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Morphological comparison of bleaks (*Alburnus*, Cyprinidae) from the Adriatic Basin with the description of a new species

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Abstract. The morphometric, meristic and phenotypical characters of the members of the genus *Alburnus* from the Adriatic Basin were analyzed on specimens from 11 localities, representing eight watersheds. The number of gill rakers, the number of lateral line scales, the number of branched anal fin rays and the coverage of the ventral keel by scales have the greatest significance in differentiating between species. Significant morphological differences exist between the *Alburnus* population from Lake Lugano (type locality for *Alborella maxima* Fatio, 1882) and all the remaining investigated populations. *Alburnus* from the Neretva River drainage is described as a new species and it is distinguished from other species of the genus *Alburnus* by the following combination of characters: $13^1/_2$-$15^1/_2$ branched anal fin rays; 17-26 gill rakers; 40-48 lateral line scales; first anal fin ray below branched dorsal rays 6-10 or located up to one scale after the last dorsal fin ray; ventral keel exposed for at least $^2/_3$ of a distance between the pelvic fin base and the anal aperture. The status of the Neretva River basin populations as a separate species was corroborated by genetic analyses, with p-distance between newly described species and *A. arborella* from the Zrmanja River 1.8-2.2%.

Key words: taxonomy, east Adriatic Basin, Neretva River basin

Introduction

Although bleaks (genus *Alburnus*) are widely spread in the waters of the Adriatic watershed, their taxonomy, systematic and actual distribution are still not well understood. Until recently, it was considered that only two widely distributed species inhabit the European waters (Kottelat 1997): *Alburnus albidus* (Costa, 1838) in the Adriatic Basin and *Alburnus alburnus* (Linnaeus, 1758) in the other parts of Europe. However, numerous subspecies, morphs and varieties of the later species were recognized by different authors (see Vuković & Ivanović 1971, Dimovski & Grupče 1975, Šorić 1980, Oliva et al. 1988) what made the taxonomy of the bleaks quite complicated and puzzling. Based on the works of Bogutskaya (1997) and Bogutskaya & Naseka (2004), as well as the systematics of Kottelat & Freyhof (2007), genera *Alburnus* and *Chalcalburnus* were merged into single genus, *Alburnus*. Recently, an intensive taxonomic work on the genus *Alburnus* has lead to a description of several new species from the Black and Aegean Sea Basins, mostly belonging to the *Alburnus chalcoides* (Gueldenstaedt, 1772) and *A. mento* (Heckel, 1836) species complexes (Freyhof & Kottelat 2007a, b, Özulug & Freyhof 2007a, b). Nowadays, 20 different species of bleaks are recognized in European freshwater systems (Kottelat & Freyhof 2007).

The region of eastern Adriatic Coast has been proved as a hotspot of ichthyological diversity for several cyprinid genera, like *Telestes*, *Scardinius* or *Delminichthys* (Kottelat & Freyhof 2007). However, the taxonomic status of the *Alburnus* populations in that area has not been particularly studied yet. Four *Alburnus* species (*A. albidus*, *A. arborella* Bonaparte, 1841, *A. belvica* Karaman, 1924, and *A. scoranza* Bonaparte, 1845) are reported to inhabit the waters of the Adriatic Basin in Italy, Switzerland, Slovenia, Croatia, Bosnia and Herzegovina, Montenegro, Macedonia, Albania and Greece (Kottelat & Freyhof 2007). Contrary to the situation with *A. alburnus* from the central and northern parts of Europe, the morphological data about many populations of bleaks in the Adriatic Basin are very scarce. That is especially true for the populations of *A. arborella*, which has the widest distribution range of all Periadriatic bleaks, occurring from northern Italy to the Neretva River drainage (Kottelat & Freyhof 2007). No other cyprinid from the Adriatic Basin – with the exception of *Phoxinus lumaireul* (Schinz, 1840) and *Squalius squalus* (Bonaparte, 1837), which probably represent complexes of several species (Kottelat & Freyhof 2007) has so wide distribution range. The aim of this work was to morphologically compare the populations of bleaks from the Adriatic Basin in order to check for morphological differences between species and to revise their taxonomic status. In order to ascertain the taxonomic status of populations from the Neretva River drainage, mitochondrial DNA analyses were also conducted, because they have proved to be a useful tool in identifying cryptic diversity among populations, especially when phenotypic plasticity is high among species and diagnostic characters are difficult to find.

Material and Methods

The specimens were collected by electrofishing from 11 localities belonging to nine river systems (Fig. 1). The examined material is listed at the end of this section and in species description. Besides the populations from the east Adriatic Coast, which are supposed to belong to the *A. arborella* (from the Vipava River in the Soča River drainage, Lake Butoniga in the Istrian peninsula, the Zrmanja River in the northern Dalmatia; and Lakes Baćinska and Kuti as well as the Mušnica River from the Neretva River drainage), we have also analyzed the specimens of *A. scoranza* from the Crni Drim and Zeta Rivers in the Ohrid-Drin-Skadar drainage and the Mat River, as well as *A. belvica* from Lake Prespa. Furthermore, the specimens from Lake Lugano (type locality of *Alborella maxima* Fatio, 1882) were included in the study. Three types of morphological characters (meristic, morphometric, and phenotypic) were examined on specimens fixed in 5% solution of formaldehyde and preserved in 70% ethanol. Meristic characters included the number of unbranched and branched fin rays in dorsal, anal, pelvic (ventral), pectoral and caudal fin. The last two branched rays in dorsal and anal fins, that are articulating on a single pterygiophore, are noted as '$1^{1}/_{2}$'. The number of gill rakers was counted on the first gill arch. The number of scales in the lateral line was counted in the complete lateral line, from the anterior scale next to the operculum to the posterior one on the caudal fin.

