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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Invasive *Coptodon* (Perciformes: Cichlidae) in southwest Turkey: Species identification using sequence data

Türkiye'nin güneybatısındaki istilacı Coptodon (Perciformes: Cichlidae): Dizi verileri kullanılarak tür tanımlaması

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Abstract: Nonnative cichlids (*Coptodon zillii*) have established populations in the Köyceğiz and Koca Lakes, located on the west coasts of Mediterranean Turkey. Conflicting species names in these lakes have been reported for many years. We studied samples from current populations of *Coptodon* in these lakes and the Pecenek canal concerning existing GenBank data. We estimated the possible ancestral population using sequence data in the mitochondrial D-loop segment. Inter and intra-population morphological variations of *Coptodon* were examined using 25 morphological and six meristic characters. Haplotype analysis revealed three unique haplotypes in three populations of *Coptodon*, indicating poor genetic diversity. Both maximum likelihood and Bayesian trees showed that these three haplotypes constitute a distinct subclade within the *Coptodon zillii* clade. This phylogenetic pattern indicates that populations of both lakes were founded by a single invasion of *C. zillii* and belong to a single species. Consistent with phylogenetic data, the populations of both lakes do not exhibit significant phenotypic divergence, though the Pecenek population is slightly divergent. Intra-population morphological variability may be due to phenotypic plasticity in response to habitat heterogeneity within the lakes.

Keywords: *Coptodon zillii*, invasive fish, mitochondrial control region, phylogeny, morphometric variation

Öz: Egzotik bir tür olarak tilapyalar (*Coptodon zillii*) Akdeniz'in batı kıyılarında bulunan Köyceğiz ve Koca Göllerinde populasyonlar oluşturmuşlardır. Bu göllerde bu familyaya ait farklı tür isimleri uzun yıllardır rapor edilmektedir. Bu çalışmada mevcut GenBank verileri referans alınarak her iki gölde ve Peçenek kanalında mevcut populasyonlar oluşturan *Coptodon* örnekleri incelenmiştir. Mitokondriyal D-loop segmentinin dizi verilerini kullanarak olası atasal populasyon tahmin edilmiştir. Ayrıca populasyonlar arası ve populasyon içi morfolojik varyasyonu, 25 morfolojik ve altı meristik karakter kullanılarak incelenmiştir. Haplotip analizi sonuçları, üç *Coptodon* populasyonunda, zayıf bir genetik çeşitliliği göstermiş ve üç benzersiz haplotip ortaya çıkarmıştır. Hem maksimum olabilirlik hem de Bayesian ağaçları, bu üç haplotipin *Coptodon zillii* kladında ayrı bir alt klad oluşturduğunu göstermiştir. Bu filogenetik model, her iki gölün populasyonlarının da *C. zillii* türüne ait olduğunu ve bölgede tek bir türün alanı istila ettiğini ortaya koymuştur. Filogenetik verilerle tutarlı olarak, her iki gölün populasyonları arasında, Peçenek populasyonu biraz farklı olsa da, önemli fenotipik varyasyon göstermediği belirlenmiştir. Populasyon içi morfolojik farklılıklar, göllerdeki habitat heterojenliğine tepki olarak fenotipik esneklikten kaynaklandığı ileri sürülebilir.

Anahtar kelimeler: *Coptodon zillii*, istilacı balık, mitokondriyal kontrol bölgesi, filogeni, morfometrik varyasyon

INTRODUCTION

Turkey harbors one of the most diverse freshwater fish fauna in the Mediterranean Basin (Ekmekçi et al., 2013). A total of 384 fish species belonging to 20 orders and 34 families have been reported in Turkish inland waters, of which 208 (54.2%) were reported as endemic and 15 (3.9%) as introduced (Çiçek et al., 2020, 2022). The available information about how and when these fish species entered and their distribution routes are pretty limited (Innal and Erk'akan 2006).

Ecosystems are threatened by global change (Linders et al., 2019). The introduction of fish species constitutes one of the most critical threats to aquatic biodiversity and ecosystem sustainability. Genus *Coptodon* and *Oreochromis* (Cichlidae, Tilapinae) are known as invasive fishes introduced to freshwaters of Turkey for aquaculture (Altun et al., 2006) and almost all over the world as well (Et et al., 2017). The General Directorate of State Hydraulic Works (DSI) started the introduction process of these fish in the 1970s. DSI brought different species of cichlid (*Oreochromis niloticus* and *Coptodon zillii* from Syria, *Coptodon rendalli* and *Sarotherodon galilaeus* from Scotland, and *Oreochromis aureus* from Israel) from other countries to research centers/institutions in the Çukurova and Hatay region, later from here to other provinces

of Turkey. After these introductions, four genera (*Coptodon, Oreochromis, Hemichromis,* and *Sarotherodon*) and five species (*C. zillii, C. rendalli, O. niloticus, O. aureus, O. mossambicus, S. galilaeus,* and *H. letourneuxi*) managed to establish populations in Turkey (Keskin et al., 2018; Innal and Sungur, 2019; Çiçek, 2021).

