5-5 or 5-6 (Table 2). The operculum bone is square-shaped; the preoperculum is "L" shaped and interoperculum is short, whereas the suboperculum is narrow and long. There are 20–39 gill rakers on first gill arch, vertebrae formula: 42–49:25–29+17–21, the lowest number of vertebrae are in *C. meandrense* (42) and the highest in *C. regium* (49) (Table 2).

External morphology and osteology of *Chondrostoma* did not support the divergence of two lineage groups, Nasus and Regium, based on molecular (16S rRNA, Cytb and COI) data. However, with a few exceptions in all Regium group species (*C. regium*, *C. ceyhanensis*, *C. kinzelbachi*, *C. toros* and *C. vardarense*); the mouth straight or slightly arched (Fig. 2c), the keratinised layer in the lower jaw and the cavity in the anterior edge of the dentary well-developed, coronoid process of dentary slightly anteriorly inclined (Fig. 3c). In the Nasus lineage group, the mouth is slightly arched but straight in *C. nasus* (Fig. 2a, b, d) the keratinised layer on the lower jaw slightly developed and dentary coronoid process slightly vertical in *C. beysehirense*, *C. cyri*, *C. meandrense*, *C. smyrnae* and *C. turnai*, albeit anteriorly inclined in *C. colchicum* and *C. holmwoodii* (Fig. 3b).

C. fahirae, which was included in the genus *Chondrostoma* by Freyhof and Özuluğ (2015), was identified as *Turcichondrostoma fahirae* (Turan et al. 2021). This new genus is clearly distinguished from other *Chondrostoma* species by their external morphology and osteology. *T. fahirae* also has reduced lateral line scales, gill rakers on first gill arch and pharyngeal teeth, 45–52, 9–14 and 5–5, respectively. Mouth arched, no keratinized layer on lower jaw; dentary short and its anterior edge not contorted, the cavity in the anterior edge of the dentary is slightly developed and coronoid prosses markedly vertical, with ascending process of premaxilla well developed and vertical, pharyngeal teeth except one row 5–5, thin-long and hooked (Turan et al. 2021), as are the genus *Alburnus*, *Pseudophoxinus* and *Squalius*, upper edge of 5th ceratobranchial not enlarged.

Table 2. Meristic characters of *Chondrostoma* species in Turkey.

Species	Lateral line scales	scales between LL-D fin origin	scales between LL.-V fin origin	D	A	Gill rakers	Pharyngeal teeth	Total vertebrae
C. colchicum (Sakarya)	59–68	9–10	5	8–9	9–11	22–25	6–5/6–6	46–47
C. colchicum (Kızılırmak)	59–66	9–10	5	9	9–11	20–25	6–6	47–48
C. colchicum (Yeşilırmak)	59–62	9	4–5	8–9	9–11	22–26	6–5/6–6	44–46
C. colchicum (Çoruh)	57–63	9–10	4–5	8–9	9–10	24–26	5–5/6–6	46
C. beysehirense (Beyşehir)	60–71	9–11	5–6	8	8–10	33–39	6–7/7–7	45
C. cyri (Aras)	54–59	8	4	8	9–10	20–24	6–5	45–46
C. cyri (Kura)	56–67	8–9	4–5	8–9	9–10	21–24	6–5/6–6	45–46
C. holmwoodii (Gediz)	60–66	9–11	5–7	8	9–10	21–24	6–6	45–46
C. ceyhanensis (Seyhan)	59–68	9–1	4–6	8–9	9–10	25–29	5–6/6–6	46–48
C. ceyhanensis(Ceyhan)	59–66	10–11	4–6	8–9	9–11	24–28	6–6	47–48
C. kinzelbachi (Gölbaşı)	59–73	10–11	5–6	8–9	10–11	32–34	6–6/7–7	46–47
C. meandrense (B.menderes)	55–60	8–10	4–5	8–9	9–10	23–28	6–5/6–6	42
C. regium (Tigris)	63–69	9–11	4–6	8–10	10–12	25–32	6–6/7–6	46–47
C. regium (Euphrates)	62–73	10–11	4–6	9	10	27–30	6–6/7–6	48–49
C. nasus (Simav)	64–72	9–10	5	8	9	24–29	6–5/7–6	46–48
C. nasus (Danube)	53–60	8–9	3–4	8–10	9–11	27–31	6–6	48
C. nasus (Rhine)	57–63	8–9	5–6	8–9	9–10	30–31	6–6	48
C. smyrnae (Tahtalı)	48–53	8–9	4	7–8	8–10	19–23	5–6/5–5	43
C. toros (Göksu)	56–64	10–11	4–6	8–9	9–11	26–29	6–6	47
C. turnai (Çine)	44–51	8–9/3–4	3–4	8	9–10	22–27	5–5/6–6	43
C.vardarense (Meriç)	57–64	8–9/4–5	4–5	8	9–10	28–31	6–6	47

