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SCARDINIUS KNEZEVICI BIANCO & KOTTELAT, 2005 AND *ALBURNUS SCORANZA* BONAPARTE, 1845: NEW SPECIES OF ICHTHYOFAUNA OF SERBIA AND THE DANUBE BASIN

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Abstract - Research into the ichthyofauna of the Vlasina Lake reservoir in south Serbia, which is part of the Danube basin, was carried out in 1993, 40 years after its formation. The results of the research reveal the presence of several species of fish belonging to the Adriatic and Aegean basin, such as *Alburnus albidus, Rutilus basak, Scardinius graecus* and *Pachychilon macedonicus*. These findings are of great importance from the aspect of conservation, because the species *Scardinius graecus* and *Alburnus albidus* are on the European list of endangered fish species. In the latest study of the Vlasina Lake reservoir ichthyofauna (70 years after its formation), the above-mentioned species were not found. However, the presence of naturalized populations of two species from the Adriatic basin were confirmed: *Scardinius knezevici* and *Alburnus scoranza.* These findings represent the first known areal expansion of these species, which are new to the ichthyofauna of Serbia, from the Adriatic into the Danube (Black Sea) basin.

Key words: New fish species, Vlasina Lake, Danube Basin Serbia

INTRODUCTION

The ichthyofauna of the Vlasina Lake reservoir (Vlasina River, Danube basin, southern Serbia) has been forming for 70 years, mostly from allochthonous species originally from the Danube, Adriatic and Aegean basin, as well as from other continents (North America and Asia).

The first report of autochthonous species of fish in the Vlasina Lake reservoir was given by Janković and Raspopović (1960), indicating the presence of populations of three species: brown trout (*Salmo trutta* Linnaeus, 1758), minnow (*Phoxinus phoxinus* Linnaeus, 1758) and brook barbel (*Barbus peloponnesius* Valenciennes, 1842). The same report provided data on the results of the first Vlasina Lake reservoir stocking. The report shows that the Ohrid trout (*Salmo letnica* Karaman, 1924) from Lake Ohrid (Adriatic basin) was introduced into the reservoir from 1953 to 1954, in a quantity of 10^6 units of fish spawn. In his work on minnow, Branković (1970) reported the first introduction of the Asian species, amur (*Ctenopharyngodon idella* Valenciennes, 1844) in a quantity of 400 units of 2+ age.

Detailed hydrobiological research of Vlasina Lake that was conducted in the period of 2001-2003 (Blaženčić, 1997) has indicated major changes in the composition and structure of the fish community. Nikčević et al. (1992) registered 11 species, of which

the autochthonous include only one unit of brook barbel.

Simonović and Nikolić (1995, 1997; Simonović, 2001) registered 16 fish species in Vlasina Lake, of which two are autochthonous for the lake itself (*Salmo trutta* and *Barbus peloponnesius*), four are autochthonous for the waters of the Danube basin in the territory of Serbia, while the others (5 species) are allochthonous and originate from: other continents, the Adriatic basin (*Alburnus albidus, Rutilus basak*) and the Aegean basin (*Pachychilon macedonicus, Scardinius graecus*).

The fish species of the Adriatic and Aegean basin that were registered in the reservoir in the period 2001-2003, have been especially focused on because of their current conservational status. According to Kottelat and Freyhof (2007), Freyhof and Brooks (2011), and IUCN (2011), *Alburnus albidus* Costa, 1838 and *Scardinius graecus* Stephanidis, 1937 have been classified as endangered species (*S. graecus* – "CR Critically Endangered"; *A. albidus* - "VU Vulnerable"). Therefore, a confirmation of the presence of these species in Vlasina Lake would be of major conservational significance.

In the work of Simonović and Nikolić (1995, 1997), the authors say that the above-mentioned species, except from *S. graecus,* arrived to the reservoir during the 1989-1991 transport of "plašica" (*Alburnus albidus*, the name used then) from Lake Ohrid with the intention of providing food for the previously introduced Ohrid trout. The origin of *S. graecus* is described by the authors as "hard to explain", considering its areal distribution.

