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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Genetic Analysis of *Carasobarbus* Karaman, 1971 (Actinopterygii Cyprinidae) in Anatolia[*]

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Abstract: We sequenced the complete mitochondrial cytochrome b gene (1141 bp) of 70 individuals from ten populations of three *Carasobarbus* species in Anatolia. Totally eight haplotypes were identified. The intraspecies genetic distance ranged from 0.00% to 0.21%, while it varied from 2.6% to 9.0% for interspecies. Except for Gaziantep samples with low sample size, high haplotype diversity (Hd= 0.590-0.833) and low nucleotide diversity (Pi= 0.05-0.65) values can be explained by the presence of small populations sensitive to genetic drift and founder effects. Phylogenetic analyses constructed with neihgbour joining, maximum likelihood and maximum parsimony generated similar topologies supported by high bootstrap values. Phylogenetic tree topologies showed that the *C. apoensis* haplotype was located in the *C. luteus* species. Therefore, the validity of species status of *C. apoensis* should be checked morphologically. On the other hand, since the Kahta population in *C. luteus* has a remarkably high genetic diversity, it must be re-evaluated morphologically. The tectonic uplift of the Anatolian Plateau between the African and European plates during the Pliocene period may have probably prevented the presence of *Carasobarbus* in the west of the Anatolian diagonal.

Keywords: Anatolia, *Carasobarbus*, mtDNA, Phylogeny.

Anadolu'daki *Carasobarbus Karaman*, 1971 (Actinopterygii Cyprinidae) Genusunun Genetik Analizi

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Öz: Anadolu'daki üç *Carasobarbus* türünün 10 populasyonundan 70 bireyin mitokondri sitokrom b geninin (1141 bp) DNA dizin analizi yapılmıştır. Toplamda sekiz haplotip tanımlandı. Sitokrom b veri analizi kullanılarak, tür içi genetik mesafenin % 0,00 ile % 0,21 arasında, türler arası için ise % 2,6 ile% 9,0 arasında değiştiği belirlenmiştir. Yeterli örneklem büyüklüğüne sahip olmayan Gaziantep örnekleri hariç yüksek haplotip çeşitlilik (Hd= 0,590-0,833) ve düşük nükleotit çeşitlilik (Pi= 0,05-0,65) değerleri, genetik sürüklenme ve kurucu etkilere karşı duyarlı olan küçük populasyonların varlığı ile açıklanabilir. Komşu birleştirme, maksimum tutumluluk ve maksimum olasılık metotları yoluyla oluşturulan filogenetik analizler yüksek güvenirlilik değerleri ile desteklenen benzer topolojiler üretmiştir. Filogenetik ağaç topolojileri gösterdi ki *C. apoensis* haplotipleri *C. luteus* haplotipleri içinde konumlandığı için türün geçerliliği morfolojik olarak kontrol edilmelidir. Diğer taraftan, *C. luteus* içindeki Kahta populasyonu dikkate değer düzeyde yüksek genetik çeşitliğe sahip olduğundan morfolojik olarak mutlaka yeniden değerlendirilmelidir. Pliyosen döneminde Afrika ve Avrupa levhaları arasındaki çarpışmadan kaynaklanan Anadolu Platosu'nun tektonik yükselişi muhtemelen Anadolu köşegeninin batısında *Carasobarbus*'un varlığını engellemiştir.

Anahtar kelimeler: Anadolu, *Carasobarbus*, Filogeni, mtDNA.

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INTRODUCTION

Members of the genus *Carasobarbus*, defined by Karaman (1971), are distributed in Southwest Asia, Northwest Africa, Mesopotamia and Southwestern Arabian peninsulas and are an important element for ichthyofauna in these regions (Froese & Pauly, 2016). *Carasobarbus* genus, which has nine species in the world, is represented with three species (*C. luteus, C. kosswigi* and *C. chantrei*) in Turkey (Borkenhagen & Krupp, 2013). One of these, *Carasobarbus luteus* (Heckel, 1843) distributed in the Euphrates, Tigris and Kueik river systems (Gökçek et al., 2008; Kuru, 1979; Ünlü, 1991), *C. kosswigi* inhabit in the Euphrates and Tigris drainages (Abdoli, 2000), and *C. chantrei* (Sauvage, 1882) distributed in the Orontes river and Dam Tahtalıköprü.