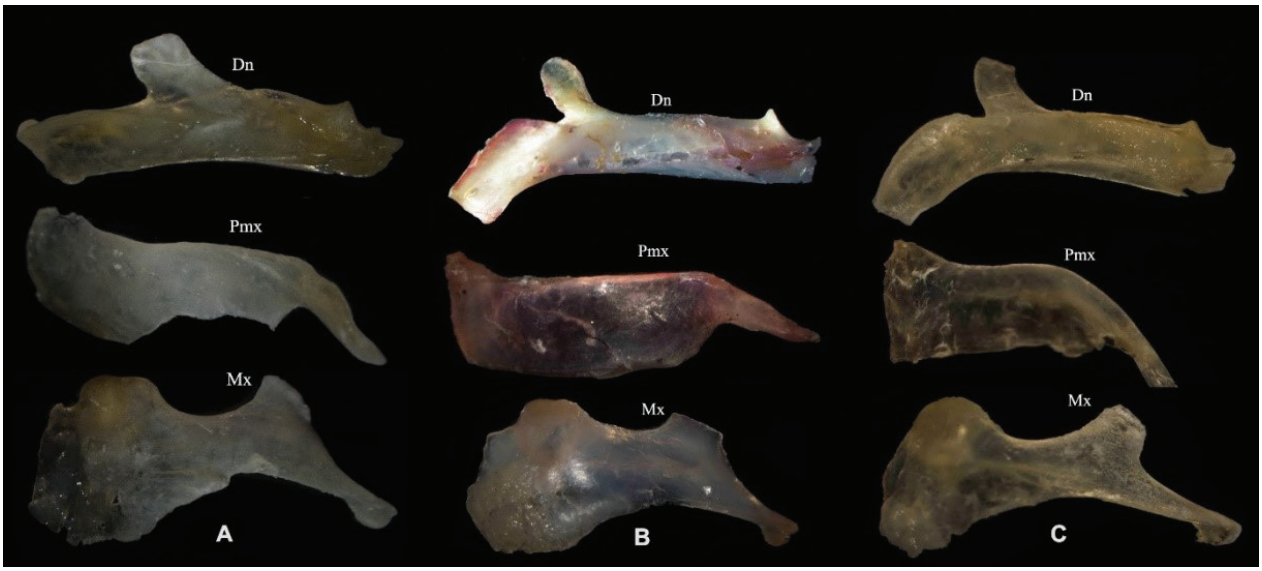

Figure 3. Dentary, premaxilla and maxilla bones of some *Chondrostoma* species (**a.** *C. nasus*; **b.** *C. meandrense*; **c.** *C. regium*).

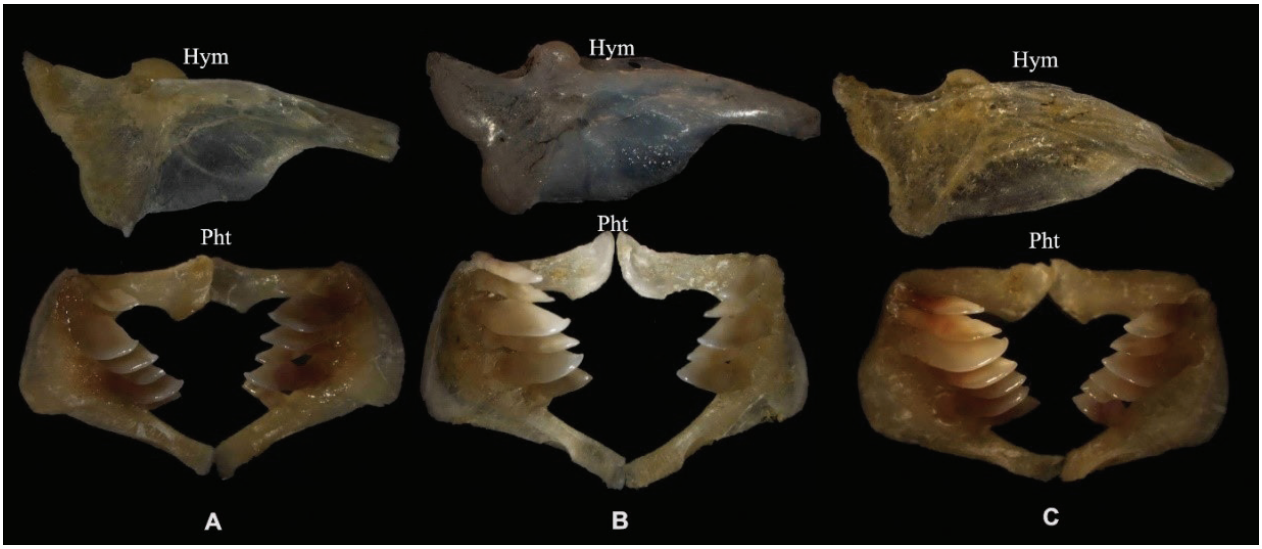

Figure 4. Hiyomandibular and pharyngeal teeth of some *Chondrostoma* species (**a.** *C. nasus*; **b.** *C. meandrense*; **c.** *C. regium*).