A total of 24 morphometric characters was measured using an electronic caliper to the nearest 0.01 mm:

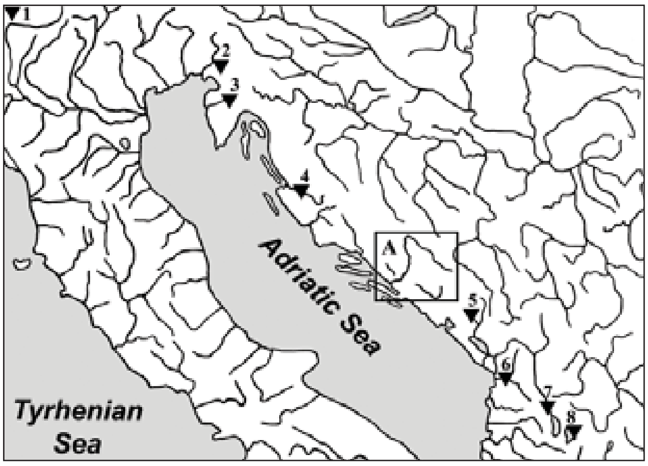

Fig. 1. The map of the Adriatic Basin.▼ collecting localities. 1 – Lake Lugano, 2 – Vipava River, 3 – Lake Butoniga, 4 – Zrmanja River, 5 – Zeta River, 6 – Mat River, 7 – Crni Drim River, 8 – Lake Prespa. Frame A shows the Neretva River drainage.

total length (TL), standard length (SL), head length (c), distance between head tip and anal aperture (pan), preanal distance (aA), preventral distance (aV), prepectoral distance (aP), predorsal distance (aD), caudal peduncle length (lpc), length of dorsal (lD), anal (lA), caudal (lC), pectoral (lP) and pelvic (ventral) fin (lV), distance between pelvic (ventral) fins and anal aperture (Van), head depth (hc), maximum body depth (H) and the caudal peduncle depth (or the minimum body height – h), head width (laco), maximum body width (lac), distance between eyes (io), eye diameter (o), preorbital distance (prO), postorbital distance (poO). The percentage ratios of morphometric characters in relations to SL, c and H were analyzed. The morphometric ratios of the populations from northern Adriatic and the Neretva River drainage, that were supposed to belong to the same species, were compared using the analysis of variance (ANOVA) with the aim to test the statistical significance of the difference between the means of each morphometric ratio at a significance level of $\alpha = 0.05$. Principal component analysis (PCA) was employed to determine whether there is a separation of the individuals from different populations of *A. arborella*, based on their morphometric ratios. All statistical comparisons were made on standardized body measures, e.g. on body ratios. The Statistica 6.0 software package was used for data analyses. The holotype as well as the paratypes of newly described species were included in morphometric analyses. In addition to meristic and morphometric characters, overall external morphology was examined.

In order to affirm the taxonomic status of *Alburnus* populations from the Neretva River drainage, mtDNA analyses were conducted on 10 specimens (two *A. scoranza* specimens from the Zeta River; four specimens of *A. arborella* from the Zrmanja River; one specimen from the Mušnica River – holotype of newly described species; and three specimens from the Neretva River). Total genomic DNA was extracted from fresh or deep-frozen muscle tissues using a standard extraction product (DNeasy tissue kit, Qiagen). Polymerase chain reaction (PCR) amplifications were performed in 50µl reaction volume containing 25µl of the HotStarTaq Master Mix (Quiagen), 2µl of each primer and 4µl of template DNA. The complete mitochondrial cytochrome *b* (cyt *b*) gene was amplified using primers L15267 and H16526 (Brito et al. 1997) and the following temperature regime: 15 min at 95ºC; 35 cycles of 30 s at 94ºC, 30 s at 50ºC and 90 s at 72ºC; 7 min at 72ºC. Sequencing was carried out by Macrogen Service Centre (Seoul, South Korea) with the internal primers. All sequences have been deposited in the GenBank under following accession numbers: GU479865-GU479874.

Pairwise comparisons of uncorrected sequence divergence (p-distances) in the cyt *b* gene were analyzed using Mega version 3.1 (Kumar et al. 2004). Two methods of phylogenetic inference were employed: maximum parsimony (MP) and maximum likelihood (ML), as implemented in PAUP (v 4.0b10, Swofford 2002). For MP analysis, the heuristic search mode with 100 replicates was used, with randomized input orders of taxa, and tree bisection-reconnection (TBR) branch swapping with all codon sites and nucleotide substitutions types weighted equally. ML analysis was performed under the heuristic search option using the TBR branch swapping algorithm. As the optimal model of sequence evolution, the Tamura-Nei plus Gamma model (TrN + G) was selected by hierarchical likelihood ratio (hLRTs) tests using the Modeltest software (version 3.06, Posada & Crandall 1998). Nonparametric bootstrapping (1000 pseudoreplicates, 10 addition-sequence replicates) was used to assess the branch support. Sequence of *Leucaspius delineatus* (Briolay 1998) was used as outgroup and previously published sequences of *A. alburnus* (Briolay 1998, Saitoh et al. 2006), *A. macedonicus*, *A. thessalicus* and *A. belvica* (Zardoya & Doadrio 1999) were also included in the analyses.

List of examined material

Institutional abbreviations: MNCN – Museo Nacional de Ciencias Naturales, Madrid; NMP – National Museum, Prague; PMF – Faculty of Science, Zagreb.

All specimens from the Neretva River basin are listed in the species description.

