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RESEARCH PAPER OPEN ACCESS

Occurrence of alien spirlin (*Alburnoides* sp.) in the Neretva river basin

Jasna Vukić[1,*], Kristýna Eliášová[2], Dario Marić[3] and Radek Šanda[2]

[1] Department of Ecology, Faculty of Science, Charles University, Viničná 7, Prague 2, 12844, Czech Republic
[2] Department of Zoology, National Museum, Václavské nám. 68, Prague 1, 115 79, Czech Republic
[3] Dobrič b.b., Široki Brijeg 88220, Bosnia and Herzegovina

Abstract – Northern Mediterranean region is characterised by an exceptional richness of the freshwater ichthyofauna. Many fish species of this region are endemic to a single or a few river basins. This is also the case of the Neretva river basin (Adriatic Sea slope), where 17 out of 34 native species are endemic solely to this river basin. However, these unique Mediterranean freshwater ecosystems are fragile and are susceptible to human-induced changes, including introduction of alien fish species. We report here a finding of the 32nd alien fish species in the Neretva river basin, spirlin *Alburnoides* sp., which was found at two localities in Bosnia and Herzegovina. The spirlin species was identified by molecular means as so far unnamed species with the native range in the Sava river basin (Danube river basin, Black Sea slope). Based on the comparison of *cytochrome b* sequences, the introduced population originated most probably from nearby rivers of the Danube basin in Bosnia and Herzegovina (Vrbas and Bosna river basins). Such a high number of alien fish species reported in a single river basin is alarming and pointing to a necessity of raising public awareness, especially among local fishermen.

Keywords: spirlin / *Alburnoides* / alien species / Neretva river / Leuciscidae

Résumé – Présence d'un spirlin non indigène (*Alburnoides* sp.) dans le bassin de la Neretva. La région de la Méditerranée septentrionale se caractérise par une richesse exceptionnelle de l'ichtyofaune d'eau douce. De nombreuses espèces de poissons de cette région sont endémiques à un ou plusieurs bassins hydrographiques. C'est également le cas du bassin de la Neretva (versant de la mer Adriatique), où 17 des 34 espèces indigènes sont endémiques uniquement à ce bassin. Cependant, ces écosystèmes d'eau douce méditerranéens uniques sont fragiles et sensibles aux changements induits par l'homme, y compris l'introduction d'espèces de poissons non indigène. Nous signalons ici une observation de la 32éme espèce de poisson non indigène dans le bassin de la Neretva, un spirlin *Alburnoides* sp. qui a été trouvé dans deux localités en Bosnie-Herzégovine. L'espèce de spirlin a été identifiée par des moyens moléculaires comme étant une espèce jusqu'à présent sans nom avec son aire de répartition indigène dans le bassin de la Sava (bassin du Danube, versant de la mer Noire). D'après la comparaison des séquences de cytochrome b, la population introduite provenait très probablement des rivières du bassin du Danube en Bosnie-Herzégovine (bassins des rivières Vrbas et Bosna). Un nombre aussi élevé d'espèces de poissons exotiques signalées dans un seul bassin hydrographique est alarmant et souligne la nécessité de sensibiliser le public, en particulier les pêcheurs locaux.

Mots-clés: spirlin / *Alburnoides* / espèce non-indigène / rivière Neretva / Leuciscidae

1 Introduction

Mediterranean rivers host considerable part of the European freshwater fish species. Majority of the freshwater fishes from this area are endemics, many of them occurring only in a few or even a single river basin (Kottelat and Freyhof, 2007). However, at the same time, the Mediterranean rivers are strongly negatively affected by human activities, including both intentional and unintentional introductions of alien fish species (Marr *et al.*, 2013).

