





Turcichondrostoma, a new genus for the Leuciscidae (Teleostei: Cypriniformes) from Southwestern Anatolia

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Abstract

Turcichondrostoma, a new genus, from the Southwestern Anatolia is distinguished by having fewer gill rakers on first gill arch and morphologies of premaxilla and dentary bones. In addition, as a result of the phylogenetic analyses based on combine data set (mtDNA COI + Cytb) sequences (1706 bp.), *Turcichondrostoma* genus was recovered with high posterior probability value (BI PP:1.0) and strong-supported bootstrap value (ML BP: 100%) among the former *Chondrostoma* groups. Also, high K2P mean genetic distance values (more than 7.84%) differentiated genus *Turcichondrostoma* from the other genera of former *Chondrostoma* group. The results of both morphological-osteological and molecular analyses are congruent with each other. The results of this study revealed that the genus *Turcichondrostoma* is easily distinguished from the genera in *Chondrostoma* group.

KEYWORDS

molecular phylogeny, new genus, osteology, southwestern Anatolia, taxonomy

1 | INTRODUCTION

Ladiges (1960) defined the fish samples collected by Curt Kosswig from Başpınar (Kırkpınar) Spring (Tefenni, Southwest Anatolia) in 1946 as *Phoxinellus fahirae* and stated that this new species was different due to having a complete lateral line from other species in Anatolia. In the revision of Bogutskaya (1992), a new classification was made in both morphological and osteological characters, and they were collected within the genus *Pseudophoxinus* belonging to species *Acanthorutilus*, *Phoxinellus*, *Pararhodeus* in Anatolia. According to this detailed study, *P. fahirae* was moved to the genus *Pseudophoxinus*. *Pseudophoxinus fahirae* is distinguished from the Asian and North African species by the connection of CPM-CIO (Bogutskaya, 1992: 265–267). Later, Bogutskaya *et al.* (2006) reported that *P. fahirae* and *P. pleurobipunctatus* (valid as *Telestes pleurobipunctatus*) (from Greece and Albania) should be kept out of the genus.

Recently, the systematic position of this species was named as *Chondrostoma fahirae* due to the fact that it had the lower jaw without a keratin layer, but the sharpened lower lip and the rostral cup were well developed. Nonetheless, it was stated that it is close to *Iberochondrostoma* (Iberian Peninsula) because it has the aforementioned morphological

characters (Freyhof & Özüluğ, 2009). Nonetheless, *Turcichondrostoma fahirae* is distinguished from *Iberochondrostoma* by having fewer gill rakers (fewer than 23 vs. more than 23).

On the contrary, in the project report of Küçük *et al.* (2014), it was stated that all morphological characters of *Cobitis fahirae* did not conform to *Chondrostoma*. In the present study, the differences between the two genera (*Turcichondrostoma* and *Chondrostoma*) are detailed in the diagnosis section.

The taxonomy of *Chondrostoma* genus is still not fully resolved despite the morphological and molecular studies carried out to date. One of the recent studies, Doadrio and Carmona (2004) mentioned the existence of seven lineage groups (nasus, soetta, toxostoma, lemmingii, polylepis, arcasii and genei) in the genus *Chondrostoma*. The lineage groups of nasus, soetta and genei include European and Asian species, whereas the lineage groups of toxostoma, lemmingii, polylepis and arcasii include species distributed in the Iberian Peninsula.

Robalo *et al.* (2007) studied the re-examination and phylogeny of the genus using morphological data as well as mtDNA (12S–16S-tRNA-Phe, Cyt b, d-loop) and nuclear DNA (beta-actin) gene data. In addition, based on the results of these data, they made the definition of five new genera from *Chondrostoma* lineages. The species

belonging to *Toxostoma* lineage group were redefined as genus *Parachondrostoma*, lemmingii lineage species as genus *Iberochondrostoma*, polylepis lineage species as genus *Pseudochondrostoma*, arcasii lineage species as genus *Achondrostoma* and genei lineage species as genus *Protochondrostoma*. The species of nasus and soetta lineages were specified as genus *Chondrostoma*. Nonetheless, in the aforementioned study, there is a lack of Balkans, Anatolia and Mesopotamia species named as nasus lineage within the genus *Chondrostoma*.

Subsequently, Çiftci *et al.* (2020) investigated the phylogeny of *Chondrostoma* species of Anatolian and Mesopotamian based on the mtDNA Cytb gene and as a result, they redefined the nasus and regium lineage groups. In addition, according to the analysis results, they stated that the *C. fahirae* is not included in these two lineage groups (nasus and regium) and reported species in between the Iberian genera and Central European species.

In the present study, the authors described a new genus, *Turcichondrostoma*, based on morphological, osteological and genetic methods.

2 | MATERIALS AND METHODS

2.1 | Sample collection

***Chondrostoma angorense*:** FFR DNA CH6, 1; Turkey: Kızılcahamam, Sakarya River drainage (GenBank accession number: MW911280).