Alburnus belvica: MNCN 121113-121128, 16 spec., SL 71.2-109.7 mm, Lake Prespa, Greece

Alburnus arborella: PMF ALZR2-21, 20 spec., SL 54.5-100.1 mm, Zrmanja River, Croatia; PMF ALBU4-25, 22 spec., SL 39.9-92.2 mm, Butoniga reservoir, Mirna River drainage, Istrian peninsula, Croatia; PMF ALVI1-10, 10 spec., SL 55.9-90.1 mm, Vipava River, Soča River drainage, Slovenia

Alburnus maxima: NMP uncatalogued, 7 spec., SL 116-128.6 mm, Lake Lugano, Italy

Alburnus scoranza: NMP P6p 84/2007, 8 spec., SL 63.2-90.0 mm, Crni Drim River in village Dobovjani, FYROM, N 41°15.219´ E 20°39.196´, elevation 688 m, 26th July 2006; NMP P6V 81887-81892, 6 spec., SL 63.8-75.9 mm, Mat River in village Milot, Albania, N 41°41´16.2´´ E 19°42´11.7´, 25th August 2004; NMP P6V 80608-80610, 3 spec., SL 84.5-107.0 mm, Zeta River in village Glava Zete, Montenegro, N 42°39.904´ E 18°59.830´, 15th July 2003

Results and Discussion

General appearance of the investigated bleaks is quite similar, while the morphometric, some meristic, and also some phenotypic features differ between the species. The variability of all – meristic, morphometric and phenotypic characters – can also be noted between the different populations of the

Table 1. *Meristic characters assessed for the investigated* Alburnus *species. N – the number of specimens used for assessment of all meristic characters, with the exception of the gill rakers count; n – number of specimens on which the number of gill rakers was counted.*

Species	Dorsal fin rays	Anal fin rays	Caudal fin branched rays	Pelvic fins rays	Pectoral fins rays	Number of gill rakers	Number of lateral line scales
A. arborella – Butoniga, Zrmanja, Vipava (N = 52; n = 17)	III + (7.5)8.5(9.5)	III + 13.5-15.5(16.5)	(16)17	I + (7)8(9)	13-16	14-22 (mean = 18)	41-48 (mean = 45)
Lake Lugano population (N = 7; n = 5)	III + 8.5	III + 13.5-15.5	17	I + 8	14-16	18-24 (mean = 20)	49-54 (mean = 51)
A. neretvae sp. nova (N = 39; n = 18)	III + 8.5-9.5	III + 13.5-15.5	17	I + 7-8	14-17	17-26 (mean = 20)	40-48 (mean = 44)
A. scoranza (N = 17; n = 17)	III + 8.5(9.5)	III + 13.5-14.5	17	I + 7-8	13-16	15-20 (mean = 18)	46-53 (mean = 50)
A. belvica (N = 16; n = 5)	III + (7.5)8.5	III + (12.5)13.5(14.5)	17	I + (7)8	13-15	29-32 (mean = 30)	52-57 (mean = 54)

same species, probably depending on the ecological factors variation in different localities.

Table 1 summarizes the results of meristic investigations. The uniformity of the number of rays in all fins, with the exception of anal fin, as well as the variability of the number of gill rakers and lateral line scales among the species, and their importance for a taxonomic identification of *Alburnus* species were already reported (Dimovski & Grupče 1975, Šorić 1980, Freyhof & Kottelat 2007a, b, Kottelat & Freyhof 2007). Although the numbers observed by above mentioned authors are not completely the same as obtained in this investigation, they fall generally within the mentioned ranges, with some exceptions (populations from Lake Lugano and the Neretva River drainage).

A. belvica is easily distinguished from all other investigated species by having higher number of gill rakers (29-32 vs. max. 26 in the remaining species) as well as the higher number of lateral line scales (52-57 vs. max. 54 in the remaining species).

A. scoranza differs from the remaining investigated species by the following combination of meristic characters: the number of lateral line scales 46-53, the number of gill rakers 15-20, and the number of branched anal fin rays $13^1/_2$-$14^1/_2$.

A. albidus, not examined in this study, differs from all other Adriatic bleaks by lower number of anal fin rays (11-$13^1/_2$) (Kottelat & Freyhof 2007) vs. $13^1/_2$-$15^1/_2$ found in other species examined in this study.

Noteworthy is the differentiation of specimens from Lake Lugano, which is the type locality for *Alborella maxima* (Fatio, 1882), currently treated as synonym of *Alburnus arborella* (Kottelat & Freyhof (2007) mention only *A. arborella* for northern Italy). The following combination of meristic characters: the number of lateral line scales 49-54, the number of gill rakers 18-24 and the number of branched anal fin rays $13^1/_2$-$15^1/_2$ clearly differentiate the population from Lake Lugano not only from the remaining investigated populations, but also from all the other European *Alburnus* species. The only species with similar (but not the same) meristic characters is *A. scoranza*. However, the ventral keel exposure (see later) is a character that differentiates between them. It is also notable that the maximum size of investigated specimens from Lake Lugano (TL up to 159 mm) was largest among all the investigated populations. Only *A. scoranza* and *A. belvica* from large Lakes Skadar, Ohrid and Prespa reach the similar size, up to 180 mm TL (Dimovski & Grupče 1971, Ivanović 1973). *A. arborella* (including the populations from the Neretva River basin) and *A. albidus* (Bianco 1980, Kottelat & Freyhof 2007) seem to attain smaller maximum size.

The populations from the northern Adriatic Basin and those from the Neretva River drainage differ from the remaining investigated populations by the lower number of lateral line scales (40-48). However, we have found a different range of number of gill rakers for *A. arborella* specimens from Butoniga, Zrmanja and Vipava (14-22) than for those from the Neretva River basin (17-26).

The coverage of ventral keel by scales also differs between species. In *A. scoranza* the whole ventral keel, from the pelvic fin base to the anal aperture, is exposed. In *A. arborella* from the Butoniga Lake, Zrmanja and Vipava Rivers, as well as in the populations from the Neretva River drainage, the ventral keel is exposed for at least $^2/_3$ of the length between the pelvic fin base and the anal aperture, although usually the anterior part of ventral keel is very thin and it only peers between the scales, while its caudal part before the anal aperture is wider. Contrary to data of Economidis (1986) and Kottelat & Freyhof (2007), most *A. belvica* specimens had the ventral keel completely covered with scales and only very rarely it was exposed for up to 3 scales in front of the anus. The specimens from Lake Lugano had the ventral keel exposed for 1-3 (exceptionally 5) scales in front of the anal aperture.

