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A Taxonomic Evaluation of *Alburnus sellal* Heckel, 1843 and *Alburnus adanensis* Battalgazi, 1944 Based on Morphological Characters and Mitochondrial DNA Sequences

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ABSTRACT

Alburnus sellal inhabits Ceyhan, Euphrates, Orontes and Tigris river basins whereas *Alburnus adanensis* is geographically restricted to Ceyhan and Seyhan river basins. Due to the very similar morphometric and meristic characteristics, discrimination of these two species is difficult. The most important diagnostic character is the scale numbers in lateral line (73-80 in *A. sellal* vs 60-66 in *A. adanensis*) and pronounced lack of the dark grey band on side of the body. In order to clarify the taxonomic status of the *A. sellal* and *A. adanensis*, a total of 27 morphological characters and the nucleotide sequence variation of mitochondrial cytochrome *b* (*cyt b*) and cytochrome oxidase I (*COI*) genes were analyzed on the specimens which were from Seyhan, Ceyhan, lower Euphrates and Orontes river basins. The results show high similarity between *A. sellal* and *A. adanensis* based on the morphometric and the morphological characters using Principal Component Analyses (PCA). In addition genetic distance of the specimens belonging to two species between 0.000 to 0.011 using *COI* and *cyt b* genes sequences. Therefore, our results based on morphology, molecules and nomenclatural priority pointed out that *A. adanensis* should be evaluated as a synonym of *A. sellal*.

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Authors' Contributions
All authors conceived and designed the study. SSB performed the experiments and wrote the article. SYY and EC supervised the work.

Key words
***Alburnus sellal, Alburnus adanensis,* bleak, *cyt b*, *COI*, morphological characters.**

INTRODUCTION

The genus *Alburnus* Rafinesque, 1820 has a wide distribution in Europe and Asia containing about 43 species, 26 of which occur in Turkey (Çiçek *et al*., 2015; Froese and Pauly, 2015). Seven *Alburnus* species, namely *A. adanensis* Battalgazi, 1944, *A. caeruleus* Heckel, 1843, *A. kotschyi* Steindachner, 1863, *A. orontis* Sauvage, 1882, *A. qalilus* Krupp, 1992, *A. mossulensis* Heckel, 1843 and *A. sellal* Heckel, 1843 are distributed in Seyhan, Ceyhan, Euphrates and Orontes river basins (Bogustkaya, 1997; Kuru, 2004; Okur *et al*., 2004; Bostancı, 2006; Dağlı and Erdemli, 2009; Sungur, 2009; Froese and Pauly, 2015).

Alburnus and *Chalcalburnus* were combined into a single genus under *Alburnus* (Bogustkaya, 1997). As expected in such a large genus, there are groups of species more similar to each other (Kottelat and Freyhof, 2007). Although bleaks are widely distributed in Turkey, their taxonomic status has not yet been well understood. *Alburnus sellal* was described from Qwaiq River, Aleppo, Syria by Heckel (1843). In Turkey, this species was reported from Orontes, Euphrates and Tigris systems (*e.g.*, Bogustkaya, 1997; Kuru, 2004; Okur *et al*., 2004; Bostancı, 2006; Dağlı and Erdemli, 2009; Sungur, 2009; Froese and Pauly, 2015). *A. sellal adanensis* was firstly published as a subspecies by Battalgazi (1944) from Seyhan River, near Adana province in Turkey, then it was additionally reported from Ceyhan river system (Kuru, 2004; Bostancı, 2006; Sungur, 2009; Erk'akan and Ozdemir, 2011; Froese and Pauly, 2015). Subsequently, the subspecies was treated as a valid subspecies of *A. sellal* by Bogustkaya (1997). However, the subspecies has been recently re-evaluated at the species level (Fricke *et al*., 2007). Discrimination of these species is not easy since the morphometric and meristic characteristics of the two species are very similar to each other. The main difference of *A. sellal* and *A. adanensis* is scale numbers in lateral line (73-80 in *A. sellal* vs 60-66 in *A. adanensis*) and the pronounced lack of the dark grey band extending on the side of the body (Battalgazi, 1944). However, intraspecific variation, habitat adaptations and seasonal variations may affect color of the fish (Björklund and Almqvist, 2010; Shen *et al*., 2012).