***Chondrostoma beysehirense*:** FFR DNA CH15, 1; Turkey: Konya Prov.: Beyşehir, Beyşehir Lake, 37° 55' 43" N, 31° 21' 24" E (GenBank accession number: MW911282).

***Chondrostoma ceyhanensis*:** FFR DNA CH27, 1; Turkey: Adana Prov.: Seyhan River (GenBank accession number: MW911285); FFR DNA CH31, 1; Turkey: Kahramanmaraş Prov.: Ceyhan River (GenBank accession number: MW911286).

***Chondrostoma fahirae*:** IFC-ESUF 03-1512, 36, 60–127 mm SL, Turkey: Burdur Prov.: Başpınar (Kırkpınar) Spring about 16 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. – FFR 2082, 10, 80–85 mm SL, Turkey: Burdur Prov.: Başpınar (Kırkpınar) Spring about 16 km south of Tefenni, 37° 11' 08" N; 29° 45' 16" E; FFR DNA CH22-23-24, 3; Turkey: Burdur Prov.: Karamanlı Village, Değirmendere Stream, 37° 23' 34.8" N 29° 50' 13.3" E (GenBank accession numbers: MW911289- MW911290- MW911291).

***Chondrostoma holmwoodii*:** FFR DNA CH1, 1; Turkey: Uşak Prov.: Gediz River at Derbent, 38° 46' 37" N, 29° 12' 4" E (GenBank accession number: MW911279).

***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 03-1521, 19, 130–231 mm SL, Turkey: Denizli Prov.: Lake Işıklı, 38° 15' 33" N; 29° 55' 24" E (GenBank accession number: MW911281).

***Chondrostoma toros*:** FFR DNA CH25-26, 2; Turkey: Göksu River (GenBank accession numbers: MW911283- MW911284).

***Chondrostoma turnai*:** FFR DNA CH33-34, 2; Turkey: Denizli Prov.: Buldan: Lake Yayla, 38° 03' 07.0" N 28° 46' 21.2" E (GenBank accession numbers: MW911287- MW911288).

2.2 | Morphological analyses

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 2010-1/015). Samples were collected by electro-shocker. After (indicate V:V ratio) 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. Methods for counts follow Kottelat and Freyhof (2007). 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 because the anteriormost rays are deeply embedded.

For osteological preparations, one specimen of a type species *Turcichondrostoma fahirae* (105.49 mm SL) and one specimen of *C. meandrense* (207.52 mm SL), the closest species geographically, were cleared and stained with alizarin red S, according to the protocol of Taylor and van Dyke (1985). The specimens were examined using a stereo microscope (Nikon SMZ1500), and photos were taken using a digital machine with a glycerol bath. The nomenclature of the skeletal elements followed Bogutskaya (1992).

2.3 | 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

2.4 | Total DNA isolation

Total DNA from fin clips of *Chondrostoma* specimens was extracted in the Qiacube Automated DNA isolation device using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The DNA concentration and purity of each specimen were assessed spectrophotometrically (Nanodrop, 2000/c, Thermo Scientific, Wilmington, USA), whereas integrity was assessed by 0.8% TAE-agarose gel electrophoresis containing 0.5 mg/L ethidium bromide (EtBr). The agarose gel carrying total genomic DNA was visualized on UV Quantum-Capt ST4 system (Vilber Lourmat, France).

2.5 | PCR amplification

The vertebrate mtDNA barcode region of cytochrome c oxidase subunit I gene (COI) was amplified using FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3'; Ward *et al.*, 2005) and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'; Ward *et al.*, 2005). PCR reactions were carried out in 50 µl total volume containing 5 µl of 10× reaction buffer; 5 µl MgCl₂ (25 mM); 5 µl of dNTPmix (10 mM); 1 µl of forward primer (10 pmol); 1 µl of reverse primer (10 pmol); 0.2 µl of Taq DNA polymerase (1 U); 2 µl of DNA template (50 ng/µl); and 30.8 µl sterilized pure water. PCR reactions were performed using a gradient thermal cycler Biorad T100™ (Bio-Rad, Hercules, USA). The PCR condition was as follows: 1 cycle at 95°C for 3 min for initial denaturation, followed by 35 cycles denaturation at 95°C for 45 s, annealing at 61°C for 30 s, extension at 72°C for 1 min, ended up with a final extension for one cycle at 72°C for 5 min. Assessment of concentrations and sizes of PCR products were performed both spectrophotometrically and by 1.2% TAE-agarose gel electrophoresis containing 0.5 mg/l EtBr.

2.6 | Sequencing

The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) according to the manufacturer's instructions. Bidirectional sequencing of PCR products was performed by an ABI PRISM 3730x1 Genetic Analyser (Applied Biosystem; www.appliedbiosystems.com) using a BigDye Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystem) at Macrogen Europe (www.macrogen.com).