Studying the *Carasobarbus* species dispersed in Iran and its neighboring regions, Borkenhagen found two different sets of haplotypes for *C. luteus* based on cytochrome b sequences. The first is widespread, consistent with the biogeographic hypothesis that points to a recent isolation between many populations as a result of rising sea levels following the recent Pleistocene glaciation. The range of the latter was limited to the Khabur River in Syria. They discussed the possibility that one of these groups corresponded to *C. albus*, but suggested that *C. luteus* was more likely to be a single species representing two different mitochondrial lineages. They concluded that the nucleotide diversity in *C. kosswigi* and *C. sublimus* was high, possibly due to the small population size and the resulting genetic shift. Borkenhagen and Krupp, (2013) revised the genus *Carasobarbus* based on comparative morphological studies of approximately 1300 preserved specimens from the collections of various museums and newly collected materials. In this study, in which *C. apoensis, C. canis, C. chantrei, C. exulatus, C. fritschii, C. harterti, C. kosswigi, C. luteus* and *C. sublimus* formed a monophyletic group, they described the species in detail and formed a diagnosis key as a result of re-evaluation of their taxonomic status. Parmaksız and Eskici, (2018) conducted a sequence analysis of the mtDNA COI locus from 4 populations to determine the genetic variation of *C. luteus* populations. They revealed the genetic diversity of the species with 9 polymorphic regions and 4 haplotypes determined for cyt b gene (625 bp) by DNA sequence analysis.

Although a limited number of taxonomic and population genetics studies based on morphological and molecular techniques have been conducted, a phylogeny study involving all *Carasobarbus* species in Turkey has not yet been conducted. The aim of this study is to reveal the phylogeny and phylogeography of valid *Carasobarbus* species using mitochondrial cytochrome b gene sequences.

MATERIAL and METHOD

Sampling: A total 70 *Carasobarbus* were sampled from ten locations in the main tributary systems in Eastern Anatolia such as Tigris, Euphrates, and Kueik River (Table 1). The sampled fish were classified and labeled according to the species identification key. Approximately 30 mg of fins was removed from each fish, stored in 96% ethanol until DNA extraction and transferred to the Genetic laboratory, Faculty of Fisheries, Recep Tayyip Erdogan University where genetic studies were performed. Sampling and experimental studies are consistent with the universal ethical standarts. The study was approved by the Ethics Committee of Recep Tayyip Erdogan University (Decision No: 2014/72).

Table 1. Sampling and location information

Species and their locations	Coordinates	N
Carasobarbus luteus		
Kueik River, Kilis	36°47'49.4"K 36°55'02.1"D	15
Anbar Stream, Tigris River, Hani, Diyarbakır	38°18'12.6"K 40°26'35.5"D	6
Merziman Stream, Euphrates River, Yavuzeli, Gaziantep	37°19'40.5"K 37°40'04.2"D	2
Karasu Stream, Euphrates River, Araban, Gaziantep	37°24'23.6"K 37°41'12.9"D	2
Çamçayı Stream, Euphrates River, Siverek, Şanlıurfa	37°39'05.5"K 39°13'50.8"D	10
Kahta Stream, Euphrates River, Kocahisar, Adıyaman	37°57'48.9"K 38°39'44.7"D	9
Çataltepe Stream, Euphrates River, Ziyaret, Kahta, Adıyaman	37°45'00.3"K 38°35'29.7"D	3
Carasobarbus chantrei		
Karasu Stream, Tahtaköprü Dam, Orontes River, Hatay	36°49'40.2"K 36°39'47.6"D	11
Muratpaşa Stream, Orontes River, Kırıkhan, Hatay	36°29'15.6"K 36°28'04.1"D	6
Karasu Stream, Orontes River, Kırıkhan, Hatay	36°27'55.0"K 36°22'45.0"D	6
Carasobarbus kosswigi		
SMF31325	KU524935	1
Total		71

DNA Extraction, Polymerase Chain Reaction and Sequencing: Genomic DNA was extracted from approximately fin tissues using the DNeasy Blood & Tissue Kit (Qiagen, USA) according to the manufacturer's protocol and stored in absolute ethanol at −20 °C for molecular studies. Samples of DNA were resolved in horizontal 0.8% agarose gels. The gels were submerged in 1x TAE (Tris-Acetate-EDTA) buffer containing 0.5 µg/ml ethidium bromide and visualized under ultraviolet light (Quantum ST4, Transilluminator + Quantum Capt Softwares, Vilber Lourmat, France).

