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The discovery of a microbialite-associated freshwater fish in the world's largest saline soda lake, Lake Van (Turkey)

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http://zoobank.org/7127F55D-ED8F-4CF6-A537-18FB4F91A765

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Abstract

Lake Van is the largest saline soda lake in the world and one of the world's few endorheic lakes of greater than 3,000 km² surface area. Despite its huge size, no fish species have so far been known to permanently occur in this lake due to its extreme environmental conditions. Here, we report the discovery of a fish population that permanently inhabits some of the unique microbialites of the lake, at a maximum depth of 13 m and about 500 m offshore. We tested whether this is an undescribed species or a new occurrence of a known species. A molecular and morphological examination showed that the newly discovered fish represents an isolated population of *Oxynoemacheilus ercisianus*, the only nemacheilid loach native to the freshwater tributaries of the Lake Van endorheic basin. Our further hypotheses on the prediction that (a) stream fishes would have a more anterior placement of fins than lake fishes were supported; but, that (b) stream fishes would be more slender bodied than their lake conspecifics was not supported. The lake dwelling population also shows very small sequence divergence (0.5% K2P distance) to its stream dwelling conspecific in the mtDNA-COI barcode region. The notable morphological difference with minute molecular divergence implies that the newly discovered population might have lost its link to freshwater during desiccation and transgressional phases of the Lake Van, and has adapted to a life on the microbialites.

Key Words

Introduction

Soda lakes are characterized as extreme environments since they are highly alkaline, originate in closed basins, and are often exposed to high evaporation rate (Jones et al. 1998; Pinti 2014). This evaporation rate results in elevated concentration of dissolved salts, especially of [CO_3^{2-}] and, in turn, increased salinity and pH levels that usually reach between 8.5 and 11.5 (Pinti 2014). Soda lakes occur all over the world with the best-known examples found along the East African rift and western USA (Schagerl and Burian 2016). However, Lake Van, which is located in the uplands of eastern Anatolia, is a less known soda lake that is famous for being the largest soda lake, and the third largest closed lake on Earth (Kempe and Kazmierczak 2011). Lake Van is also unique because it possesses the largest recent organosedimentary deposits (microbialites) on earth (Kempe et al. 1991). The lake is located over 1640 m above sea level and has a maximum depth of 450 m, 9.7–9.9 pH and 22‰ total salinity (Degens et al. 1984; Reimer et al. 2009). Due to these extreme living conditions, none of the fish species in the endorheic lake basin is expected to enter the lake itself. Indeed, all the fish species recorded in the basin, namely *Alburnus timarensis*, *Barbus lacerta*, *Capoeta damascina*, *Oxynoemacheilus ercisianus* and non-native *Salmo trutta*,

permanently inhabit the freshwater tributaries and streams outside the lake (Elp et al. 2016; Kaya 2020). One exception to this is the diadromous population of *Alburnus tarichi* (Tarek), which is able to enter the estuaries and the lake, but still migrates to freshwater tributaries on which it relies to spawn in the reproduction season (Sarı 2008). Our knowledge in this regard has become enhanced after an exploration in a microbialite area in the south of the lake by scuba-diving operations approximately 500 m offshore. Here, we surprisingly discovered a nemacheilid fish occupying the branches and holes of a 13-meter high tower-like microbialite in 15-meters depth of the lake. The fish seemed morphologically different from *Oxynoemacheilus ercisianus*, the only nemacheilid loach species in the Lake Van basin. Therefore, our main hypothesis was to test if the newly-found lake population of nemacheilid fish is conspecific with *O. ercisianus*, or it might represent an undescribed species. This was addressed by producing mtDNA-COI barcode sequences and by examining some morphological traits.

Nemacheilid loaches are typically found in shallow waters and associated with fast to moderate flowing stretches of the streams, and to a lesser extent, large rivers and lake shores. Habitat associated divergence in morphological traits has been documented in a great number of studies with the best known examples including species from Characidae, Cichlidae (Langerhans et al. 2003; Costa-Pereira et al. 2016; Perazzo et al. 2019); Cyprinidae (Haas et al. 2010; Franssen et al. 2013); Galaxiidae (Dunn et al. 2020) and Centrarchidae (Brinsmead and Fox 2002). To the best of our knowledge, no study has yet addressed this topic in nemacheilids, and this study is also the first one to document morphological differences between two groups to see if the differences are due to a response to the contrasting habitats (lentic vs. lotic), or imply species-specific characters.