2.7 | Molecular data analyses

The raw COI sequences were initially analysed in Bioedit 7.2.5 (Hall, 1999) by comparing to sequences in GenBank database (<http://www.ncbi.nih.gov>). In addition, mtDNA cytochrome b (Cytb) data of each species used in the study was downloaded from GenBank and combined with the COI sequence. Then, all sequences were aligned by the Clustal W method (Thompson *et al.*, 1994), trimmed from the ends and converted to Fasta file. The mean genetic distances among and within genera *Achondrostoma*, *Chondrostoma*, *Iberochondrostoma*, *Parachondrostoma*, *Pseudochondrostoma*, *Protochondrostoma* and *Turcichondrostoma* (new genus) were calculated according to the Kimura 2-parameter (K2P; Kimura, 1980) distance model in MEGA X programme (Kumar *et al.*, 2018). Phylogenetic relationships among the genera were estimated using Bayesian analysis (BI) in MrBayes v3.2.1 programme (Ronquist *et al.*, 2012) and maximum likelihood (ML) algorithms in MEGA X programme. ML tree was generated according to GTR + I + G model that the best-fit evolution model was selected by the lowest AIC score in jModeltest 0.1.1 (Posada, 2008). The ML tree was generated using 1000 bootstrap replicates to estimate phylogenetic relationships of the mtDNA lineages.

BI tree was generated by MrBayes v3.2.1 (Ronquist *et al.*, 2012) according to the GTR + I + G model that the best-fit evolution model

was selected by the lowest BIC score in jmodelTest 0.1.1. For BI, analyses were run for 1×10^6 generations with Metropolis coupled Monte Carlo Markov Chains (MCMC) sampled every 1000 generations. A conservative 25% of the trees were discarded as burn-in. Visualization of the BI tree was performed by iTOL (Interactive Tree Of Life; <https://itol.embl.de/>), a web-based software.

The author also included COI and Cytb sequences of species of *Rutilus rutilus*, *Alburnus alburnus* and *Alburnoides eichwaldii* (for COI/Cytb GenBank accession numbers: MG806877/Y10440, MG806821/MG806649 and KX189523/MK860061, respectively) as outgroups to the phylogenetic analysis (Supporting Information Table TABLE S1).

3 | RESULTS

Key to *Chondrostoma* group genera (Modified from Robalo *et al.*, 2007).

1a- Lower lip with horny layer.....	2
1b- Lower lip without horny layer on.....	5
2a-Premaxilla without ascendent process.....	<i>Chondrostoma</i>
2b-Premaxilla with ascendent process.....	3
3a- Mouth arched, fewer than 18 gill rakers on first gill arch.....	4
3b-Mouth straight, more than 19 gill rakers on first gill arch.....	
<i>Pseudochondrostoma</i>	
4a-Ascendent process of premaxilla inclined forward.....	
.....	<i>Protochondrostoma</i>
4b-Ascendent process of premaxilla vertical.....	<i>Parachondrostoma</i>
5a-More than 23 gill rakers on first gill arch.....	<i>Iberochondrostoma</i>
5b-Fewer than 23 gill rakers on first gill arch.....	6
6a-Dentary coronoid process posteriorly inclined, anal-fin with seven branched rays.....	<i>Achondrostoma</i>
6b- Dentary coronoid process vertical, anal-fin with 8-9 branched rays.....	<i>Turcichondrostoma</i>



FIGURE 1 (a) *Turcichondrostoma fahirae*, FFR 2082, 85 mm SL, Turkey: Başpınar (Kırkpınar) Spring, Tefenni, (b) IFC-ESUF 03-1512, 121 mm SL, Turkey: Başpınar (Kırkpınar) Spring, Tefenni

Turcichondrostoma, new genus

Type species. *Turcichondrostoma fahirae* (Ladiges 1960) (Figure 1).

3.1 | Diagnosis

The new genus *Turcichondrostoma* is distinguished from other genus of *Chondrostoma*, *Achondrostoma*, *Iberochondrostoma*, *Pseudochondrostoma*, *Protochondrostoma* and *Parachondrostoma* by having fewer gill rakers on the first gill arch (9–14, vs. in *Chondrostoma* 19–40, in *Iberochondrostoma* 24–27, in *Pseudochondrostoma* 19–35,

in *Protochondrostoma* 14–18, except *Achondrostoma* and *Parachondrostoma*). In addition, morphologies of premaxilla and dentary bones of *Turcichondrostoma* are different compared to other genus. In *Turcichondrostoma*, ascending process of premaxilla bone which is vertically well developed (vs. absent in *Chondrostoma*, inclined anteriorly in *Protochondrostoma* and *Parachondrostoma*, and inclined posteriorly in *Pseudochondrostoma*). In *Turcichondrostoma*, dentary bone deep, its coronoid process located in the middle in vertical position (vs. inclined anteriorly in *Chondrostoma*, *Pseudochondrostoma*, *Protochondrostoma* and *Parachondrostoma*, inclined posteriorly in *Achondrostoma* and *Iberochondrostoma*) (Figure 2).