The aim of this work was to establish the exact taxonomy, origin and population status of the gathered specimens of fish originating from the Adriatic and Aegean basin, during 2003 and 2011 fishing from Lake Vlasina reservoir.

MATERIALS AND METHODS

The research into the ichthyofauna of the Vlasina Lake reservoir (Fig. 1) was performed with the purpose of producing a program of sustainable use of the fish stocks of the protected area during the months of July and August 2003 and 2011.

Experimental fishing was carried out with a fishing net with a 10 to 140 m diameter, total length 1000 m at a depth of 1.5-4 m. The fishing nets were active in the period from 18:00 h to 08:00 h the next day, for 7 days. The catch was examined every morning. Electrofishing was being done at 0.2 ha near the confluences of tributaries.

The purpose of the research was in the domain of sustainable use of fish stocks, so the majority of the units were routinely determined on the field and returned to the water or handed out to fish keepers if dead. This is also the reason why, for a detailed taxonomic analysis of (seemingly unknown) taxa, only 10 units of *Alburnus* sp. (all from 2011) and 5 units of *Scardinius* sp. (four from 2003 and one from 2011) were preserved.

The identification of fish suspected of originating from the Adriatic and Aegean basins was carried out primarily on the basis of characteristics given by Kottelat and Freyhof (2007) and Bianco and Kottelat (2005), but also by using: Banarescu (1964), Ladiges and Vogt (1965); Vuković (1977), Maitland (1977) and Simonović (2001).

Quantitative representation of species of the Adriatic and Aegean basins was presented as the catch per unit of effort (CPUE) for the period of research in question.

RESULTS

Data from the literature and the records of users of the fishing area on the stocking of the Vlasina Lake reservoir are presented in Table 1. Analysis of this data can provide a relative reconstruction of the origin of the fish that populate this reservoir nowadays.

Analysis of the data from the above-mentioned table shows that the origin of Adriatic and Aegean

Table 1. Known data on the reintroduction of fish into the Vlasina Lake reservoir from 1954-2010.

Introduction -period	Target fish species Accidental	Quantity	Basin-area	Primary habitat	References
1953-1954	*Salmo letnica* *Oncorhynchus mykiss*	10^6 units of fish spawn	Adriatic	Ohrid lake	Janković & Raspopović, (1960)
1968, 1971, 1991	*Ctenopharyngodon idella*	-	Asia	Danube, fishponds in Vojvodina	Janković (1994)
	Tinca Tinca Cyprinus carpio Carassius carassius *Perca fluviatilis* *Abramis brama*		Danube		Simonović & Nikolić (1997)
	Carassius auratus *Lepomis gibbosus* *Ictalurus nebulosus*	-	Asia North America		
1971, 1977	*Salmo sp.?* *Leuciscus sp.?* *Alburnus sp ?*	-	Adriatic Aegean	Dojran lake, Ohrid lake Skadar lake	Report of the users of Fishing area
1973	*Thymallus thymallus*	10^4	Danube –Black Sea	Bohinj lake, Slovenia.	Simonović & Nikolić (1997)
1989	*Anguilla anguilla*	200 pieces	Adriatic	Ohrid lake	
1989-1991	*Alburnus albidus*	10^6	Adriatic	Ohrid lake	Simonović & Nikolić (1997)
1993-1997 1998-2010	*Salmo trutta* (st) *Cyprinus carpio* (cc) *Abramis brama* (ab)	$2x10^6$ pieces 1+ i 2+ (st). 10^3 pieces 2+ (cc, ab)	Danube –Black Sea	Basin of Vlasina (st) (Danube, Tisa, fish pond-Mošorin (cc, ab)	Simić & Simić (2003, 2007)

Table 2. Fish species of the Adriatic and Aegean basin and their quantitative presence in the Vlasina Lake during the 1993, 2003 and 2011 research.