The biodiversity of the Köyceğiz-Dalaman river basin, declared a Special Environmental Protection Area, contributes significantly to individual and social welfare. Unfortunately, this unique ecosystem is also under the influence of introduced fish, especially species of the Cichlidae, and these invasive populations increase in size year by year (observation of local fishermen). While there is no report on the fish fauna of Koca Lake, conflicting cichlid species have been reported for the Köyceğiz Lake. For example, Çalışkan and Yerli (1999) identified *Oreochromis mossambicus* as the only species in Köyceğiz Lake, while Akın et al. (2005) reported the existence of 3 species: *Coptodon zilli, Oreochromis aureus,* and *Oreochromis nilotica*. Hereafter, studies in this lake reported only a single genus and species, *Coptodon zillii*, adhering only to morphology without any genetic research (Balık et al., 2005; Yılmaz et al., 2006; Tarkan et al., 2015; Çoban, 2018). A distinctive character (dark tilapia spot on the dorsal fin) distinguishes the genera *Coptodon* and *Oreochromis* (except for some species) from each other. However, it isn't easy to determine red-breasted tilapia, *C. rendalli,* from red-bellied tilapia, *C. zillii* (Froese and Pauly, 2019).

The morphology-based cichlid taxonomy has been revised using molecular phylogenetic data (Dunz et al., 2013), and this has returned to standard practice for the invasive species of *Coptodon* such as *C. zillii* and *C. rendalli* (Nagl et al., 2001; Szitenberg et al., 2012; Gu et al. 2016; Kide et al., 2016; Colihueque et al., 2019). Developing functional conservation approaches and strategies requires the identification of the invasive cichlid fish species that have established dense populations in the Köyceğiz and Koca Lake systems. The present study has two main goals. The first is to identify the species of *Coptodon* in these lakes and estimate the source population using sequences of the mitochondrial D-loop by reference to existing sequences of the genus in GenBank. The second is to reveal the morphological diversity of the determined species within and between lakes in the context of metric and meristic morphological characters. As no sequence-based studies have been conducted to identify the invasive members of *Coptodon* from Turkey, the present study is the first on this subject in Turkey.

MATERIAL AND METHODS

Study area and sampling

The research was carried out in two coastal lake systems (Koca Lake, Köyceğiz Lake, and the Peçenek Drainage Canal connected to the second lake) in the southwest of Anatolia (Figure 1, Table 1). The length of the Köyceğiz Lake is approximately 12-13 km, and its width is 5-6 km. It is connected to the Mediterranean Sea by a natural canal. The lake's surface area is 5500 ha, the average depth is 2.5 m, and the maximum depth is 60 m (Ayaz et al., 2013). Koca Lake is located within the borders of Kapukargın Village, about 6 km away from the Dalaman district of Mugla (Figure 1, Table 1).

Figure 1. Location of study sites (arrows pointed to study sites).

The lake is also located 35 km southeast of Köyceğiz Lake, and they are not hydrologically linked. Depth varies between 1 and 20 m, and the lake's surface area is 260 ha (Ayaz et al., 2013). The northwest part of Koca Lake is shallow and covered mainly by submerged plants and reeds. The lake water is rapidly warming up in the spring, is highly productive with abundant vegetation, and provides suitable breeding habitat for cichlid fish (Emre, Y., unpublished report).

Table 1. Study sites with coordinates, and number (N), weight and standard length of each fish.

Study sites	N	Coordinate	Weight (g)		Standard length (mm)	
			Min-Max	Mean	Min-Max	Mean
Koca Lake	96	36°54'49.24"K - 28°41'29.34"D	5.48 -152.11	48.82	57.99-166.00	108.04
Köyceğiz Lake	64	36°41'38.96"K - 28°49'12.95"D	6.96 - 216.86	69.95	61.29-184.50	112.87
Pecenek Channel	10	36°51'18.32"K - 28°41'1.96"D	13.97-108.70	31.61	79.61-108.26	90.82

The fish communities of Köyceğiz Lake are dominated numerically by cyprinids (*Vimba vimba*, *Capoeta aydinensis,* etc.), while cichlids (*Coptodon* sp.) and mugilids (*Mugil cephalus, Lisa ramada*, etc.) for Koca Lake (Emre, Y., unpublished report). The Pecenek drainage canal was created around Köyceğiz Lake for agricultural irrigation and water drainage (Figure 1, Table 1). Its water was very turbid due to heavy domestic waste (sewer and garbage) and the mud on the ground. Fish were caught using fyke (15m x 1.7cm) and gill nets (30m x 1.5 cm) in October 2019 and June 2020, representing different micro-habitats from the densely vegetated littoral and open-water pelagic habitats.

Morphological studies

Morphological identification of *Coptodon* individuals was made using standard identification keys (Teugels and Thys van den Audenaerde, 2003; Gu et al., 2016; Kide et al., 2016). Then, twenty-nine morphometric and six meristic characters were measured per specimen according to Boussou et al. (2010); Kide et al. (2016) (for the character list, see Table 2). All measurements were taken to the nearest 0.1 mm with a digital caliper. To minimize any variation resulting from allometric growth, data was standardized according to the following formula (Elliott et al., 1995):

$$Madj = M(Ls / Lo)b$$

where M: actual measurement, Madj: size adjusted measurement, Lo: standard length of fish, Ls: overall mean of standard length for all fish from all samples in each analysis. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all samples. This transformation best reflects shape variation among groups independently of size factors. Therefore, each specimen's total length, standard length, fork length, and weight were excluded from the final analysis. On the other hand, meristic characters are not standardized as they do not show a significant correlation with the body size of fish individuals (Turan et al., 2006).