The Neretva river basin (Adriatic Sea slope, Bosnia and Herzegovina and Croatia), including the endorheic karstic field drainages connected only underground with the main river, is

*Corresponding author: jvukic@seznam.cz

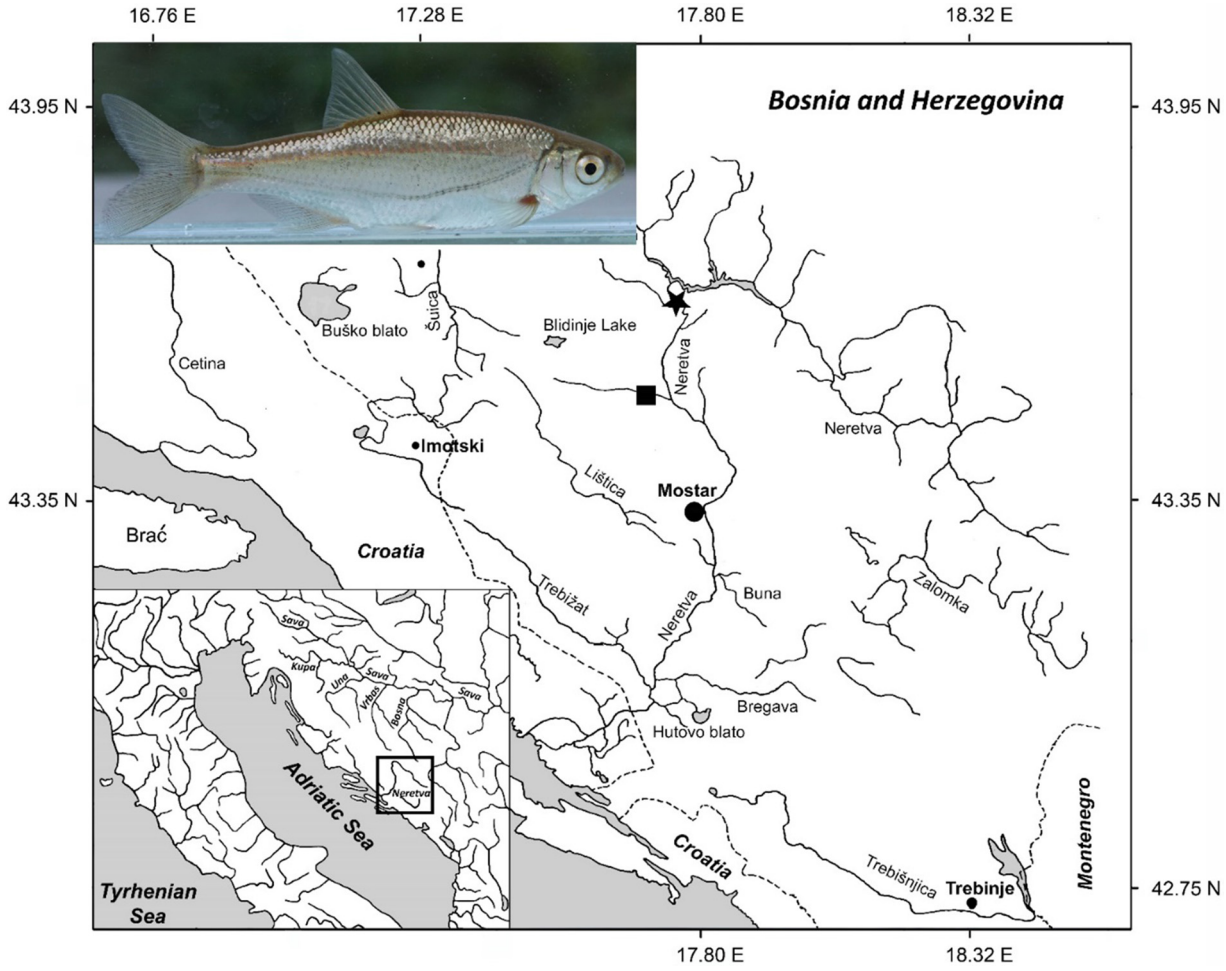

Fig. 1. Map of the Neretva river basin. Localities with *Alburnoides* sp. indicated: Neretva river in Jablanica (★) and the Drežnica river (tributary of the Neretva river) in Donja Drežnica (■). Upper left corner: a photo of alive specimen of *Alburnoides* sp. from the Drežnica river.

characterised by a high degree of endemism of freshwater fishes. It is inhabited by 34 native freshwater species (Tab. S1), 17 of which are endemic solely to this basin and some of them were described only recently (Bogutskaya and Zupančič, 2003; Kovačić, 2005; Kottelat and Freyhof, 2007; Buj *et al.*, 2010, 2014; Bogutskaya *et al.*, 2012; Glamuzina *et al.*, 2013; Tutman *et al.*, 2017a). Unfortunately, also alien species have been recorded from this river basin, and their number is as high as 31 (Tab. S2) (Glamuzina *et al.*, 2013, 2017; Dulčić *et al.*, 2017; Tutman *et al.*, 2017b; Šukalo *et al.*, 2018). The majority of the introduced species are considered to have a strong negative impact on the native fishes (Glamuzina *et al.*, 2017). Moreover, their number still increases.

