Hydrobiologia (2018) 807:297–312
https://doi.org/10.1007/s10750-017-3405-y

CrossMark

PRIMARY RESEARCH PAPER

Systematic evaluation of the genus *Alburnus* (Cyprinidae) with description of a new species

F. Mangit · S. V. Yerli

Received: 24 March 2017 / Revised: 29 September 2017 / Accepted: 4 October 2017 / Published online: 12 October 2017
© Springer International Publishing AG 2017

Abstract The genus *Alburnus*, a member of the Cyprinidae family, includes 43 species that are widely distributed in Europe and the northern part of Western Asia. To date, inter-specific relationships within the genus have not been assessed in detail. The main objective of this research was to assess phylogenetic relationships of the genus and solve taxonomic uncertainties. For this purpose, the mitochondrial gene cytochrome c oxidase subunit I (COI) was selected and analyzed by Bayesian and maximum-likelihood approaches. Inter- and intra-specific genetic distances of the putative species were calculated. In addition, body shape was quantified by landmark-based geometric morphometrics on the available material from Turkey in order to determine whether the emerging patterns of shape are congruent with the COI phylogeny. Our data suggest multiple synonymies within the genus and the addition of a new species, *Alburnus kurui* sp. n., from the Dalaman River. We conclude that by including this new species and considering the synonymies, the genus *Alburnus* now comprises 36 species.

Keywords Phylogeny · MTDNA · COI · Geometric morphometrics · *Alburnus kurui* sp. n

Introduction

The genus *Alburnus* (Rafinesque, 1820) had formerly been placed in the subfamily Leuciscinae (Bonaparte, 1835). Because of the identification of synapomorphic characters that are shared with other taxa and the formation of a monophyletic group, it is now considered to belong in the subfamily Alburninae (Girard, 1858) (Winfield & Nelson, 2012; Froese & Pauly, 2015) and it has been synonymized with the genus *Chalcalburnus* by Bogutskaya (1997).

With the description of *Alburnus amirkabiri* Mousavi-Sabet et al., 2015 from Iran, *Alburnus selcuklui* Elp et al., 2015 from Turkey, and synonymization of five species (Parin et al., 2014) with *Alburnus mento* (Heckel, 1837), the genus *Alburnus* comprised 43 species (Eschmeyer et al., 2016). According to Kuru et al. (2014), our own unpublished data, and including the extinct *A. akili* Battalgil, 1942 and *A. nicaeensis* Battalgil, 1941, 27 of them are distributed in Turkey.

Electronic supplementary material The online version of this article (doi:10.1007/s10750-017-3405-y) contains supplementary material, which is available to authorized users.

Handling editor: Christian Sturmbauer

F. Mangit (✉) · S. V. Yerli
Biology Department, SAL, Hacettepe University, Beytepe, Ankara, Turkey
e-mail: fatihmangit@gmail.com

Systematic studies regarding genus *Alburnus* in Turkey date back to Steindachner (1897), a study in which *Alburnus escherichii* was described near Ankara. Following this publication, ichthyological studies from the region remained scarce (Boulenger, 1896; Devedjian, 1915; Hanko, 1924) until the 1940s. To our knowledge, only Devedjian (1915) reported some members of the genus (with its local name) from various localities through Turkey.

Fahire Battalgil (later F. Battalgazi) described six new subspecies from the genus (Battalgil, 1941, 1942; Battalgazi, 1944). Four of these described taxa were considered as subspecies of *A. chalcoides* (Güldentädt, 1772) by various authors (Bogutskaya, 1997; Kottelat, 1997). However, Özuluğ & Freyhof (2007b) validated three of them as *A. carinatus* Battalgil, 1941, *A. istanbulensis* Battalgil, 1941 and *A. nicaeensis*, and synonymized *Alburnus chalcoides sapancae* as *A. istanbulensis*. Another subspecies *A. sellal adanensis* was validated as *A. adanensis* Battalgazi, 1944 by Fricke et al. (2007). Aside from these subspecies, *Alburnus mossulensis delineatus* is considered as uncertain as *A. mossulensis* Heckel, 1843 according to Bogutskaya (1997). In addition to these subspecies, Battalgil also described four species (Battalgil, 1941, 1942, 1944), which are now considered as valid species except for *A. kosswigi* Battalgil, 1941 which is a synonym of *A. escherichii* Steindachner, 1897 according to Bogutskaya (1997). Valid species described by the author are *A. akili*, *A. heckeli* Battalgil, 1943, and *A. nasreddini* Battalgil, 1943.

Another example for the raising of the subspecies is *A. chalcoides derjugini* (Berg, 1923), which was validated as *A. derjugini* by Özuluğ & Freyhof (2007b) but later suggested as synonymous with *A. mento* by Parin et al. (2014), with four more species. On the other hand, *A. nasreddini* and *A. escherichii* were once synonymized with *A. orontis* Sauvage, 1882 (Ladiges, 1960; Kuru, 1982; Erk'akan, 1983) but later, all of them were treated as valid species (Bogutskaya, 1997).

Some of the species that are distributed in the eastern parts of Anatolia, such as *A. sellal* Heckel, 1843, *A. mossulensis*, and *A. kotschyi* Steindachner, 1863, are listed as uncertain (Bogutskaya, 1997) but the latter has been accepted as a valid species by Fricke et al. (2007).

In addition to the synonymization, validation, and uncertainties, relatively new species, including *A. baliki* Bogutskaya, Küçük & Ünlü, 2000, *A. attalus* Özuluğ & Freyhof, 2007, *A. battalgilae* Özuluğ & Freyhof, 2007, *A. demiri* Özuluğ & Freyhof, 2008, and *A. selcuklui*, have been described from various locations of Turkey.

Some members of the genus have been subjected to phylogenetic studies concerning higher taxonomical issues of the Cyprinidae family (Perea et al., 2010), local relationships among genus (Ketmaier et al., 2009), and barcoding (Triantafyllidis et al., 2011; Keskin & Atar, 2013; Geiger et al., 2014; Knebelsberger et al., 2015). However, inter-specific relationships among members of the genus have not been discussed to date. The main objective of this research was to assess the phylogenetic relationships of the genus and to solve the above-mentioned systematic uncertainties. For this purpose, the mitochondrial gene cytochrome c oxidase subunit I (COI) was selected, which has been proven to be useful to discriminate and barcode many animal groups (Avise, 1994; Hebert et al., 2003). In addition to this, the body shape of the species was quantified by landmark-based geometric morphometrics from available material from Turkey in order to determine whether the shape was congruent with the COI phylogeny.

Materials and methods

Sampling

Sampling was conducted in 21 drainage basins across Turkey using electrofishing, various nets (18 × 18 mm−22 × 22 mm), and fishing lines from 2011 to 2015. Specimens were fixed in 4% buffered formaldehyde and, after a few days, they were cleansed and transferred to a 70% alcohol solution for morphological analysis. Identification of the specimens was conducted following relevant literature (Battalgil, 1941, 1944; Battalgazi, 1944; Bogutskaya, 1997; Bogutskaya et al., 2000; Geldiay & Balık, 2007; Özuluğ & Freyhof, 2007a, b). The materials used in this study are summarized in Table 1 and details of the GenBank samples are given as Supplementary Material (Appendix 1—Supplementary Material).