It was observed that some of the morphological characters (KYY, KFU, IOM, PDM, DYTU, AYTU, PYU1, POKU, and SDPG; see Table 2 for abbreviations) exhibited normal distribution (ND). However, the rest of the characters did not show ND. A one-way analysis of variance (ANOVA) was used to look for differences between populations based on morphological traits. The Tukey Posthoc test was used for data with ND, and Kruskal-Walsh and Dunn were used for data with no ND.

Principal component analysis (PCA, variant-covariant matrix) was used to test the contribution of twenty-five morphological characters to the configuration of variance. A discriminant analysis (DA) was performed, which linearly combined a selection of body size measurements to produce a mathematical function that could categorize individuals into groups. Wilks' lambda (λ) was used to detect morphological variation between the three populations. Past 4.04 and R programs were used for all analyses.

Molecular studies

DNA extraction and amplification

We preserved 50 mg of muscle tissue in 95% ethanol for each fish. Genomic DNA was extracted using the PureLink Genomic DNA Extraction Kit (Invitrogen, Thermo Fischer Scientific) with validated modifications to the protocol. We studied the mitochondrial control region (D – loop), as in other studies of cichlid populations (Szitenberg et al., 2012). A fragment of the app. 472 bp was amplified using primers Ormt-449UP (5'-CTAACTCCCAAAGCTAGGATTCT-3') and Ormt-917LP (5'-CTTATGCAAGCGTCGATGAAA-3') (Nagl et al., 2001). PCR amplicons with adequate amplification were sequenced via an intake service from Macrogen Europe (Macrogen Inc.), and sequences were deduced from the obtained AB1 files for computer-based analysis.

Sequence data analysis

Consensus sequences were formed by aligning the sequences produced with forward and reverse primers using the Sequencher v.4.01 (Gene codes Corp.). The unique haplotypes and their frequencies among 93 samples were detected by DNASP v.5 (Librado and Rozas 2009). The characteristics of the matrix, such as the nucleotide composition, the number of variables, indels, and parsimony-informative sites were calculated in MEGA v.X (Kumar et al., 2018). Then, a second matrix was established by combining the obtained unique haplotypes with sequences of *Coptodon* species downloaded from GenBank (Table 2). One sequence per *Pelmatolapia mariae*, *Oreochromis niloticus*, *Oreochromis sp*., and *Cyprichromis leptosoma* were selected as outgroups (see Table 2 for accession numbers). This matrix's multiple sequence alignment was done using MAFFT v.7 (Katoh et al., 2019) with an auto-alignment strategy, and a data matrix was created. The unique haplotypes it contains and the sequence characteristics were determined with the DnaSP v.5 and MEGA v.X, respectively. This second matrix was used in phylogenetic analyzes.

Before the phylogenetic analysis, the substitution model of the data set was estimated using PartitionFinder v.1.1.1 (Lanfear et al., 2012). Phylogenetic relationships among haplotypes were estimated using the maximum likelihood (ML) and Bayesian (BI) phylogenetic algorithms. The ML phylogenetic analysis was conducted using RAxML v.8.0.9 (Stamatakis 2006) implemented in to Geneious v.9.0.5 with a 1000 non-parametric bootstrap (Felsenstein 1985) and GTR+I+G substitution model suggested by PartitionFinder. BI analysis was conducted using MRBAYES v.3.2.2 (Ronquist et al., 2012) with two independent runs, four Markov chains, for 10 million generations, sampling every 1000th generation using the GTR+I+G model proposed by PartitionFinder. The first 25% of trees were discarded as burn-in, and a majority-rule consensus tree was generated from the remaining trees. BI analysis was monitored by TRACER v.1.7 (Rambaut et al., 2018), and trees were visualized using FIGTREE v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 2. *Coptodon* samples were used in phylogenetic analyses, including GenBank accession number (AN), species, location, and outgroups (shown in bold).