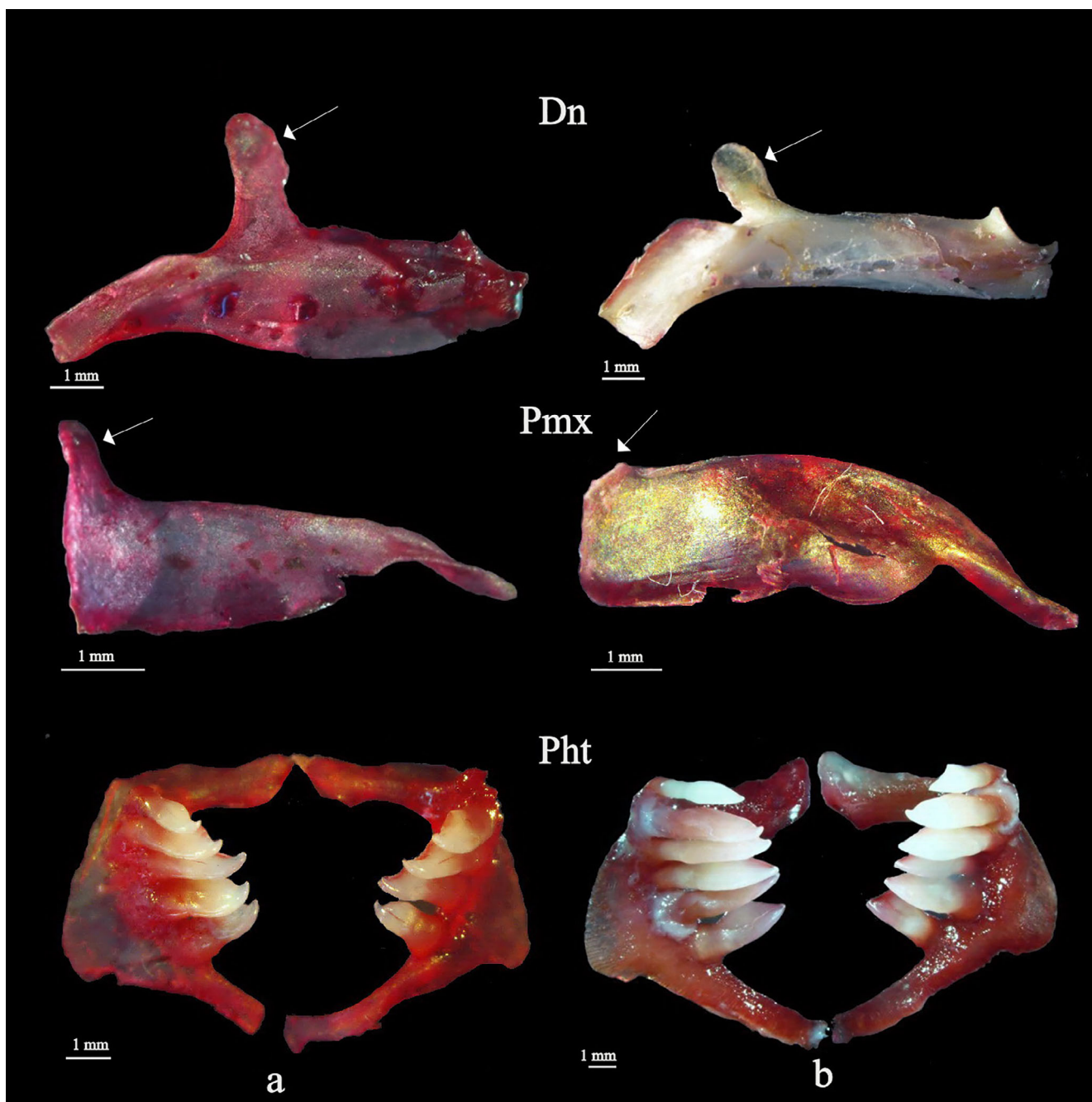


FIGURE 2 Jaw bones of *Turcichondrostoma fahirae* (a) and *Chondrostoma meandrense* (b) (Dn: dentary, Pmx: premaxilla, Pht: pharynx teeth)



FIGURE 3 Type locality of *Turcichondrostoma fahirae*; Turkey: Başpınar (Kırkpınar) Spring, Tefenni

It can be contributed to the separation of the other genus in the following combination of characters: a small-sized fish, maximum total length 135 mm (Figure 2); the horny layer on the lower lip is slightly developed; the mouth arched; lateral line with 44–50 + 1–2 pored scales, 8–10 scale rows between lateral line and dorsal-fin origin; 4–6 scale rows between the lateral line and anal-fin origin below the lateral line; dorsal fin with 7–8 branched rays; anal fin with 8–9 branched rays; the lower jaw short and thick, with the coronoid process vertical and very long; the premaxilla thin, with ascending process well developed and vertical, posterior edge short and straight; pharyngeal teeth 5–5, hooked and serrated inner surface; the hyomandibular long and narrow; total vertebrae 40–42 (43), abdominal vertebrae 24 (25) and caudal vertebrae 17–18; the preopercular-mandibular canal (CPM) connects temporal portion of the infraorbital canal (CIO).