The information on genetic diversity of wildlife is necessary to ascertain the genetically deteriorated populations so that better management plans can be established for their conservation (Arif *et al*., 2011). Due to the complexity and limitations of morphological characters used in traditional taxonomy, several PCR-based methods of genotype analysis have been developed for the identification of fish species, particularly for eggs, larvae, and commercial products (Zhang, 2011).

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Sequence analysis of species-specific DNA fragments (often mitochondrial or ribosomal genes) and multiplex PCR of species-conserved DNA fragments are efficient for fish species identification (Zhang, 2011). There is a growing frequency of fish phylogenetic studies that are using DNA sequence datasets analyzed with species tree methods. Such phylogenetic approaches use sequences from a single gene, multiple genes, or the complete mitochondrial genome (mitogenome) (Kartavtsev *et al*., 2007). Compared to 12S and 16S rDNAs, the mitochondrial protein coding genes evolve much faster and therefore regarded as powerful markers for inferring evolutionary history in lower categorical levels such as families, genera, and species. This feature of mtDNA in phylogeny is suitable for resolving taxonomic uncertainties in conservation genetics (Arif *et al*., 2011). The *cyt b* and *COI* genes are widely used for phylogenetic analyses and one of the most reliable mitochondrial markers for phylogeny including Cyprinids (Folmer *et al*., 1994; Briolay *et al*., 1998; Zardoya *et al*., 1999; Finamore *et al*., 2004; Durand *et al*., 2002; Guo and Zhang, 2005; Ketmaier *et al*., 2009; Mayden *et al*., 2009; Perea *et al*., 2010; Imoto *et al*., 2013). Although many phylogenetic studies have been conducted on Cyprinidae family there has been a few study carried out on genus *Alburnus* (Briolay *et al*., 1998; Zardoya *et al*., 1999; Durand *et al*., 2002; Mayden *et al*., 2009; Imoto *et al*., 2013).

The aim of the study is to clarify the taxonomic status of two fish species *A. sellal* and *A. adanensis* based on DNA sequence comparison of mitochondrial *cyt b* (partial) and *COI* gene regions and morphometric and meristic characters.

MATERIALS AND METHODS

Fish sampling and morphological analyses

The specimens were collected by electrofishing (SAMUS 725 MP) from 63 sampling stations belonging to Ceyhan, Orontes, Seyhan and lower Euphrates river basins on June-August 2014 (Table I). A total of 403 *Alburnus* specimens fixed and preserved in 96% ethanol and transferred to Ichthyology Laboratory of Nevsehir Hacı Bektaş Veli University. Of these 159 specimens were *A. sellal*, 28 *A. adanensis*, 54 *A. qalilus*, 81 specimens were *A. caeruleus* and 81 *A. mossulensis*.

Two types of morphological characters were examined; meristic traits and morphometric measurements (Buj *et al*., 2010; Zareian *et al*., 2013). Meristic traits were: the number of un-branched and branched fin rays in dorsal (D), anal (A), ventral (V), pectoral (P) and caudal (C) fins, the number of scales in lateral line (LL), the number of scales between lateral line and the base of the first dorsal and anal fin elements (LT). Morphometric characters were measured using 0.1 mm precision digital caliper. Twenty morphometric characters include total length (TL), standard length (SL), fork length (FL), head length (HL), eye diameter (ED), snout length (SnL), pre-dorsal distance (PreD), pre-pelvic distance (PrePe), pre-anal distance (PreA), pre-pectoral distance (PreP), body depth (BD), caudal peduncle depth (CPD), depth of dorsal (DFD), ventral (VFD), anal (AFD), pectoral (PFD) fins, fin base of dorsal (DFB), ventral (VFB), anal (AFB) and pectoral (PFB) fins. The percentage ratios of morphometric characters in relations to standard length and head length were analyzed and Principal Component Analyses (PCA) was employed (R, Version 2.3; R Core Team) to determine whether there is a separation of the species.

