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Article

New localities of *Scardinius elmaliensis* Bogutskaya, 1997 (Teleostei: Cyprinidae) and its phylogenetic relationship based on mtDNA Cytb region sequences

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Abstract

This study was conducted to report five new localities out of its type locality for *Scardinius elmaliensis* in the Western Mediterranean Basin of Turkey by providing their morphological characteristics, and their phylogenetic relationship based on mtDNA Cytb region. The results revealed significant differences of all studied populations in terms of the morphometric characters despite their low genetic differences, but their meristic characters were not different. All six studied populations of *S. elmaliensis* including that of type locality formed a monophyletic group with *S. erythrophthalmus* as sister group. The molecular result confirmed distinction of *S. elmaliensis* from *S. erythrophthalmus* based on Cytb genetic distance of 1.6-1.8%. The occurrence of *S. elmaliensis* out of type locality was firstly reported in this study. Such knowledge is important for future conservation strategies and habitat management of this species.

Keywords: Turkey, Cytochorome b, Morphometry, Phylogenetic, Phenotypic plasticity.
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Introduction

The rudds, members of the family Cyprinidae, are found in Europe and the northern parts of Asia. They are small to medium size fishes adapted to temperate waters with submerged vegetation, such as lowland lakes, and lentic parts of rivers and streams (Valic et al. 2013). According to Berthou and Amich (2000), they are littoral species, and closely associated to vegetation related to their high degree of herbivory (Niederholzer and Hofer 1980; Prejs 1984). The genus *Scardinius* consists of approximately 10 species (Eschmeyer et al. 2016) that 2 of them are found in Turkish inland waters. *Scardinius erythrophthalmus* (Linneus, 1758) has a wide distribution from lake, to reservoirs and rivers in Aegean, Marmara and Black Sea basins of Turkey, and *S. elmaliensis* Bogutskaya, 1997 is endemic to Anatolia and only known from Elmali, Antalya (Bogutskaya 1997). *Scardinius elmaliensis* is listed as endangered based on the IUCN red list (Freyhof 2014). However, little is known about its habitat requirement, distribution, and bioecological properties.

It is inevitable to observe morphological variations in fishes with wide distribution area as a result of different ecological conditions of their habitats. Morphological variations was reported in populations of the fish species from different localities even in the same river system (Nakamura 2003; Kara and Alp 2007; Çiçek 2009) showing their adaptive responses to environmental factors due to phenotypic plasticity (Mittelbach et al. 1999).

Under the above pretext, the present study was aimed to report five new localities of *S. elmaliensis* in the Western Mediterranean Basin of Turkey by providing their morphological characteristics and comparing them with those of type locality population, and their phylogenetic relationship based on mitochondrial Cytb region.

Material and Methods

A total of 40 specimens of *S. elmaliensis* were sampled from its type locality (Karagöl channels, Elmali) in September 2013, July-September 2014 and April-July 2015 using electrofishing device. In addition, 57 specimens of *S. elmaliensis* were collected from five new localities, including Lake Gölhisar (37°06′52″N, 29°35′59″E), Çayboğazı Dam Lake (36°31′33″N, 29°41′00″E), Osmankalfalar Dam Lake (37°06′49″ N, 29°53′09″E), Yapraklı

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Dam Lake (37°01′47″N, 29°27′08″E) and Çavdır Dam Lake (37°04′19″N, 29°43′46″E) in the Western Mediterranean basin using gill nets with mesh sizes of 10-50 mm. Caudal fin clips of three specimens from each population were cut, fixed in 99% ethanol and stored at -40°C for DNA extraction. Then, the collected specimens were preserved in 10% buffered formaldehyde after anaesthesia by over anaesthetization using MS222 and transferred to laboratory for further examinations.