Results of molecular analyses

A total of 146 specimens from Anatolian *Chondrostoma* species were sequenced and deposited in GenBank with accession numbers ON796577–ON796688 for 16S rRNA and OL870982–OL871061 for Cytb gen regions. In total, 77 published cyt b sequences and 63 published COI sequences from GenBank were downloaded (Suppl. material 1: Table S1). The 16S rRNA, Cytb and COI sequences of each species were concatenated to generate a combined data set. The final data set had a total of 2369 (for 16S rRNA: 577 bp, Cytb: 1140 bp and COI: 652 bp) nucleotide positions without insertion, deletion, gap and stop codon. Phylogenetic relationships between sequences were reconstructed for the combined data set using the BI and ML method. The phylogenetic trees (BI and ML) showed congruent topologies with high posterior probability (PP) and bootstrap (BP) values ranging from 0.51 to1.0 and 60.5% to 100%, respectively and were consistently divided into two major lineages (Regium and Nasus lineages) (Fig. 5). Regium lineage at the base of the tree represents sixteen localities (locality codes: A, Ma, Q, U, Z, FIR, E, P, GO, D, H, J, M, Sr, BE and ME) from the Tigris and Euphrates rivers to Ceyhan, Seyhan, Berdan and Göksu rivers, the Orontes River and its main branches. Nasus lineages consists of seventeen localities (locality codes: Ak, V, Y, CO, KI, YE, I, S, T, F, O, R, L, K, C, B and G) from rivers draining into the Sea of Marmara, the southwest of the Anatolian Peninsula, the Black Sea Basin and Caucasian basin from north-eastern Turkey. Within Regium lineage, there were three well-supported clades. The first clade (CI) contains species (*C. vardarense*) from the Meriç River (Evros), the longest river flowing entirely within the borders of the Balkan region, eventually emptying into the Aegean Sea near Enez in Turkey. The second clade (CII) includes the monotypic species (*C. regium*) from the Tigris and Euphrates River basins in the eastern part of Turkey. The third clade (CIII) comprises

the species of the eastern Mediterranean represented by three monophyletic, easily distinguished subclades, namely *C. ceyhanensis*, *C. kinzelbachi* and *C. toros*. Within the Nasus lineage there were four clades. The first clade (CIV) includes *C. beysehirense* from Lake Beyşehir in the provinces of Isparta and Konya in south-western Turkey. The second clade (CV) includes *C. cyri* from Lake Çıldır and the tributaries of the Kura River in Ardahan Province. The third clade (CVI) consisted of three subclades of Aegean species; the first subclade included only *C. holmwoodii* from Lake Marmara and Gediz River, the second subclade consisted of *C. smyrnae* from the Şaşal Stream, which was recently described (Küçük et al. 2021), and the third subclade was formed only by *C.turnai* from the Çine Stream. The last clade (CVII) included three species, *C. colchicum*, *C. meandrense* and *C. nasus*, from rivers draining into the Sea of Marmara, the southwest of the Anatolian Peninsula and the Black Sea basin.

For the combined mitochondrial genes (16S rRNA, Cytb and COI), interspecific and intraspecies genetic distance values (K2P) are given in Table 3. The molecular analysis in this study shows that the interspecific genetic distances between the analysed *Chondrostoma* species ranged from 0.866% (between *C. colchicum* and *C. meandrense*) to 3.911% (between *C. toros* and *C. smyrnae*). On the other hand, intraspecific genetic distances within *Chondrostoma* species ranged from 0.015% for *C. holmwoodii* and 0.664% for *C. kinzelbachi* (Table 3).

Table 3. Average intergeneric and intrageneric distance of Cyt b, 16S rRNA and COI combined data set for *Chondrostoma* species based on 1,000 bootstrap replications using K2P distance method; values in lower left cells = percent difference among taxa and diagonal = percent difference within taxa.

		N	1	2	3	4	5	6	7	8	9	10	11	12	13
1	***C. colchicum***	16	0.275												
2	***C. meandrense***	9	0.866	0.162											
3	***C. nasus***	10	1.051	1.041	0.324										
4	***C. turnai***	7	1.868	1.942	1.836	0.228									
5	***C. smyrnae***	4	1.922	2.004	1.975	1.574	0.087								
6	***C. holmwoodii***	8	1.740	1.950	2.042	2.002	2.057	0.015							
7	***C. cyri***	8	1.763	1.808	1.850	2.315	2.432	2.285	0.301						
8	***C. beysehirense***	2	1.731	1.685	1.644	2.160	2.124	2.282	1.867	0.4091					
9	***C. ceyhanensis***	16	2.852	2.394	2.783	3.588	3.489	3.509	2.903	2.664	0.464				
10	***C. toros***	4	3.202	2.872	3.130	3.967	3.911	3.635	3.272	3.174	1.792	0.058			
11	***C. kinzelbachi***	3	3.023	2.731	3.121	3.884	3.663	3.523	3.041	3.001	1.685	1.948	0.664		
12	***C. regium***	22	2.642	2.643	2.673	3.232	3.224	2.981	2.562	2.467	1.777	2.276	1.932	0.438	
13	***C. vardarense***	3	3.060	3.196	3.282	3.849	3.678	3.527	3.366	3.439	3.015	3.187	3.158	2.845	0.078

Zoogeography of *Chondrostoma* species in the inland water of Turkey

Anatolia is well isolated from its surroundings. Therefore, it has complex lentic, lotic systems and endorheic basins for the diversification of fish taxa. The Anatolian fish species are Tethys Sea origins, Glacier relicts, Central and Western Europe origins, Central and Western Asia origins, Mesopotamia origins, Sarmatian Sea origins and have entered Inland Waters from the Seas (Kuru et al. 2014), but the diversification centre of many species is Anatolia, for example, genus *Pseudophoxinus* (Hrbek et al. 2002, 2004).