Material and methods

Fish sampling and measurements

Fish were collected by scuba diving using a small dip net with 4 mm mesh size, by two sampling events carried out in January 2018 and January 2020. After anaesthesia, fish were fixed in 5% formaldehyde and stored in 70% ethanol, or directly stored in 99% ethanol. Measurements were made with a digital calliper and recorded to 0.1 mm. All measurements were made point-to-point, never by projections. Methods for counts and measurements follow Kottelat and Freyhof (2007). Standard length (SL) is measured from the tip of the snout to the posterior extremity of the hypural complex. The length of the caudal peduncle is measured from behind the base of the last anal-fin ray to the posterior extremity of the hypural complex, at mid-height of the caudal-fin base. The last two branched rays articulating on a single pterygiophore in the dorsal and anal fins are counted as 1½.

Simple rays of dorsal and anal fins are not counted as they are deeply embedded.

Our hypothesis does not include testing for the variations in body shape. Therefore we used only linear measurements to identify morphological differences between the two groups of fish collected from stream and lake. All body measurements were standardized by individuals' SL, and the measurements taken from the head region were standardized by individuals' head length. To reduce the effect of allometric growth on some ratios, we selected similar size range for comparison (i.e., 30.0 mm to 39.2 mm for lake and 30.7 mm to 42.4 mm for stream groups). We also examined univariate patterns to test for significant effect of the explanatory variable as habitat type (stream vs. lake) on each of the response variables (morphometric measurement) using regression model with analysis of covariance (ANCOVA). In this procedure, SL was used as a covariate to control for variation due to fish size (all dependent variables and the covariate were log-transformed) (Zar 2010). Statistical effects evaluated at $\alpha = 0.01$.

The water physicochemical parameters including temperature, pH, dissolved oxygen and salinity were measured at the sampling locations (both in streams and in the lake) using a multiparameter instrument (YSI ProDS, Yellow Spring Instruments, Yellow Springs, OH, USA). The lake measurements were taken during scuba diving, just near the surface of the microbialites where the fish were collected, and not from the open water.

Abbreviations used: SL, standard length; HL, head length. Collection codes: FFR, Recep Tayyip Erdogan University Zoology Museum of the Faculty of Fisheries, Rize, Turkey.