Molecular analyses

Eight specimens for each species (*A. sellal*, *A. adanensis*, *A. qalilus*, *A. caeruleus* and *A. mossulensis*) were screened for sequence polymorphism of the mitochondrial DNA region encoding for a fragment of 664 base pairs (bp) of *cyt b* gene and a fragment of 547 bp of *COI* gene. Eight sequences were obtained for each gene. *Alburnoides bipunctatus* and *Squalius cephalus* were used as out groups. Molecular analyses were applied by Iontek Laboratory (Istanbul). Mitochondrial DNA was extracted from muscle tissues by Qiagen Mini DNA Kit procedure. Primers for the *cyt b* gene region were:
L15267: 5'-ATGGCAAGCCTACGAAAAAC-3',
H15891: 5'-TCGGATTACAAGACCGATGCTT-3'
(Briolay *et al*., 1998; Ketmaier *et al*., 2009), primers for the *CO I* gene region were:
LCO1490: 5'-
GGTCAACAAATCATAAAGATATTGG-3',
HCO2198: 5'-
TAAACTTCAGGGTGACCAAAAAATCA-3'
(Folmer *et al*., 1994). Thermal cycle amplifications were performed in a 25.0 µl tube containing 12.5 µl PCR Mix, 2.5 µl of each primer, 2.5 µl dH_2O and 5 µl template mtDNA. Thermal cycle comprised 15 min at 95°C (1 cycle); 45 seconds at 94°C, 45 seconds at 60°C, 1 min at 72°C (40 cycles), 5 min at 72°C (1 cycle). Polymerase chain reaction (PCR) products were purified from 2% agarose (High Pure PCR Product Purification Kit, Roche) and sequencing (BigDye Terminator Cycle Sequencing Kit, Applied Biosystems) with an automated DNA sequence following the instructions.

Sequences were aligned using MEGA version 6 (Tamura *et al*., 2013) software and they were checked for stop codons using ExPASy Bioformatics Resources Portal (Artimo *et al*., 2012). Sequence divergences were

Table I.- Sampling localities for *Alburnus* genus.