The *Chondrostoma* species showing allopatric speciation in inland waters of Turkey have diversified in all river basins (except between the Göksu and Eşen rivers in the Mediterranean region) (Fig. 1). Of these species, *C. regium* is the most widely distributed species, including the Tigris, Euphrates basins and Sinnep Stream (upper basin of the Quveik River, Northern Syria). There are three *Chondrostoma* species in the Eastern Mediterranean rivers' basin; these are *C. kinzelbachi* (Asi River and Balıklı Lake), *C. ceyhanensis* (Ceyhan, Seyhan and Berdan rivers) and *C. toros* (Göksu River) (Fig. 6).

C. angorense was described by Elvira (1997) from Sakarya River. The same researcher also reported this species from Kızılırmak and Yeşilırmak rivers. This species is distributed between Sakarya and Kızılırmak rivers drainage in Black Sea basin. Therefore, it is possible to see morphological variations among the populations of *C. angorense*; for example, the number of the lateral line scales (57–68), the number of gill rakers on first gill arch (20–26) (Table 2), and the development of the keratinised layer in the lower jaw.

We compared *C. angorense* specimens from Sakarya (Porsuk River and Akin Stream) and Kızılırmak rivers with *C. colchicum*. No morphological or genetic differences were found between these populations (Çiftçi et al. 2020). The number of scales on the lateral line (59–68 in Sakarya, 59–66 in Kızılırmak, 59–62 in Yeşilırmak and 57–63 in Çoruh), scales rows between lateral line and dorsal-fin origin (9–10 in Sakarya, Kızılırmak and Çoruh, 9 in Yeşilırmak) and scales rows between lateral line and pelvic-fin origin (5 in Sakarya and Kızılırmak, 4–5 in Yeşilırmak and Çoruh), gill rakers on first gill arch (20–25 in Kızılırmak and Sakarya, 22–26 in Yeşilırmak and Çoruh) were similar. However, the number of vertebrae was slightly different (46–48 in Kızılırmak and Sakarya, and 44–46 in Yeşilırmak and Çoruh) (Table 2). Furthermore, it has been reported that any differentiation did not exist between these populations at molecular level. There-