Molecular data analysis

Genomic DNA was extracted from fin tissue using Macherey and Nagel NucleoSpin Tissue kits following the manufacturer's protocol on an Eppendorf EpMotion® pipetting-roboter with vacuum manifold. The standard vertebrate DNA barcode region of COI was amplified using a M13-tailed primer cocktail including FishF2_t1 (5'TGTAAAACGACGGCCAGTCGACTAATCATAAAGA-TATCGGCAC), FishR2_t1 5'CAGGAAA- CAGC-TATGACACTTCAGGGTGACCGAAGAATCAGAA), VF2_t1 (5'TGTAAAACGACGGCCAGTCAAC-CAACCACAAAGACATTGGCAC) and FR1d_t1 (5'CAGGAAACAGCTATGACACCTCAGGGTGTCC-GAARAAYCARAA) (Ivanova et al. 2007). PCR were performed using Qiagen Multiplex taq as follows: 15min at 95 °C; 10 cycles of 35 s at 94 °C, 90 s at 52–49 °C (touch-down) and 90s at 72 °C followed by 25 cycles of 35 s at 94 °C, 90s at 55 °C and 90s at 72 °C with final elongation for 10min at 72 °C and hold at 10 °C. Sequencing of the ExoSAP-IT (USB) purified PCR product in both directions was conducted at Macrogen Europe Laboratories with forward sequencing primer M13F
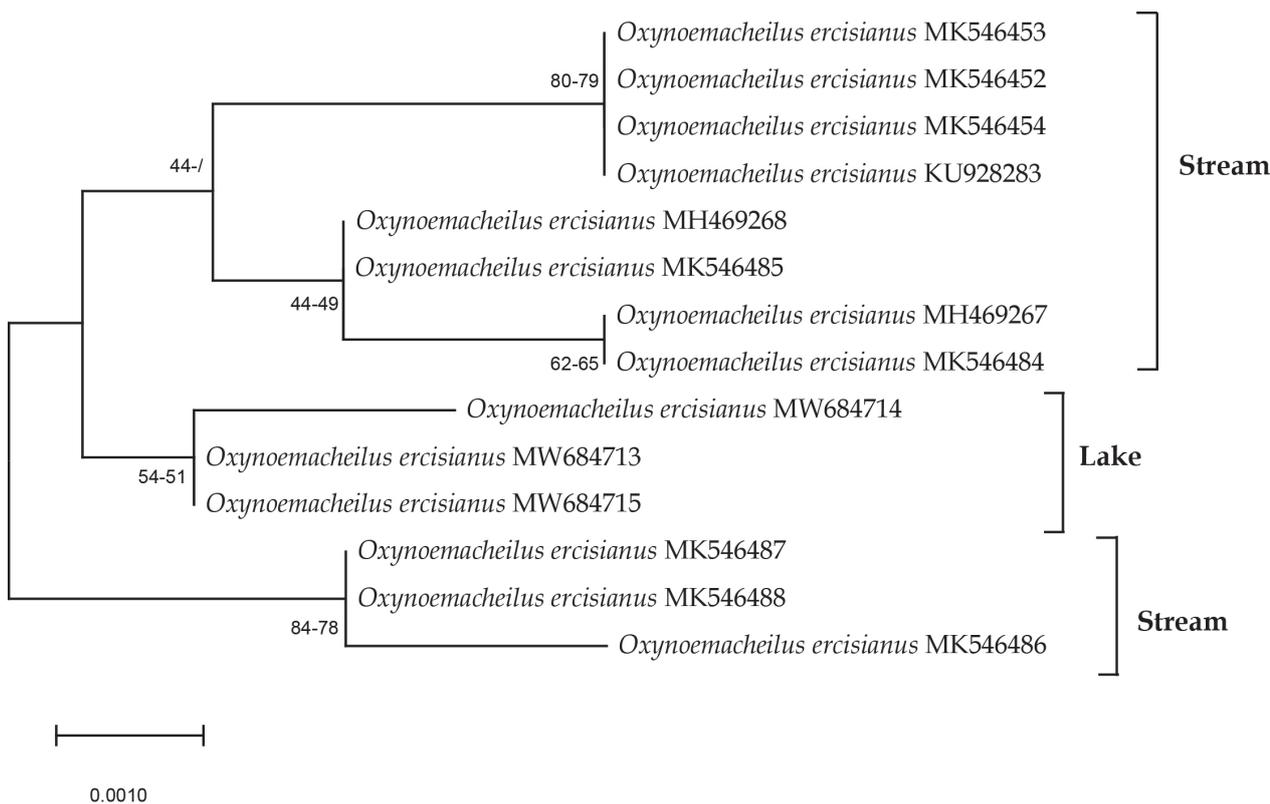
Figure 1. Maximum likelihood estimation of the phylogenetic relationships based on the mitochondrial COI barcode region (K2P model, discrete Gamma distribution for rate differences with 3 categories (+*G*, parameter = 0.0500)). Nucleotide positions with less than 98% site coverage were eliminated, resulting in 570 analysed positions. Numbers of major nodes indicate bootstrap values from 1000 pseudoreplicates from the NJ and ML method.

(5'GTAAAACGACGGCCAGT) and reverse sequencing primer M13R-pUC (5'CAGGAAACAGCTATGAC).