The character often used for diversification of *Alburnus* species is also the position of the anal fin. In the investigated *A. arborella* populations (from the Butoniga Lake, Zrmanja and Vipava Rivers) the beginning of the anal fin was located below 4-9th branched dorsal ray, very rarely immediately behind the last dorsal fin ray. In the populations from the Neretva River drainage the first anal ray was located below 6-10th or behind the last dorsal ray (up to one scale behind). In the specimens from Lake Lugano as well as in *A. scoranza* specimens, the anal fin origin was located below 6-9th or immediately behind the last dorsal ray. In *A. belvica* the first anal fin ray was located usually for 1-$1^1/_2$ scales behind the base of the last dorsal ray. However, we also found a small number of specimens where the anal fin originated immediately after the base of the last dorsal fin ray or only $^1/_2$ scale behind it. In *A. albidus* the anal fin begins below or behind base of last dorsal ray (Kottelat & Freyhof 2007). Thus, these results suggest that this character is highly overlapping among the Adriatic bleaks.

Besides the number of gill rakers, their length is also sometimes used as a diagnostic character (Kottelat & Freyhof 2007). However, since we examined more than one population of some species, we were able to conclude that the length of gill rakers varies between different populations of the same species, probably depending on the specific ecological factors or a diet of a certain population and cannot be used for the differentiation of the species. Namely, in the *A. arborella* population from the Zrmanja River, the maximum gill raker length (measured at the angle between upper and lower gill arch limb) was for about 30% smaller than the maximum gill filaments length (measured at the same point), while in the populations from Butoniga Lake and the Neretva River drainage the maximum gill raker length was smaller, about 50% of the maximum gill filaments length. In the population from Baćinska Lakes, the largest gill rakers were almost as long as the largest gill filaments, while in the specimens from the Mušnica River they were for about 50% smaller. However, we have found differences in the morphology of gill rakers that might be species-specific. In *A. arborella* populations from the northern Adriatic basin (Vipava, Butoniga, Zrmanja) the gill rakers appeared as thin, flexible projections with a hook at the top and with a slightly serrated inner side. In the populations from the Neretva River drainage, they were more solid and not as thin as in *A. arborella*, but they were also slightly serrated on the inner side. In *A. scoranza* the gill rakers were both thin and short, but without serration. The gill rakers of the Lake Lugano population ended with a large, pointed buckled hook. Moreover, the gill rakers in the middle part of a gill arch were often forked, a character found in a few specimens of *A. belvica* and not in any other species.

Table 2 comprises morphometric data for the investigated species. The morphometric ratios were mostly similar and with great overlap between different species. Nevertheless, five morphometric ratios pan/SL, aA/SL, aV/SL, hco/H and h/lpc were found significantly different (ANOVA, $p < 0.05$) between populations supposed to belong to *A. arborella*, but not among populations from the northern Adriatic basin (Vipava, Butoniga, Zrmanja) nor from those from the Neretva River basin (Lakes Kuti and Baćinska, Mušnica). Furthermore, lpc and lA in relation to SL as well as prO, poO and hc in relation to c, vary between populations from the northern Adriatic area and those from the Neretva River drainage (Table 2), although the differences are not significant.

Fig. 2 represents the result of the PCA analysis that included specimens from the populations that were until now considered as *A. arborella* (Lake Lugano; Lake Butoniga, Vipava and Zrmanja Rivers; Lakes Baćinska and Kuti and Mušnica River from the Neretva River drainage). The separation of the specimens from Lake Lugano is obvious. However, the remaining populations are not so clearly separated although grouping of the specimens from Butoniga Lake and the Zrmanja and Vipava Rivers on one hand, and those from Baćinska and Kuti Lakes and the Mušnica River on other hand, is apparent, but with overlapping between these two groups. It suggests that although *A. arborella* and *Alburnus* from the Neretva River drainage are quite similar in their morphometric ratios and there is a certain overlap, they demonstrate some differences in morphometric features. Furthermore, there is no separation of the specimens from different populations belonging to the same geographic area, which suggests that they are more similar to each other then to the remaining investigated populations.

General characteristics of external morphology show great similarities between investigated populations. All the investigated *Alburnus* species have elongated body with a pointed head, forked caudal fin and slightly superior mouth. The colouration of all the investigated species (preserved specimens) is also quite similar; the dorsal part of the body and head bears a dark stripe, below it on each side there is a lighter area, then a blue or grey stripe and the ventral part is usually whitish to yellow. However, some differences in colouration exist between the species. *A. scoranza* and the populations from the Neretva River drainage have darker upper half of the body, while in *A. arborella* from the Zrmanja and Vipava Rivers and Butoniga Lake, the dorsal half of the body is lighter. In live specimens of *A. arborella* a greenish colouration can often be seen on top of the blue stripe, while in the populations from the Neretva River drainage, there is usually a red tone.

Although morphological differences between bleaks from the northern Adriatic Basin and those from the Neretva River drainage are not very pronounced, phylogenetic analyses corroborated their taxonomic value and separation of specimens from two geographically isolated areas in two independent lineages. The entire cyt *b* gene (1140 bp) was obtained from 10 specimens. The sequences showed 59 variable sites, 44 of them being parsimoniously informative. Uncorrected pairwise sequence divergence (p-distance) between specimens from the Zrmanja River and those from the Neretva River drainage ranged between 1.8 and 2.1%, with a mean value of 1.9%. Among the haplotypes from the Zrmanja River the p-distance was between 0.1 and 0.3% (mean = 0.2%), while for those from the Neretva River basin it was 0.4-0.7% (mean = 0.5%).