Eight meristic characters, including D: dorsal, A: anal, P: pectoral, PV: pelvic and C: caudal fin rays, GR: gill rakers, PT: pharynx teethes, and LL: scales of lateral line were counted (Table 1). A total of 26 morphometric characters were measured using digital callipers to the nearest 0.01 mm (Table 2). Counts and measurements were made point to point according to Kottelat and Freyhof (2007). The percentage ratios of morphometric characters in relations to standard length (SL), head length (HL), caudal peduncle length and maximum body depth were calculated.

An allometric method was used to remove size-dependent variation in morphometric characters using following formula (Elliott et al. 1995): $M_{adj} = M(L_s/L_0)^b$

Where M is the original measurement, M_{adj} the size adjusted measurement, L_0 the standard length of the fish, L_s the overall mean of the standard length for all fish from all samples in each analysis, and *b* was estimated for each character from the observed data as the slope of the regression of log M on log L_0 using all fish in any group. The adjusted morphometric characters of the studied populations were analysed using Principal Component Analysis (PCA) and compared by Non-Parametric Multivariate Analysis Of Variance (NPMANOVA) based on *P*-values obtained from permutation test with 1000 replicates in PAST software (version 2.14). Every meristic characters of six populations were compared using Ttest in SPSS software for Windows (version 17).

DNA was extracted from fin clips using DNeasy Blood and Tissue Kit (Sigma) following the manufacturer's instructions. Amplification of the Cytb gene was carried out in PCR master mix tip NAD-K0171 (Fermantas) using primers L15267 and H16526 (Briolay et al. 1998). PCR cycle conditions were: 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s; and finally 72°C for 7 min. The amplification products were sequenced in both directions using the primers used in the PCR reactions by Macrogen Inc. (Korea).

The forward and reverse nucleotide sequences were assembled, edited and aligned using CodonCode Aligner 3.5.6 (CodonCode Corporation, Centerville, MA, USA). The best-fit model of DNA substitution and the parameter estimates used for tree constructions were selected according to the Akaike Information Criterion (AIC) as implemented in JModeltest 2.1.4 (Darriba et al. 2012). Maximum Likelihood (ML) method were applied to infer phylogenetic relationship using “construct ML tree” option implemented in the MEGA version 6 (Tamura et al. 2013). In addition to our produced dataset, the related gene regions of seven species of the genus *Scardinius* and one outgroup (*Tinca tinca*) were retrieved from NCBI annotated database (see Table 3 for details of the species).

Results

A total of 97 specimens from six populations of *S. elmaliensis* were considered for morphometric measurements and meristic counts. All measured meristic characters are presented in Table 1. Ratio of the morphometric characters (Mean±SD) are shown in Table 2.

The result of PCA analysis showed that all specimens explained 50.34% of morphometric variations by the first two PC axes extracted from the variance-covariance matrix (PC1=33.19% and PC2=17.15%). Plotting of first and second PCs displayed a complete segregation of the Yapraklı Dam population from others. In addition, NPMANOVA showed significant differences of all studied populations in terms of the morphometric characters ($P<0.001$) (Fig. 1). The results also showed no significant differences in terms of meristic characters between the studied populations ($P>0.05$).

Table 1. Meristic characters of the studied populations (D: dorsal, A: anal, P: pectoral, PV: pelvic and C: caudal fin rays, GR: gill rakers, PT: pharynx teethes, and LL: scales of lateral line).

Sampling sites	P	D	PV	A	C	LL	GR	PT
Karagöl Channels (n: 17)	I 13-15	III 9	II 8-9	III 9-10	22-25	39-42	15-18	5.3-3.5
Çayboğazı Dam (n: 10)	I 12-14	III 8	II 8	III 9-10	25-26	38-41	17-20	5.3-3.5
Lake Gölhisar (n: 40)	I 12-14	III 8-9	II 8-10	III 9-11	22-25	39-42	18-21	5.3-3.5
Osmankalfalar Dam (n: 10)	I 13	III 8-9	II 8	III 9	24-26	39-41	16-18	5.3-3.5
Çavdır Dam (n: 10)	I 14-15	III 8-9	II 8	III 9-10	27	40-42	16-19	5.3-3.5
Yapraklı Dam (n: 10)	I 15	III 8-9	II 8	III 9-10	26	42-43	15-17	5.3-3.5