Molecular analysis involved 16 nucleotide sequences. Among these, we newly generated 3 DNA barcodes from lake dwelling specimens and included already published data from NCBI GenBank for 11 specimens from Ilıca (Zilan) stream, a northern tributary in Erciş (Geiger et al. 2014) (Table 1). The Muscle algorithm (Edgar 2004) was used to align the DNA barcodes after manually screening for unexpected indels or stop codons. The sequence evolution model test implemented in the MEGA X software (Kumar et al. 2018) was used to determine the most appropriate evolution model for the given data and to reconstruct the mitochondrial relationships between the studied groups. The model with the lowest BIC scores (Bayesian Information Criterion) is considered to best describe the substitution pattern. Initial tree(s) for the heuristic search was obtained by applying the Neighbour-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0,0500)). We generated neighbour-joining (Saitou and Nei 1987) and maximum likelihood (ML) phylogenetic trees with 1000 bootstrap replicates to explore phylogenetic affinities of the mitochondrial lineages. The tree is drawn to scale, with branch lengths measured in the

Table 1. List of COI sequences downloaded from NCBI GenBank with information on drainage and country of origin.

Species	Drainage	Country	Genbank	Reference
Oxynoemacheilus ercisianus	Ilıca Stream (Van)	Turkey	MH469267	Turan et al. 2019
			MH469268	
			MK546484	Geiger MF (unpublished)
			MK546485	
			MK546486	
			MK546487	
			MK546488	
	Mağara Stream (Van)		MK546452	
			MK546453	
			MK546454	
			KU928283	Freyhof et al. 2016

number of substitutions per site. All codon positions were included and positions with less than 98% site coverage were eliminated, resulting in 570 analysed nucleotide positions. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018).

Results

In total, 44 fish from microbialites and 33 fish from streams were collected and examined. The standard length (SL) of the *Oxynoemacheilus* individuals from microbialites ranged from 17.8 mm to 39.2 mm, whereas the size of the stream group ranged from 30.7 mm to 66.7 mm.

Figure 2. A lake-resident individual of *Oxynoemacheilus ercisianus* on the microbialite (left). Views from microbialites in Lake Van (right).

Molecular and morphological assessments

The analysis of the nucleotide sequences of the COI barcode region resulted in a mean 0.5% K2P distance between the stream and the lake-resident groups, both are separated by 7 variable nucleotide substitutions one of which is unique to the lake group. We treated, therefore, the lake-resident group as conspecific to *O. ercisianus*.

Despite the low molecular distance, several significant differences were found between the morphological characters of the two populations. The lake-resident population is differentiated from the stream population by the following combination of morphological characters: longer pre-dorsal length (52–56% SL vs. 50–52), longer pre-anal length (78–83% SL vs. 73–77), longer pre-pelvic length (58–61 %SL vs. 52–55) and greater distance between pectoral and pelvic fin origin (33–36% SL vs. 26–31), shorter pelvic fin length (11–13% SL vs. 14–16) and shorter caudal peduncle length (14–16% SL vs 16–18). The pectoral fin length is significantly greater in the stream dwelling population, yet the range overlapped with lake-resident population. Similarly, the snout, post-orbital and inter-orbital distances were significantly greater in the stream population, whereas all overlapped by means of minimum and maximum ratio. See Figs 2, 3 and Table 2 for general appearance and comparison with stream population and other morphometric measurements, respectively.

The lake-resident population is further differentiated from the stream population by having a shorter lateral line with 5–7 pores reaching up to vertical of pectoral fin midline (vs. 9–12 pores reaching up to pectoral fin tip or dorsal fin origin). Usually, there are none, or just one faint lateral pore in supratemporal canal (vs. two or three apparent pores). All other meristic traits including fin ray numbers overlapped between lake and stream dwelling populations.

Table 2. Nucleotide substitutions in the variable sites of the mitochondrial COI gene (570 bp) of *Oxynoemacheilus ercisianus* from lake and stream populations.