AN	Species/ Locality	AN	Species/ Locality	AN	Species/ Locality
KU180527.1	*C. zillii Chinese*	KU180510.1	*C. zillii Chinese*	KU180531.1	*C. zillii Chinese*
KU180619.1	*C. zillii Chinese*	KY587518.1	*C. zillii Japan*	KU180564.1	*C. zillii Chinese*
KU180516.1	*C. zillii Chinese*	KY587521.1	*C. zillii Japan*	KU180618.1	*C. zillii Chinese*
KU180608.1	*C. zillii Chinese*	KY465487.1	*C. zillii Egypt*	KU180620.1	*C. zillii Chinese*
KU180515.1	*C. zillii Chinese*	KY465486.1	*C. zillii Egypt*	KU180605.1	*C. zillii Chinese*
KU180595.1	*C. zillii Chinese*	KY465488.1	*C. zillii Egypt*	KY587519.1	*C. zillii Japan*
KU180558.1	*C. zillii Chinese*	KY465484.1	*C. zillii Egypt*	KY587522.1	*C. zillii Japan*
KU180606.1	*C. zillii Chinese*	KY465482.1	*C. zillii Egypt*	KY587517.1	*C. zillii Japan*
KU180629.1	*C. zillii Chinese*	FJ613474.1	*C. zillii Egypt*	KY587516.1	*C. zillii Japan*
KU180512.1	*C. zillii Chinese*	KY465489.1	*C. zillii Egypt*	KY587523.1	*C. zillii Japan*
KU180520.1	*C. zillii Chinese*	KY465485.1	*C. zillii Egypt*	KY587520.1	*C. zillii Japan*
KU180596.1	*C. zillii Chinese*	KY465483.1	*C. zillii Egypt*	KX523912.1	*C. deckerti*
KU180519.1	*C. zillii Chinese*	EU163723.1	*C. zillii Israel*	MH644435.1	*C. rendalli Tanzania*
KU180522.1	*C. zillii Chinese*	KU180602.1	*C. zillii Chinese*	AF296503.1	*C. rendalli Africa-Egypt*
KU180607.1	*C. zillii Chinese*	KU180599.1	*C. zillii Chinese*	AF296505.1	*C. rendalli Africa-Egypt*
KU180523.1	*C. zillii Chinese*	KU180601.1	*C. zillii Chinese*	AF296504.1	*C. rendalli Africa-Egypt*
KU180592.1	*C. zillii Chinese*	KU180627.1	*C. zillii Chinese*	AF296498.1	*C. bemini*
KU180600.1	*C. zillii Chinese*	KU180559.1	*C. zillii Chinese*	AF296.500.1	*C. discolor*
KU180616.1	*C. zillii Chinese*	FJ613477.1	*C. zillii Israel*	AF296499.1	*C. guineensis*
KU180628.1	*C. zillii Chinese*	EU163719.1	*C. zillii Israel*	AF296506.1	*Tilapia ruweti*
KU180528.1	*C. zillii Chinese*	FJ613479.1	*C. zillii Israel*	AF296497.1	*Pelmatolapia mariae*
KU180617.1	*C. zillii Chinese*	EU163717.1	*C. zillii Israel*	MG728003.1	*Oreochromis niloticus*
KU180604.1	*C. zillii Chinese*	EU163718.1	*C. zillii Israel*	AF296491.1	*Oreochromis sp.*
				AY740331.1	*Cyprichromis leptosoma*

RESULTS

Morphological results

In total, 170 individuals of *Coptodon* from Koca and Köycağiz lakes and the Pecenek drainage canal were examined for morphometric and meristic analysis. The univariate analysis results revealed individuals of the Koca Lake population had a significantly bigger head, larger mouth and split eyes, smaller pharyngeal bone length and width, eye diameter, and pelvic fin length than those of the other two sites (Table 3 and 4). Individuals had larger body height and weight from populations of Köyceğiz and Koca Lake than those of the Peçenek canal (Table 3 and 4).

There were significant differences in only two meristic characters (dorsal-fin rays and scales along the lower lateral line) between the populations of Koca and Köyceğiz Lake ($F=13.51$; $P<0.001$, $F=21.89$; $P<0.001$, respectively).

Table 3. Mean and standard deviations (SD) of the transformed morphometric measurements, and mode, minimum and maximum value of meristic characters of each character of each population.

Morphometric Trait(mm)	**Code**	**Koca Lake**		**Köyceğiz Lake**		**Pecenek**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Body Height	VY	44.14	2.69	46.19	3.71	40.60	2.52
Body Width	VG	17.92	1.87	17.81	1.54	15.25	1.92
Caudal Peduncle Depth	KSD	14.45	1.40	14.24	1.30	14.38	0.47
Caudal Fin Length	KYY	34.09	6.27	36.60	5.94	35.49	3.10
Head Length	KFU	35.20	1.41	34.50	1.31	34.40	0.81
Head Depth	KFD	23.22	1.23	23.59	0.91	24.07	0.69
Interorbital Distance	İOM	11.63	0.68	11.23	0.62	11.26	0.38
Diameter of eye	GC	8.21	0.60	8.52	0.48	9.26	0.19
Snout Length	BU	6.82	0.89	7.03	0.49	6.91	0.21
Mouth Width	AG	8.43	0.91	8.01	0.45	8.01	0.43
Mouth Depth	AD	9.76	1.31	9.02	0.45	9.11	0.42
Predorsal Distance	PDM	35.14	1.96	32.39	1.71	32.53	0.73
Preanal Distance	PAM	75.73	7.33	76.62	2.39	74.97	1.70
Prepectoral Distance	PPM1	39.05	2.15	38.53	2.02	37.73	1.22
Prepelvic Distance	PPM2	35.90	2.01	34.25	1.29	34.59	1.59
Base length of dorsal fin	DYTU	60.75	1.90	61.24	2.18	61.58	1.30
Base length of anal fin	AYTU	18.18	1.05	18.02	0.93	18.55	0.76
Length of pectoral fin	PYU1	30.84	2.35	31.90	1.94	30.66	1.47
Length of pelvic fin	PYU2	34.32	2.83	36.34	1.75	36.48	1.80
Caudal peduncle height	KSP	16.44	1.27	16.26	0.77	16.43	0.60
Length of the first spine of the dorsal fin	EUDY	28.29	3.51	27.46	2.40	27.29	2.51
Length of the third spine of the anal fin	ATIU	23.11	2.80	23.49	1.49	23.29	1.63
Preorbital distance	POKU	12.72	1.26	12.30	0.80	12.35	0.45
Width of pharyngeal bone	SDPG	13.39	0.88	13.86	0.60	13.55	0.67
Length of pharyngeal bone	FKU	10.51	0.93	11.23	0.57	11.25	0.54