Sampling permissions for this study were granted by the Republic of Turkey Ministry of Food and Agriculture and Livestock (B.12.0.BSU.0.10.03.00/330.07.03-538) and the procedures were approved by

Table 1 Species used in analysis

Abbreviations	Species	GMM	COI This study	COI NCBI
Albadan	*Alburnus adanensis*	43	4	2[a]
Albalbi	*Alburnus albidus*	–	–	7
Albalbu	*Alburnus alburnus*	9	3	53
Albarbo	*Alburnus arborella*	–	–	21
Albatta	*Alburnus attalus*	6	–	5[a]
Albbali	*Alburnus baliki*	14	3	3
Albbatt	*Alburnus battalgilae*[a]	24	–	3[a]
Albbelv	*Alburnus belvica*	–	–	6
Albcaer	*Alburnus caeruleus*	5	–	1
Albcari	*Alburnus carinatus*[a]	–	–	3[a]
Albchal	*Alburnus chalcoides*[a]	138	22[a]	–
Albaral	*Alburnus chalcoides aralensis*	–	–	1
Albdemi	*Alburnus demiri*	5	2	3[a]
Albesch	*Alburnus escherichii*	143	11	7
Albfili	*Alburnus filippii*	11	0	2
Albheck	*Alburnus heckeli*	7	2	0
Albista	*Alburnus istanbulensis*[a]	–	0	6[a]
Albkots	*Alburnus kotschyi*	–	1	7[a]
Albmace	*Alburnus macedonicus*	–	–	3
Albment	*Alburnus mento*	–	–	6[a]
Albnasr	*Alburnus nasreddini*	23	1	5
Alboron	*Alburnus orontis*	–	–	2
Albqali	*Alburnus qalilus*	–	–	5[a]
Albscor	*Alburnus scoranza*	–	–	6
Albsell	*Alburnus sellal*	36	8	1
Albkuru	*Alburnus* sp nova 'Kurui'[a]	–	2	–[a]
Albvolvi	*Alburnus* sp nova 'Volvi'	–	–	5
Albtari	*Alburnus tarichi*	36	4	6
Albthes	*Alburnus thessalicus*	–	–	6[a]
Albvist	*Alburnus vistonicus*[a]	–	–	4[a]
Albvolv	*Alburnus volviticus*[a]	–	–	1[a]

GMM sample size used in geometric morphometric analysis, *COI This study* sample size of newly created sequences in this study, *COI NCBI* sample size of sequences from NCBI

[a]Changes offered following the analyses

the Hacettepe University Animal Experimentations Ethic Board (B.30.2.HAC.0.05.06.00/62).

Molecular analyses

The right pectoral fins of the specimens were placed in a 99% alcohol solution and kept at −20°C. Genomic DNA was extracted using an E.Z.N.A.® Easy DNA Tissue Kit (Omega Bio-tek, Norcross, Georgia) following the manufacturer's instructions. The cytochrome oxidase I (COI) region was amplified by polymerase chain reaction following the protocol provided by Ivey & Santos (2007), using the LCO1490A and HCO2198A primer pair (Tang et al., 2010). Amplicon purification and sequence analysis were performed by Macrogen (Seoul, South Korea).

All raw sequences were edited with the CodonCode Aligner© software (CodonCode Corporation) and aligned together with the sequences retrieved from GenBank (Appendix 1—Supplementary Material) by Clustal X 2.0 software (Larkin et al., 2007). *Squalius anatolicus* (Bogutskaya, 1997) and *Alburnoides* sp. sequences were used as outgroups.

Phylogenetic analyses

Maximum-likelihood (ML) and Bayesian Inference (BI) analyses were conducted to infer phylogenetic

relationships within the genus. The nucleotide substitution model used in the ML and BI analyses was determined according to 'Akaike Information Criterion' (AIC) using JModelTest2.1.4 (Darriba et al., 2012) and the GTR + G + I model was selected. The ML analysis was conducted by RaxML (Stamatakis, 2006). A Bayesian Markov Chain Monte Carlo analysis was conducted using Beast 2.0.3 (Bouckaert et al., 2014) in three runs, with tree and parameter values sampled every 5,000 steps over a total of 50 million generations. The quality of the analysis was checked by comparing likelihood values and parameter estimates from different runs in Tracer v.1.6 (Rambaut et al., 2014) and 25% of the trees (2,500) were discarded as burn-ins. The remaining trees were summarized by maximum clade credibility using TreeAnnotator (Drummond & Rambaut, 2007). Nodes with a posterior probability higher than 0.80 are indicated by gray to black dots in the consensus tree. Bootstrap scores for these nodes are given as numbers below these nodes.

Haplotype analyses

Haplotype diversity (Hd), nucleotide diversity (Pi), and the average number of pairwise differences (K) were calculated using DnaSP version 5.10.1 (Librado & Rozas, 2009). In order to visualize genealogical relationships of haplotypes within putative species, a TCS network (Clement et al., 2002) was constructed using PopART version 1.7 (http://popart.otago.ac.nz).

Genetic distance analyses

Sequences were grouped according to prior knowledge from previous analyses and intra- and interspecific genetic distances between these groups were assessed. K2P (Kimura, 1980), JC (Jukes & Cantor, 1969), and simple p-distances were calculated using Mega7 (Kumar et al., 2016). The K2P and JC distance values and their standard errors were larger (Appendix 2—Supplementary Material) and, therefore, p-distances were reported and used in further analyses. A species delimitation tool, Automatic Barcode Gap Discovery (ABGD), was used on the web interface (Puillandre et al., 2012) using the p-distance metric with a relative gap (X) value of 1 and species delimitation results were summarized.

Geometric morphometrics

Specimens were fixed to a plate with a millimeter scale and photographed from their left side using a Canon EOS 450D camera. Insect needles were used for positioning of the specimens and for accurate determination of landmarks (Fig. 1). Thin-plate spline (TPS) file creation and landmark digitization were conducted using TPS series software TPSUtil and TPSDig2 (Rohlf, 2015). Specimens showing deformations due to fixation were either removed from the analyses or corrected with the unbent option in TPSUtil.

Following the Procrustes superimposition, routine analysis of morphometric data (PCA, ANOVA, and others) was conducted. All analyses were conducted using the 'geomorph package' (Adams et al., 2015) implemented in R 3.2.3 (R Core Team, 2015). Mean shapes of species were calculated and the degree of morphological inter-specific variation was assessed in a phylogenetic context. For this, the cytochrome oxidase I tree, which is presented in this study, was used. The degree of phylogenetic signal was estimated using the 'physignal' function of the geomorph package, which is a multivariate version of the K-statistic (Adams, 2014).

Results

Molecular diversity within genus

The final alignment consists of 245 sequences, excluding the outgroups, and 653 base pairs with 132 variable sites (121 parsimony informative). A total of 63 sequences were generated in this study and these were deposited in GenBank.

COI phylogeny

Bayesian analysis revealed three distinct lineages (nodes B, C, and E) supported by high posterior probabilities (PP $>$ 0.95) (Fig. 2): Lineage I (supported by node E), Lineage II (supported by node C), and Lineage III (supported by node B). Maximum-likelihood analysis returned an identical tree in terms of these lineages; however, they had lower bootstrap scores (83, 100, and 62 for lineages I, II, and III, respectively). Tree topology was found to be more

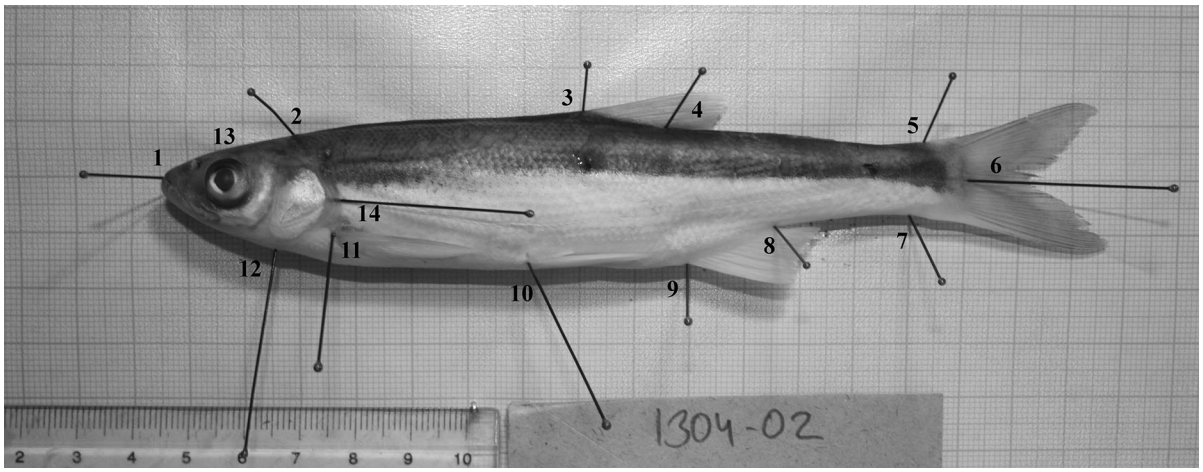

Fig. 1 Landmarks used in this study (01, tip of mouth; 02, beginning of scales; 03 and 04, anterior and posterior base of dorsal fin; 05 and 07, dorsal and ventral base of caudal fin; 06, last scale of lateral line; 08 and 09, posterior and anterior base of the anal fin; 10, anterior base of first pelvic fin ray; 11, anterior base of first pectoral fin ray; 12, intersection of branchiostegal rays; 13, middle of eye; 14, tip of operculum)