			Nucleotide position				
				1	3	4	4
			3	4	3	8	8
Individuals	8	3	5	1	0	2	5
Oxynoemacheilus ercisianus MW684715 (Lake)	T	C	C	C	T	G	A
Oxynoemacheilus ercisianus MW684714 (Lake)	.	.	.	T	.	.	.
Oxynoemacheilus ercisianus MW684713 (Lake)	.	.	.	.	.	.	.
Oxynoemacheilus ercisianus KU928283 (Stream)	C	.	.	.	.	.	G
Oxynoemacheilus ercisianus MK546487 (Stream)	.	A	A	.	.	.	.
Oxynoemacheilus ercisianus MH469267 (Stream)	.	.	A	.	C	.	G
Oxynoemacheilus ercisianus MH469268 (Stream)	.	.	A	.	.	.	G
Oxynoemacheilus ercisianus MK546488 (Stream)	.	A	A	.	.	.	.
Oxynoemacheilus ercisianus MK546486 (Stream)	.	A	A	.	.	A	.
Oxynoemacheilus ercisianus MK546485 (Stream)	.	.	A	.	.	.	G
Oxynoemacheilus ercisianus MK546484 (Stream)	.	.	A	.	C	.	G
Oxynoemacheilus ercisianus MK546454 (Stream)	C	.	.	.	.	.	G
Oxynoemacheilus ercisianus MK546453 (Stream)	C	.	.	.	.	.	G
Oxynoemacheilus ercisianus MK546452 (Stream)	C	.	.	.	.	.	G

Figure 3. *Oxynoemacheilus ercisianus,* left from top: FFR 01403, 32.3 mm SL, 31.1 mm SL, 36.8 mm SL, 37.3 mm SL, Edremit (Lake-resident population); right from top: 37.4 mm SL, 42.0 mm SL, 41.0 mm SL, 33.4 mm SL, Bendimahi River (Stream population)

The SL of the lake-resident population ranged from 17.8 mm to 39.2 mm, suggesting smaller maximum size compared to the stream population (ranged from 30.7 mm to 66.7 mm SL).

Material examined

Oxynoemacheilus ercisianus (stream dwelling population): FFR 15533, 3, 35–48 mm SL, Turkey: Van prov.: Ilıca stream at Erciş, 2 km northwest to Ulupamir, 39.1813, 43.3019. –FFR 15534, 3, 38–49 mm SL; Turkey: Van prov.: Ilıca stream at Erciş Örene, under the bridge at Bitlis-Tatvan Road, 39.0063, 43.3180. –Uncat., 27, 31–67 mm SL, Turkey: Van prov.: Ilıca stream 20 km north of Erciş, 39.2264, 43.3887.

Oxynoemacheilus ercisianus (lake-resident population)–Uncat., 19, 18–36 mm SL, Turkey: Van prov.: gulf near Gevaş in the southern Lake Van, 38.3168, 43.1149. –FFR 01403, 25, 19–39 mm SL, Turkey: Van prov.: shore of Edremit, Lake Van, 38.4250, 43.2319.

Material used in molecular genetic analysis

Uncat. Turkey: Van prov.: gulf near Gevaş in the southern Lake Van, 38.3168, 43.1149 (GenBank accession numbers: MW684713, MW684714, MW684715).