Finding year	Fish of the Adriatic / Aegean basin	Literature	Total Number of Units CPUE
1993	*Pachychilon macedonicus* (Staindachner, 1892)	Simonović & Nikolić (1995, 1997)	1
	Rutilus basak (Heckel, 1843)		1
	Scardinius graecus (Stephanidis, 1837)*		1
	Alburnus albidus (Costa, 1838)		4
2003	*Scardinius knezevici* Bianco & Kottelat 2005	Unpublished data	2
	Alburnus scoranza Hechel & Kner,1858		34
2011	*Scardinius knezevici*		7
	Alburnus scoranza		18

*original nomenclature has been used, as given in the work (Simonović & Nikolić 2005, 2007).

fish species is mostly from the territory of FYROM (Lake Ohrid, Dojran Lake) and Montenegro (Lake Skadar). The data also show that the target species for reintroduction were Ohrid trout (*Salmo letnica* Karaman, 1924) and later (probably in the period 1989-1991), bleak, under its previous name *Alburnus albi-*

Table 3. Comparative review of key characteristics for the identification of the genus Alburnus in Europe: Atlantic, Western Mediterranean and Adriatic basins (Kottelat & Freyhof, 2007) and specimens of Alburnus from the Vlasina Lake reservoir.

characteristics for distinguishing (Kottelat & Freyhof 2007) / species	*A. scoranza*	*A. alburnus*	*A. albidus*	*A. arborella*	Vlasina Lake reservoir *A. scoranza*
Lateral line scales	47-53+2	45-48+3	39-45+3	40-47+3	47-51
Branched anal rays	13-14½	17-20½	11-13½	13-16½	14½
Gill rakers	17-22	16-22	13-18	16-22	18-20
Ventral keel exposed for at least 2/3 of distance from anus to pelvic base	+	-	-		+
Anal origin below or immediately behind base of last branched dorsal rays	+	-	-	-	+
Anal origin below branched dorsal rays 4-8				+	-
Anal origin below or behind base of last dorsal ray			+	-	-
Very contrasted dark midlateral stripe when preserved	-	-	-	+	-

Table 4. Comparative review of key characteristics for the identification of genus Scardinius in Europe, according to Kottelat & Freyhof (2007) (a part of Italy, Greece, Macedonia, Serbia) and the units of roach from the Vlasina Lake reservoir.

characteristics for distinguishing (Kottelat & Freyhof 2007) species	*S. knezevici*	*S. scardafa*	*S. graecus*	Vlasina Lake reservoir n=5 *S. knezevici*
Lateral line scales	34-37+2-3	37-40	38-43+2-3	35- 37 +3
Branched anal rays	9½	9-10½	11½	9½
Articulation of lower jaw below or behind anterior margin of eye	+	-	+	+
Head length			24-35% SL	24% SL
Gill rakers	10-12	12-14	17-24	10-14
Body depth		31-36%	20-30% SL	33% SL
Color of fins	grayish	dark grey		grayish
Dorsal profile of head conspicuously concave, tip of snout about level of middle of eye			+	tip of the snout above the level eye -

dus alborella (de Filippi, 1841) or "plašica" (popular local name) from Lake Ohrid, and earlier (uncertain source) *Alburnus alburnus alborella* (de Filippi, 1844) or "ukljeva" (local name) from Lake Skadar.

During the ichthyologic research of the Vlasina Lake reservoir in 1993, 2003 and 2011, the presence of fish species from the Adriatic and Aegean basins was recorded in qualitative and quantitative composition, shown in Table 2.

The results of the analysis of morphomeristic and phenotype characteristics according to Kotellat and Freyhof (2007), which are crucial for the differentia-

Table 4a. Comparative review of meristic characteristics of *S. knezevici* from lakes Skadar and Ohrid, according to Bianco & Kottelat (2005) and specimens of roach from the Vlasina Lake reservoir.