Included species. *Turcichondrostoma fahirae*

Distribution: Başpınar (Kırkpınar) Spring near Karamusa village, 16 km south of Tefenni, the source of Dalaman River (Figure 3) and Değirmendere Stream and Karamanlı Dam Lake near Karamanlı (Burdur). Its stream existence in Karataş Lake is unknown, because there has been no water connection between Değirmendere and Karataş Lake for a long time.

3.2 | Results of molecular data analyses

In the present study, the authors sequenced a total of 13 specimens from Anatolia *Chondrostoma* species (*Chondrostoma angorensis*, *Chondrostoma beysehirensis*, *Chondrostoma*

holmwoodii, *Chondrostoma meandrense*, *Chondrostoma toros*, *Chondrostoma turnai* and *Turcichondrostoma fahirae*). The newly generated 13 COI sequences were deposited in GenBank accession numbers MW911279–MW911291. In total, 41 published COI sequences and 54 published Cytb sequences from GenBank were downloaded (Supporting Information Table TABLE S1). The COI and Cytb sequences of each species were added end-to-end to generate a combined data set. The final data set had a total of 1706 (for COI: 618 bp. and Cytb: 1088 bp.) nucleotide positions without insertion, deletion, gap and stop codon.

According to the results of the BI and ML methods, *Turcichondrostoma* (new genus) was monotypic and not testable as *Protochondrostoma* was represented by a single tip. Other genera (*Chondrostoma*, *Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma* and *Iberochondrostoma*) are monophyletic (Figure 4).

In the present study, the authors identified eight major lineages, and when the interrelationships between them were examined, the BI analysis result strongly supported that eight major lineages were formed among former *Chondrostoma* species, with posterior probability (PP) values between 0.98 and 1.0. Nonetheless, the ML analysis result could not resolve the occurrence of these eight major lineages with bootstrap (BP) values between 11% and 100%. Only, *Turcichondrostoma* (new genus) was resolved from other groups (genera) with strong-supported bootstrap (ML BP: 100%; Figure 4) value. Other lineages could not be recovered with weak bootstrap values ranging from 11% to 62%. The genus *Turcichondrostoma* was positioned as the outermost of all *Chondrostoma* genera in both analyses based on this data set.

The ML tree topology indicated the existence of two groups (*Chondrostoma nasus*-regium lineage and soetta lineage) within the