The polymerase chain reaction (PCR) was used to amplify the mtDNA cytochrome *b* (1141 bp) with the universal cyt *b* primers: L14724 (5'-GTG ACT TGA AAA ACC ACC GTT G-3') and H15915 (5'-CAA CGA TCT CCG GTT TAC AAG AC-3') published by Anderson et al.,

(1981). The volume of the PCRs was 50 µl and contained 5 µl 10× reaction buffer, 2 µl dNTPs (10 mM), 0.2 µl of each primer (10 pmol/µl), 3 µl DNA-extract (50 ng/ml), 0.2 µl *Taq* polymerase (5 U/µl), and 26.8 µl ddH_2O. The PCR conditions consisted of preheating at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 61°C for 30 s, and 72°C for 60 s, finalised at 72°C for 5 min, with a final 5 min extension at 72°C on T100TM PCR Gradient Thermal Cycler (Bio-Rad, Hercules, USA). PCR products were displayed under the UV Quantum–Capt ST4 system (Vilber Lourmat, Marne-La-Vallee, France). PCR products were purified with the Qiagen purification kit. Automated bi-directional sequencing was performed using the Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) and an ABI PRISM 3730x1 Genetic Analyser (Applied Biosystem, USA) sequencer.

Data Analyses: MtDNA cytochrome *b* sequences were aligned and corrected manually with Bioedit 7.0.0 (Hall, 1999). The number of haplotype, number of polymorphic sites, the nucleotide composition, haplotype and nucleotide diversity were calculated using the software DNASP v.5.10.01 (Librado & Rozas, 2009). Interspecific and intraspecific pairwise distances were calculated using Kimura two-parameter model (K2P; Kimura, 1980) implemented in MEGA version X (Kumar et al., 2018).

Phylogenetic analyses were performed by using neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses. NJ trees were generated using MEGA X (Kumar et al., 2018) with 1000 bootstrap replicates. A maximum parsimony analysis was estimated using heuristic searches, as implemented in PAUP* v.4.0b10 (Swofford, 2003). A heuristic search for the most parsimonious trees was implemented using with random addition sequence (100 repitions) and tree bisection–reconnection (TBR) branch-swapping procedure. A majority-rule consensus tree was constructed and bootstrap re-sampling (Felsenstein, 1981) based on 1000 replicates was used to assess support of relationships. According to the Akaike information criterion (AIC), jModeltest v.0.1.1 (Posada, 2008) selected the HKY+G as the best model evolution for the cyt *b* dataset. ML analysis with 100 bootstrap replicates implemented in PhyML ver. 2.4.4 (Guindon & Gascuel, 2003). For all phylogenetic analyses, *Barbus tauricus* (GenBank accession number MH010350) was used as outgroup.

RESULTS

Genetic Diversity: The complete cyt *b* sequences were sequenced for 70 individuals belonging to three *Carasobarbus*. Cytochrome *b* (1141 bp) had 114 variable sites, 39 of which were parsimony informative. The overall nucleotide composition of the mitochondrial cyt *b* gene is A: 30.3%, G: 13.6%, T: 28.6% and C: 27.5%, respectively, with an A + T-rich feature (55.72%).

In total, nine haplotypes were observed from *C. luteus* (7), *C. chantrei* (1) and *C. kosswigi* (1) (Table 2). For *C. luteus*, high haplotype diversity (h) and low nucleotide diversity (π) were calculated as h=0.7040 and π=0.0026, respectively while *C. chantrei* and *C. kosswigi* have only one haplotype each (h=0.00 and π=0.00; Table 2).