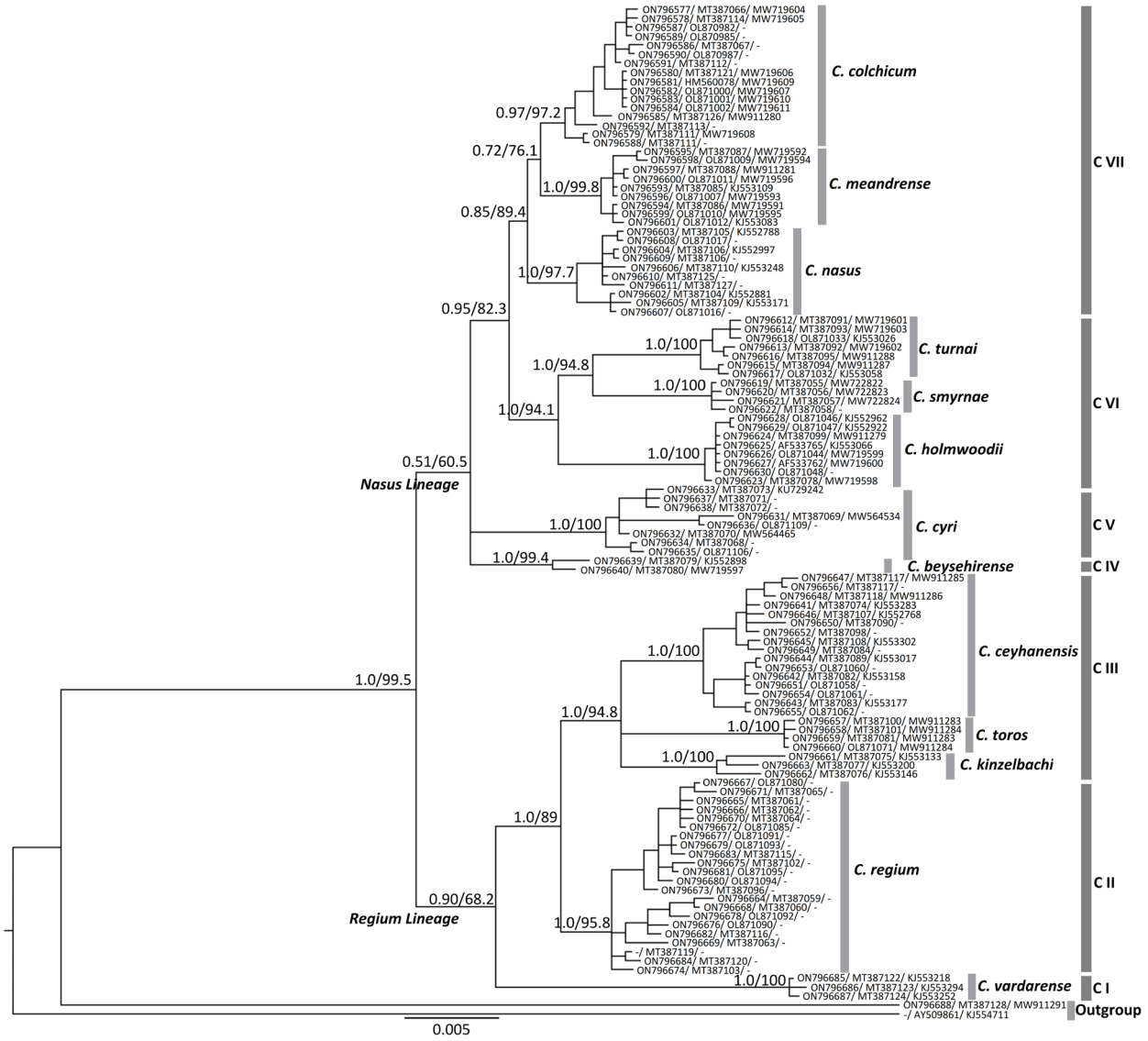

Figure 5. Bayesian inference tree of *Chondrostoma* species based on the combined data set. Bayesian and ML methods yielded identical topologies and so only the Bayesian tree is shown. The numbers above nodes are Bayesian posterior probabilities and maximum likelihood (ML) bootstrap values, respectively (those above 50% are shown).

fore, Çiftçi et al. (2020) proposed that *C. colchicum* specimens from Yeşilırmak and Çoruh rivers are similar to *C. angorense* (Sakarya and Kızılırmak). We recommend that this species is re-examined at morphological and genetic levels by collecting samples of all populations(Tuapse, Çoruh, Yeşilırmak, Kızılırmak and Sakarya rivers).

Kura River is the type location of *C. cyri*, which is only distributed in the Aras and Kura rivers basin. In our study, two localities were sampled; these were the upper tributary of Kura River (Çakır Dere near Göle) and Lake Çıldır (Aras basin). Kaya et al. (2020) reported from Lake Çıldır, Kars Stream, B-20 Canal at Aralık (Aras basin) and Stream Çakır (Kura basin). However, the fossil record of *C. cyri* was found in the Erzurum region during the Pleistocene (1.55–1.0 Mya) period (Böhme and Ilg 2003). *C. beysehirense* is distributed across Beyşehir Lake basin (Beyşehir and Suğla lakes, and Apa reservoir). This species is consumed in limited quantities by the local people in the Beyşehir region (Konya province) (Fig. 7).

Four *Chondrostoma* species are distributed in the Aegean Region rivers (Bakırçay, Gediz, Büyük Menderes and Tahtalı reservoir, near Küçük Menderes basin) of Turkey. *C. holmwoodii* is distributed in the Gediz and Bakırçay rivers basin. This species differs from other Anatolian *Chondrostoma* species by the pale-pink thin band between the operculum and the caudal fin base. The lower basin of the Gediz River is highly polluted so that *C. holmwoodii* populations exist only in the upper basin of this river (upper region of Demirköprü reservoir). On the other hand, İlhan et al. (2020) reported that this species is widespread in all basins of Bakırçay. Two species are diversified in Büyük Menderes, the largest river in the Aegean region. Of them, *C. meandrense* is found in the

middle and upper river basin, and *C. turnai* in the Çine Stream (lower river basin). In previous studies, it was reported that only *C. meandrense* was distributed in this river basin (Elvira 1997; Fricke et al. 2007). However, Geiger et al. (2014) and our project results (TÜBİTAK, KBAG-111T900) led to a new unnamed species different from *C.meandrense* being recorded in the Büyük Menderes River. The new species *C. turnai*, which was described by Güçlü et al. (2018), is known from the lower and middle branches of the Büyük Menderes basin (Çine Stream, Akçay Stream near Nazilli, Yenicekent near Sarayköy) (Fig. 8).

Figure 6. Eastern Mediterranean and South-eastern Anatolia *Chondrostoma* species (all Turkey); **a.** *C. toros*, IFC-ESUF 03-1555, 142.76 mm SL; Mersin prov.: Hamamköy Village, Mut, Göksu River; **b.** *C. ceyhanensis*, IFC-ESUF 03-1556, 208.49 mm SL; Kahramanmaraş prov.: Sır reservoir, Ceyhan River; **c.** *C. kinzelbachi* IFC-ESUF 03-1518, 214.6 mm SL; Hatay prov.: Lake Gölbaşı, Asi River; **d.** *C. regium*, IFC-ESUF 03-1533, 214.7 mm SL; Sivas prov.: Kangal, Euphrates River.

Distribution area of *C. nasus* and *C. vardarense* in Turkey and its taxonomic status

C. nasus has a wide distribution area that spans from the Black Sea basin to the southern Baltic Sea and southern North Sea. It has also been recorded as an invasive species or introduced in some streams in France, Italy and Slovenia, although it has not been recorded from Meriç River (Turkey and Greece) which originates from Europe and flows into the Aegean Sea (Kottelat and Freyhof 2007). However, *C. nasus* is distributed in Simav and Kocaçay rivers (Susurluk River basin) in the Northern Aegean and Marmara regions (Geiger et al. 2014; Çiftçi et al. 2020), and has an interesting zoogeographical distribution pattern.