No	Date	Locality	River basın	Coordinate
1	10-06-2013	Gaziantep-Araban	Lower Euphrates	37º38’33” N 37º47’49” E
2	10-06-2013	Gaziantep-Araban	Lower Euphrates	37º28’27” N37º37’31” E
3	11-06-2013	Gaziantep-Yavuzeli	Lower Euphrates	37º17’45” N 37º30’54” E
4	11-06-2013	Gaziantep-Şehitkamil	Lower Euphrates	37º18’29” N 37º24’45” E
5	11-06-2013	Gaziantep-Yavuzeli	Lower Euphrates	37º19’04” N 37º34’06” E
6	11-06-2013	Gaziantep-Yavuzeli	Lower Euphrates	37º19’31” N 37º38’40” E
7	11-06-2013	Gaziantep-Nurdağı	Orontes	37º7’59” N 36º52’38” E
8	11-06-2013	Gaziantep-Nurdağı	Orontes	37º6’56” N 36º52’1” E
9	11-06-2013	Gaziantep-İslahiye	Orontes	37º1’34” N 36º52’57” E
10	11-06-2013	Gaziantep-Nurdağı	Orontes	36º59’21” N 36º53’29” E
11	12-06-2013	Gaziantep-Nurdağı	Orontes	37º9’31” N 36º51’17”E
12	12-06-2013	Gaziantep-İslahiye	Orontes	36º54’21” N 36º44’44” E
13	13-06-2013	Kahramanmaraş	Ceyhan	37º48’20” N 36º47’21” E
14	13-06-2013	Kahramanmaraş-Elbistan	Ceyhan	38º16’9” N 37º22’51” E
15	13-06-2013	Kahramanmaraş-Elbistan	Ceyhan	38º12’37” N 37º10’52” E
16	13-06-2013	Kahramanmaraş-Elbistan	Ceyhan	38º14’34” N 37º6’42” E
17	14-06-2013	Kahramanmaraş-Afşin	Ceyhan	38º9’56” N 36º53’13” E
18	14-06-2013	Kahramanmaraş-Afşin	Ceyhan	38º9’7” N 36º47’53” E
19	14-06-2013	Kahramanmaraş-Göksun	Ceyhan	38º4’35” N 36º31’27” E
20	14-06-2013	Kahramanmaraş-Göksun	Ceyhan	38º5’4” N 36º27’41” E
21	14-06-2013	Kahramanmaraş-Andırın	Ceyhan	37º44’22” N 36º27’53” E
22	15-06-2013	Kahramanmaraş-Çağlayancerit	Ceyhan	37º38’9” N 37º27’19” E
23	15-06-2013	Kahramanmaraş-Çağlayancerit	Ceyhan	37º45’2” N 37º25’26” E
24	15-06-2013	Kahramanmaraş-Çağlayancerit	Ceyhan	37º47’42” N 37º28’13” E
25	15-06-2013	Kahramanmaraş-Nurhak	Ceyhan	37º51’39” N 37º28’9” E
26	15-06-2013	Adıyaman-Gölbaşı	Ceyhan	37º36’52” N 37º28’36” E
27	15-06-2013	Gaziantep-Araban	Lower Euphrates	37º24’53” N 37º27’55” E
28	22-06-2013	Kahramanmaraş-Pazarcık	Ceyhan	37º26’58” N 37º10’21” E
29	22-06-2013	Kahramanmaraş- Emiroğlu	Ceyhan	37º20’9” N 37º2’47” E
30	22-06-2013	Kahramanmaraş- Emiroğlu	Ceyhan	37º20’22” N 37º2’58” E
31	22-06-2013	Kahramanmaraş- Türkoğlu	Ceyhan	37º25’48” N 36º50’30” E
32	22-06-2013	Kahramanmaraş- Türkoğlu	Ceyhan	37º21’11” N 36º54’57” E
33	22-06-2013	Kahramanmaraş- Türkoğlu	Ceyhan	37º19’35” N 36º52’23” E
34	22-06-2013	Gaziantep- Nurdağı, Balıkalan	Orontes	37º17’5” N 36º55’8” E
35	22-06-2013	Gaziantep- Nurdağı	Orontes	37º12’13” N 36º57’48” E
36	24-06-2013	Adana-Karaisalı	Seyhan	37º18’36” N 35º15’38” E
37	24-06-2013	Adana-Karaisalı	Seyhan	37º11’57” N 35º5’55” E
38	24-06-2013	Adana-Yumurtalık	Seyhan	36º51’53” N 35º33’19” E
39	25-06-2013	Hatay-Arsuz	Orontes	36º24’19” N 35º53’16” E
40	25-06-2013	Hatay-İskenderun	Orontes	36º39’51” N 36º12’53” E
41	25-06-2013	Osmaniye-Toprakkale	Ceyhan	37º4’47” N 36º6’43” E
42	25-06-2013	Osmaniye-Toprakkale	Ceyhan	37º6’4” N 36º2’57” E
43	25-06-2013	Adana-Ceyhan	Ceyhan	37º6’58” N 35º58’50” E
44	25-06-2013	Adana-Ceyhan	Ceyhan	37º14’55” N 35º50’17” E
45	25-06-2013	Osmaniye-Kadirli	Ceyhan	37º21’23” N 36º0’47” E
46	26-06-2013	Osmaniye-Bahçe	Ceyhan	37º10’26” N 36º30’23” E
47	26-06-2013	Osmaniye-Kadirli	Ceyhan	37º11’34” N 36º5’27” E
48	26-06-2013	Osmaniye-Düziçi	Ceyhan	37º9’36” N 36º24’33” E
49	27-06-2013	Kilis	Lower Euphrates	36º51’17” N 37º20’13” E
50	27-06-2013	Kilis	Lower Euphrates	36º48’27” N 37º18’4” E
51	27-06-2013	Kilis	Lower Euphrates	36º46’3” N 37º14’58” E
52	27-06-2013	Kilis	Orontes	36º48’44” N 36º59’9” E

Continued

No	Date	Locality	River basın	Coordinate
53	27-06-2013	Kilis-Musabeyli	Orontes	36º52’24” N 36º51’40” E
54	27-06-2013	Antakya-Hassa	Orontes	36º51’34” N 36º37’37” E
55	27-06-2013	Antakya-Hassa	Orontes	36º43’56” N 36º31’43” E
56	27-06-2013	Kırıkhan	Orontes	36º26’6” N 36º23’47” E
57	27-06-2013	Kırıkhan	Orontes	36º15’42” N 36º23’27” E
58	27-06-2013	Samandağ	Orontes	36º8’47” N 36º4’8” E
59	29-06-2013	Adıyaman-Yarbaşı	Ceyhan	37º45’18” N 37º44’52” E
60	29-06-2013	Adıyaman-Besni	Ceyhan	37º44’16” N 37º52’28” E
61	29-06-2013	Şambayat	Lower Euphrates	37º40’49” N 38º05’14” E
62	29-06-2013	Adıyaman	Lower Euphrates	37º43’09” N 38º09’19” E
63	29-06-2013	Adıyaman-Kahta	Lower Euphrates	37º45’42” N 38º20’36” E

calculated using the Kimura two parameter (K2P) distance model (Kimura, 1980). Neighbor Joining (NJ) (Saitou and Nei, 1987) and maximum likelihood (ML) (Felsenstein, 1985) methods implemented by the program MEGA version 6 (Tamura *et al*., 2013), were applied for phylogenetic tree reconstruction. Bootstrapping was performed in MEGA 6 (Tamura *et al*., 2013) with 1000 replicates. All of the DNA sequences generated in this study were submitted to GenBank (KT220597–KT220616).