Table 2. Morphometric characteristics of the studied *Scardinius elmaliensis* populations (1: Karagöl Channels; 2: Çayboğazı Dam; 3: Lake Gölhisar; 4: Osmankalfalar Dam; 5: Çavdır Dam; 6: Yapraklı Dam; SD: Standard deviation).

Characteristics	1 (n=40)	2 (n=10)	3 (n=10)	4 (n=17)	5 (n=10)	6 (n=10)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
%Standard length						
Head Depth	11.89±0.35	13.12±0.26	11.11±0.42	13.41±0.58	12.76±0.57	12.35±0.66
Head Length	27.56±0.39	26.47±0.94	26.15±0.80	24.92±0.39	25.78±0.75	25.80±0.92
Dorsal Head Length	17.79±0.71	19.20±0.90	16.89±1.05	17.72±0.71	19.79±0.60	18.24±0.92
Max. Body Depth	29.08±0.93	29.63±0.96	30.87±1.21	26.43±1.63	27.39±1.62	30.24±1.49
Predorsal Distance	57.34±0.84	58.07±1.58	57.45±1.07	57.18±2.05	57.57±1.19	53.17±1.46
Postdorsal Distance	32.48±0.76	31.60±2.17	32.64±1.60	30.23±1.64	31.27±0.37	34.27±2.20
Caudal Peduncle Depth	10.78±0.30	10.40±0.45	10.30±0.50	10.31±0.50	11.26±0.39	11.57±0.64
Caudal Peduncle Length	16.99±0.66	16.65±0.41	17.93±0.89	18.76±1.06	16.71±0.37	17.57±0.64
Preventral Distance	51.29±0.72	51.24±1.39	50.77±1.13	50.23±2.04	51.81±2.09	51.47±2.02
Preanal Distance	72.53±0.97	70.76±1.41	71.54±1.55	71.60±1.56	72.95±1.37	69.70±4.49
Distance Between Pectoral-ventral	25.23±0.78	25.19±1.98	26.27±0.83	26.44±1.65	26.27±1.17	27.51±1.74
Distance Between Ventral-anal	22.36±0.83	21.64±1.68	22.50±1.15	23.17±2.08	23.38±1.22	23.73±2.39
Dorsal Fin Height	19.85±0.86	19.63±0.96	20.26±0.81	18.76±1.06	20.36±0.85	18.67±1.20
Dorsal Fin Base Length	12.59±0.56	11.72±0.95	12.07±0.66	11.68±0.96	12.35±0.62	10.46±0.59
Anal Fin Height	16.22±0.79	16.66±0.99	16.78±0.79	16.30±0.63	15.94±0.57	17.10±1.30
Anal Fin Base Length	12.45±0.65	13.29±0.95	12.29±0.60	11.88±0.92	10.73±3.38	10.44±1.21
Pectoral Fin Length	18.43±0.76	19.39±1.05	20.15±0.89	18.24±0.71	18.45±0.77	19.01±0.75
Ventral Fin Length	15.78±0.99	15.26±0.95	17.53±0.54	15.13±0.80	16.06±0.59	14.93±0.60
%Head Length						
Head Depth	43.12±0.93	44.95±1.56	42.47±1.15	43.82±2.94	44.32±3.19	43.66±2.75
Preorbital Distance	25.13±1.60	25.63±1.28	24.62±0.94	25.10±1.72	26.01±0.83	27.68±3.88
Snout Length	16.47±1.01	15.46±0.99	15.85±1.15	13.75±1.20	17.73±1.59	17.73±1.77
Eye Diameter	21.89±1.56	24.17±1.67	22.93±0.90	22.71±1.92	25.08±1.65	22.42±1.47
Post-orbital Distance	53.85±1.98	49.89±1.88	53.69±2.10	52.03±1.85	54.90±2.37	54.31±3.89
Interorbital Distance	32.27±1.29	32.08±1.31	32.31±1.34	34.13±2.13	36.43±1.49	30.54±2.11
Lower jaw length	24.91±0.88	26.07±1.20	24.91±1.23	23.41±1.37	26.96±1.44	24.10±1.69
%Caudal Peduncle Length						
Caudal Peduncle Depth	63.49±2.49	61.37±2.07	57.56±3.82	58.97±7.94	59.61±3.76	58.40±3.25
%Max Body Depth						
Caudal Peduncle Depth	37.08±1.08	36.55±3.10	33.39±1.64	33.67±5.12	38.86±3.37	37.08±2.42