Based on the cyt b analysis, the sequence divergence in the three *Carasobarbus* species (*C. chantrei*, *C. luteus* and *C. kosswigi*) distributed in the Euphrates, Tigris, Orontes and Kueik rivers, which are important water resources of Anatolia, were ranged from 0.026 to 0.090, whereas lower sequence divergence were between the *C. luteus* and *C. chantrei* (0.026) and maximum divergence were between the *C. luteus* and *C. kosswigi* (0.090) (Table 3).

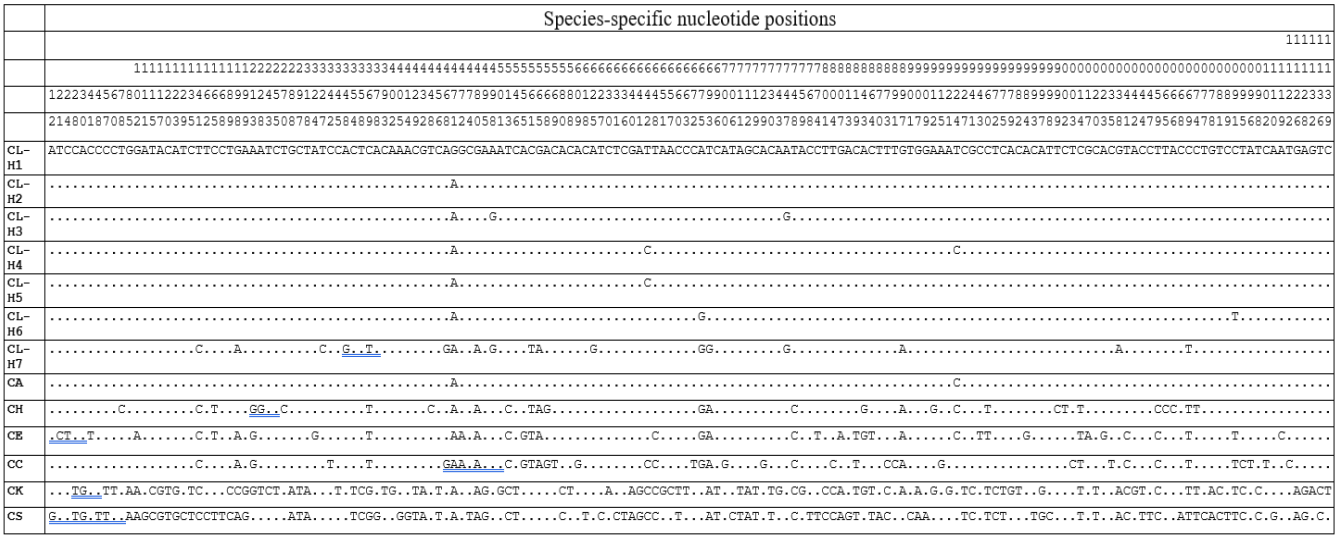
On the other hand, intraspecific sequence divergence values range from 0.00% (*C. kosswigi*) to 0.21% (*C. luteus*) (Table 3). Also, the genetic distances between these three *Carasobarbus* species (*C. chantrei*, *C. luteus* and *C. kosswigi*) and other species in Genbank range from 0.35% to 10.92% (Table 3).

Species-specific nucleotide positions in the mtDNA cyt *b* gene of *Carasobarbus* species are given in Figure 1. Seven *Carasobarbus* species differ from each other at 166 different nucleotide positions (Figure 1).

Table 2. Origin and number of individuals from Carasobarbus species sequenced for mtDNA cytochrome b gene. Sample numbers (N), Haplotype codes (Hc), Haplotype numbers (Hn), Haplotype diversity (Hd), Nucleotide diversity (Pi).