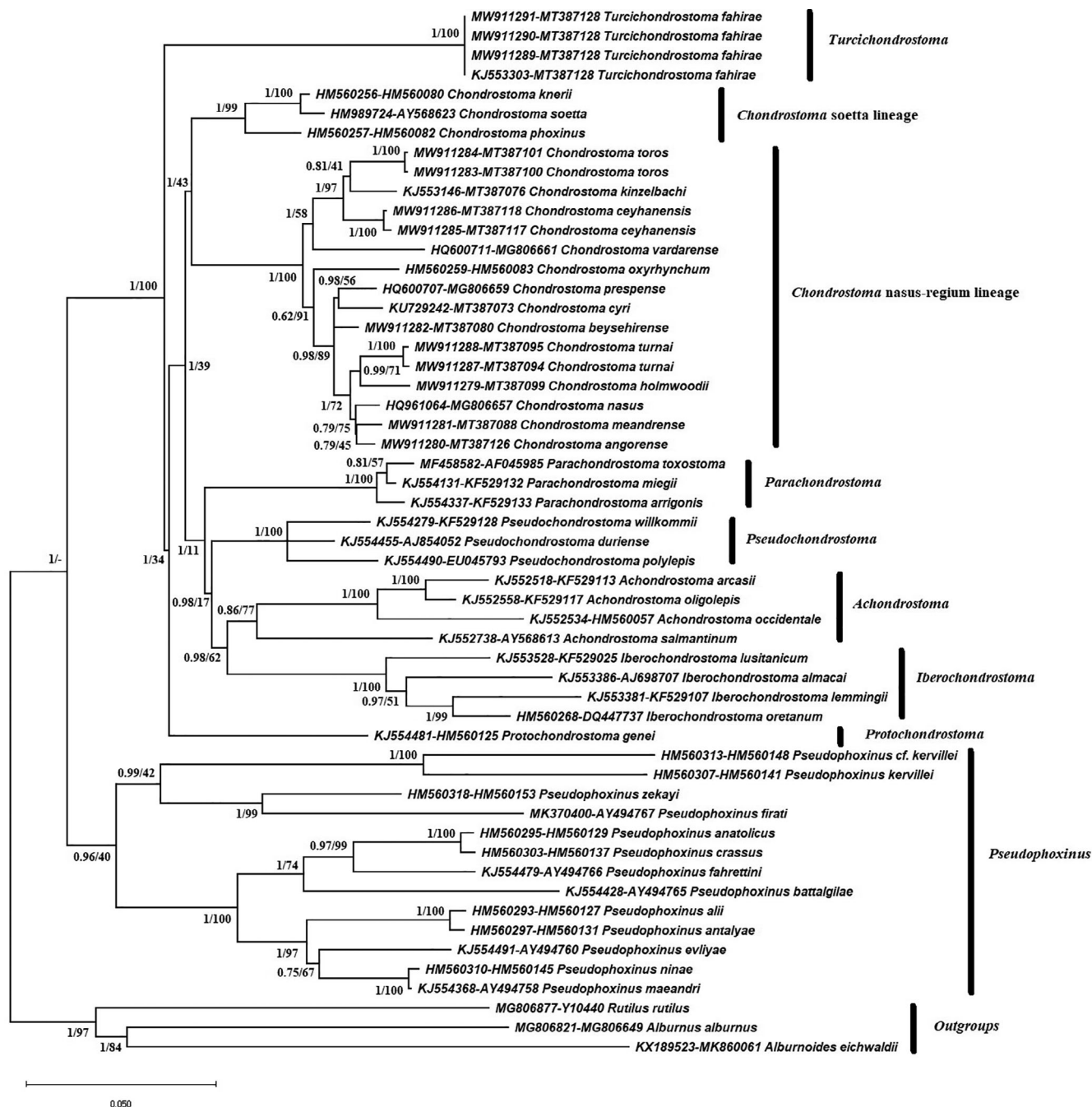


FIGURE 4 Maximum likelihood (ML) phylogenetic tree generated based on the mitochondrial combined data set (COI + Cytb). ML and BI methods yielded the same topologies, and therefore only the ML tree is shown. The bootstrap values of ML and posterior probability values of BI are indicated on nodes (BI/ML)

genus *Chondrostoma*, although it did not recover a weak-supported bootstrap value (BP: %43; Figure 4). In the BI method, phylogenetic relationships between these two groups within the genus *Chondrostoma* were well resolved with a high posterior probability value (PP:1.0; Figure 4).

Monophyles of seven *Chondrostoma* lineages, except *Achondrostoma* lineage (0.86 PP/77 BP; Figure 4), were strongly supported by posterior probability and bootstrap values (1.0 PP/99–100 BP; Figure 4).

The highest mean genetic distance within the groups was *Achondrostoma* with 5.64%, whereas the lowest *Turchondrostoma* were calculated as 0%. The mean genetic distance among the groups ranged from 5.78% (*Chondrostoma soetta* lineage – *C. nasus-regium* lineage) to 10.12% (*Iberochondrostoma* – *Turchondrostoma*). The next lowest mean between groups' genetic distance value is 5.81% (*Pseudochondrostoma* – *C. soetta* lineage). The mean genetic distances between new genus *Turchondrostoma* and the other groups ranged

TABLE 1 The mean genetic distances among and within groups calculated based on COI + Cytb

Groups	N	1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Turcichondrostoma</i>	4	0.00											
2 <i>Chondrostoma</i> (nasus-regium lineage)	16	8.85	2.96										
3 <i>Chondrostoma</i> (soetta lineage)	3	7.84	5.78	2.12									
4 <i>Achondrostoma</i>	4	9.97	8.01	7.15	5.64								
5 <i>Iberochondrostoma</i>	4	10.12	8.53	8.04	9.07	5.32							
6 <i>Pseudochondrostoma</i>	3	8.57	6.42	5.81	7.07	8.42	3.11						
7 <i>Protochondrostoma</i>	1	8.48	7.28	6.25	8.64	9.67	7.11	-					
8 <i>Parachondrostoma</i>	3	8.95	7.08	6.49	8.15	9.22	6.54	7.14	1.11				
9 <i>Pseudophoxinus</i>	13	12.09	11.00	10.56	11.92	12.45	11.04	10.64	11.44	9.70			
10 <i>Rutilus</i>	1	12.74	11.65	11.91	12.40	13.14	11.22	12.66	12.32	13.45	-		
11 <i>Alburnus</i>	1	14.65	14.26	13.33	14.34	14.87	13.24	14.12	14.88	15.22	13.87	-	
12 <i>Alburnoides</i>	1	16.90	15.06	15.03	14.90	15.23	15.05	15.02	15.07	15.17	14.45	15.62	-

Note: Genetic distances are given in percentage (%). The parts marked in grey indicate the mean genetic distances within the genus. "N" indicates the sample numbers.

from 7.84% to 10.12%. The mean genetic distance values within and among groups are given in Table 1.

Etymology: Referring to Turkey where this genus is distributed.

4 | DISCUSSION

In recent years, both morphological-osteological and molecular studies have tried to solve the complex structure of the genus *Chondrostoma*, and even new species have been identified (Güçlü *et al.*, 2018; Küçük *et al.*, 2017, 2021). In the present study, the authors examined the genetic relationships of *C. fahirae* species by including all *Chondrostoma*, *Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma*, *Iberochondrostoma* and *Protochondrostoma* species in the analyses. The mtDNA COI and Cytb gene regions are frequently used in studies to explain phylogenetic approaches (Hebert *et al.*, 2003; Johns & Avise, 1998). In the present study, the authors performed a phylogenetic analysis based on the combined data set (mtDNA COI + Cytb), using both their own data and the available genetic resources in the gene bank. They evaluated the species for which they could access both COI and Cytb data in the analysis.