Both employed methods of phylogenetic inference resulted in similar topology of the phylogenetic trees. MP analysis revealed one tree (tree length = 244, consistency index (CI) = 0.7951, homoplasy index (HI) = 0.2049, retention index (RI) = 0.7547, rescaled consistency index (RC) = 0.5992). ML analysis also resulted in a single tree (-Ln likelihood = 2725.92080). The only difference between the

Table 2. *Morphometric ratios assessed for the investigated* Alburnus *species. N = the number of specimens used for morphometric analyses.*

	Lake Lugano specimens (N = 7)		*A. scoranza* (N = 17)		*A. belvica* (N = 16)		*A. arborella* (N = 52)		*A. neretvae* sp. nova (N = 39)	
	mean min-max	SD	mean min-max	SD	mean min-max	SD	mean min-max	SD	mean min-max	SD
SL	121.4 116.0-128.6	4.2	76.0 63.2-107.0	11.4	99.7 71.2-109.7	8.8	64.9 39.9-101.0	16.9	78.8 50.2-108.9	13.2
SL/TL	0.82 0.81-0.84	0.01	0.82 0.80-0.84	0.01	0.81 0.80-0.84	0.01	0.81 0.78-0.84	0.01	0.82 0.79-0.85	0.02
in % of SL:										
c	24.1 22.9-25.0	0.7	25.2 23.1-28.0	1.3	28.0 26.4-30.3	1.0	25.1 22.4-28.5	1.4	25.2 22.8-27.4	1.1
pan	65.2 64.4-66.6	0.7	64.2 62.4-66.3	1.3	66.9 65.0-68.6	1.0	64.7 61.3-68.0	1.5	66.1 63.0-68.0	1.3
aA	66.6 65.3-67.4	0.8	65.0 62.9-66.7	1.5	67.6 66.3-69.5	0.9	65.6 62.1-69.5	1.6	67.2 64.4-71.5	1.4
aV	47.4 46.1-49.3	1.0	46.7 45.4-48.1	0.8	49.4 47.2-50.8	1.0	46.6 43.7-49.6	1.3	48.0 44.9-50.3	1.3
aP	23.2 22.2-24.2	0.7	25.7 23.0-27.7	1.3	26.8 25.7-28.0	0.7	25.2 22.3-29.3	1.5	25.2 23.5-27.2	1.1
aD	56.8 54.9-57.8	1.3	55.8 52.8-57.1	1.2	56.5 55.6-57.9	0.7	56.8 53.2-61.3	1.4	57.2 53.5-61.1	1.5
lpc	16.9 15.4-18.6	1.0	18.2 16.1-19.8	1.3	19.1 17.6-20.7	0.9	18.4 15.4-23.7	1.6	16.3 13.0-19.6	1.3
Van	18.9 17.3-21.2	1.4	18.5 16.4-20.3	1.3	19.0 17.8-20.2	0.8	19.0 14.1-22.7	1.7	19.9 17.2-22.7	1.5
lD	10.6	0.5	10.3	0.6	9.7	0.8	10.4	1.1	10.5	0.8

Table 2. continued

	9.9-11.2		9.3-12.0		8.0-11.5		8.5-12.6		8.9-11.8	
lA	15.2 14.0-17.1	1.1	15.9 13.6-18.1	1.1	13.9 12.9-15.0	0.6	16.7 14.8-19.5	1.1	15.6 13.2-17.9	1.3
lC	23.0 20.9-25.4	1.9	23.0 20.6-26.1	1.4	23.6 21.5-25.5	1.2	25.2 20.6-30.4	1.9	24.4 19.9-29.4	2.5
lP	19.5 17.4-23.0	1.8	19.2 17.6-20.9	1.0	19.7 18.3-20.8	0.6	20.2 17.9-22.9	1.3	19.5 16.4-22.4	1.4
lV	13.7 13.0-14.5	0.6	14.9 13.5-15.8	0.7	15.2 13.9-16.3	0.6	15.3 13.2-18.9	1.2	15.2 12.7-16.8	1.0
lac	11.5 10.4-12.0	0.5	11.3 9.3-12.7	0.8	12.4 11.1-13.3	0.6	12.4 8.0-15.1	2.0	12.2 9.1-15.6	1.5
laco	10.1 9.7-10.6	0.3	11.3 10.1-12.4	0.5	11.9 11.1-13.1	0.5	11.5 9.6-13.9	1.0	11.3 9.2-13.2	1.0
H	21.5 20.7-22.8	0.9	21.2 17.7-24.6	1.9	20.5 18.9-22.3	0.9	23.8 20.3-26.7	1.6	23.7 19.1-28.8	2.5
h	8.7 8.3-9.2	0.3	9.4 8.3-10.3	0.6	9.3 8.6-9.7	0.3	9.5 7.6-10.7	0.7	9.3 7.8-10.8	0.8
in % of c:										
io	29.5 27.2-34.4	2.3	31.6 26.1-36.3	2.8	27.6 25.1-29.9	1.3	32.2 26.7-37.8	2.2	30.8 24.6-35.5	2.3
Oh	24.6 23.6-26.0	0.7	27.5 23.2-31.7	2.3	23.8 22.5-26.7	1.2	28.8 23.0-34.1	2.8	27.2 23.7-30.2	1.6
prO	26.1 24.8-28.4	1.1	28.8 25.5-33.3	2.0	26.6 23.9-28.8	1.3	26.8 21.9-46.1	3.2	27.7 25.0-31.9	1.6
poO	47.8 46.1-51.1	1.7	43.9 40.2-50.0	2.6	48.9 46.5-51.1	1.4	43.1 37.8-47.9	2.5	46.2 41.1-52.1	2.7
	41.9		44.6		42.4		45.9		45.0	

Table 2. *continued*

hc	54.7 52.0-57.4	2.0	51.5 47.4-56.9	2.5	48.0 44.5-52.5	2.1	56.6 50.3-68.2	3.7	54.6 47.1-58.7	2.9
in % of H:										
lac	53.4 50.2-57.1	3.0	53.5 49.2-57.5	2.5	60.6 53.8-63.9	2.5	51.9 38.2-61.1	6.4	52.8 44.1-59.0	3.5
hc	61.2 57.1-65.0	2.6	61.6 48.9-71.4	5.7	65.7 58.1-70.5	3.6	60.0 49.9-74.2	5.7	58.4 48.9-73.2	6.0
h	40.5 36.5-43.5	2.4	44.4 41.0-49.6	2.2	45.4 38.4-48.3	2.4	40.1 34.8-46.3	2.6	39.8 34.4-45.2	2.6
in % of lpc:										
h	51.3 44.8-57.3	4.5	51.7 44.4-59.9	4.4	48.8 42.7-55.3	3.0	52.0 40.5-65.6	5.3	57.4 46.7-74.8	6.1

tree obtained by ML analysis and that recovered by MP analysis is the position of *A. belvica* lineage, that, however, has no importance in revealing the taxonomic status of the Neretva River drainage populations. Both employed phylogenetic methods have verified the genetic distinctiveness of the *Alburnus* from the Neretva River drainage (Fig. 3). There is a clear separation of the haplotypes from the Neretva River drainage and they form an independent lineage in the phylogenetic tree. This separation and clustering is very well supported by MP and ML bootstrap values.