	S. knezevici L. Skadar (MNE) L. Ohrid (FYROM)	*S. knezevici* Vlasina Lake (SRB)
SL (mm)	35-190	112-118
Scales		
Lateral line	36-38	35-37
Above lateral line	7½	7½
Below lateral line	3½	3½
Circumpedicular	14	14
Gil rakers		
Epibranchial	(2-3)3	3
Ceratobranchial	(8-9)9	(8-11-10)
Total	(11-12)12	10-14
Branched rays		
Dorsal fin	8	8
Anal fin	9	9
Pelvic fin	8	8

tion of species within genera from the same or different ecoregions or basin areas, applied to species of the Adriatic and Aegean basins found in Vlasina Lake reservoir, are shown in Tables 3, 4 and 4a.

The analysis of key characteristics for the differentiation of species of the genus *Alburnus* according to Kottelat and Freyhof (2007) and the specimens of *Alburnus* from the Vlasina Lake reservoir was conducted in the same way as in the previous case, and the results are shown in Table 3.

The results showing the differences between the characteristics of taxa *Scardinius*, described in the area of Italy, Greece, FYROM and Montenegro, and samples of Vlasina Lake reservoir are presented in Table 4.

The presented results of the comparative analysis of morphomeristic and phenotypic characteristics for the differentiation of the species of genera *Alburnus* and *Scardinius* indicate that specimens of the genus *Scardinius* captured in the Vlasina Lake reservoir during 2003 and 2011 belong to the species *Scardinius knezevici* Bianco and Kottelat, 2005 (Figs. 2 and 2a), while species of the genus Alburnus belong to *Alburnus scoranza* Bonaparte, 1845 (Fig. 3). Bianco and Kottelat (2005) have described *S. knezevici* as a new species for Lake Skadar. In our subsequent analysis, we have compared the meristic characteristics of *S. knezevici* from the Skadar and Ohrid lakes with the meristic characteristics of specimens of roach from the Vlasina Lake reservoir. Results are shown in Table 4a.

DISCUSSION

The Vlasina Lake reservoir was formed on the upper reach of river Vlasina (belonging to the Danube basin), by flooding a large mountain peat bog. Since its formation, it has been a place of active anthropogenic introduction of fish species with the purpose of increasing its fishing attractiveness. Interestingly, since the first stock, no concern has been given to the origin of the introduced species and the preservation of autochthonous populations of fish, which, according to Janković and Raspopović (1960), consisted of three species: *Salmo trutta, Barbus peloponnesius* and *Phoxinus phoxinus*. The geographical location (south Serbia) and ecological status (coldwater oligotrophic accumulation) of the new reservoir probably contributed to the decision to perform the first