Based on the results of both morphological-osteological and molecular data, they redefined *C. fahirae* as *T. fahirae*. As a result of the phylogenetic tree generated by the BI and ML methods, *T. fahirae* is a genetically different genus and is easily distinguished from the remaining ones (Figure 4). In addition, high genetic distance values compared to other genera support the phylogenetic tree results and are easily differentiated from other genera. The morphological-osteological differences between *Turcichondrostoma*, *Chondrostoma*, *Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma*, *Iberochondrostoma* and *Protochondrostoma* are given in detail in the diagnosis section. Moreover, a key was generated to all *Chondrostoma* groups.

T. fahirae, which was first identified as *P. fahirae* (Ladiges, 1960) and later as *P. fahirae* (Bogutskaya, 1992) by previous morphological and osteological studies, did not cluster together with *Pseudophoxinus*

species in phylogenetic trees generated according to both methods in the present study. Kırkpınar (Korkuteli) specimen north of Söğüt was analysed as *P. fahirae* in the genetic study of Hrbek *et al.* (2004), and in the results of the analysis, it was reconstructed as nested in a clade of the genus *Pseudophoxinus*. The authors of the study think that such a result is due to misidentification of the specimens. Later on, Freyhof and Özüluğ (2009) defined from a small stream near Söğüt specimens as a new species, *Pseudophoxinus evliyai*, in their study. Therefore, Perea *et al.* (2010) included specimens of *P. fahirae* of Hrbek *et al.* (2004) as *P. evliyai* in their genetic analysis. The result of Hrbek *et al.* (2004) is consistent with this result because it was evaluated with a misidentification. According to the BI and ML results, *T. fahirae* is clearly a different clade from *Pseudophoxinus* (Figure 4).

In addition, Freyhof and Özüluğ (2009) redefined *P. fahirae* as *C. fahirae* according to analysis results based on morphological characters. Nonetheless, in the project report of Küçük *et al.* (2014), it was stated that all morphological characters of *C. fahirae* did not conform to *Chondrostoma*.

When evaluated according to phylogenetic results, the distribution of groups on the phylogenetic tree indicated the same topology according to the two methods. Two phylogenetic trees that were exactly congruent with each other were generated (Figure 4). The BI results indicated the existence of strong-supported eight major lineages (groups) with posterior probability values between 0.98 and 1.0. These values are above the conventional threshold value (PP: 0.95; Alfaro & Holder, 2006) in Bayesian systematics. These well-supported eight groups are genera of *Achondrostoma*, *Parachondrostoma*, *Protochondrostoma*, *Pseudochondrostoma*, *Iberochondrostoma*, soetta and nasus-regium lineages of genus *Chondrostoma* and new genus *Turcichondrostoma*. The BI method, which analysed with the combined data set, well resolved the phylogenetic relationships of the major groups. Nonetheless, eight groups with weak-supported (BP: less than 50%), moderately supported (BP: between 50% and 70%) and strongly supported (BP: over 70%) bootstrap values (La Farge *et al.*, 2002) can be mentioned in the

ML results (Figure 4). Deep relationships among *Chondrostoma* groups (*Chondrostoma*, *Protochondrostoma*, *Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma* and *Iberochondrostoma*), except for *Turcichondrostoma*, could not be well resolved with weak-supported (less 50% for five groups) and moderately supported (62% for two groups) bootstrap values in the ML results. The *Turcichondrostoma* group was placed outside of the remaining seven groups in the analysis results. The *Turcichondrostoma* group formed a well-supported lineage by both methods, and their phylogenetic relationships are well resolved.

In the results of Robalo *et al.* (2007), they stated that BI analysis indicated high posterior probability values, but ML and MP analyses could not resolve certain relationships and many clades by showing low bootstrap support values. Nonetheless, they stated that results of ML and MP do not contradict with result of BI. Also, in previous studies, Bayesian analysis has been experimentally proven to be the most effective character-based method for accurately reconstructing a phylogeny (Simmons & Miya, 2004). In the study of Robalo *et al.* (2007), the distribution of *Chondrostoma* genera in the phylogenetic tree topology and the results in the present study were resulted generally in accordance with each other. Only, not exactly the same regarding some interrelationships: Robalo *et al.* (2007) recovered ((*Achondrostoma*, *Pseudochondrostoma*), *Iberochondrostoma*), whereas the present study recovered ((*Achondrostoma*, *Iberochondrostoma*), *Pseudochondrostoma*).

Doadrio and Carmona (2004) examined phylogeny and biogeography of the species of the genus *Chondrostoma* based on mitochondrial cytochrome b gene. The results in their study indicate less-supported *Chondrostoma* lineages. The distribution of all *Chondrostoma* genera in the present study on the phylogenetic tree is partially consistent with their results. The distribution of Iberian genera (*Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma* and *Iberochondrostoma*) on the tree differed from the results of the present study. In addition, both studies recovered the Iberian lineages as a monophyle, as the *C. nasus*-regium location differed in the level of resolution.