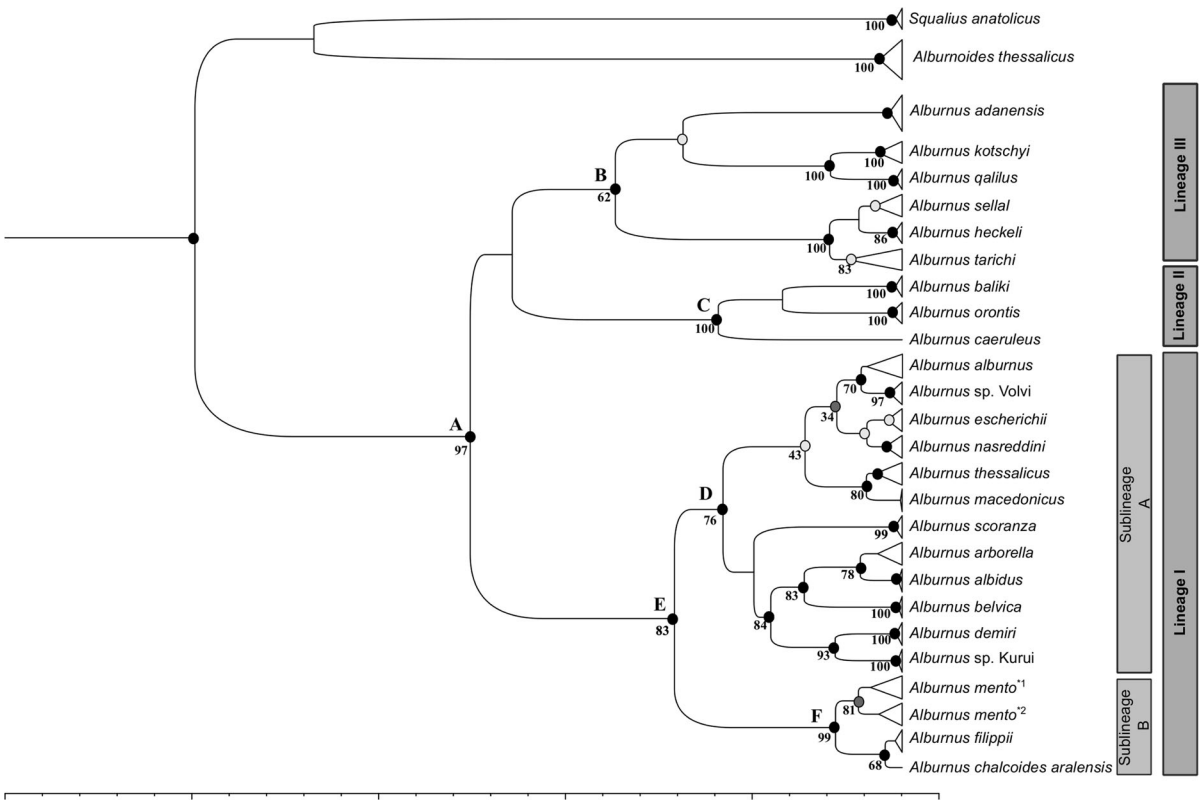

Fig. 2 Genus *Alburnus* phylogeny based on mtDNA COI region (Posterior probabilities higher than 0.80 are illustrated as colored dots on nods; black: >0.95, dark gray: >0.90, gray: >0.80. Maximum-likelihood bootstrap scores are given under the node if node posterior probability is over 0.80) *Alburnus mento*[1]: *A. attalus*, *A. battalgilae*, *A. mento*, and *A. schischkovi*; *Alburnus mento*[2]: *A. derjugini*, *A. vistonicus*, *A. carinatus*, *A. volviticus*

successful with Bayesian inference than the maximum-likelihood approach. Bootstrap scores were generally low and failed to distinguish most of the putative species. Therefore, identification of lineages followed the posterior probability scores.

Excluding the outgroups, the final tree consists of a total of 31 putative species (Table 1). Species delineation resulted in Lineage II and III being supported with high posterior probabilities; however, some taxa failed to be discriminated in Lineage I. In Sublineage A, delineation of *Alburnus* sp. 'Volvi' from *A. alburnus*, *A. macedonicus* from *A. thessalicus*, and the position of *A. scoranza* are not resolved. In Sublineage B, a total of eight taxa are clustered into the two main ones and both of them are not strongly supported. These clusters are indicated with superscripts for *A. mento*. Also, *A. chalcoides aralensis* and *A. filippii* are clustered together.

Haplotype analyses

Using the same taxa, two TCS networks were separately constructed for Lineage I (Fig. 3) and Lineage II and III (Fig. 4). Haplotypes are colored according to putative species; however, because of the large number of them presented in the figure, the legend is written with the haplotype numbers.

Lineage I is the most diverse lineage and it consists of 193 sequences from 22 putative species with 88 variable sites (S) and 49 distinct haplotypes. Haplotype diversity (Hd) of this lineage was found to be 0.936. According to the haplotype network results, *A. scoranza* (Hap_27) seems to be related to both *A. alburnus* and the *A. albidus-A. arborella* group, thereby preventing further delineation of the sublineage. Putative species from the *A. mento*[1] and *A. mento*[2] (Fig. 2) groups formed 2 clusters. One cluster consists of *A. attalus* and *A. battalgilae* (Hap_39–40) and is positioned four steps away from the central haplotype (Hap_43). Another cluster (dotted line in Fig. 3) consists of *A. schischkovi*, *A. mento*, *A. istanbulensis*, *A. volviticus*, *A. derjugini*, *A. carinatus*, and *A. vistonicus* (Hap_41–48). Also, *A. filippii* formed another cluster with *A. chalcoides aralensis* (Hap_49), five steps away from the main haplotype (Hap_43) (Fig. 3).

Lineage II consists of five distinct haplotypes from three species, *A. baliki*, *A. orontis*, and *A. caeruleus* (Fig. 4). Haplotype diversity of this lineage is 0.722. Lineage III consists of 22 distinct haplotypes from six species and haplotype diversity of this lineage is 0.934. *A. sellal* and *A. tarichi* were each represented by six haplotypes and both were found to be closely related with *A. heckeli*. Other informative data about the lineages are summarized in Table 2.

Genetic distance analyses

Sequences were grouped following the previous analyses (*A. attalus* and *A. battalgilae* as *A. attalus*; *A. schischkovi*, *A. mento*, *A. istanbulensis*, *A. volviticus*, *A. derjugini*, *A. carinatus*, and *A. vistonicus* as *A. mento*) and inter- and intra-specific *p*-distances (%) are summarized in Table 3 (Lineage I) and Table 4 (Lineage II and III).

Intra-specific *p*-distances in Lineage I varied from 0.00 to 0.51%, while inter-specific distances varied between 0.21% (*A. nasreddini*–*A. escherichii*) and 6.82% (*A. demiri*–*A. attalus*) with a median value of 3.23%. Intra-specific *p*-distances in Lineage II varied from 0.05 to 0.34%. Inter-specific distances in Lineage II were higher than in Lineage I, ranging from 2.85 to 4.73%, with a median value of 4.01%. Intra-specific *p*-distances in Lineage III varied between 0.06 and 1.21%, whereas inter-specific distances varied between 0.38% (*A. sellal*–*A. heckeli*) and 6.05% (*A. kotschyi*–*A. heckeli*) with a median value of 4.59%. The ABGD species delineation results according to these *p*-distances are given in Table 5.

In Lineage I, recursive partitioning discovered 11 operational taxonomic units (OTUs) with these settings and the delineation results were stable until intra-specific divergence values (*P*) rose to 0.001668 (data not shown). According to these results, *A. alburnus*, *A. escherichii*, *A. nasreddini*, *A. thessalicus*, and *A. macedonicus* are clustered together. Species delineation results showed no difference for Lineage II and these results were stable until $P = 0.0215$. In Lineage III, a total of eight OTU clusters were discovered; however, in addition to the splitting of the *A. tarichi* into four groups, *A. sellal*, *A. heckeli*, and *A. tarichi* are clustered together.