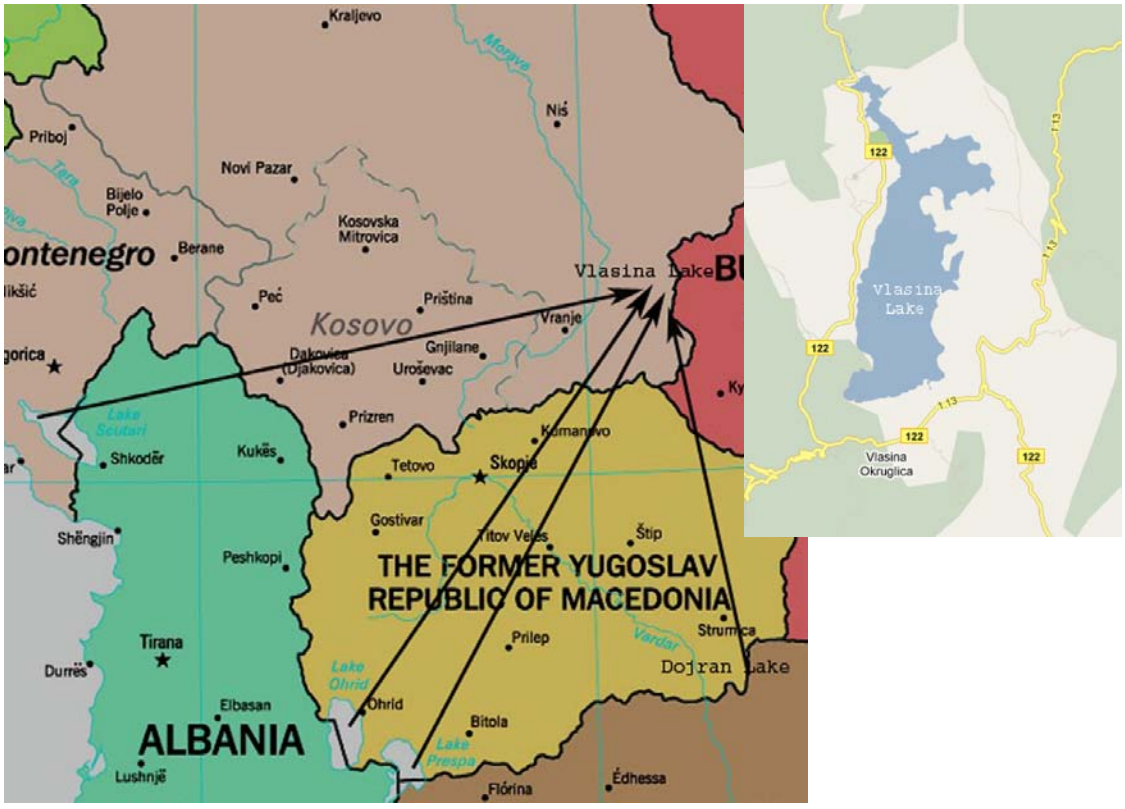

Fig. 1. Courses of anthropogenic spreading fish species of the Adriatic and the Aegean basin in the Danube Basin - Vlasina Lake reservoir.
Abiotic parameters and trophic level of Vlasina Lake reservoir: coordinates 42°42′N 22°20′E, max. length - 10.5 km, max. width - 3.5 km, surface area - 16km^2, average depth - 10.3 m, max. depth - 34 m, water temp. (°C) – min- 0.5; max- 18.8, trophic level – oligotrophic (Blaženčić, 1997).

and subsequent stockings with species from the territory of Macedonia and Montenegro (Blaženčić, 1997), namely the Ohrid-Drim-Skadar hydrosystem, belonging to the Adriatic basin (Fig. 1). The first reintroduction of allochthonous species was performed during 1953-1954, when the Ohrid trout (*Salmo letnica*) was introduced from Lake Ohrid. According to the report of the authors of this project (Janković and Raspopović, 1960), a successful acclimatization has been recorded, along with an even better growth of this species in the Vlasina Lake reservoir than in its original habitat. However, in later research (Nikčević et al., 1992; Janković 1994; Simonović and Nikolić 1995, 1997; Simić and Simić, 2003, 2007, 2011), the specimens of trout, which were found individually and rarely, were closest to *Salmo trutta* from the Danube basin by their morphology and meristics, according to the systematization of Kottelat and Freyhof (2007), as well as the views of Simonović et al. (2007). This fact is justified by at least two phenomena Firstly, a hybridization between the autochthonous and the introduced trout probably occured in the reservoir. Secondly, in the succeeding period, stockings were performed exclusively with autochthonous trout from the Vlasina River (Black Sea basin) (Simić and Simić, 2003), so the Danube (Black Sea) basin form probably prevailed in the reservoir. A genetic analysis of the trout population planned for the upcoming period will most likely provide more reliable information on the origin and genetic status of the trout in the Vlasina Lake reservoir.

Unlike the trout, the presence of fish from the Cyprinidae family in the Vlasina Lake reservoir,

Fig. 2. Specimen of roach (*Scardinius knezevici*) caught in 2003 (Vlasina Lake reservoir).

Fig. 2a. Specimen of roach (*Scardinius knezevici*) caught in 2011(Vlasina Lake reservoir).