Habitat characteristics

The lake-resident populations of *O. ercisianus* were found from two microbialite areas on the south-eastern coast of the Lake Van (Fig. 4), from approximately 0.5 km offshore to the west of Edremit and from 0.3 km offshore to the north of Gevaş in the south-eastern Anatolia. The specimens were collected from a 13-meter-high tower-like microbialite under 15-m depth from the surface in Edremit and from a 3-meter-high tower-like microbialite under 6-m depth in Gevaş. The microbialites are covered by several groups of algae, and they have several holes, cavities and indented portions where fish are found. The water coming out of the cracks in the lake bottom follows through the microbialites and leaks at the top like a spring water coming out of the crevasse. Leaked water has formed a microhabitat on the microbialite surface and the water physico-chemical parameters fluctuated in parallel with the amount of this leakage. However, we were able to measure some of the water parameters during scuba-diving for sampling (Table 3). During the daytime dives, very few fish were able to be seen on the microbialite, while hundreds of fish were seen in the night dives. Based on this observation, it might be concluded that the fish exhibits a nocturnal activity. All the fish were observed just on the near surface branches of the microbialites; and they were restricted to maximum ca. 1 m diameter radius of movement range. No individual was observed at the bottom surface of the microbialites or in
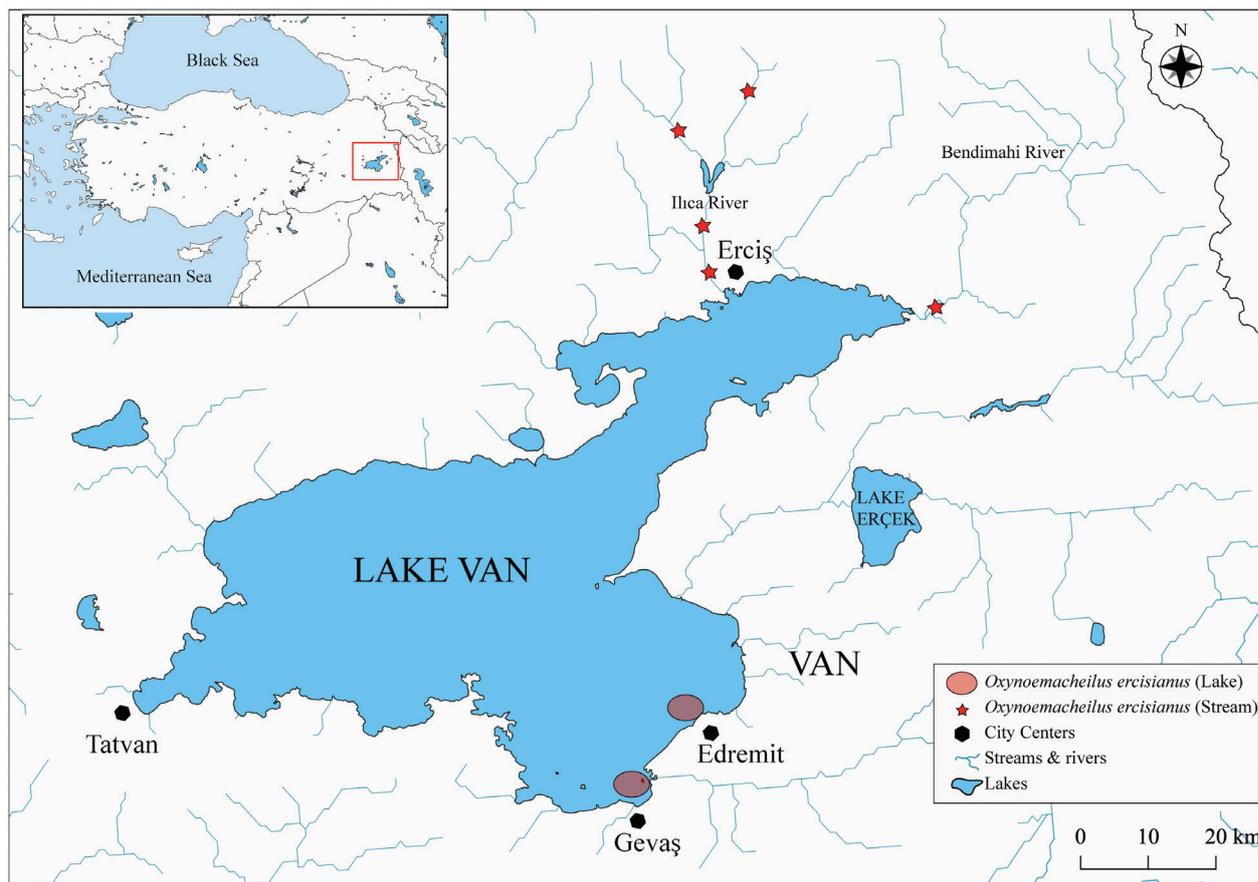
Figure 4. Map of the study area and sampling locations in the Lake Van basin.

Table 3. Morphometric data of *Oxynoemacheilus ercisianus* from lake and stream populations (Lake, FFR 01403, n = 12; Stream, FFR 15533, n = 10). Bold text – both significant at *p* < 0.01 (ANCOVA) and non-overlapping ranges.