The aim of this work is to provide the information on a new alien species record from the Neretva river basin, spirlin *Alburnoides* sp. (Leuciscidae *sensu* Schönhuth *et al.*, 2018), and its probable origin, based on a molecular identification.

2 Materials and methods

Several specimens of *Alburnoides* were obtained on 4–5 October 2016, from two localities within the Neretva river basin: the Neretva river in Jablanica (N 43°39′55″ E 17°45′47″) and the Drežnica river (a tributary of the Neretva river) in Donja Drežnica (N 43°31′31″ E 17°42′51″), Bosnia and Herzegovina (Fig. 1). All specimens were collected by a local sport fisherman, either by a small lift net or by fly fishing. The specimens were sacrificed by over-anaesthetisation by quinaldine. A piece of fin tissue was stored in 96% ethanol for subsequent molecular analyses. The voucher specimens were preserved in 4% solution of formaldehyde immediately after the collection, and later transferred to 70% ethanol and stored in the ichthyological collection of the National Museum in Prague (collection numbers NMP P6V 144776–144793). Since the taxonomy of *Alburnoides* in Europe, and especially in the Balkans, is still unresolved, *i.e.* the genus includes cryptic species (Stierandová *et al.*, 2016), and morphological data enabling identification are missing for many species/populations, we used molecular data for identification.

The DNA extraction, PCR reactions, amplification protocol and PCR product purification follow Šanda *et al.* (2008a). Sequencing was carried out by the Macrogen Service Centre Europe (Amsterdam, Netherlands) using the internal primers from Stierandová *et al.* (2016).

The sequences were aligned manually and revised in Geneious R9.1. The final alignment included 1140 base pairs of *cytochrome b*. Previously published sequences of