Based on morphological differences as well as genetic distinctiveness, we conclude that the bleaks from the Neretva River drainage represent a separate species. Furthermore, we have found great morphological differences between the population from Lake Lugano and all the remaining investigated populations suggesting that *A. maxima* should be considered as a valid species. Nevertheless, a detailed study is needed to compare the populations of *A. arborella* throughout its rage, from central Italy to the Zrmanja River basin, especially with respect to the lacustrine populations from northern Italy.

Alburnus neretvae Buj, Šanda et Perea, sp. nova (Fig. 4)

Holotype: NMP P6V 83 139, 101.1 mm SL, Bosnia and Herzegovina, the Mušnica River by the village Avtovac in Gatačko polje, N 43°08.290′, E 18°34.343′, elevation 960 m, 12th July 2006, leg. Šanda R., Kohout J. & Šedivá A., Fig. 4.

Paratypes: NMP P6V 83 140 and 83 141, 106.7 and 65.8 mm SL, respectively, the same data as for the holotype; PMF BAC 1-5, 5 spec., 88.0-118.8 mm SL, Croatia, Lakes Baćinska jezera, 27th June 1997, leg. Mrakovčić M., Schneider D., Mustafić P. & Vajdić S.

Additional material: NMP P6p 84/2007, 10 spec., SL 58.8-108.9 mm, same data as holotype; PMF BAC 6, 8, 10, 14, 15, 17 and 20, 7 spec., SL 71.3-84.0 mm, same data as paratypes PMF BAC 1-5; PMF KUT 1-14, 14 spec., SL 50.2-88.1 mm, Lake Kuti, Croatia, 27th June 1997, leg. Mrakovčić M., Schneider D., Mustafić P. & Vajdić S.

Diagnosis: Alburnus neretvae is distinguished from other species of the genus *Alburnus* by the following combination of characters: $13^{1}/_{2}$-$15^{1}/_{2}$ branched anal fin rays; 17-26 gill rakers; 40-48 lateral line scales; first anal fin ray below branched dorsal rays 6-10 or located up to one scale after the last dorsal fin ray;