Figure 7. Central Anatolia, Marmara and Western Black Sea regions *Chondrostoma* species; **a.** *C. beysehirense*, IFC-ESUF 03-1505, 265.3 mm SL, Turkey: Konya prov.: Lake Beyşehir; **b.** *C. cyrii*, IFC-ESUF 03-1511, 144.2 mm SL; Turkey: Kars prov.: Lake Çıldır, Aras River; **c.** *C. colchicum*, IFC-ESUF 03-1501, 169.4 mm SL; Turkey: Eskişehir prov.: Porsuk Stream; **d.** *C. nasus*, IFC-ESUF 03-1536, 256.8 mm SL; Turkey: Kütahya prov.: Yanıkburnu Stream, Simav River; **e.** *C. nasus* FSJF-781, ZFMK-ICH 84668-94679, 221 mm SL; Germany: Rhine River.

We compared the morphological characters of *C. nasus* specimens from Rhine (Germany) and Danube (Romania) rivers with those of Simav River specimens. The Simav River specimens are distinguished from Danube River specimens by having more lateral line scales (64–72, vs. 53–60), more scale rows between lateral line and pelvic-fin origin (4–5, mode 5, vs. 3–4, mode 4), a more slender body (body depth at dorsal-fin origin 22–26% SL, mean 24.0, vs. 25–28, mean 27), a more slender caudal peduncle (9–11% SL, vs. 11–12), a longer snout (33–38% HL, vs. 28–32) and a smaller eye diameter (17–24% HL, vs. 25–27). In addition to the above-mentioned differences, the Simav River specimens are distinguished from Danube River specimens by the shape of the head (the upper profile of the head is convex in interorbital space, vs. straight or slightly convex) and markedly concave at level of nostrils (vs. straight or slightly concave). The Simav River specimens are distinguished from Rhine River specimens by having more lateral line scales (64–72,

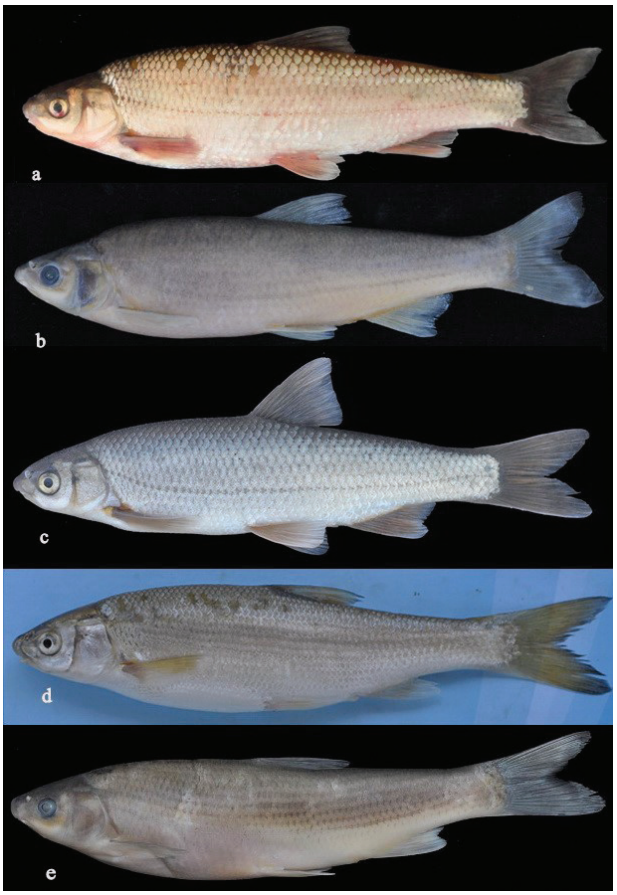

Figure 8. Aegean and Thrace region *Chondrostoma* species (all Turkey); **a.** *Chondrostoma smyrnae*, IFC-ESUF 03–1567, 167 mm SL, İzmir prov.: Tahtalı reservoir; **b.** *C. turnai*, IFC-ESUF 03-1557, 197 mm SL, Aydın prov.: Çine Stream in the lower Büyük Menderes River; **c.** *C. meandrense*, IFC-ESUF 03-1519, 180.21 mm SL; Denizli prov.: Işıklı Spring in the Upper Büyük Menderes watershed; **d.** *C. holmwoodii*, IFC-ESUF 03-1513, 149.5 mm SL; Manisa prov.: Gediz River; **e.** *Chondrostoma vardarense*, IFC-ESUF 03-1534, 195 mm SL; Edirne prov.: Meriç River.

vs. 57–63), a longer head (22–26, mean 24.4% SL, vs. 21–23, mean 21.5), a more slender body (body depth at dorsal-fin origin 22–26% SL, mean 24.6, vs. 25–29, mean 27.3), a more slender caudal peduncle (caudal peduncle depth 9–11% SL, vs. 11–12), a narrow interorbital distance (34–40% HL, mean 38.0, vs. 39–45, mean 41.6) and a smaller mouth gape (26–30, mean 29.4% HL, vs. 29–34, mean 30.2) (Tables 1, 2).