Meristic Traits (mm)	**Code**	**Koca Lake**			**Köyceğiz Lake**			**Pecenek**		
		Mode	**Min**	**Max**	**Mode**	**Min**	**Max**	**Mode**	**Min**	**Max**
Dorsal-fin rays	SYIS	11,0	9,0	15,0	12,0	10,0	13,0	12,0	11,0	13,0
Dorsal-fin spines	SYDS	15,0	14,0	16,0	15,0	15,0	16,0	15,0	15,0	16,0
Anal-fin rays	AYIS	8,0	7,0	9,0	9,0	8,0	10,0	9,0	8,0	9,0
Anal-fin spines	AYDS	3,0	2,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Scales along the lower lateral line	YCPS	12,0	9,0	14,0	13,0	11,0	14,0	12,0	11,0	13,0
Gill rakers on the first ceratobranchial gill arch	1SYDS	11,0	9,0	13,0	11,0	11,0	12,0	11,0	11,0	11,0

The results for the DA showed that the scatter plot for DF1 and DF2 generated a clear separation only between the populations of Pecenek and the two lakes. At the same time, there was a partial overlap between the two lake populations (Figure 2). The percentages of morphological differences among the three populations indicated highly significant differences (Wilks' lambda = 0.018; F= 264.44; P < 0.001). The first DF accounted for 81.20%, and the second accounted for 18.08% of the between-group variability, explaining 100% of the total between groups variability. The characters of primary importance in distinguishing between the groups were body height (0.49), interorbital (-0.43), predorsal (-0.52) and

preorbital distance (-0.60), and length of pharyngeal bone (0.46) variables for the first canonical variable, and body height (-0.61), body width (-0.50) and eye diameter (0.68) variables for the second one. Each individual could be classified correctly into the three populations with an accuracy of 95.9%. Additionally, it was seen that only 2 variables (dorsal-fin rays (0.63) and scales along the lower lateral line (0.75) out of 6 meristic characters were important in the formation of DF1, which explained 96.6% of the total variance (Wilks' lambda = 0.77; F= 43.13; P < 0.01) (Figure 2).

Table 4. Summary of Kruskal-Wallis (Chi2) and ANOVA (F) results for 25 morphological characters of *C. zillii* from three site. Significance levels; * P < 0.05; ** P < 0.01; *** P < 0.001.

Trait	Chi2	*P*	Population	
			Dunn's Post hoc test	
VY	27.87	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
VG	13.88	<0.001	Koca Lake- Peçenek. Köyceğiz Lake - Peçenek	*
KSD	2.28	0.32		
KFD	7.39	<0.05	Koca Lake - Peçenek	*
GC	30.52	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
BU	0.85	0.65		
AG	17.21	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
AD	17.18	<0.002	Koca Lake - Köyceğiz Lake	***
PAM	4.95	0.08		
PPM1	5.69	0.08		
PPM2	37.54	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
PYU2	28.29	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
KSP	5.61	0.06		
EUDY	2.76	0.25		
ATIU	0.54	0.76		
FKU	25.99	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	
Trait	**F**	***P***	**Population**	
			Tukey Post hoc test	
KYY	3.39	0.04	Koca Lake - Köyceğiz Lake	*
KFU	5.96	<0.001	Koca Lake - Köyceğiz Lake	***
İOM	8.06	<0.001	Koca Lake - Köyceğiz Lake	***
PDM	46.86	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
DYTU	1.64	0.20		
AYTU	1.38	0.25		
PYU1	1.38	0.25		
POKU	3.03	0.06		
SDPG	6.99	0.00	Koca Lake - Köyceğiz Lake	***