RESULTS

Morphological analyses

The results of meristic investigations have been summarized in Table II and morphologic characters and ratios of specimens are summarized in Table III. *A. adanensis* has II-III un-branched, 7-9 branched fin rays in dorsal fin, III un-branched, 8-11 branched fin rays in anal fin and 44-59 scales in lateral line (Fig. 1A). *A. sellal* has II-III un-branched, 7-9 branched fin rays in dorsal fin, III un-branched and 9-12 branched fin rays in anal fin and 49-63 scales in lateral line (Fig. 1B). Principal Component Analysis (PCA) was applied to find clusters in a set of data (Ozdemir, 2015). PCA scores of specimens were calculated using a total of 27 morphological characters. The first and second principle component axes explained 82.12 and 4.93 percent of total variation, respectively. The cumulative variation of these two axes was 87.09% (Fig. 3).

Molecular analyses

A 547 bp DNA fragment of *COI* gene and 664 bp of *cytb* gene were sequenced from the 5 *Alburnus* species. Base composition of these sequences were low G content (mean 17% for *cyt b* and 18.3% for *COI*), nearly equal A, C, T contents (mean 24.7%, 29.2% and 29.2% respectively for *cyt b*, 24.3%, 28.2% and 29.3% respectively for *COI*) for investigated *Alburnus* species. The ratios were similar to those previously reported fish sequences (Briolay *et al*., 1998; Durand *et al*., 2002).

Based on the analysis of the *COI* data the genetic distance between *A. sellal* and *A. adanensis* was 0.000. The smallest genetic distance was between *A. qalilus* and *A. sellal, A. adanensis* (both 0.051) and the largest genetic distance was between *A. caeruleus* and *A. mossulensis* (0.097). Based on the analysis of the *cyt b* data the smallest genetic distance was among *A. qalilus, A. adanensis* and *A. sellal* (0.054 vs. 0.060) and the largest genetic distance was between *A. caeruleus* and *A. qalilus* (0.111). Based on the analysis of the *cyt b* data the intraspecies genetic distance was between; 0.002 and 0.009 for *A. mossulensis*, 0.002 and 0.009 for *A. sellal*, 0.002 and 0.004 for *A. adanensis* populations analyzed. The genetic distance between *A. sellal* and *A. adanensis* populations was 0.002-0.011.

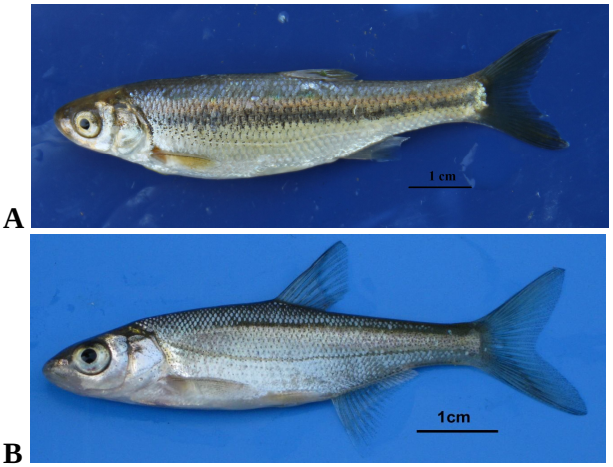

Fig. 1. A, *Alburnus adanensis* collected from Ceyhan river basin; B, *Alburnus sellal* collected from lower Euphrates river basin.

ML and NJ analyses based on mtDNA sequences resulted in resolved trees. The NJ and ML dendograms of *cyt b* and *COI* for five *Alburnus* species and haplotypes

showed that the two bleak (*A. sellal* and *A. adanensis*) species group together (Fig. 3).