C. nasus (Simav, Danube and Rhine rivers) is distinguished from *C. colchicum* from Sakarya and Kızılırmak rivers by having more gill rakers on the outer side of the first gill arch (24–31, mode 27.8, vs. (20) 22–25, mode 23.5) and a wider mouth gape (24–34% HL, vs. 24–26). Moreover, the lower jaw in *C. nasus* is characterized by a well-developed keratinized edge (vs. slightly developed) and slightly arched (vs. straight). In addition to the above-mentioned differences, it is distinguished from *C. colchicum* by having a deeper body (body depth at dorsal fin origin 22–29% SL, mean 26.2, vs. 21–25, mean 22.9) and a slightly wider head (interorbital distance 34–45% HL, mean 39.2 vs. 33–39, mean 35.3) (Tables 1, 2).

Moreover, the molecular structure of the Meriç River samples has been compared with that of the Aoos River in north-western Greece, the Erzen River in central Albania, the Lepenac River in northern Macedonia, and the Angitis River in northern Greece, where the distribution of *C. vardarense* was established by the same researchers (Çiftçi et al. 2020). They found that the samples from the Meriç River were closer to the samples from the Angitis and Lepenac rivers (Aegean region) than the samples from the Aoos and Erzen rivers (Adriatic region). Moreover, intraspecific and interspecific genetic distance estimates do not support the current classification of *C. vardarense*. Meriç River population with a very high intraspecies heterogeneity was recorded as *Chondrostoma* sp. (Çiftçi et al. 2020). However, we assumed that this species could be *C. vardarense* since a new taxonomic revision is currently unavailable.

Vardar (Axios) River (Aegean Sea region) is the type locality of *C. vardarense*. However, this species has been documented from the rivers Pinios, Aliakmon, Strymon, Nestos and Meriç (Crivelli 2006). In addition, some studies reported that this species inhabits the Aoos River, which is a part of the Adriatic Sea basin. However, we have no adequate information as to whether this species is present in other rivers. Therefore, determining its precise distribution regions merits further investigation.

Conclusion

We here reviewed *Chondrostoma* species in Turkish inland waters using external morphology, osteology and molecular data. As stated by Elvira (1997), taxonomical status of *Chondrostoma* species is quite complex. Some morphological characters are functional in taxonomy, among which are the gill rakers' first gill arch and lateral line scales. Although these characters do not apply to many species, they are useful in identifying species together with osteology (dentary and premaxilla) and molecular data. In conclusion, our study has confirmed the existence of 13 valid species, namely *C. colchicum*, *C. beysehirense*, *C. ceyhanensis*, *C. cyri*, *C. holmwoodii*, *C. kinzelbachi*, *C. meandrense*, *C. nasus*, *C. regium*, *C. smyrnae*, *C. toros*, *C. turnai* and *C. vardarense* in Turkish inland waters. On the other hand, the taxonomic status of *C. angorense* remains uncertain. We consider that re-examination of all *Chondrostoma* populations between Sakarya (Turkey) and Tuapse (Russia), where the species is dispersed, could resolve this uncertainty.

Comparison material

***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 Eğrigöz, 39°28'10"N, 29°15'17"E.—IFC-ESUF 03-1538, 3, 137–185 mm SL; Turkey: Balıkesir prov.: Bigadiç Stream west of Bigadiç, 39°23'48"N, 28°04'50"E.—IFC-ESUF 03-1549, 1, 264 mm SL; Turkey: Afyonkarahisar prov.: Kali Stream 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 ceyhanensis*:** IFC-ESUF 03-1556, 208.49 mm SL; Turkey: Kahramanmaraş prov.: Sır Dam Lake, Ceyhan River, 37°34'30.09"N, 36°45'43.60"E.—IFC-ESUF 03-1545, 22, 191.76–264.77 mm SL; Turkey: Kahramanmaraş prov.: Sır Dam Lake, Ceyhan River, 37°34'30.09"N, 36°45'43.60"E.—IFC-ESUF 03-1546, 26, 77.76–143.15 mm SL; Turkey: Osmaniye prov.: Tecirli Bridge, Ceyhan River drainage, 37°11'41.4"N, 36°04'59.3"E.—IFC-ESUF 03-1539, 14, 140.85–205.36 mm SL; Turkey: Adana prov.: Seyhan Dam Lake, 37°03'58.9"N, 35°17'46.7"E.—IFC-ESUF 03-1540, 3, 59.54–97.73 mm SL; Turkey: Adana prov.: Çakıt Stream, Seyhan River drainage, 37°06'10.4"N, 35°06'34.7"E.—IFC-ESUF 03-1541, 16, 71.42–81.88 mm SL; Turkey: Adana prov.: Eğlence Stream, Seyhan River drainage, 37°17'30.8"N, 35°13'28.7"E.

Chondrostoma colchicum: IFC-ESUF 03-1506, 9, 228.74–277.73 mm SL; Turkey: Erzurum prov.: İspir, Çoruh River, 40°31'50.0"N, 41°02'19.2"E.—IFC-ESUF 03-1507, 7, 191.71–242.77 mm SL; Turkey: Artvin prov.: Borçka, Çoruh River, 41°21'53.4"N, 41°40'38.1"E.