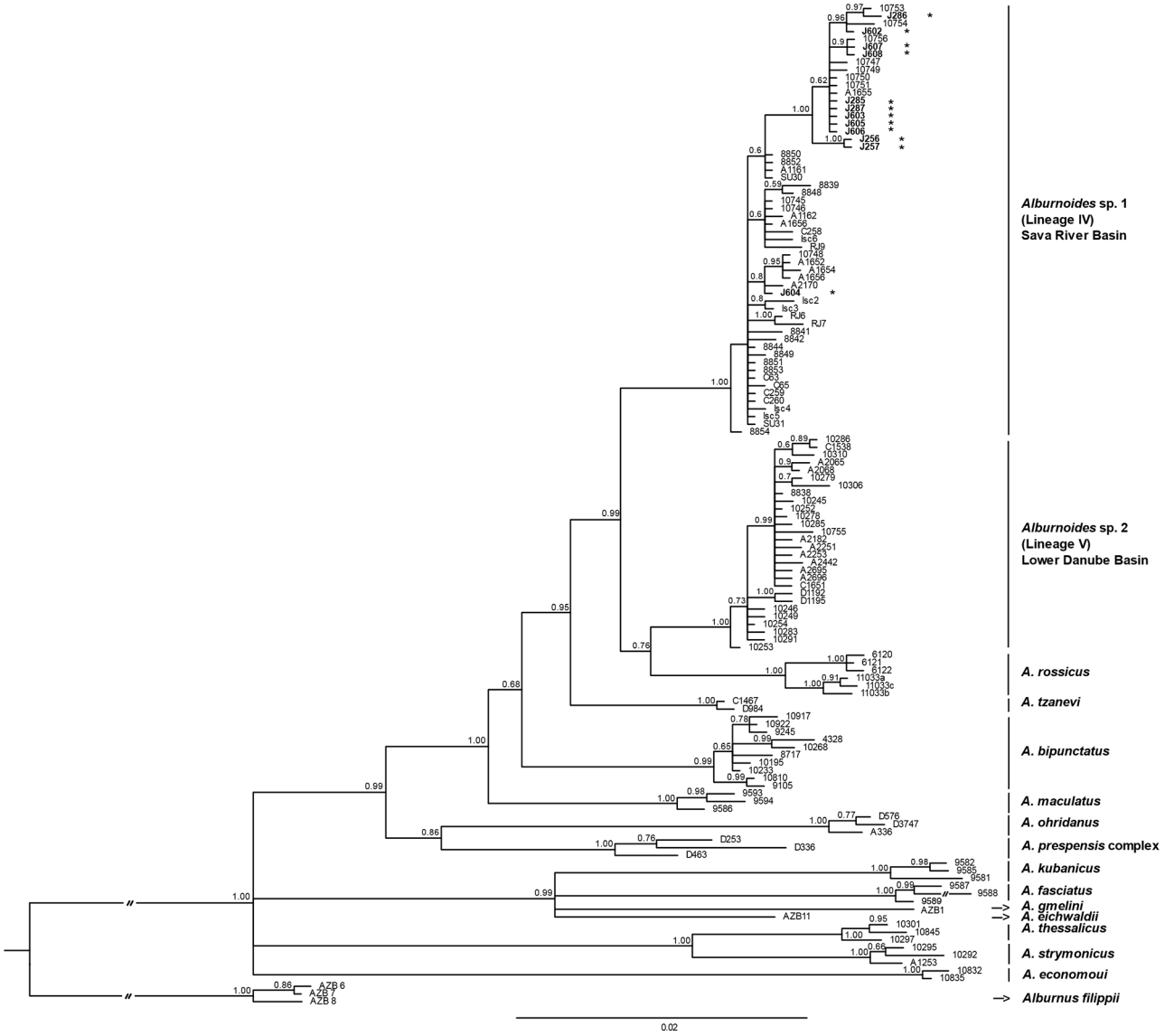

Fig. 2. Phylogenetic tree of *cytochrome b* sequences of *Alburnoides* spp. based on Bayesian inference method. Samples from the Neretva river basin are indicated by asterisk. Numbers on the branches indicate the posterior probability support. Lineages named according to Stierandová *et al.* (2016).

cytochrome b of the available European species/clades of *Alburnoides* (Stierandová *et al.*, 2016) were included in the analyses. *Alburnus filippii* was used as outgroup. New sequences were deposited in GenBank under the accession numbers MH656906–MH656910. The details about the samples used for the molecular data analyses are included in Table S3.

The best fitting model of nucleotide substitution was estimated using JModelTest 0.1.1 (Posada, 2008). The GTR+G model was selected using Akaike information criterion. A Bayesian analysis was conducted using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). Two runs, each consisting of four Monte Carlo Markov Chains, were run simultaneously for 10 000 000 generations with sampling trees every 100 generations. The first 25% of trees were discarded as burn-in and the remaining trees were used to construct a 50% majority-rule consensus tree. The posterior probabilities indicate the branch supports in the final tree.

A detailed reconstruction of relationships of the haplotypes of *Alburnoides* population from the Neretva river basin and other most closely related populations was performed by a statistical parsimony method under a 95% connection limit, using TCS v. 1.21 (Clement *et al.*, 2000).

3 Results

The size of the 18 collected specimens ranged from 26 mm to 91 mm standard length. Based on the molecular analyses of *cytochrome b* gene of 12 specimens (Fig. 2), the population from the Neretva river basin belongs to *Alburnoides* sp. 1 *sensu* Stierandová *et al.* (2016) inhabiting the Sava river basin (Danube river basin, Black Sea slope). Some of the observed