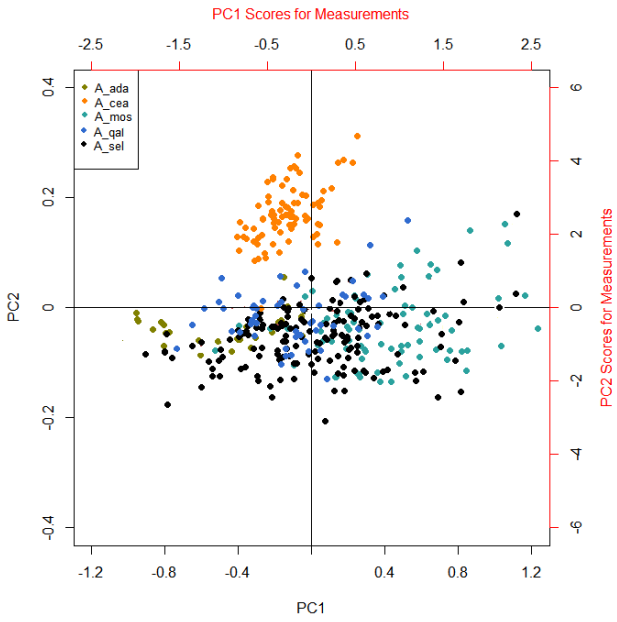

Fig. 2. PCA models of *Alburnus* species collected from Seyhan, Ceyhan, Orontes and lower Euphrates river basins (A_ada: *A. adanensis* (n=28), A_cea: *A. caeruleus* (n=81), A_mos: *A. mossulensis* (n=81), A_qal: *A. qalilus* (n=54), A_sel: *A. sellal* (n=159)).

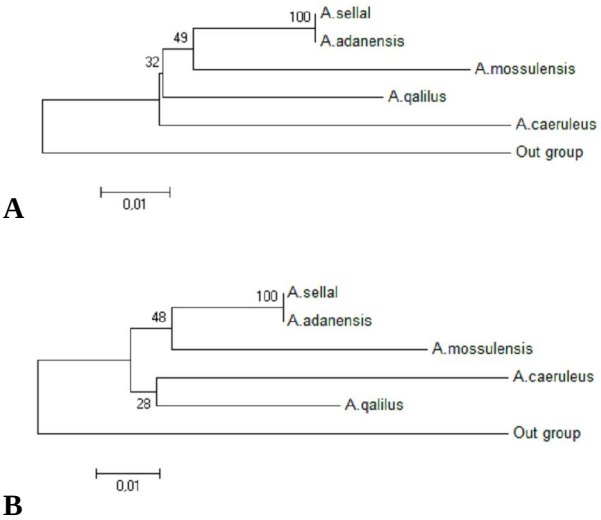

Fig. 3. Phylogenetic tree of *Alburnus* species constructed phylogenetic tree using neighbor-joining method for *COI* (A), using maximum likelihood method for *COI* (B).

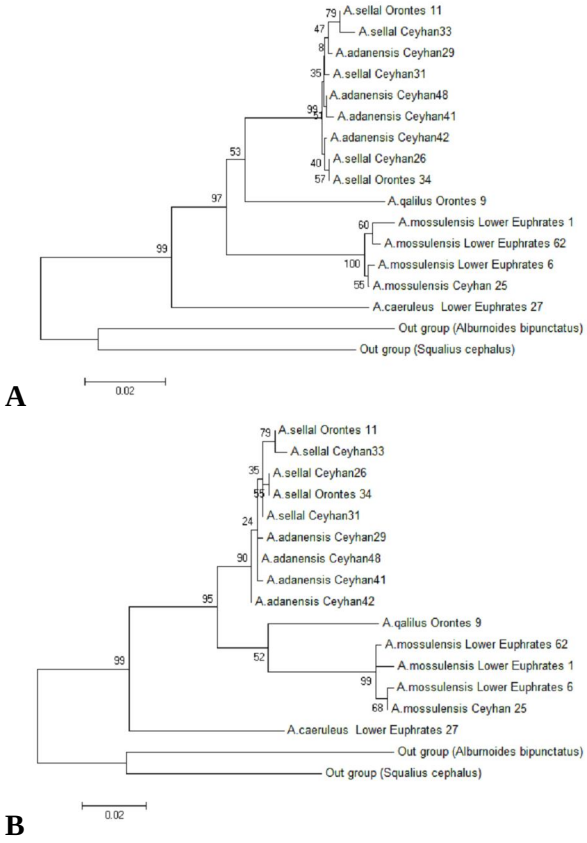

Fig. 4. Phylogenetic tree of *Alburnus* species constructed phylogenetic tree using neighbor-joining method for *cyt b* (A), and maximum likelihood method for *cyt b* (B).