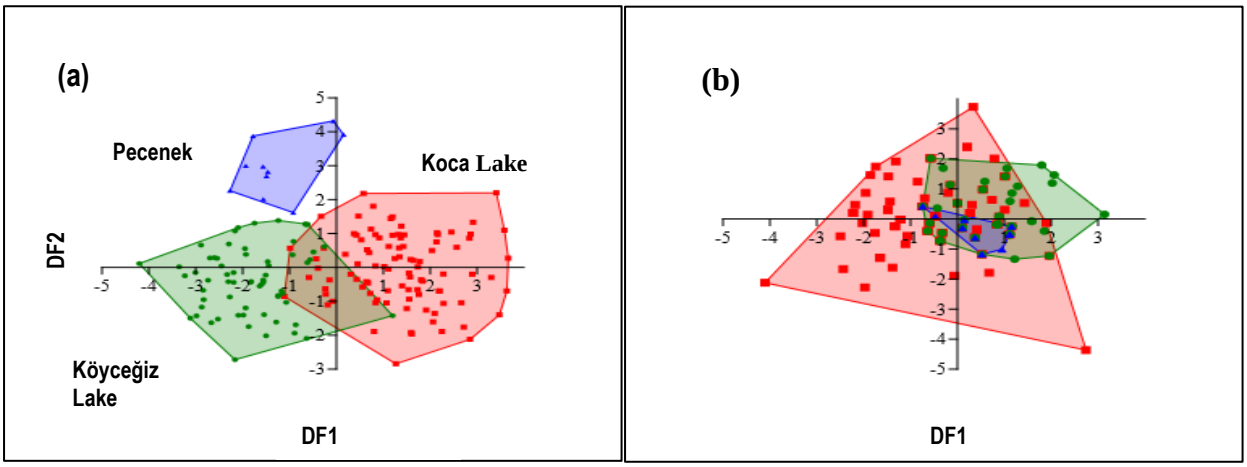

Figure 2. Scatter plot of the DF1 and DF2 axes of the DA of (a) morphometric; (b) meristic characters of *Coptodon zillii* collected at three sites.

Intra-population morphological differences were explained by one to five functions in the discriminant analyses (Figure 3). Plots of DF1 and DF2 for the individuals from the populations illustrate a noticeable variation in the morphological traits between microhabitats (DF2 scores) and sampling years (DF1 scores) in the Koca Lake population (Wilk's Lambda = 0.007; F= 442.4; P<0.000). While individuals from the littoral habitat in the Koca Lake population generally had longer pelvic fins, those from the pelagic habitat had a larger head, nose, mouth, eyes, and pharynx bone. Individuals caught in 2019 have a larger mouth and the dorsal fins located further back than those in 2020. The morphological variation within the population of Köycegiz Lake appeared both in the micro-habitat and sampling years (Wilk's Lambda = 0.016; F= 200.9; P<0.000). In 2020, the caudal peduncle was high, and in 2019, the snout length was high. It was determined that the lengths of the pelvic and pectoral fins and the distance between the anal and caudal fins of pelagic individuals were longer than those living in the littoral habitat (Figure 3).

There was no significant intra-population meristic variation in the two lakes (Wilk's Lambda = 0.99; F= 1.69; P=0.90).

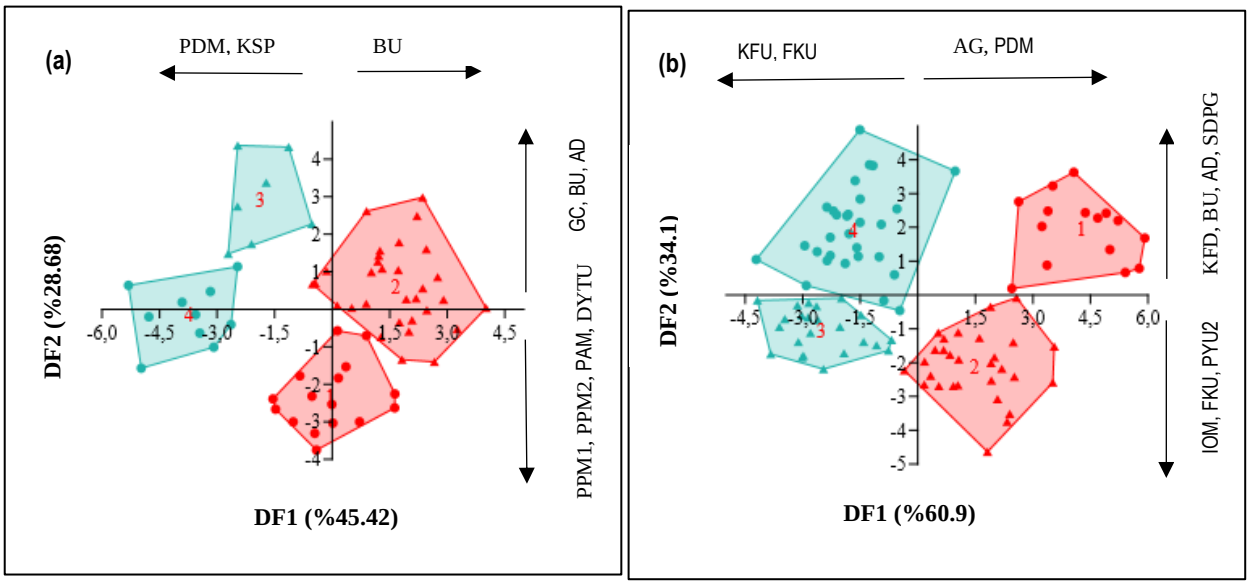

Figure 3. Plot of discriminant function one and two for the different individuals (●: pelagic habitat (1-4), ▲ : littoral habitat (2-3); red color represent October 2019, blue represents June 2020) for each of the two populations of *C. zillii*: (a) Köycegiz Lake; (b) Koca Lake

Molecular results

Three unique haplotypes were recognized among the D-loop sequences of a total of 93 *C. zillii* samples. The sequences have been deposited in the GenBank database and were assigned H1-ON337141, H2-ON337142, H3-ON337143. Haplotypes H2-H3 were obtained from Köyceğiz and Koca lakes, and H1 only from Pecenek Canal. The most common haplotype was H3 (73 individuals; 78.5%), then H2 (18 individuals; 19.35%), and last was H1 (2 individuals; 2.15%). We have not calculated genetic diversity parameters since only three unique haplotypes were detected. A data matrix was established using these three unique haplotypes plus 70 sequences of *Coptodon* species downloaded from GenBank (Table 2). After alignment and trimming, the final length of the sequences was 472, of which 270 were constant and 200 variables. In total, 73 unique haplotypes were detected, 68 representing *Coptodon* members as ingroup and four representing the outgroup.