Species, River, and Locations	N	Hc	Hn	Hd	Pi
Carasobarbus luteus					
Kueik River, Kilis	15	H1, H2, H3	3	0.590	0.0006
Anbar Stream, Tigris, Hani, Diyarbakır	6	H1, H3	2	0.600	0.0005
Merzimen Stream, Euphrates, Yavuzeli, Gaziantep	2	H4	1	0.000	0.0000
Karasu Stream, Euphrates, Araban, Gaziantep	2	H3	1	0.000	0.0000
Çamçayı Stream, Euphrates, Siverek, Şanlıurfa	10	H1, H3, H4	3	0.600	0.0006
Kahta Stream, Euphrates, Kocahisar, Adıyaman	9	H1, H3, H6, H7	4	0.833	0.0065
Çataltepe Stream, Euphrates, Kahta, Adıyaman	3	H1, H5	2	0.666	0.0005
Total	**47**		**7**	**0.704**	**0.0020**
Carasobarbus chantrei					
Karasu Stream, Tahtaköprü Dam, Orontes, Hatay	11	H8	1	0.000	0.0000
Muratpaşa Stream, Orontes Kırıkhan, Hatay	6	H8	1	0.000	0.0000
Karasu Stream, Orontes Kırıkhan, Hatay	6	H8	1	0.000	0.0000
Total	**23**		**1**	**0.000**	**0.0000**
	70	**8**			

Ingroup					
Carasobarbus kosswigi					
Karkheh River, Khuzestan, Iran	**1**	KU524935	**1**	**0.000**	**0.0000**
Carasobarbus luteus					
Khābūr River, Ghawat, Syria		KU524927			
Khābūr River, Ash Shaddādah, Syria		KU524928			
Khābūr River, Tall Tamr, Syria		KU524929			
Carasobarbus apoensis					
Saudi Arabia museum no SMF33517		KU525007			
Carasobarbus chantrei					
Syria, museum no SMF31016		KU524914			
Syria, museum no SMF31309		KU524921			
Carasobarbus exulatus					
Saudi Arabia, museum no SMF30774		KU524903			
Carasobarbus canis					
Israel, museum no SMF31314		KU524926			
Carasobarbus sublimus					
Iran, museum no SMF31319		KU524930			
Carasobarbus harterti					
Moorocco, meseum no SMF33366		KU524975			
Carasobarbus fritschii					
Morocco, museum no SMF33481		KU524988			
Outgroup					
Barbus tauricus					
Terme Stream, Turkey		MH010348			



CL: *C. luteus*, CA: *C. apoensis*, CH: *C. chanteri*, CE: *C. exulatus*, CC: *C. canis*, CK: *C. kosswigi*, CS: *C. sublimus*

Figure 1. Species-specific nucleotide positions of seven Middle Eastern species belonging to the *Carasobarbus* genus.

Table 3. Mean interspecific and intraspecific genetic distances of *Carasobarbus*.

	Species	1	2	3	4	5	6	7	8	9
1	*C. luteus*	0.0021								
2	*C. apoensis*	0.0035	0.0013							
3	*C. chantrei*	0.0260	0.0263	0.0000						
4	*C. exulatus*	0.0340	0.0324	0.0275	0.0010					
5	*C. canis*	0.0360	0.0341	0.0348	0.0313	0.0006				
6	*C. harterti*	0.0666	0.0635	0.0662	0.0636	0.0606	0.0000			
7	*C. fritschii*	0.0760	0.0737	0.0764	0.0739	0.0708	0.0317	0.0066		
8	*C. kosswigi*	0.0900	0.0890	0.0883	0.0864	0.0907	0.1092	0.1085	0.0000	
9	*C. sublimus*	0.0966	0.0957	0.1023	0.0940	0.1006	0.1063	0.1036	0.0516	0.0024

The neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) phylogenetic methods resulted in similar trees topologies, supported by high bootstrap values (NJ:100-66, MP:100-80 and ML:100-68; Figure 2).