We generated the nucleotide information of Cytb sequences (1140 bp) for 6 specimens of *S. elmaliensis* collected from six studied populations. The phylogenetic relationships based on the Cytb sequences indicated that all members of the six populations were placed in the same lineage. All analysed populations of *S. elmaliensis* were corresponded to a distinct clade, forming a monophyletic group with *S. erythrophthalmus* (Fig. 2). Based on the analysis of the Cytb data, the pairwise distances of the six studied populations were between 0.000-0.005 and the interspecies genetic distance was 0.016-0.018 between *S. elmaliensis* and *S. erythrophthalmus* as its sister group.

Table 3. List of species used in this study with GenBank accesion numbers.

Species	Accesion Number	Reference
Scardinius acarnanicus	AY509831	Ketmaier et al.. 2004
Scardinius dergle	JF727576	Valic. 2011 (unpublished)
Scardinius elmaliensis Karagöl Channels. Elmali	KY288477	This study
Scardinius elmaliensis Lake Gölhisar	KY288478	This study
Scardinius elmaliensis Çayboğazı Dam Lake	KY288479	This study
Scardinius elmaliensis Osmankalfalar Dam Lake	KY288482	This study
Scardinius elmaliensis Yapraklı Dam Lake	KY288480	This study
Scardinius elmaliensis Çavdır Dam Lake	KY288481	This study
Scardinius erythrophthalmus	HM560171	Perea et al.. 2010
Scardinius graecus	AY509832	Ketmaier et al.. 2004
Scardinius hesperidicus	HM560174	Perea et al.. 2010
Scardinius plotizza	HM560176	Perea et al.. 2010
Scardinius scardafa	AY509833	Ketmaier et al.. 2004
Tinca tinca	HM167957	Lajbner and Kotlik. 2011

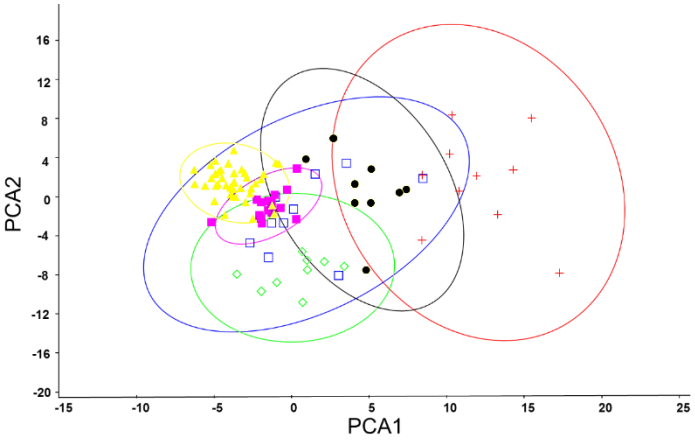

Figure 1. Comparison based on 26 morphometric characters specimens from type locality (■=Elmali) and new localities (◊=Çayboğazı Dam; ▲=Lake Gölhisar; □=Osmankalfalar Dam; ●=Çavdır Dam; += Yapraklı Dam) by Principal Component Analysis.