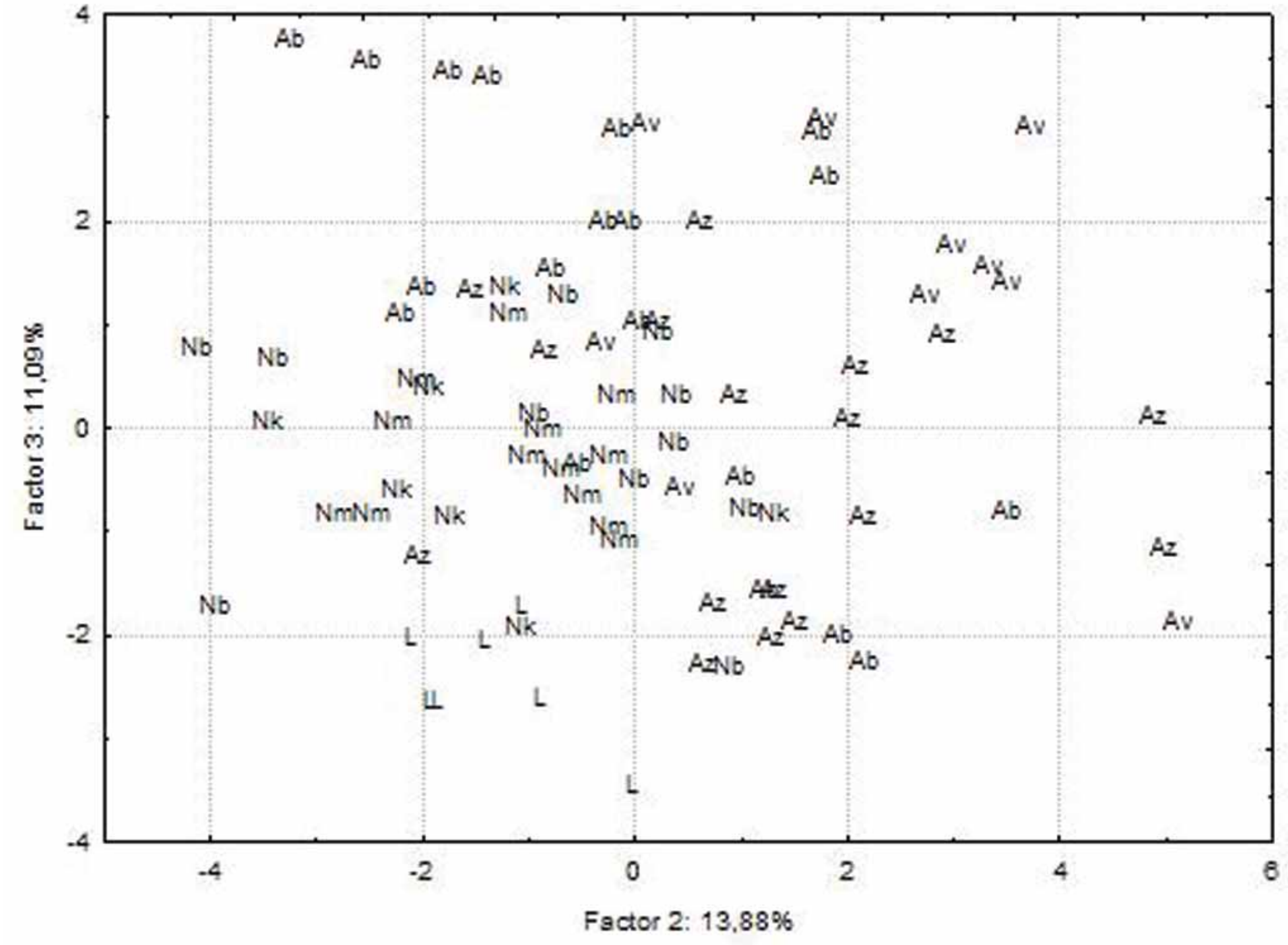

***Fig. 2**. Plot of the factor scores for factors 2 and 3 of all morphometric ratios for six investigated populations previously supposed to belong to* A. arborella*. L – Lake Lugano population; Az –* A. arborella *population from the Zrmanja River; Ab –* A. arborella *population from Lake Butoniga; Av –* A. arborella *population from the Vipava River; Nb –* A. neretvae*, sp. nova from Lakes Baćinska; Nk –* A. neretvae*, sp. nova from Lake Kuti; Nm –* A. neretvae*, sp. nova from the Mušnica River.*

ventral keel exposed for at least $^{2}/_{3}$ of the distance between the pelvic fin base and the anal aperture.

Description: D III $8^{1}/_{2}$-$9^{1}/_{2}$, A III $13^{1}/_{2}$-$15^{1}/_{2}$, P 14-17, V I 7-8, C 17, ll 40-48 scales, 1-3 located on the caudal fin. Body is medium sized (62 up to 129 mm TL; 50 up to 109 mm SL; morphometric characters are summarized in Table 2), elongated, slightly to moderately high and moderately compressed. The maximum body depth is between the pectoral and ventral fins. The head is elongated and slightly pointed. The head depth is 47-59% of the head length and 49%-73% of a maximum body depth. The mouth is quite large and slightly superior. The lower lip has a small, pointed projection, in the middle. The lateral line is complete, with the beginning located in the level of the upper third of the eye, than it is descending to the end of the ventral fins, where it reaches the lowest point. Afterwards, the lateral line is slightly rising until the caudal peduncle. Pectoral fins are ending $^{1}/_{2}$-5 scales before the beginning of the pelvic fins. Pelvic fin end is located $^{1}/_{2}$-5 scales before the anal aperture. Ventral keel is exposed for at least $^{2}/_{3}$ of the distance between the pelvic fin base and anal aperture; however, usually it is wider just before the anal aperture (up to 3 scales before). The first anal fin ray is most often located below the last dorsal fin ray or immediately after it, but its position varies. It can also be positioned below 6-10th dorsal fin ray or up to one scale behind the base of the last dorsal fin ray. The maximal size of gill rakers varies between the populations from reaching the half of the size of the gill filaments (in the population from the Mušnica River) to being almost as long as the gill filaments (in the population from Baćinska Lakes), all measured at the angle between the upper and lower limb of the first gill arch.

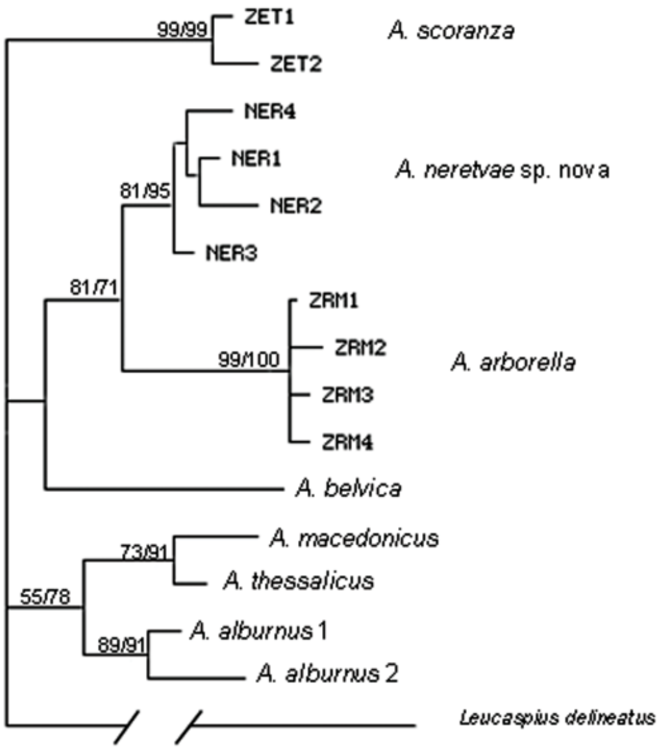

***Fig. 3**. Phylogenetic tree derived from the ML analysis of the investigated* Alburnus *specimens. Numbers at nodes represent ML and MP bootstrap values. Legend: ZET1 and ZET2 – haplotypes of* A. scoranza *from the Zeta River; NER1, NER2 and NER3 – haplotypes of* Alburnus *specimens from the Neretva River in Metković; NER4 – haplotype of the holotype of the newly described species; ZRM1, ZRM2, ZRM3 and ZRM4 – haplotypes of* A. arborella *from the Zrmanja River.*

Colouration: Dorsal part of the body and head is dark brown to black, with a brownish to dark reddish colouration below. Underneath that there is a blue line (dark blue to dark grey in preserved specimens). Ventral parts of the body are silvery (white or yellow in preserved specimens) with few very small dark spots located below the blue line and on the pectoral and ventral fins.