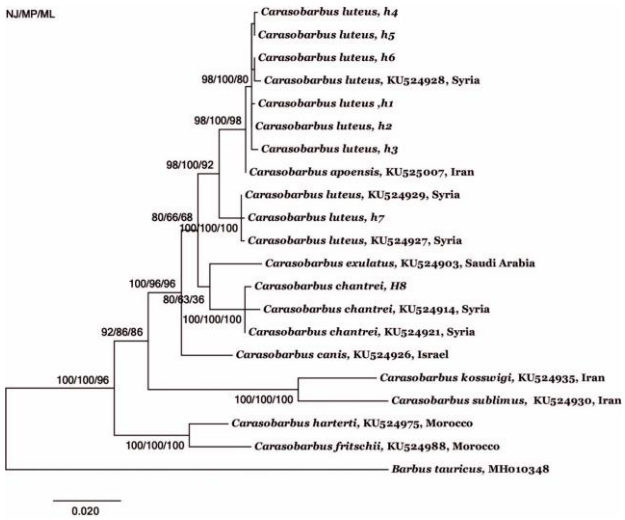


Figure 2. Maximum likelihood tree of *Carasobarbus* species based on the cyt *b* gene. NJ, MP and ML methods produced identical topologies and therefore only the ML tree is shown. The numbers above the branches are bootstrap values

DISCUSSION

Anatolia, the Asian part of Turkey, is in the region of intersection of the important biodiversity hotspots such as Caucasus, Iran-Anatolia and Mediterranean basin. One of the most distinctive biogeographical features determining the biodiversity level of Anatolia is the Anatolian diagonal, which is considered to be the biogeographical boundary between the central and eastern Anatolian fauna. During the last interglacial cycle, it is suggested the most of the populations or taxa isolated on both sides of the Anatolian diagonal diverged over time under the influence of environmental factors. (Gür, 2016). As stated in the literature (*Carasobarbus luteus* Heckel, 1843, Borkenhagen, 2005; Kaya et al., 2016, *C. kosswigi*, Borkenhagen et al., 2011; Esmaeili et al., 2006; Kaya et al., 2016 and *C. chantrei*, Borkenhagen et al., 2011; Esmaeili et al., 2006), the distribution of genus *Carasobarbus* Karaman, 1971 is limited to the Tigris, Euphrates, Kueik and Orontes river basin in the east of Anatolia diagonal as well as southwestern Asia and northwestern Africa. Therefore, the geographical distribution of *Carasobarbus* supports Anatolian diagonal model. The base composition of the cyt *b* gene of three *Carasobarbus* species (lower G and equal C, A and T) is very similar to that previously reported for *Carasobarbus* (Borkenhagen et al., 2011) and other some fish species (Briolay et al., 1998; Cantatore et al., 1994; Tang et al., 2006).

Species identification depends on the detection of species-specific genetic differences in the DNA (Liu & Cordes, 2004), and cyt *b* sequences show significant differences even for closely related species as they have relatively high interspecies and low intraspecies variation (Aranishi et al., 2005). The presence of species-specific nucleotide positions in mitochondrial DNA cytochrome *b* gene sequences should provide evidence for the genetic identification of three *Carasobarbus* species in Turkish freshwater fauna (Table 4), while *C. apoensis* and *C. luteus* species could not be distinguished. On the contrary, a significant number of specific mutations (4 SNPs, Table 4) in a haplotype H7 identified from the *C. luteus* population in Kahta Stream requires a morphological review of this population.

The number of haplotypes determined for *C. luteus* (7), *C. chantrei* (1) and *C. kosswigi* (1) by analysis of mDNA cyt *b* gene is quite low (Table 2). In addition, the high haplotype diversity (Table 2; Hd= 0.590-0.833) and low nucleotide diversity values (Table 2; Pi= 0.05-0.65) determined for *Carasobarbus* species may be explained to some extent by the presence of rare small populations sensitive to genetic drift and founder effects. A similar situation was previously reported by Borkenhagen et al. (2011) for *C. kosswigi* and *C. sublimus* in the Iranian basin. *Carasobarbus kosswigi* inhabits in small rivers and mountain streams and is less probably to migrate than *C. luteus*, which occupy in the lowland areas of rivers. This results in much lower gene flow among *C. kosswigi* populations. The low haplotypic and nucleotide diversity values (Table 2; Hd= 0.00 ve Pi= 0.00) detected in the Merzimen and Karasu river samples can be attributed to the small sample size. Studies with larger sample sizes can provide useful information about population dynamics of *C. kosswigi*. Molecular genetic studies using mitochondrial DNA marker variation to characterize the genetic diversity of existing species are known to be particularly sensitive to sample size (Aksu & Bektas, 2019). As a result, the small sample sizes do not have the discriminatory power needed to accurately identify samples on the basis of mtDNA genetic polymorphism (Phillips et al., 2019).