The BF and ML trees produced from this matrix differed in topology. ML tree supported the monophyly of *Coptodon* haplotypes with a bootstrap support value of 70 (Figure 4a), but a haplotype of *Tilapia ruweti* was nested in the *Coptodon* haploclade. The single haplotypes of *Coptodon bemini* branch off at the base of the *Coptodon* haploclade, leading to all others, and the monophyly of this later haploclade was supported with 84% bootstrap values. The later haploclade consists of three subhaploclades: (i) *C. rendalli* (1 Tanzanian + 2 Egyptian haplotypes), (ii) *T. ruweti* + *C. deckerti,* and (iii) the clade including one haplotype per *C. discolor* and *C. guineensis* plus 60 haplotypes belonging to *C. zillii. Coptodon discolor* + *C. guineensis* constitute a sister clade to the clade, including haplotypes of *C. zillii* and the *C. zillii* haploclade received 85 bootstrap support. Relationships between haplotypes of *C. zillii* are mainly unresolved, but the three haplotypes obtained from Koca and Köyceğiz lakes plus Pecenek formed an internal clade within the *C. zillii* lineage (Figure 4a).

The BI tree supported the monophyly of *Coptodon zillii* when a haplotype from Egypt was omitted (Figure 4b). BI tree occurs with a basal trichotomy, and one haplotype per *Coptodon deckerti* and *Tilapia ruweti* constitute independent branches, while all others as a single haploclade supported by 0.99 posterior probability. The last clade includes two sister haploclades, which we defined as C1 and C2 (Figure 4b). The C1 consists of all outgroup haplotypes plus one haplotype of *C. zillii* and three haplotypes of *C. rendalli*.

Coptodon discolor + *C. guinensis* branch off basally, leading to the C2_2 haploclade including only haplotypes of *C. zillii,* which is supported by 1.0 posterior probability support. The C2_2 haploclade has 25 branches in polytomy, and three haplotypes obtained from Mugla make up one of these 25 branches.