Comparativere marks: A. neretvae differs from *A. belvica* by the number of gill rakers (17-26 in *A. neretvae* vs. 29-32 in *A. belvica*) and the number of lateral line scales (40-48 in *A. neretvae* vs. 52-57 in *A. belvica*). *A. neretvae* can be distinguished from *A. scoranza* by the number of gill rakers (17-26 in *A. neretvae* vs. 15-20 in *A. scoranza*) and by the lateral line scales number (40-48 in *A. neretvae* vs. 46-53 in *A. scoranza*). *A. neretvae* is different from *A. albidus* by the number of branched anal fin rays ($13^{1}/_{2}$-$15^{1}/_{2}$ in *A. neretvae* vs. 11-$13^{1}/_{2}$ in *A. albidus*, Kottelat & Freyhof 2007) and the number of gill rakers (17-26 in *A. neretvae* vs. 13-18 in *A. albidus*, Kottelat & Freyhof 2007). *A. neretvae* differs from *A. arborella* by the number of gill rakers (17-26 in *A. neretvae* vs. 14-22 in *A. arborella*) and by the position of the first anal fin ray (in *A. neretvae* located below 6-10^{th} or up to one scale behind the last dorsal ray, while in *A. arborella* it is located below 4-9^{th} branched dorsal ray, only exceptionally immediately behind the last dorsal fin ray). *A. neretvae* is different from *A. alburnus* by the number of branched anal fin rays ($13^{1}/_{2}$-$15^{1}/_{2}$ in *A. neretvae* vs. 17-$20^{1}/_{2}$ in *A. alburnus*, Kottelat & Freyhof 2007), the number of lateral line scales (40-48 in *A. neretvae* vs. 48-51 in *A. alburnus*, Kottelat & Freyhof 2007) and the position of the anal fin origin (in *A. neretvae* located below 6-10^{th} branched dorsal fin ray or up to one scale behind the base of the last dorsal fin ray, while in *A. alburnus* it is located below 4-5^{th} branched dorsal fin ray, Kottelat & Freyhof 2007).

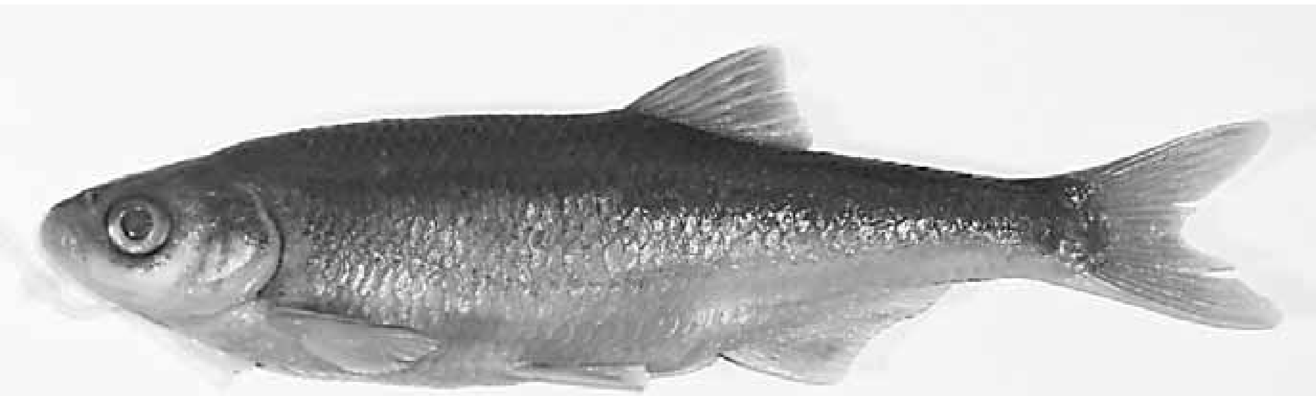

Fig. 4. Alburnus neretvae*, holotype – NMP P6V 83 139, 101.1 mm SL, the Mušnica River (Bosnia and Herzegovina).*

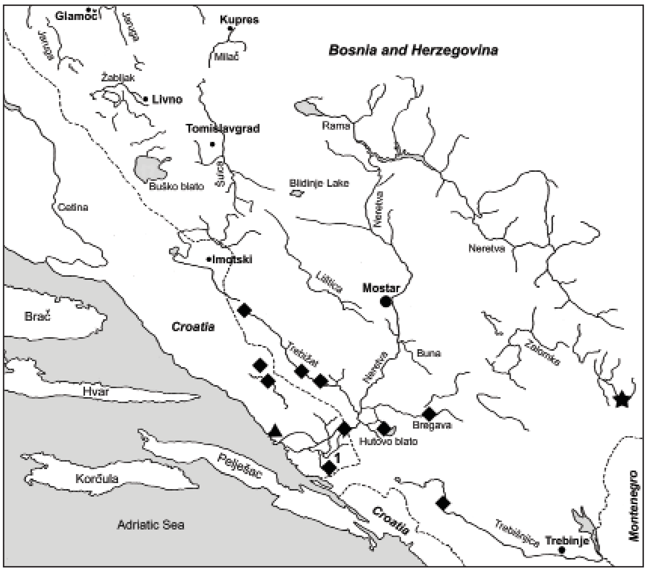

Fig. 5. *The map of the Neretva River drainage with indication of localities with the recorded presence of* A. neretvae. *★ – type locality, Gatačko polje, Mušnica River; ▲ – Baćinska Lakes; ◆ – other localities (1 – Lake Kuti).*

Distribution: Endemic to the Neretva River drainage in Croatia and Bosnia and Herzegovina. Recorded in Lakes Kuti and Baćinska Lakes, the Neretva River in Metković, the Bregava River, wetland Hutovo blato, waters of karstic fields Rastoke and Jezero near Vrgorac, in the Mušnica River (Gatačko polje), the Trebišnjica River (Popovo polje) and Tihaljina/Trebižat River system (Fig. 5). It occurs also in the Buna River (Kosorić & Vuković 1966) and in lower and middle section of the Neretva River, from the mouth of the River Buna downstream to the mouth into the sea (Kosorić & Vuković 1966, Kosorić et al. 1989, Mrakovčić et al. 1995). After formation of the systems of the artifitial reservoirs on the upper and middle part of the Neretva River, it was recorded also in the reservoir Salakovac, aproximately 20 km upstream of Mostar (Škrijelj 2002).

Etymology: Named after the Neretva River (the Croatian name), because it is endemic to the Neretva River drainage.

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