The relative genetic affinity (2.63%, Table 3) between *C. luteus* and *C. chantrei*, determined based on pairwise sequence divergence, can be explained by the proximity of the sampling locations. On the other hand, the relatively high genetic distance between *C. luteus* and *C. kosswigi* (8.98%, Table 3) with overlapping geographic distribution can be explained by their different habitat preferences, as mentioned above. In order to understand the phylogenetic relationships in the genus of *Carasobarbus*, analyzes were carried out using Genbank data belonging to *Carasobarbus* species from other regions. Inter-species genetic distances ranged from 0.44% (*C. luteus* and *C. apoensis* in rivers in southwest Saudi Arabia that drain into the Red Sea) to 10.91% (between *C. harterti*; Morocco, nortwestern Africa and *C. kosswigi*; Persian Gulf Basin) (Table 3). In accordance with Wright's (1943) hypothesis, our results showed that genetic distances between species are directly proportional to their geographic distances.

Phylogenetic analysis of mtDNA cytochrome *b* haplotypes of *Carasobarbus* species including *C. luteus*, *C. chantrei* and *C. kosswigi*, which are distributed in Anatolia, based on NJ, MP and ML methods, showed a monophyletic structure with high reliability (Figure 1). Similarly, Borkenhagen, (2017) had reported that *C. apoensis*, *C. canis*, *C. chantrei*, *C. exulatus*, *C. fritschii*, *C. harterti*, *C. kosswigi*, *C. luteus* and *C. sublimus* form a monophyletic group, based on mitochondrial cytochrome *b* and ND4 genes. Borkenhagen, (2017) reported that the *Carasobarbus* genus is classified under two species groups as eastern *(C. canis*, *C. exulatus*, *C. chanteri*, *C. luteus* and *C. apoensis*) and western (*C. harherti* and *C. fritschii*), *C. kosswigi* and *C. sublimus* clustered together in the phylogenetic tree. Looking at the phylogenetic tree topologies obtained from the analysis of the species belonging to the Eastern group, It will be seen that the haplotype H7 (*C. luteus*) obtained from Anatolia clustered together with the Dicle-Fırat haplotypes reported by

Borkenhagen et al., (2011). What is interesting here is not the existence of two groups within the *C. luteus* species, but the fact that *C. apoensis* in the rivers draining into the Red Sea in Saudi Arabia is located between these groups (Figure 1). Contrary to what is expected in the obtained phylogenetic tree topologies, the merging of *C. apoensis* haplotypes into the *C. luteus* group requires a morphological review *of C. apoensis* with a large sampling group (Figure 1). In addition, the remarkable genetic distance between *C. luteus* haplotype (H7) obtained from Kahta Stream (Euphrates River) and other homologous haplotypes (H1, H3 and H6) determined in the same location requires a morphological revision of Kahta Stream samples. According to the phylogenetic tree topologies we obtained in present study (Figure 1), *C. kosswigi* and *C. sublimus* can be considered as a paraphyletic group since they contain only a part of *Carasobarbus* species originating from a common ancestor. Moreover, this suggestion had suggested by Borkenhagen et al., (2011), which previously made the molecular systematics of *Carasobarbus*, based on the mitochondrial genome.

Wang et al., (2013) suggested that the Torini group, which includes the *Carasobarbus* lineage, arised in the east about 9.94 million years ago (mya), and the *Carasobarbus* lineage diverged from others by about 7.7 mya. The Anatolian diagonal extending in the north-east south-west direction in Anatolia, which is the source region of European colonization, is an obstacle to the colonization of Europe by organisms from the Near East (Ansell et al., 2011). As a result of the collision of the African and European plates, the rise of the Pliocene Anatolian plateau (orogenic activity) and global climatic changes probably (Durand et al., 2002; Fairbridge et al., 1997) caused the disconnection of the wide river connections in Anatolia and thus prevented the presence of *Carasobarbus* in the fauna in the west of the Anatolian diagonal. The fact that the genus *Carasobarbus* has no distribution in the east of the Anatolian diagonal can be explained by the paleogeographic history of the region.

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