DISCUSSION

Many fish species (as well as other creatures) have been named and described more than once (Parenti and Poly, 2004). This is especially true for widely distributed species, commercially important species, or those that change markedly through ontogeny or differ between male and female phase (Parenti and Poly, 2004). In the last decades, several long-forgotten names have been rediscovered by many authors (Cohen *et al.*, 1991; Randall and Khalaf, 2003; Parenti and Poly, 2004; Motomura *et al.*, 2010; Parenti, 2013). According to Escmeyer and Fricke (2015) the number of available names for use at the species level as of 3 February 2015 is 58.298 and the number of valid species is 33.377.

One of the aims at this study was to determine correct taxonomic status of two fish species *A. adanensis* and *A. sellal*. It is determined that the morphological diagnostic characters of *A. sellal* and *A. adanensis* were in wide range, questionable and similar to each other

Table II.- Meristic characteristics of *Alburnus sellal* and *Alburnus adanensis.*

Species	n	Dorsal fin rays	Anal fin rays	Pelvic fin rays	Pectoral fin rays	Caudal fin rays	Number of lateral line scales	Number of lateral line scales to dorsal and ventral fin margins
Alburnus adanensis	28	II-III 7-9	III 8-11	II 7-8	I 10-14	18-23	44-59	4-6/10-12
Alburnus sellal	159	II-III 7-9	III 9-12	II 7-8	I 11-15	18-23	49-63	4-7/9-13
Alburnus mossulensis	81	II-III 7-9	II-III 11-13	I-II 7-8	I 12-15	18-22	66-89	11-16/4-8
Alburnus qalilus	54	II-III 8-9	II-III 10-11	I-II 7-8	I 11-14	16-22	42-56	8-10/4-5
Alburnus caeruleus	81	II-III 8-10	III 13-16	I-II 6-7	I 12-14	20-24	46-59	10-12/4-6

Table III.- Morphometric characteristics of *Alburnus sellal* and *Alburnus adanensis.*

	Alburnus adanensis		*Alburnus sellal*	
	Mean±SD	**Min-Max**	**Mean±SD**	**Min-Max**
Standard length (%)				
Head length	26.09±3.75	21.21-32.26	24.86±1.05	22.47-27.85
Pre-dorsal distance	56.34±8.82	50.00-86.05	54.75±2.39	50.00-59.65
Pre-pelvic distance	50.20±7.89	42.42-55.00	48.92±1.91	44.59-52.33
Pre-anal distance	65.59±10.69	60.61-74.19	68.07±3.23	64.04-76.62
Pre-pectoral distance	27.39±5.28	22.73-44.19	25.46±1.03	23.60-30.49
Body depth	26.67±4.61	23.64-41.86	25.32±2.24	22.22-31.65
Depth of dorsal fin	11.55±2.55	8.33-19.23	11.10±0.82	8.45-12.70
Base length of dorsal fin	19.46±3.97	15.09-30.23	16.10±1.37	8.86-19.12
Depth of anal fin	12.83±2.44	8.57-20.93	11.90±0.62	10.00-13.41
Base length of anal fin	15.06±2.83	12.12-23.26	12.63±1.03	10.61-16.90
Depth of pelvic fin	3.50±1.25	1.89-6.98	4.18±0.84	3.08-9.46
Base length of pelvic fin	14.81±3.45	11.48-25.58	13.27±0.72	7.59-14.71
Head length (%)				
Eye diameter	26.74±3.17	20.00-40.00	29.02±2.25	22.73-33.33
Snout length	27.36±2.53	25.00-40.00	25.87±3.40	18.75-31.25

Table IV.- Meristic characteristics of *Alburnus sellal* and *Alburnus adanensis* given in the previous studies

Dorsal fin rays	Anal fin rays	Pelvic fin rays	Pectoral fin rays	Caudal fin rays	Number of lateral line scales	Number of lateral line scales to dorsal, ventral margins	Author
Alburnus sellal							
II-III 7-9	III 9-12	II 7-8	I 11-15	18-23	49-63	4-7/9-13	This study
I 8	II 9-10	I 7-8	I 10-12		60-64		Okur *et al.* 2004
II 8	III 11-13	I 9	I 16-18		65-74		Bostancı, 2006
III 8-9	III 13-16	I 7	I 13-14		48-59		Sungur, 2009
Alburnus adanensis							
II-III 7-9	III 8-11	II 7-8	I 10-14	18-23	44-59	4-6/10-12	This study
II-8	III 10-11	I 8	I 12-13		50-53		Bostancı, 2006
II-III 8-9	II-III 10-13	I 8	I 12-15		57-63		Sungur, 2009