Chondrostoma cyri: IFC-ESUF 03-1508, 7, 129.05–159.85 mm SL; Turkey: Ardahan prov.: Çakır Stream, Kura River, 40°58'01.4"N, 42°35'12.7"E.—IFC-ESUF 03-1509, 53, 118.70–155.51 mm SL; Turkey: Ardahan prov.: Göle, Kura River, 40°54'32.6"N, 42°39'08.9"E.—IFC-ESUF 03-1510, 4, 95.38–128.96 mm SL; Turkey: Kars prov.: Akçalar Creek, Arpaçayı Stream, Aras River, 40°46'20.8"N, 43°17'40.6"E.—IFC-ESUF 03-1511, 3, 130.20–153.47 mm SL; Turkey: Kars prov.: Çıldır Lake, Aras River, 41°02'32.3"N, 43°13'15.5"E.

Turcichondrostoma 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 Gölmarmara, 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 kinzelbachi: IFC-ESUF 03-1518,19, 173.6-220.58 mm SL, Turkey: Hatay prov.: Gölbaşı Lake, 36°30'13.0"N, 36°29'45.3"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.: Küfi Stream 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.: Karasandıklı Stream 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.: Suçıkan 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 nasus: IFC-ESUF 03-1536, 17, 138.62–170.62 mm SL, Turkey: Kütahya prov.: Yanıkburnu Stream about 25 km east of Dursunbey, 39°33'04"N, 28°56'55"E.—IFC-ESUF 03-1537, 23, 151–216 mm SL; Turkey: Kütahya prov.: Yanıkburnu Stream about 25 km east of Dursunbey, 39°33'04"N, 28°56'55"E.

***Chondrostoma regium*:** IFC-ESUF 03-1527, 21,177.96–263.07 mm SL; Turkey: Siirt prov.: Botan Stream, Tigris River drainage, 37°51'09"N, 41°53'14"E.—IFC-ESUF 03-1528, 20, 151.97–196.37 mm SL;Turkey: Batman prov.: Botan Stream, Tigris River drainage, 37°51'09"N, 41°53'14"E.—IFC-ESUF03-1529, 15, 151.43–228.79 mm SL; Turkey: Batman prov.: Botan Stream, Tigris River drainage, 37°51'09"N, 41°53'14"E.—IFC-ESUF 03-1530, 9, 181.67–204.74 mm SL; Turkey: Diyarbakır prov.: Ongözlü Bridge, Tigris River drainage, 37°53'13.6"N, 40°13'42.4"E.—IFC-ESUF 03-1531, 9, 157.68–202.35 mmSL; Turkey: Ilısu Village, Kaplıcalar, Tigris River drainage, 37°31'06.3"N, 41°50'17.5"E.—IFC-ESUF03-1533, 20, 137.06–235.95 mm SL; Turkey: Sivas prov.: Kangal, Delihacı Village, Euphrates River drainage, 39°17'45.5"N, 37°28'47.4"E.—IFC-ESUF 03-1552, 1, 70.82 mm SL; Turkey: Kilis prov.: Sinnep (Kuveik) Stream, 36°44'50.2"N, 37°14'40.4"E.

Chondrostoma smyrnae: IFC-ESUF 03-1566, 190 mm SL; Turkey: İzmir prov.: Tahtalı reservoir about 2 km north of Değirmendere, 38°08'19"N, 27°07'10"E.—IFC-ESUF 03-1567, 22, 152–205 mm SL; Turkey: İzmir prov.: Tahtalı reservoir about 2 km north of Değirmendere, 38°08'19"N, 27°07'10"E.—IFC-ESUF 03-1568, 22, 181–272 mm SL; Turkey: İzmir prov.: Tahtalı reservoir about 2 km north of Değirmendere, 38°08'19"N,

27°07'10"E.—IFC-ESUF 03-1550, 2, 92.68–109.02 mm SL; Turkey: İzmir prov.: Şaşal Stream about 1 km south of Küner, 38°11'57"N, 27°08'09"E.

***Chondrostoma toros*:** IFC-ESUF 03-1555, 142.76 mm SL; Turkey: Mersin (İçel) prov.: Hamamköy Village, Mut, Göksu River, 36°37'51.74"N, 33°22'03.18"E.—IFC-ESUF 03-1547, 34, 54.95–163.77 mm SL; Turkey: Mersin (İçel) prov.: Hamamköy Village, Mut, Göksu River, 36°37'51.74"N, 33°22'03.18"E.—IFC-ESUF 03-1554, 10, 142.37–189.92 mm SL; Turkey: Mersin prov.: Eustarin Zone, Silifke, Göksu River drainage, 36°20'51.0"N, 34°01'09.9"E.

Chondrostoma turnai: IFC-ESUF 03-1524, 44, 75–210 mm SL; Turkey: Aydın prov.: Çine Stream 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.

Chondrostoma vardarense: IFC-ESUF 03-1534, 26, 195–210 mm SL; Turkey: Edirne prov.: Meriç River, 41°39'39.1"N, 26°33'41.1"E.

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Supplementary material 1

Table S1

Authors: Fahrettin Küçük, Yılmaz Çiftçi, Salim Serkan Güçlü, Ayşe Gül Mutlu, Davut Turan

Data type: MS Word file

Explanation note: **Table S1.** List of 16S rRNA, Cytb and COI sequences used in molecular data analyses.

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