	Lake-resident population				Stream population				Significance
	mean	min	max	SD	mean	min	max	SD	p-Value
Standard length (mm)		30	39			31	42		
In percent of standard length									
Head length	24.7	23.3	26.4	1.0	25.7	24.0	27.6	1.0	0.020
Body depth at dorsal–fin origin	15.9	14.0	17.3	1.3	17.2	16.3	20.1	1.2	0.266
Pre-dorsal length	**53.8**	**52.1**	**56.0**	**1.3**	**50.8**	**49.8**	**51.7**	**0.8**	**< 0.001**
Pre-pelvic length	**59.2**	**58.2**	**60.7**	**0.8**	**53.2**	**51.9**	**54.7**	**1.0**	**< 0.001**
Pre-pectoral length	28.9	28.4	29.6	0.4	28.3	27.1	29.5	0.9	0.233
Pre-anal length	**80.0**	**77.8**	**82.8**	**1.5**	**74.9**	**73.1**	**77.0**	**1.2**	**< 0.001**
Post-dorsal length	35.1	33.8	37.9	1.0	37.0	35.0	38.6	1.3	0.003
Distance between pec. and pel-fin origins	**34.6**	**33.0**	**35.7**	**1.0**	**29.2**	**26.4**	**30.6**	**1.3**	**< 0.001**
Distance between vent and anal-fin origin	2.9	2.3	3.3	0.3	3.2	2.8	3.7	0.3	0.490
Distance between pel. and anal-fin origins	21.2	19.2	21.9	0.8	21.4	20.1	22.1	0.7	0.638
Dorsal-fin length	17.5	16.2	19.5	1.0	20.2	19.1	21.7	0.9	< 0.001
Anal-fin base length	7.5	6.7	9.0	0.6	8.2	6.9	9.0	0.6	0.012
Pectoral-fin length	17.6	16.0	19.7	1.3	20.2	18.2	22.0	1.4	0.006
Pelvic-fin length	**12.2**	**11.2**	**13.3**	**0.7**	**14.8**	**13.6**	**15.9**	**0.8**	**< 0.001**
Length of caudal peduncle	**14.5**	**13.5**	**16.1**	**0.8**	**17.0**	**16.2**	**18.2**	**0.6**	**< 0.001**
Depth of caudal peduncle	9.1	8.2	10.1	0.7	10.2	9.6	10.8	0.5	0.012
In percent of head length									
Snout length	35.9	34.4	37.5	1.0	38.0	36.3	40.0	1.5	0.003
Eye diameter	22.1	19.9	25.0	1.3	20.4	18.6	22.6	1.4	0.565
Interorbital width	33.2	30.6	35.1	1.4	35.3	32.7	37.5	1.8	< 0.001
Postorbital distance	45.0	42.3	47.4	1.7	47.2	45.7	48.6	1.1	0.001
Maximum head width	63.4	61.8	65.4	1.2	64.6	61.8	70.2	2.8	0.065
Head depth at eye	45.9	43.8	48.0	1.2	49.0	45.2	51.7	2.4	0.005
Length of inner rostral barbel	17.1	16.3	19.8	1.0	16.4	14.4	18.2	1.3	0.832
Length of outer rostral barbel	19.2	17.3	21.2	1.3	17.8	16.3	19.6	1.2	0.363
Length of maxillary barbel	22.2	20.7	23.6	1.0	20.1	18.4	22.7	1.4	0.070

Table 4. Water physico-chemical parameters measured in two microbialite areas (in Edremit and Gevaş and in Bendimahi river.

Parameter	Edremit (Lake)	Gevaş (Lake)	Bendimahi (Stream)
Water temperature (C°)	11	12.3	6.8
Salinity (‰)	18.1	18.3	0.4
pH	9.1	9.2	7.9
Dissolved Oxygen (mg/L)	7.6	7.9	10.2
Total Dissolved Solids (g/L)	19.3	20.1	15.6

open water. More explorations are needed to demonstrate whether the species is restricted to two microbialite areas in the lake or it is more common on this type of groundwater-fed microbialites.