Shape differences

Geometric morphometric analysis revealed significant differences between the shapes of species according to selected landmarks. However, results of these analyses

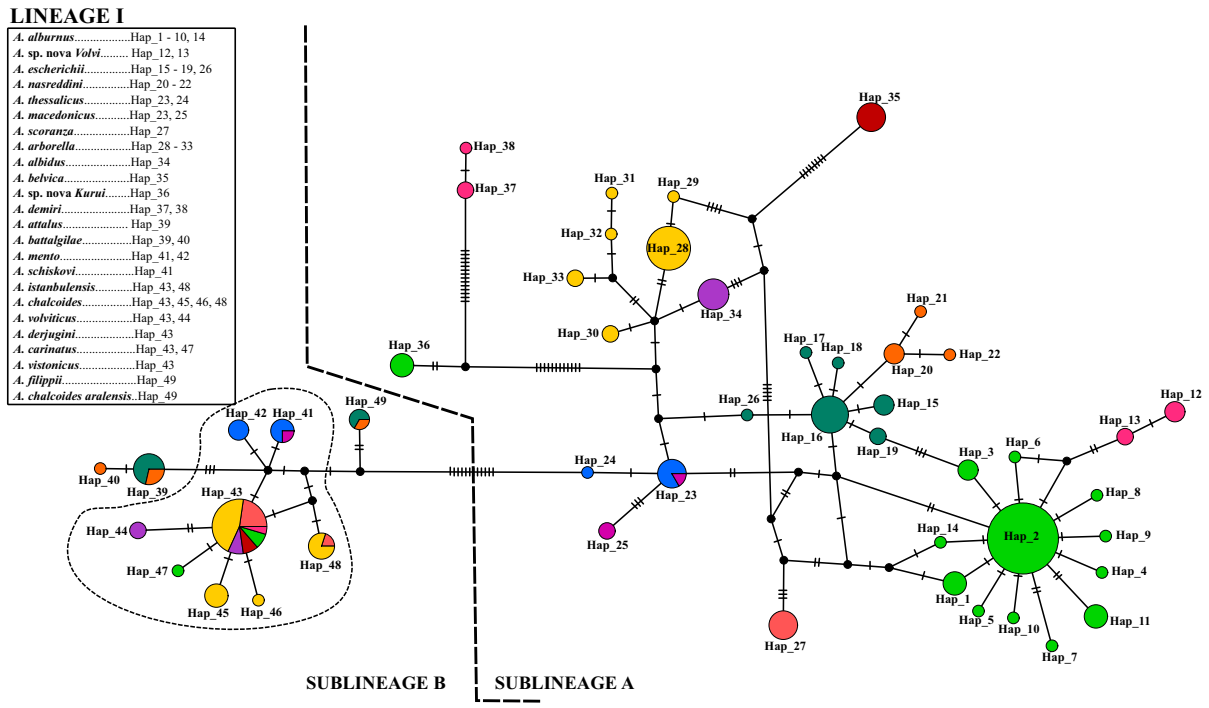

Fig. 3 Haplotype network of Lineage I

have not been given in detail in this study (Mangıt, 2014). Procrustes-aligned specimens were used with the phylogenetic tree and the degree of phylogenetic signal in the data was estimated for 13 species from Turkey (Fig. 5).

Analysis with all lineages revealed a significant low K value (0.438; $P = 0.005$) indicating moderate agreement. When Lineage I was separated from the rest, the molecular data of Lineage II and III gave an almost perfect agreement (K: 0.815; $P = 0.003$) with shape. In addition to this, K scores of Lineage I also increased (K: 0.478) after separation; however, this result was not significant ($P = 0.204$).

Morphological examination of the population from the Dalaman River revealed differences in important characters that are used to distinguish species of the genus. Therefore, it was treated as a separate taxon throughout the analysis. With validation of this difference using the COI data, a new species is described.

Genus *Alburnus* (Rafinesque, 1820)

Alburnus kurui sp. nov. (Fig. 6)

Holotype. HUSal 480301, 65 mm SL, Dalaman River, Muğla, Turkey, 4 km SE of Ortaca 1 km W of bridge D400 (36°48.880′N, 28°47.607′E). Collected June 15, 2012, by F. Mangıt, U. Sü, M. Korkmaz.

Paratypes. HUSal 480302–480307, six specimens, 50−69 mm SL. Same data as for holotype.

Etymology. The name of the new species is dedicated to Prof. Dr. Mustafa Kuru, recognizing his contributions to ichthyological research on Turkey fish fauna.

Diagnosis. *Alburnus kurui* is distinguished from all other species of *Alburnus* by the following characters: anal fin origin below branched dorsal fin ray 5−6, 12½−13½ branched anal fin rays; 14 gill rakers and 43−46 + 1 lateral line scales and faint dark lateral stripe on body. And morphometric features: head length 23–25% standard length (SL), predorsal length 55−59% SL, caudal peduncle length 18−22% SL, caudal peduncle depth 9−10% SL, caudal peduncle depth 1.99−2.32 times the caudal peduncle length, eye diameter 7−8% SL, eye diameter 1.03–1.20 times the interorbital distance.

Description. Holotype medium-sized, compressed body, dorsal and ventral profiles slightly convex (Fig. 6). Lower jaw slightly projecting beyond upper jaw causing oblique mouth to be positioned subterminally; eye diameter bigger than interorbital

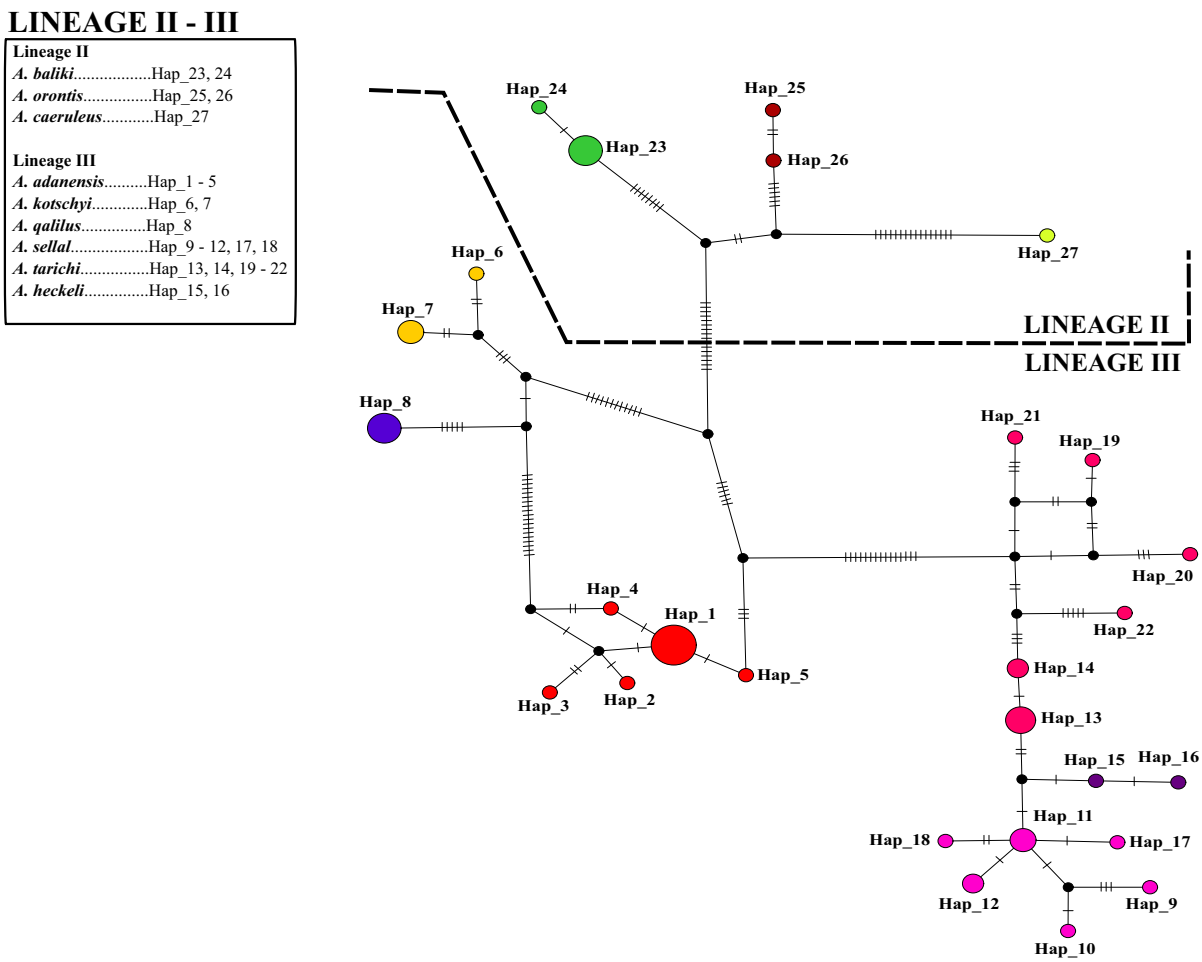

Fig. 4 Haplotype network of Lineages II–III

Table 2 Summary of mtDNA sample data for genus *Alburnus* based on 653-bp COI region