The distribution of species in the clade *Chondrostoma* in the phylogenetic tree based on Cytb of Hrbek *et al.* (2004) is partially congruent with the result of the present study. Although the distribution of *Chondrostoma* lineages (*nasus* and *soetta*) is congruent with the present study, it is not congruent with the closely together species of the other four genera (*Achondrostoma*, *Parachondrostoma*, *Pseudochondrostoma* and *Iberochondrostoma*). In the results of both methods in the present study, out-groups and *Pseudophoxinus* group clearly differentiated from the *Chondrostoma* groups are shown. Similarly, in the results of Hrbek *et al.* (2004), *Pseudophoxinus* genus and *Chondrostoma* groups are clearly separated.

Perea *et al.* (2010) aimed to resolve the phylogenetic relationships of the sub-family Leuciscinae based on mitochondrial genes (Cytb, COI) and nuclear genes (RAG1, S7), and the multi-gene analysis. Tan and Armbruster (2018) redefined the sub-family Leuciscinae as the family Leuciscidae. The distribution of *Chondrostoma* groups in the phylogenetic tree topology of the present study did not yield results congruent with their results. Similarly, in the results of Perea

et al. (2010), *Pseudophoxinus* genus and *Chondrostoma* groups are clearly distinguished.

In the results of their study Çiftci *et al.* (2020) stated that the *C. fahirae* species is positioned between the Central European and Iberian clusters. Contrary to Çiftci *et al.*'s (2020) results, according to phylogenetic tree results of this study, *C. fahirae* (new genus *T. fahirae*) is positioned at the outermost of the groups of *Chondrostoma* and is also clearly recovered with high posterior probability (PP:1.0) and strongly-supported bootstrap (BP:100%) values. The results of study of Çiftci *et al.* (2020) also indicate that *C. fahirae* (new genus *T. fahirae*) are distinctly differentiated from the genus *Chondrostoma* based on genetic results. Çiftci *et al.* (2020) stated that *C. fahirae* can be defined as a member of the genus *Protochondrostoma* in their results. Nonetheless, according to morphological, osteological and molecular results of this study, the genus *Turcichondrostoma* can be easily distinguished from the genus *Protochondrostoma*.

By the present study, the authors attribute the different positioning of the *Chondrostoma* groups in the phylogenetic trees in the results of Hrbek *et al.* (2004), Doadrio and Carmona (2004), Perea *et al.* (2010) and Çiftci *et al.* (2020) to the fact that the analysed data sets are not the same. There was no agreement between the results of analyses using the Cytb gene alone (Çiftci *et al.*, 2020; Doadrio & Carmona, 2004; Hrbek *et al.*, 2004; Perea *et al.*, 2010) and the results of analyses with multiple gene regions (Robalo *et al.*, 2007; the present study). It is known that the rate of molecular evolution and gene length increase the possibility of better explaining phylogenetic relationships (Mueller, 2006). The success of resolving deep relationships can be increased by using longer gene regions. Moreover, because the evolutionary rates between mitochondrial and nuclear markers are different, there may be differences in the distribution of clades on the phylogenetic tree. Even evolutionary rate differences between mitochondrial markers may show discordant results. In addition, the number of taxa used in the analysis, incomplete lineage sorting, introgression and recombination may cause inconsistency in the results of phylogenetic analysis methods (Degnan & Rosenberg, 2006; Zwickl & Hillis, 2002).

The genetic distance analysis results based on K2P method supported the phylogenetic tree results and indicated eight different groups within the former genus *Chondrostoma*, with values ranged from 5.78% to 10.12%. *T. fahirae* is distinguished from other groups by its high genetic distance values (Table 1). The closest related taxon to the new genus *Turcichondrostoma* is the *C. soetta* lineage with a genetic distance value of 7.84% (Table 1). Nonetheless, the phylogeny result indicated the *C. soetta* lineage to be the sister of the *C. nasus*-regium lineage. Therefore, the phylogeny result contradicts the genetic distance result. Besides, in between taxa are phylogenies, which are the inferences of evolutionary relationships, not genetic distances that indicate relatedness.

In addition, another remarkable situation in phylogenetic results is that the *C. soetta* lineage (*C. soetta*, *C. phoxinus* and *C. knerii* species) is positioned as a distinctly different clade from lineages of *C. nasus* and regium. Although the *C. nasus*-regium and *soetta* lineages were not recovered in the ML tree, but generated based on combined data set

(COI + Cytb), with a weak bootstrap value (BP: 43%), the BI result indicated that these two groups were recovered with a strong-supported posterior probability value (PP: 1.0). In their results Çiftçi et al. (2020) showed that the soetta and nasus-regium lineages were recovered with strong-supported bootstrap values according to Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) analyses at the first bifurcation, and the soetta lineages did not recover from *C. fahirae*–*C. genei* with moderate bootstrap values at subsequent bifurcation. The authors suggest that the species belonging to the *C. soetta* lineage should be re-evaluated in taxonomic terms.

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