(Table III and IV). Although the numbers observed by above mentioned authors are not completely the same as obtained in this study, they fall generally within the mentioned ranges. Therefore, classification of these species is very complicated. According to Bogustkaya (1997), the number of scales in the lateral line ranged from 66 to 80 for *A. sellal* and 60 to 66 for *A. adanensis* (Battalgazi, 1944). However, previous studies showed that number of lateral line scales show wide range of 48-74 for *A. sellal* and 44-63 for *A. adanensis* (Bogustkaya, 1997; Kuru, 2004; Okur *et al*., 2004; Bostancı, 2006; Fricke *et al*., 2007; Daglı and Erdemli, 2009; Sungur, 2009; Erk'akan and Ozdemir, 2011). Morphologic ratios (Table III) and morphometric characters (Table II) of the two bleaks are mostly similar. Absence of the extending dark grey band on side of the body is an other morphological character (Battalgazi, 1944). However, the specimens collected from Orontes and Ceyhan rivers basins have nearly the same scale numbers and similar morphologic characters (Table II and III). Indeed according to Bogustkaya (1997) and Krupp (1985) the taxonomic assignment of *A. sellal adanensis* is questionable. This similarity or differences of morphologic characters closely related due to differences in habitat. In deed Björklund and Almqvist (2010) reported the genetic structuring and some morphological characters can evolve within a short time for *Neogobius melanostomus* in the Baltic Sea. The morphologic characters variabilities between species can also be noted between the different populations of the same species, probably depending on the ecological factors variation in different localities (Björklund and Almqvist, 2010). Heckel (1843) describe four different species namely *A. sellal, A. pallidus, A. hebes* and *A. microlepis.* Subsequently; it was determined that *A. pallidus, A. hebes* and *A. microlepis* were junior synonym to *A. sellal* (Froese and Pauly, 2015).

The fast rate of evolution of mtDNA compared to nuclear DNA makes mtDNA useful for high-resolution analyses of recent evolutionary events. This fast rate of mtDNA evolution coupled with maternal inheritance have made mtDNA an extremely popular genetic system with which to study gene flow, hybrid zones, population structure and other population level questions (Meyer, 1993). However, it may not be suitable for inferring phylogenetic relationships among distantly related groups (Kikugawa *et al.*, 2004). The mtDNA *cyt b* and *COI* genes are very useful molecular markers for understanding the phylogenetic and taxonomic relationships at the species level and higher taxonomic levels (Folmer *et al*., 1994; Shen *et al*., 2012).

The limit values for the genetic distance between fish species have been reported in previous studies conducted in mtDNA can vary between 2% and 4% (Hebert *et al*., 2004; Ward *et al*., 2005; Zhang, 2011; Ozdemir, 2013). This value may vary according to different taxonomic groups. For example the limit values for genetic distance on species for genus *Capoeta* are listed as 2% by Ozdemir (2013). Genetic distance between *A. sellal* and *A. adanensis* was found considerably below 2% as 0.002-0.011 for *cyt b* and 0.000 for *COI*. Additionally the NJ and ML dendograms of *cyt b* and *COI* of *Alburnus* species showed that the two bleak (*A. sellal* and *A. adanensis*) branched out together (Figs. 3, 4).

This study was aimed at clarifying the taxonomic status of *A. sellal* and *A. adanensis* both morphological and molecular characters. Analyses of 27 morphological characters and PCA overlaps revealed no separate clusters for the two taxa. Genetic distance between two species was found intraspecific limits. The NJ and ML dendograms of *cyt b* and *COI* of *Alburnus* species showed that the two bleak (*A. sellal* and *A. adanensis*) branched out together. These data let us conclude that between the two first taxa the differentiation is minor and they fall within the limits of intraspecies variation (Kartavtsev *et al*., 2007; Hsu *et al*., 2009). Consequently, the morphological and mtDNA sequence analyses provide evidence that *A. adanensis* is a junior synonym of *A. sellal.*

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