Range	*N*	*S*	PIS	*K*	π	*H*	HD
All	245	132	121	21.92	0.04256	76	0.958
Lineage I	193	88	76	13.94	0.02701	49	0.936
Lineage II	9	34	16	11.56	0.02417	5	0.722
Lineage III	43	67	59	21.43	0.03903	22	0.934

N sample size, *S* number of polymorphic (segregating) sites, *PIS* parsimony informative sites, *K* average number of pairwise differences, π nucleotide diversity, *H* number of haplotypes, *HD* haplotype diversity

distance and 2.85−3.35 times the head length. Fin formula of taxonomically important anal fin III 12½−13½. Lateral line complete and slightly curved downward with 43−46 scales on body and one on caudal fin. Between dorsal fin origin and lateral line 7½−8½ rows of scales, 2−3 scale rows between lateral line and pelvic fin origin. Pharyngeal teeth in two rows, with 5.2−2.5 set up. Gill raker count 14, according to 2 paratype specimens.

Remarks. Genetic distance (*p*) between *Alburnus kurui* and its sister species *A. demiri* is 2.46%. And *A. kurui* can easily be distinguished from *A. demiri* by having 14 gill rakers (vs. 18−21). It is hard to distinguish *Alburnus kurui* from *A. escherichii* and *A. nasreddini*, which are distributed in neighboring basins. It can be distinguished by having 7½−8½ rows of scale between dorsal fin origin and lateral line (vs. 8½−10½ in other mentioned species). *Alburnus kurui* is further distinguished from *A. escherichii* by having a caudal peduncle depth 1.99−2.32 times the

Table 3 Inter-specific and intra-specific (bold) *p*-distance (%) between putative species from Lineage I (see Table 1 for species abbreviations)

	Albalbi	Albalbu	Albarbo	Albatta	Albbelv	Albdemi	Albesch	Albfili
Albalbi	**0.00**							
Albalbu	3.02	**0.23**						
Albarbo	0.77	2.65	**0.35**					
Albatta[a]	5.39	3.90	5.07	**0.04**				
Albbelv	2.61	3.78	2.15	5.54	**0.00**			
Albdemi	4.61	4.43	3.89	6.82	4.76	**0.10**		
Albesch	2.74	0.60	2.34	3.30	3.17	4.47	**0.13**	
Albfili[b]	4.98	3.33	4.56	1.19	4.92	6.25	2.75	**0.22**
Albkuru	3.23	3.34	2.49	4.68	3.38	2.46	3.05	4.50
Albmace	3.23	1.46	2.81	3.64	3.53	5.22	0.87	3.05
Albment[c]	4.95	3.41	4.56	0.66	5.08	6.53	2.86	0.71
Albnasr	2.92	0.73	2.52	3.31	3.38	4.61	0.12	2.85
Albscor	2.56	1.72	2.24	3.95	3.39	4.32	1.50	3.39
Albthes	2.92	1.17	2.53	3.45	3.38	4.92	0.61	2.84
Albvolvi	3.38	0.82	3.17	4.54	4.33	4.79	1.21	3.96
Outgroup	10.53	9.32	10.14	10.17	9.49	9.99	9.44	9.89

	Albkuru	Albmace	Albment	Albnasr	Albscor	Albthes	Albvolvi	Outgroup
Albalbi								
Albalbu								
Albarbo								
Albatta[a]								
Albbelv								
Albdemi								
Albesch								
Albfili[b]								
Albkuru	**0.00**							
Albmace	3.38	**0.51**						
Albment[c]	4.52	3.28	**0.32**					
Albnasr	3.23	1.06	2.92	**0.31**				
Albscor	3.08	2.12	3.44	1.66	**0.05**			
Albthes	3.07	0.31	2.99	0.77	1.81	**0.10**		
Albvolvi	3.72	2.09	4.01	1.32	2.30	1.78	**0.31**	
Outgroup	9.34	9.64	9.74	9.18	9.73	9.64	9.60	**2.36**

[a] *A. attalus* and *A. battalgilae*

[b] *A. filippii* and *A. chalcoides aralensis*

[c] *A. carinatus*, *A. chalcoides* (later treated as *A. derjugini*), *A. mento*, *A. schischkovi*, *A. vistonicus*, and *A. volviticus*

caudal peduncle length (vs. 2.17−2.82 times). Comparative material: *A. demiri* Dem Stream, Küçük Menderes Basin, İzmir; *A. nasreddini* Pazarağaç Stream, Akarçay Basin, Afyon; *A. escherichii* Porsuk Reservoir, Sakarya Basin, Eskişehir.

Distribution. *Alburnus kurui* was collected in the Dalaman River which discharges to the Aegean Sea from the southwestern part of Turkey. Species is possibly endemic to the southwestern region, which is dominated by the Büyük Menderes and Dalaman rivers. It is geographically and phylogenetically closely related to *A. demiri* from the Küçük Menderes Basins to the north.

Discussion

In this study, we used mitochondrial COI sequences and morphometrical data to evaluate the relationships between species of genus *Alburnus*. Our results revealed three distinct lineages supported by high posterior probabilities. *Leucaspius delineatus*

Table 4 Inter-specific and intra-specific (bold) *p*-distance (%) between putative species from Lineage II–III (see Table 1 for species abbreviations)

	Albadan	Albbali	Albcaer	Albheck	Albkots	Alboron	Albqali	Albsell	Albtari	Outgroup
Albadan	**0.21**									
Albbali	6.32	**0.05**								
Albcaer	6.68	4.73	–							
Albheck	4.57	8.30	8.92	**0.15**						
Albkots	4.40	7.17	7.43	6.05	**0.41**					
Alboron	6.61	2.85	4.01	8.61	7.19	**0.34**				
Albqali	4.35	7.19	7.06	5.84	1.59	7.21	**0.06**			
Albsell	4.29	7.78	8.34	0.38	5.74	8.09	5.59	**0.45**		
Albtari	4.01	7.85	8.50	0.65	5.86	8.33	5.60	0.65	**1.22**	
Outgroup	9.91	10.12	11.53	10.66	9.77	10.69	10.32	10.37	10.40	**2.36**

(Heckel, 1843), which is widely distributed in Europe, and Iberian species *Anaecypris hispanica* (Steindachner, 1866) separate Lineage I from the rest of the lineages (data not shown), giving *Alburnus* a paraphyletic status. Whether to include or exclude Lineage II and Lineage III in genus *Alburnus* is still a question (Perea et al., 2010). According to our results, we propose to exclude these lineages from the rest of genus *Alburnus*. In addition to geographic and phylogenetic differences, the meristic characters of these lineages are different from those of Lineage I (*Alburnus* sensu stricto) and include a high lateral line count of *A. sellal*, *A. tarichi*, and *A. heckeli* (66−90) and a low gill raker count for *A. baliki*, *A. orontis*, and *A. caeruleus* (9−14). However, it is not the aim of this study to offer a new genus for these lineages as we believe a detailed study about them might reveal more than one genus.

Lineage I

There are two distinct sublineages in Lineage I. Sublineage A distributes throughout Europe, while Sublineage B consists of species related to Black Sea and Caspian Sea species. The phylogenetic position of *A. scoranza* remained unclear based on the available data. Use of the nuclear rhodopsin gene as a marker, which was shown to be capable of improving species delimitation (Behrens-Chapuis et al., 2015), could resolve this polytomy and reveal an Aegean–Adriatic lineage.