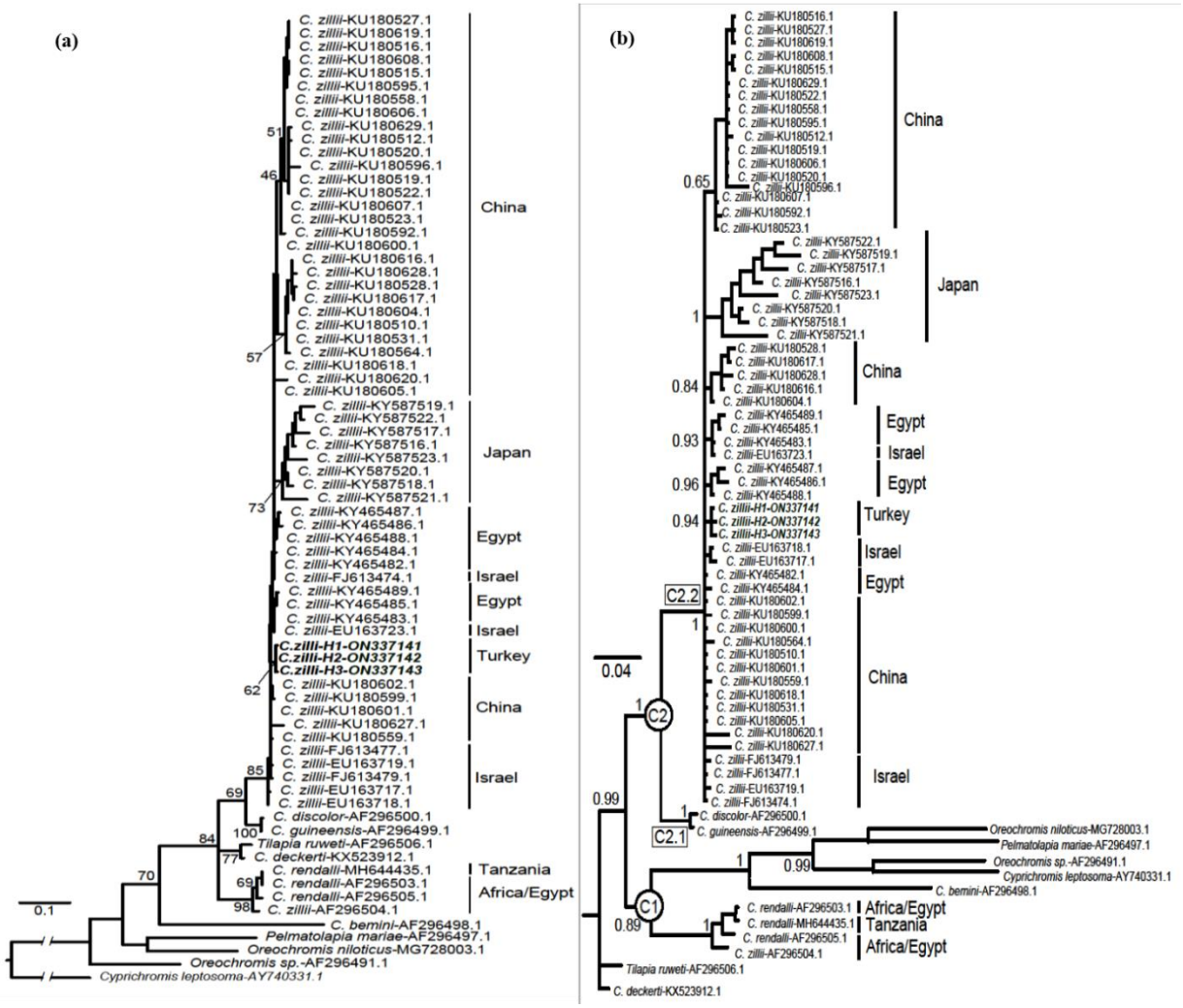

Figure 4. Phylogenetic tree from (a) ML and (b) BI analysis with D-loop dataset

DISCUSSION

Three unique haplotypes detected from the study area constitute a haploclade within *C. zillii*. These results suggest that a single species, *C. zillii*, occurs in Koca and Köyceğiz lakes and the Pecenek drainage canal.

However, this three-haplotype clade constitutes an independent branch within the *C. zillii* haploclade with no relationship to any other geographically specific haplotypes. Therefore, it was not possible to determine the origins of the populations that invaded these systems. As these three haplotypes differed by a single base position from each other and formed a monophyletic haploclade, we concluded that the population in all systems was established by a single introduction. Further, the population's genetic diversity was low for the same reasons (Freeman and Herron 2007).

In this study, we also aimed to reveal the inter-and intra-population morphological differences of *C. zillii* in the context

of metric and meristic morphological characters. Although Wilk's Lambda test showed that the morphometric and meristic differences observed between populations were statistically significant, this difference was not supported by the discriminant and PC analysis results. The observed similarity of morphometric traits based on DA between the populations of Köyceğiz Lake, Koca Lake, and Pecenek suggest individuals belonging to the same source population were recently introduced in both lakes. Thus, the results of morphological analyses are consistent with genetic results.

Although the populations exhibited similar morphological characteristics, fish from the Pecenek population had the greatest eye diameter, thus distinguishable from the populations of the other two lakes (Figure 2). Jawad et al. (2018) presented evidence supporting this phenomenon for *C. zillii* and *O. aureus*. Variation in eye size can result from differences in water transparency (as in the Pecenek canal) or differences in the size of available food (Solem et al., 2006). These results suggest that the large eyes of nonnative fish such as *C. zillii* make them superior predators or competitors, even in anthropogenically modified systems (Moran et al., 2018).

Morphometry of lake can also predict the likelihood of habitat coupling between littoral and pelagic zones by a mobile fish (Chavarie et al., 2015). Overall, we obtained different morphological patterns in both lakes about their area and time of capture. When we examined micro-habitats, it was seen that the *C. zillii* from the pelagic habitat of the Koca Lake typically exhibited morphological differences in head traits and conspecifics from Köyceğiz Lake in terms of fin traits. Lakes can represent a rich source of environmental gradients (e.g., size, depth, temperature, light, amount of vegetation cover, salinity, types of predators, and competitors) associated with different prey species and habitat characteristics that have the potential to promote ecological segregation (Chavarie et al., 2015). This study's morphological pattern observed in head and fin morphology suggests related to feeding and swimming traits based on the heterogeneity of habitat and season. This might be due to phenotypic plasticity, not genetic differences.

CONCLUSION

In the light of these data, the following conclusions were reached: (i) The Koca and Köyceğiz lakes, and Pecenek drainage canal in Muğla Province – Turkey, were invaded at once by a single founder population of *C. zillii*, (ii) this population contains a poor genetic diversity, due to recent foundation, (iii) determining origin population requires richer genetic data and (iv) there is no significant inter-lake morphological difference, but there is a significant intra-lake difference, possibly due to local ecological condition.

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AUTHORSHIP CONTRIBUTIONS

Dilara Sarıbaş: Analyzed the collected sampling and wrote the first draft of the manuscript. Nehir Kaymak: Corrected the draft and built the final version of the manuscript. Özgül Yahyaoğlu: Provided assistance and guidance throughout the molecular analysis. Battal Çıplak: Contributed to the interpretation of molecular data and reviewed the manuscript. All authors have read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no known financial or personal conflict that may affect the research (article)

ETHICS APPROVAL

Approval was granted by the Ethics Committee of Burdur Mehmet Akif Ersoy University (Date: 19.07.2018 / Approval Number: 93773921).

DATA AVAILABILITY

For questions regarding datasets, the corresponding author should be contacted

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