Fig. 3. Specimen of bleak (*Alburnus scoranza*) caught in 2003 (Vlasina Lake reservoir).

which originate from the Adriatic basin, has been clearly confirmed by the analysis of morphomeristic parameters, according to Kottelat and Freyhof (2007), in the form of naturalized populations *Alburnus scoranza* and *Scardinius knezevici.* On the other hand, the findings of the species *Alburnus albidus*, *Rutilus basak* (Adriatic basin) and *Scardinius graecus* and *Pachychilon macedonicus* (Aegean basin), described by Simonović and Nikolić (1997) and Simonović (2001), have not been confirmed in this research. The specimens of all species mentioned above from the period of 1992-1993 (a period of deep economic crisis) have unfortunately been lost. Therefore, no check or sample comparison could be done in this work.

The species *A. scoranza* was probably introduced into the Vlasina Lake reservoir from Lake Ohrid in 1989, under the name used then, *A. albidus* (plašica), with the purpose of providing natural food for the Ohrid trout (*Salmo letnica*) introduced earlier. During the study of the reservoir in 1992-1993, Simonović and Nikolić (1995, 1977) and Simonović (2001) quote findings of 4 units of "plašica" determined as *A. albidus* (Costa, 1838) and quote the following characteristics: D 3+7-9; A 3+10-17; Llat 45-56; sLlat 8-11; iLlat 3-5; vertebrae 38-42; DF 5,2-2,5; HL 17,3-34 SL; between the ventral fin and the anus there is a narrow ventral ridge, which is bare and without scales. Based on the analysis of the units of roach captured during 2003 and 2011, using the characteristics given by Kottelat and Freyhof (2007), the presence of *A. scoranza* has been confirmed, while *A. albidus* has not been recorded or confirmed according to the above mentioned characteristics (Simonović, 2001). According to our results, *A. scoranza* from the Vlasina Lake reservoir is different from *A. albidus* primarily by the number of scales along the lateral line, the number of branched rays in the anal fin, the number of gill rakers and anal origin below or behind the base of the last dorsal ray (Table 3). Compared to *A. arborella*, which is the most commonly used name of this species from the Drim basin or Ohrid-Drim-Skadar basin (*A. alburnus alborella* Dimovski and Grupče (1971, 1972), *A. arborella* (Šorić, 2001) (the accepted valid nomenclature is *A. arborella* Kottelat and Bianco, 2005), it differs in a greater number of scales along the lateral line and ventral keel scales that are exposed for at least 2/3 of the distance from the anus to the pelvic base; the anal origin is below or immediately behind the base of the last branched dorsal rays (*A. scoranza*), unlike the origin below the base of the branched dorsal rays 4-8 from *A. arborella* (Kottelat and Freyhof, 2007) (Table 3).

Besides bleak ("plašica"), during their research of the Vlasina Lake reservoir, Simonović and Nikolić (1997) and Simonović (2001) describe finding 1 specimen (male) of rudd, determined as the Greek roach *Scardinius graecus* (Stefanidis, 1837). The authors give the following characteristics: D 2+9; A 3+10; P 1+14; V 1+8; Llat 35-36; sLlat 6; iLlat 5; gill rakers 15; DF 5.2-2.5. By analysis of the roaches captured during 2003 and 2011 in the Vlasina Lake reservoir, certain differences were found compared to the specimen from 1993. These include branched anal rays 9½ and gill rakers 10-14 (Tables 4 and 4a). The captured specimens of roach from the Vlasina Lake reservoir mostly match the characteristics described for specimens from lakes Skadar and Ohrid given by Bianco and Kottelat (2005), primarily in the number of scales on the lateral line, above the lateral line, below the lateral line, circumpedicular and branched rays. The only difference is in the number of gill rakers.