The distance-based Automated Barcode Gap Discovery (ABGD) tool failed to discriminate *A. alburnus*, *A. escherichii*, *A. nasreddini*, *A. thessalicus*, and *A. macedonicus* due to the small inter-specific distances between them (highest *p*-distance: 1.46%). Among these species, *Alburnus alburnus* is the most abundant representative of the lineage throughout Europe (Hap 1–10, 14), whereas its distribution is limited to the Susurluk Basin in Anatolia (Hap 11). Distribution of this species in Anatolia is possibly anthropogenic as this species is commonly used by local fishermen as bait for catching predator fish species (e.g., *Esox lucius*) and can be translocated. The neighboring basins of Susurluk are Sakarya B. (east) and Akarçay B. (southeast), which are inhabited by *A. escherichii* (Hap 15–19, 26) and *A. nasreddini* (Hap 20–22), respectively. *A. escherichii* and *A. nasreddini* were synonymized with *A. orontis* (Ladiges, 1960; Kuru, 1982; Erk'akan, 1983) due to similar characteristics, such as line lateral scale count. According to the results presented in this study, they are significantly different from *A. orontis*. However, the differences between them are not very significant. According to Gülle et al. (2017), *A. nasreddini* is distinguished from *A. escherichii* by having a deeper body, a shorter and more pointed snout, a large eye, and a larger ventral keel exposure rate (by scale count). However, the low genetic distance (0.12%) between them questions not only the validity of *A. nasreddini* but also the meristic characters used to distinguish them.

Table 5 ABGD species delimitation results according to prior intra-specific divergence (P) of 0.001 and relative gap width (X) of 1)

	$P = 0.001$
Lineage I	
Albalbu	1[a]
Albvolvi	1
Albesch	1[a]
Albnasr	1[a]
Albthes	1[a]
Albmace	1
Albscor	1
Albarbo	1
Albalbi	1
Albbelv	1
Albdemi	1
Albkuru	1
Albment	1
Albatta	1
Albfili	1
Total clusters	11
Lineage II	
Albbali	1
Alboron	1
Albcaer	1
Total clusters	3
Lineage III	
Albadan	1
Albkots	1
Albqali	1
Albsell	1[a]
Albheck	1[a]
Albtari	4[a]
Total clusters	8

Numbers indicate the number of clusters the sequences spread

[a]Which species are clustered together in each large lineage (see Table 1 for species abbreviations)

Balkanian species which are closely related with *A. alburnus* are *A. macedonicus* (Hap 23, 25), *A. thessalicus* (Hap 23, 24), *Alburnus* sp. nov. 'Volvi' (Hap 12, 13), and *A. scoranza* (Hap_27). ABGD analysis could resolve delineation; however, another polytomy is represented by Bayesian analysis of *A. macedonicus* and *A. scoranza*. *Alburnus* sp. nov. 'Volvi' has been described in Kottelat & Freyhof, 2007 but, interestingly, it has not been validated since. According to our analysis, this new species is closely related with Lineage I and is a sister species with *A. alburnus*. In addition to that, it seems some GenBank specimens of *A. thessalicus* are confused with *Alburnoides thessalicus* Stephanidis, 1950 or there has been a hybridization event between the aforementioned species. These specimens were kept in the analysis as an outgroup.

Closely related *A. belvica* (Hap_35) is distributed throughout the Balkan Peninsula, *A. arborella* (Hap 28–33) and *A. albidus* (Hap 34) in Italy, and *A demiri* (Hap_37, 38) and the newly described *A kurui* (Hap_39) in Anatolia. Distribution patterns and phylogenetic relationships of these taxa indicate that they might be Paratethyan-Messinian relics that separated from the Danubian forms (Lineage I, *A. alburnus*) (Por & Dimentan, 1985; Bianco, 1990). However, it is not easy to discuss distribution patterns of the genus until the phylogenetic relationships of *A. scoranza* are resolved.

Sublineage B of Lineage I is composed of 12 species with 12 distinct haplotypes and it is probably the most problematic one. One clade in this sublineage is given as *A. mento* with a superscript one (*A. mento*[1]) in the tree and it is made up of *A. attalus*, *A. battalgilae*, *A. mento*, and *A. schischkovi*. The population distributed in the Bakır River Basin was identified as *A. attalus* and the population from the Gediz River Basin was identified as *A. battalgilae* (Özuluğ & Freyhof, 2007b). However, as can be seen in Fig. 3, most of the specimens from these mentioned basins belong to Hap_39 of the lineage. Only one specimen from Gediz differs from them with one step. According to Özuluğ & Freyhof (2007b), *A. battalgilae* differs from *A. attalus* by having four scale rows on its caudal fin base (vs. three), three scale rows between the pelvic fin origin and the lateral line (vs. four), a slight difference in the anal fin position (in individuals larger than 90 mm), and a more slender body. Therefore, considering these differences were insufficient for discrimination of these species, we propose *A. battalgilae* as a synonym to *A. attalus* until further evidence from osteological features are presented or evidence from additional genes is reported.

A similar case can be seen for *A. mento* and *A. schischkovi* (Hap_41 and Hap_42). Although the Thracean population was formerly defined as *A.*

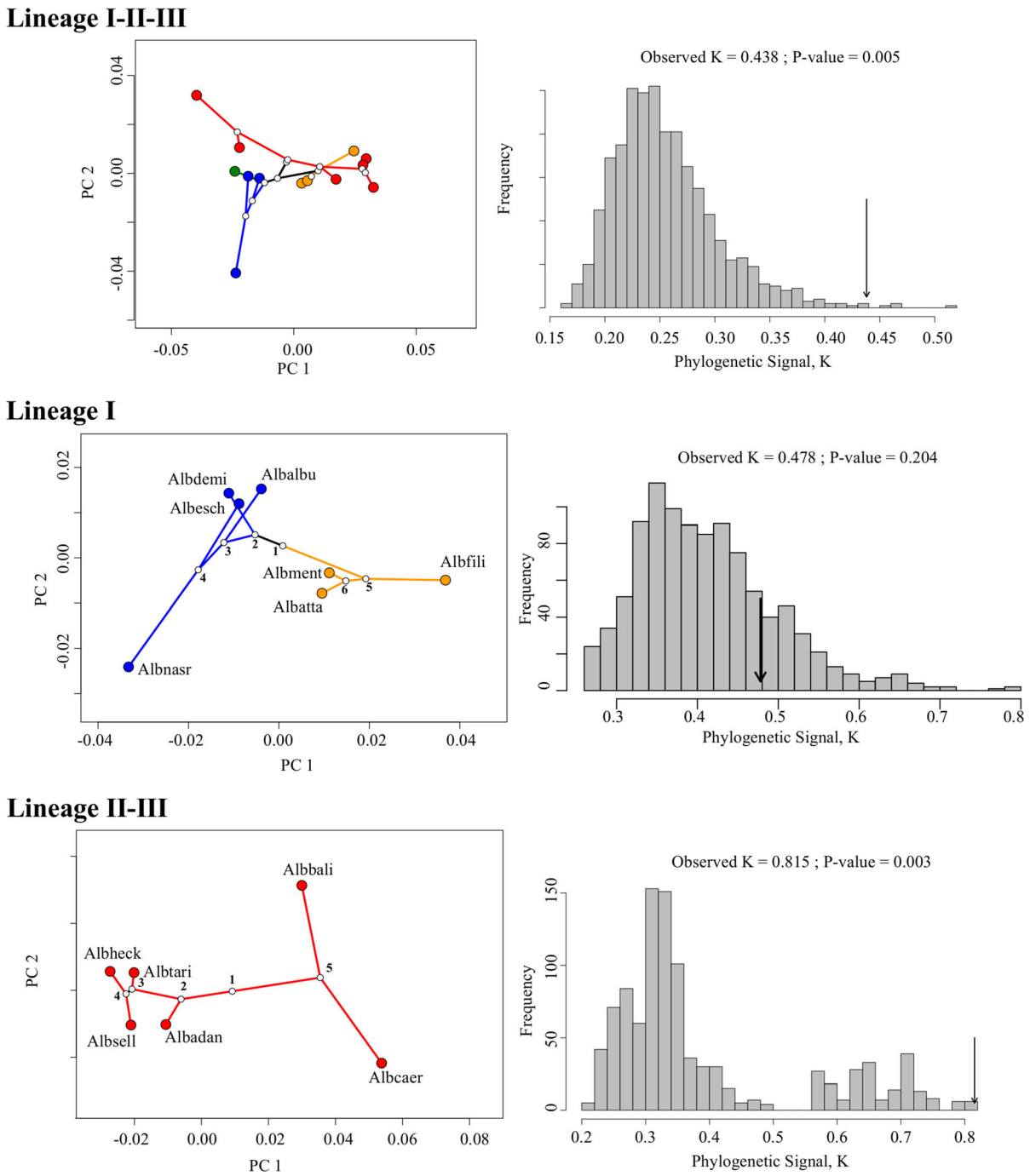

Fig. 5 COI phylogeny superimposed to specimens in tangent space and degree of phylogenetic signal present in shape for different sets of lineages (see Table 1 for species abbreviations)

schischkovi, our data support synonymy of this species with *A. mento*, as previously suggested by Parin et al. (2014).