Discussion

The findings of the present study have entirely changed the generally accepted knowledge that no fish species is found to permanently occur in Lake Van. Our discovery of the first fish, a nemacheilid loach, permanently inhabiting the lake, triggered the question whether it might be a new or undescribed species. Our hypothesis testing resulted in recognizing the fish as a distinct and isolated population of *Oxynoemacheilus ercisianus*, the only nemacheilid species in the endorheic Lake Van basin. Despite considerable differences in some of the morphometric characters between the newly found lake-resident population and its stream conspecific, the two groups are superficially very similar to each other with also very small K2P distance in their COI barcode region. We follow Freyhof et al. (2018) and Yoğurtçuoğlu et al. (2020) treating populations without clear morphological differences as conspecific, if they have K2P distances smaller than 2% separating them. However, if diagnostic differences are observed, then small K2P distance is violated and the entities are treated as separate species. In the present study, the morphological differences between the two groups indicated a response to the contrasting habitats (lentic vs. lotic), rather than being species-diagnostic difference. Indeed, several studies have demonstrated that populations of the same species inhabiting different flow regimes (e.g. lentic vs. lotic) may have a different set of morphological characters. For example, it has been hypothesised that the lateral fins of lake populations will be more posteriorly positioned than those of stream populations of a fish species (Webb 1984; Swain and Holtby 1989; Langerhans 2008). This prediction is supported by the pelvic fins of the lake-resident population being remarkably more posteriorly positioned than in the stream population. This was not supported for the pectoral fins, and it resulted in greater distance between pectoral and pelvic fins in lake-resident population. McGuian et al. (2003) demonstrated that some lake-dwelling rainbow fish species (*Melanotaenia* spp.) had significantly more posteriorly positioned dorsal fins relative to their stream dwelling conspecifics. This was also supported in *O. er-*

cisianus, as the dorsal fin of the lake-resident population was significantly more posteriorly positioned relative to stream dwelling population. According to Webb (1984) the more posterior position of the lateral fins allows for additional manoeuvrability in fishes. Yet, McGuian et al. (2003) accepted that their findings (more posterior dorsal fin in lake populations) were inconsistent with the expectations of hydromechanical evolution, and they avoided proposing a hypothesis of divergence in this trait of fin positioning, as it might have been driven by complicated factors such as predator occurrence or genetics. We partly excluded this explanation as the lake is free of any predators, and also our genetic analysis is not able to support or oppose this prediction. According to the hydrodynamic theory, stream fishes would be more slender-bodied than their lake conspecifics to minimize drag induced by the water current (Webb 1984; McLaughlin and Grant 1994). This prediction was not supported by our dataset, as we found no significant difference in body depth between two compared populations. A possible explanation for this might be that this prediction has been generally tested and associated with fishes that maintain sustained swimming, like salmonids; whereas *Oxynoemacheilus* species generally lack sustained swimming ability.

To answer how *O. ercisianus* might have been locked in the microbialites of the Lake Van is not easy. However, according to the geological support, the Lake Van had been exposed to a combination of rapid desiccation and transgressional phases throughout the Holocene and late Pleistocene (Reimer et al. 2009), and we propose that the newly found lake-resident population might have lost its link to freshwater during these historical water level fluctuations. These rapid changes in the lake's level have also been demonstrated to lead to the development of relic deltas 40–60 km away from the present river mouths (Degens et al. 1984), which overlap the limits of the microbialite range. As a result, one possible scenario explaining the confinement of *O. ercisianus* in the microbialites is that, during these lake level fluctuations in the past, some freshwater populations might have been stuck in one or more of the coastal aquifers that were further inundated by the lake, on which the microbialites were developed. As a primary freshwater group, nemacheilids are intolerant to high range of water salinities. Therefore, the lack of connection between the microbialites to the freshwater streams is hindering fish to migrate through the extreme ionic gradient. Indeed, we observed individuals in only a very restricted range of motion where they rely upon the freshwater seepage on the microbialites to survive. They cannot even move to the base of the microbialites, where no or little freshwater comes out. The occurrence of juveniles caught in January (probably the young of the year) also supports the case that the fish has become well-adapted to a microbialite-associated life. Its nocturnal behaviour, small size and a very restricted occurrence also might have impeded its discovery by the earlier explorations. Further research is needed to better understand the hydromechanical and physiological adaptation of this lake population.

Acknowledgements

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