On the described specimens of holotype and paratype *S. knezevici* from lakes Skadar and Ohrid, the total number of gill rakers is 12 (11-12), while on the specimens from Vlasina Lake, the number of gill rakers varies from 10 to 14. However, taking into account the variability of the morphology of *S. knezevici* from earlier descriptions (Ivanović, 1973; Knežević 1981; Vuković and Ivanović, 1971) from Lake Skadar and from lakes Skadar and Ohrid (Grupče and Dimovski, 1984), the number of gill rakers of roach from Lake Ohrid can vary within the range of 8-14, which is within the results obtained for the specimens of roach in the Vlasina Lake reservoir. This fact may imply that the population of *S. knezevici* in the Vlasina Lake reservoir originates from Lake Ohrid, but also that this character is conditioned by the ecological characteristics of the habitat (the trophic level, etc.)

A significant indicator that the naturalized populations of *A. scoranza* and *S. knezevici* originate from the hydrologic system of Ohrid-Drim-Skadar is seen through new information on the taxonomy and distribution of species of the genera *Alburnus* and *Scardinius* from the Adriatic and Aegean basins, which were obtained on the basis of morphomeristic, phenotype and genetic characteristics (Marić and Kažić, 1990; Rakaj and Flloko, 1995; Iliadou et al., 1996; Iliadou and Anderson 1998; Bianco et al., 2001, 2004, 2005; Bianco, 2004a; Ketmaier et al., 2003, 2004, 2009, Finamore et al., 2004; Kottelat and Freyhof 2007; Talevski et al., 2009; Buj et al., 2010). From the works of the above-mentioned authors, it is clear that the hydrological system of Ohrid-Drim-Skadar, which spreads through the territory of FYROM, Montenegro, Albania and Kosovo, is populated by both *A. scoranza* and *S. knezevici*, unlike other species of these genera which are widespread outside this hydrological system, such as *A. albidus* (Italy), *A. arborella* (Italy, Slovenia, Bosnia and Herzegovina, Croatia), *S. scardafa* (Italy), *S. graecus* (Greece, Lake Yliki, Parlimni).

The finding of populations of *A. scoranza* and *S. knezevici* in the Vlasina Lake reservoir (south Serbia) represents an area expansion (the only one known so far) of these species from the Adriatic into the Danube (Black Sea) basin.

The presence of species from the Adriatic and Aegean basins that were recorded in 1992-1993 in the Vlasina Lake reservoir, such as *Alburnus albidus, Scardinius graecus, Pachychilon macedonicus* (Steindachner, 1892) and *Rutilus basak* (Heckel, 1843), has not been confirmed, which implies that either the identification was unclear in that period because of individual findings, or that they did not acclimatize in the new environment after the accidental introduction, which is less likely to be true.

From the conservation aspect, the fact that there has been no record of a population (or individual specimens) of species of the genera *Alburnus* and *Scardinius* from the Danube basin, such as *Scardinius erythrophthalmus* and *Alburnus alburnus* in the latest study of the Vlasina Lake reservoir (2011) may be important, indicating that hybridization with the species introduced from the Adriatic basin, *A. scoranza* and *S. knezevici,* has not occured so far. Therefore, new populations can serve as a source of genetic variability to the autochthonous

populations from the Adriatic basin. This can be especially important for the populations of *S. knezevici*, whose endangerment status in Lake Ohrid has been declared to be Vulnerable (VU D2*) (Kottelat and Freyhof , 2007), but the endangerment status on a global level is assessed to be Least Concern (LC) (IUCN, 2011).

However, further expansion (intentional or unintentional) of these species from the Vlasina Lake reservoir into the waters of the Danube basin could be negative for autochthonous populations of *S. erythrophthalmus* and *A. alburnus* in the Danube. It is advisable to prevent the introduction of the Danube species of roach and bleak into the reservoir (since they have not been introduced so far) and to explore the genetic structure of the introduced populations, especially the population of *S. knezevici.* This species was introduced accidentally, during the intentional introduction of bleak (plašica); therefore, it is possible that the number of founders was small, which can negatively reflect on the genetic structure of the population.

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