Formerly, all specimens from the Black Sea basin of Turkey were identified as *A. chalcoides* (Kuru, 1982; Bogutskaya, 1997; Geldiay & Balık, 2007). Therefore, we also followed these authors and

Fig. 6 *Alburnus kurui* sp. n

conducted our analysis accordingly (Hap_43, 45, 46, and 48). However, according to Kottelat & Freyhof (2007), *A. chalcoides* is restricted to the Caspian Basin. Therefore, all specimens which were initially identified as *A. chalcoides* have been reassessed as *A. derjugini,* as between the Çoruh River and the Biga Peninsula (all of the Black Sea coast), Hap_43 is shared. According to the Bayesian analysis results, *A. carinatus*, *A. istanbulensis*, *A. vistonicus*, and *A. volviticus* are polytomic, and taking into account their similar meristic characteristics as well, we propose that all of these species should be synonyms with *A. derjugini*. In addition to that, the differences of all these mentioned species with *A. mento* are few, as can be seen in the haplotype analysis (2 steps). Therefore, we support the synonymy of *A. derjugini* as *A. mento* as it was previously proposed by Parin et al. (2014). According to data from Kottelat & Freyhof (2007), the line lateral scale count for *A. mento* following this suggestion is between 52−69 and the gill raker count is between 18 and 39. Before this suggestion, the line lateral scale count range for *A. mento* was 52−65 and the gill raker count range for *A. istanbulensis* was 24−35. Similarly, the anal fin soft ray count ranges are not changed significantly.

To summarize, we propose that all Black Sea specimens (Hap41−Hap48) be synonymized with *Alburnus mento* and *A. battalgilae* as a synonym to *A. attalus*. By accepting this proposal, the distribution pattern of this lineage will be congruent with the population continuum phenomenon described by Mayr (1963). When we treat this lineage as a cluster of populations divided mainly by the Black Sea, a highly variable species, *A. mento*, resides in the middle. Steady dispersal of *A. mento*, which possibly shows tolerance to salinity, resulted in continuous gene exchange among populations, thus limiting speciation. As a result, *A. mento* and the terminal populations, here *A. attalus* in the west and *A. chalcoides* and *A. filippii* in the east, deserve recognition as a species.

Lineage II

This lineage consists of *A. orontis*, *A. baliki*, and *A. caeruleus* and is strongly supported. *A. caeruleus* which distributes in the Euphrates-Tigris river system is at the basal position of the lineage. Species of this lineage were discriminated successfully and are morphologically different from others according to the shape of *A. baliki* and *A. caeruleus*.

Lineage III

One sublineage in Lineage III consists of *A. adanensis*, *A. kotschyi*, and *A. qalilus*. According to Geiger et al. (2014), the Arsuz and Ceyhan populations have been identified as *A. kotschyi* and the Seyhan population has been identified as *A. adanensis*. First, the NCBI record for *A. kotschyi* was given by Perea et al. (2010) (HM560249) from the Ceyhan River. Later, Geiger et al. (2014) defined this species from the Ceyhan River and the Arsuz drainage basin and identified the Seyhan population as *A. adanensis* (KJ552680, KJ552703, and KJ552508). The description of *A. kotschyi* seems to be insufficient in that it can be mistaken with *A. adanensis* (Battalgazi, 1994) and *A. qalilus* (Krupp, 1992). However, our results suggest that specimens from the Asi Basin are *A. kotschyi* (*Alburnus* sp., KJ552654, KJ552696; *A. qalilus*,

KT220599), whereas specimens from three small Syrian basins are *A. qalilus*.

Another problematic species from this lineage is *Alburnus adanensis*. The main problem is the similarity of the lateral line scale counts of geographically close species, as described in detail by Birecikligil et al. (2016). Our data showed that specimens from the Euphrates-Tigris river system are identical and clearly distinct from the Seyhan, Ceyhan, and Arsuz specimens. Following Bogutskaya (1997), they need to be treated as *A. sellal* rather than *A. mossulensis* and this will lead to validity for *A. adanensis*.

Conclusion

Some species of the genus *Alburnus* show great geographic variation and some of them have indistinct characters. We believe this study and the suggestions herein will not solve all problems of the genus. However, they will serve as a basis for future studies.

The genetic distance between some of the species, especially the ones closely related to *A. alburnus*, *A. mento*, and *A. sellal*, was found to be below 1%. However, before further evidence is presented, synonymy is not suggested for the species which have unique haplotypes.

The use of geometric morphometric methods looks promising for the genus. With the addition of more species and specimens, a more realistic phylogenetic signal might be obtained.

Before this study, the *Alburnus* genus comprised 43 species. A total of 31 taxa were subjected to this study and a total of seven synonymies are proposed. As a result, the genus now comprises 36 species and 21 of them are distributed in Turkey (Appendix 3—Supplementary Material). The distribution of the species in this study was drawn with QGis (GIS Development Team, 2017), following our results for Turkey, Kottelat & Freyhof (2007), and from others, and is given separately based on lineages (Appendix 4—Supplementary Material).

Acknowledgements This study was supported by the Hacettepe University Scientific Research Projects Coordination Unit, Sampling Project No. 011 D06 601 007, Sequence Analyses Project No. 1448, and Software Project No. 014 D01 601 015-489. This study is a part of the Ph.D. thesis of Fatih Mangıt. The authors would also like to thank Hacettepe Technology Transfer Center for proof reading service.

References

Adams, D. C., 2014. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. Systematic Biology 63: 685–697.

Adams, D. C., M. L. Collyer & E. Sherratt, 2015. Geomorph: software for geometric morphometric analyses. R Package version 3.0.0. http://cran.r-project.org/web/packages/geomorph/index.html.

Avise, J. C., 1994. Molecular Markers, Natural History and Evolution: Natural History and evolution. Chapman-Hall, New York.

Battalgazi, F., 1944. Poissons nouveaux et peu connus de la Turquie. Revue de la Faculté des Sciences de l'Université d'Istanbul, Série B, Sciences Naturelles 9: 299–305.

Battalgil, F., 1941. Les poissons des eaux douces de la Turquie. Revue de la Faculté des Sciences de l'Université d'Istanbul, Série B, Sciences Naturelles 6: 170–186.

Battalgil, F., 1942. Contribution a la connaissance des poisons des eaux douces de la Turquie. Revue de la Faculté des Sciences de l'Université d'Istanbul, Série B, Sciences Naturelles 7: 287–306.

Battalgil, F., 1944. Nouveaux poisons des eaux douces de la Turquie. Revue de la Faculté des Sciences de l'Université d'Istanbul, Série B, Sciences Naturelles 9: 126–133.

Behrens-Chapuis, S., F. Herder, M. F. Geiger, H. R. Esmaeili, N. A. Hamidan, M. Özuluğ & R. Šanda, 2015. Adding nuclear rhodopsin data where mitochondrial COI indicates discrepancies – can this marker help to explain conflicts in cyprinids. DNA Barcodes 3(1): 187–199.

Bianco, P. G., 1990. Potential role of the palaeohistory of the Mediterranean and Paratethys basin on the early dispersal of Euro-Mediterranean freshwater fishes. Ichthyological Exploration of Freshwaters 1: 167–184.

Birecikligil, S. S., Ş. Y. Yücel & E. Çiçek, 2016. A taxonomic evaluation of *Alburnus sellal* Heckel, 1843 and *Alburnus adanensis* Battalgazi, 1944 based on morphological characters and mitochondrial DNA sequences. Pakistan Journal of Zoology 48: 465–473.