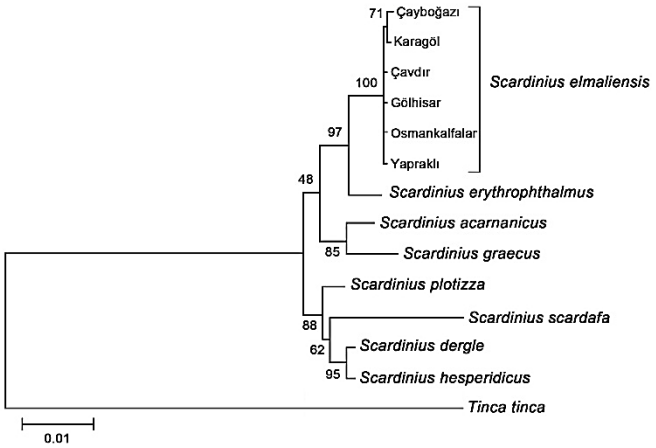

Figure 2. ML phylogenetic tree of *Scardinius elmaliensis* based on the mitochondrial cytochrome b nucleotide sequences.

Discussion

Scardinius elmaliensis was originally described from Elmali, Antalya by Bogutskaya (1997) as subspecies of *S. erythrophthalmus* using preserved materials of museum collection (Holotype: ZMH 8863, Elmali, Taurus, coll. C. Kosswig, 04.04.1957). *Scardinius elmaliensis* has following diagnosis: D III 8-9; A III 9-11; scales of lateral line 38-43; gill rakers 15-20; pharyngeal teeth 5.3-3.5; and differs from *S. erythrophthalmus* by having a lower number of branched rays in the anal fin (vs 11-13) and a larger number of gill rakers (vs 9-12) (Bogutskaya 1997). According to identification key given by Bogutskaya (1997), all collected specimens from five new locations were identified as *S. elmaliensis* showing its range extension. The results showed no significant differences in terms of meristic characters between the studied populations. However a higher number of gill rakers (15-22) was found in studied specimens compared to that given by Bogutskaya (1997). This is logic since this species described based on holotype and two paratypes specimens, whereas we examined a higher number of specimens (n=97).

The results showed that morphometric characters of the studied populations are significantly different despite their low genetic differences. Such differences have been observed in *S. erythrophthalmus* from two locations of the Szczecin Lagoon, one site with warmer water from cooling system of a power plant and another one from inside of Lagoon (Krzykawski et al. 1997). Rudd from warmer waters had the longer head, lower body depth,

narrower body, shorter predorsal and postdorsal distances (Szlachciak and Strakowska 2010). Morphological variations have been reported in populations of the fish species from different localities as adaptive responses to environmental factors due to phenotypic plasticity (Mittelbach et al. 1999). Phenotypic plasticity as an important mechanism of adaptation provides various phenotypes of the same genotypes in response to different environmental conditions (Çiçek 2009) as observed in the present study.

The molecular result confirmed the distinction of *S. elmaliensis* from *S. erythrophthalmus* based on Cytb genetic distance with 1.6-1.8%. In addition, the genetic distances among the studied populations of *S. elmaliensis* were ranged from 0.00% to 0.50%, showing that they belong to same species i.e. this genetic distance can be explained as intra-species variations.

As given above, *S. elmaliensis* was originally described as subspecies of *S. erythrophthalmus* from Elmali. Thereafter, Fricke et al. (2007) was considered *S. elmaliensis* as distinct species based on morphological characteristics without any explanations. Addition to morphometric distinction of *S. elmaliensis* from *S. erythrophthalmus*, our molecular results confirmed that *S. elmaliensis* is distinct and valid species. By the way, this study showed that distribution range of this species has not restricted to its type locality, Elmali region. It has wide distribution range in the northern part of the western Mediterranean basin.

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