Bogutskaya, N., 1997. Contribution to the knowledge of Leuciscinae fishes of Asia Minor. Part 2. An annotated checklist of Leuciscinae fishes (Leuciscinae, Cyprinidae) of Turkey with descriptions of a new species and two new subspecies. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 94: 161–186.

Bogutskaya, N. G., F. Küçük & E. Ünlü, 2000. *Alburnus baliki*, a new species of cyprinid fish from the Manavgat River system, Turkey. Ichthyological Exploration of Freshwaters 11: 55–64.

Bouckaert, R., J. Heled, D. Kühnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut & A. J. Drummond, 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PLoS Computational Biology 10: e1003537.

Boulenger, G. A., 1896. On freshwater fishes from Smyrna. Annals and Magazine of Natural History 6: 153–154.

Clement, M., Q. Snell, P. Walke, D. Posada & K. Crandall, 2002. TCS: estimating gene genealogies. In IEEE International Parallel Distribution Symposium, p. 0184.

Darriba, D., G. L. Taboada, R. Doallo & D. Posada, 2012. JModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 772.

Devedjian, K., 1915. Fish and Fishery in Turkey. Düyun-u Umumiye-i Osmaniye Varidat-ı Mahsusa İdare-i Merkeziyesi Matbaası, İstanbul.

Drummond, A. & A. Rambaut, 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7(1): 214.

Erk'akan, F., 1983. The fish species of the Sakarya basin and their abundance. Hacettepe Bulletin of Natural Sciences & Engineering 12: 21–38.

Eschmeyer, W. N., R. Fricke & R. van der Laan (eds), 2016. Catalog of Fishes: Genera, Species, References. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Fricke, R., M. Bilecenoğlu & H. M. Sarı, 2007. Annotated checklist of fish and lamprey species (Gnathostomata and Petromyzontomorphi) of Turkey, including a Red List of threatened and declining species. Stuttgarter Beiträge zur Naturkunde 706: 1–169.

Froese, R. & D. Pauly (eds), 2015. Fishbase. World Wide Web Electronic Publication. www.fishbase.org, version (03/2017).

Geiger, M. F., F. Herder, M. T. Monaghan, V. Almada, R. Barbieri, M. Bariche, P. Berrebi, J. Bohlen, M. Casal-Lopez, G. B. Delmastro, et al., 2014. Spatial heterogeneity in the Mediterranean biodiversity hotspot affects barcoding accuracy of its freshwater fishes. Molecular Ecology Resources 14: 1210–1221.

Geldiay, R. & S. Balık, 2007. Freshwater Fishes of Turkey. Ege University Press, Bornova.

GIS Development Team, 2017. QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://www.qgis.org/.

Gülle, İ., F. Küçük & S. S. Güçlü, 2017. Re-description and new distribution area of an endemic anatolian fish species, *Alburnus nasreddini* Battalgil, 1944. Turkish Journal of Fisheries and Aquatic Sciences 17(5): 863–869.

Hanko, B., 1924. Fische aus Kleinasien. Annales Musei Historico-Naturalis Hungarici 21: 137–158.

Hebert, P. D. N., A. Cywinska, S. Ball & J. Dewaard, 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B – Biological Sciences 270: 313–321.

Ivey, J. L. & S. R. Santos, 2007. The complete mitochondrial genome of the Hawaiian anchialine shrimp *Halocaridina rubra* Holthuis, 1963 (Crustacea: Decapoda: Atyidae). Gene 394: 35–44.

Jukes, T. H. & C. R. Cantor, 1969. Evolution of protein molecules. In Munro, H. N. (ed.), Mammalian Protein Metabolism. Academic Press, New York.

Keskin, E. & H. H. Atar, 2013. DNA barcoding commercially important fish species of Turkey. Molecular Ecology Resources 13: 788–797.

Ketmaier, V., F. Finamore, C. Largiader, M. Milone & P. Bianco, 2009. Phylogeography of bleaks *Alburnus* spp. (Cyprinidae) in Italy, based on cytochrome b data. Journal of Fish Biology 75: 997–1017.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120.

Knebelsberger, T., A. R. Dunz, D. Neumann & M. F. Geiger, 2015. Molecular diversity of Germany's freshwater fishes and lampreys assessed by DNA barcoding. Molecular Ecology Resources 15: 562–572.

Kottelat, M., 1997. European freshwater fishes. An heuristic checklist of the freshwater fishes of Europe (exclusive of former USSR), with an introduction for non-systematists and comments on nomenclature and conservation. Biologia, Bratislava 52: 1–271.

Kottelat, M. & J. Freyhof, 2007. Handbook of European Freshwater Fishes. Cornol/Freyhof, Switzerland/Berlin.

Kumar, S., G. Stecher & K. Tamura, 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874.

Kuru, M., 1982. Türkiye Tatlısu Balıkları Kataloğu. Hacettepe Üniversitesi Fen Fakültesi Basımevi, Beytepe.

Kuru, M., S. V. Yerli, F. Mangıt, E. Ünlü & A. Alp, 2014. Fish biodiversity in inland waters of Turkey. Journal of Academic Documents for Fisheries and Aquaculture 1: 93–120.

Ladiges, W., 1960. Süsswasserfische der Türkey, 1. Teil Cyprinidae. Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 58: 105–150.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson & D. G. Higgins, 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

Librado, P. & J. Rozas, 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Mangıt, F., 2014. Morphometric and Phylogenetic Studies on Genus *Alburnus* (Teleostei: Cyprinidae). PhD Thesis, Hacettepe University Graduate School of Science and Engineering, 82 pp. (In Turkish).

Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge, MA.

Özuluğ, M. & J. Freyhof, 2007a. *Alburnus demiri*, a new species of bleak from western anatolia, Turkey (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 18: 307–312.

Özuluğ, M. & J. Freyhof, 2007b. Rediagnosis of four species of *Alburnus* from Turkey and description of two new species (Teleostei: Cyprinidae). Ichthyological Exploration of Freshwaters 18: 233–246.

Parin, N. V., S. A. Evseenko & E. D. Vasili'eva, 2014. Fishes of Russian Seas: Annotated Catalogue. KMK Scientific Press, Moscow.

Perea, S., M. Böhme, P. Zupančič, J. Freyhof, R. Šanda, M. Özuluğ, A. Abdoli & I. Doadrio, 2010. Phylogenetic relationships and biogeographical patterns in circum-mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. BMC Evolutionary Biology 10: 265.

Por, F. D. & C. Dimentan, 1985. Continuity of Messian biota in the Mediterranean basin. In Stanley, D. J. & F. C. Wezel (eds.), Geological Evolution of the Mediterranean Basin. Springer, New York.

Puillandre, N., A. Lamber, S. Brouillet & G. Achaz, 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21: 1864–1877.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/.

Rambaut, A., M. A. Suchard, D. Xie & A. J. Drummond, 2014. Tracer v1.6 [Computer Software and Manual]. http://beast.bio.ed.ac.uk/Tracer.

Rohlf, F. J., 2015. The tps series of software. Hystrix, the Italian Journal of Mammalogy 26: 9–12.

Stamatakis, A., 2006. Raxml-vi-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Steindachner, F., 1897. Bericht über dei von Dr. Escherich in der umgebung von Angora gesammelten fische und reptilian. Denkschriften (Akademie der Wissenschaften in Wien) 64: 685–699.

Tang, K. L., M. K. Agnew, M. V. Hirt, T. Sado, L. M. Schneider, J. Freyhof, Z. Sulaiman, E. Swartz, C. Vidthayanon & M. Miya, 2010. Systematics of the subfamily Danioninae (Teleostei: Cypriniformes: Cyprinidae). Molecular Phylogenetics and Evolution 57: 189–214.

Triantafyllidis, A., D. Bobori, C. Koliamitra, E. Gbandi, M. Mpanti, O. Petriki & N. Karaiskou, 2011. DNA barcoding analysis of fish species diversity in four north Greek lakes. Mitochondrial DNA 22: 37–42.

Winfield, I. & J. S. Nelson (eds), 2012. Cyprinid Fishes: Systematics, Biology and Exploitation, Vol. 3. Springer, Dordrecht.