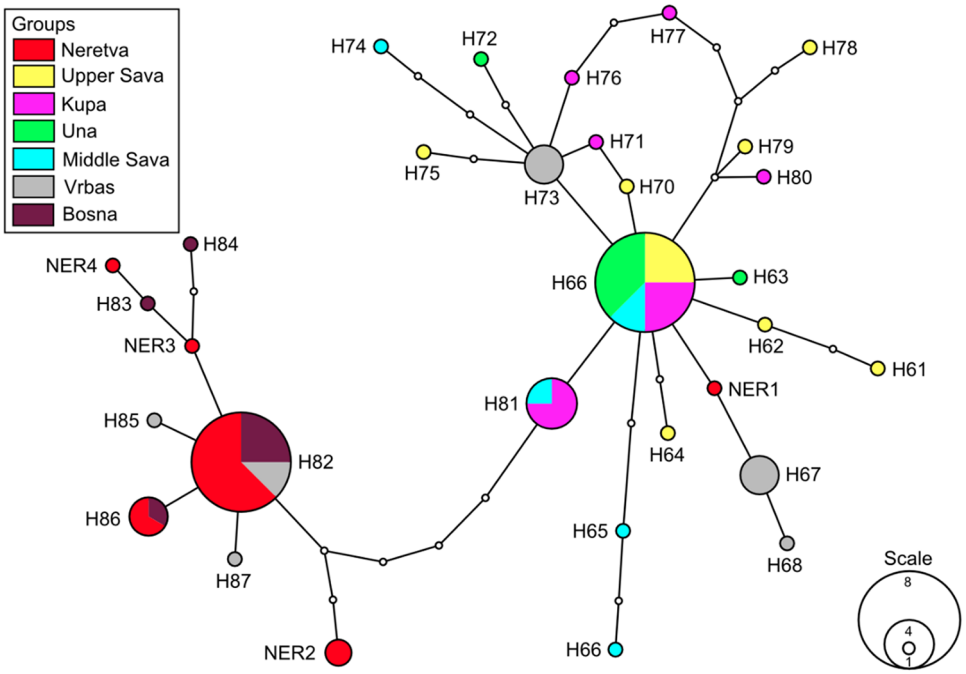

Fig. 3. Haplotype network of *cytochrome b* sequences of *Alburnoides* sp. 1 *sensu* Stierandová *et al.* (2016) constructed by statistical parsimony method under a 95% connection limit. Haplotype codes correspond to information on samples in Table S3.

haplotypes are identical to already published ones in Stierandová *et al.* (2016), several are new (NER1-4, Fig. 3), but all of them are either identical to (including the most frequent one, H82) or greatly similar to the ones belonging to the specimens originating from the Vrbas and the Bosna river basins (Fig. 3); both rivers are tributaries of the Sava river (see Fig. 1).

4 Discussion

The knowledge on the diversity of spirlin (*Alburnoides*) in Europe was updated only very recently, mostly owning to analyses of DNA sequence data (Geiger *et al.*, 2014; Stierandová *et al.*, 2016). One decade ago only three species of spirlin were recognised (Kottelat and Freyhof, 2007). Later, three new species were described, two of which from Albania (Bogutskaya *et al.*, 2010) and one from Greece (Barbieri *et al.*, 2017). However, the molecular data revealed a presence of altogether 17 evolutionary lineages of *Alburnoides* in Europe (Stierandová *et al.*, 2016). Eleven of these were possible to assign to the previously described taxa (Levin *et al.*, 2016; Stierandová *et al.*, 2016; Barbieri *et al.*, 2017), two are considered to represent yet undescribed species, while the *Alburnoides prespensis* complex requires further taxonomic clarification (Stierandová *et al.*, 2016).

No *Alburnoides* species has been known from the Neretva river basin (Glamuzina *et al.*, 2013). Several species of the genus occur in adjacent regions. In the Ohrid-Drin-Skadar basin and some other Albanian rivers, *A. ohridanus* is present (Stierandová *et al.*, 2016). Further to the south, the Albanian basins host *A. prespensis* complex (*A. prespensis*, *A. fangfangae* and *A. devolli*) (Kottelat and Freyhof, 2007; Bogutskaya *et al.*, 2010; Stierandová *et al.*, 2016). Geographically very close Danubian basin rivers from Bosnia and Herzegovina and Croatia, all tributaries of the Sava river, are inhabited by *Alburnoides* sp. 1 *sensu* Stierandová *et al.* (2016), whereas Danubian rivers located more easterly (Serbia, Bulgaria and Romania) are inhabited by *Alburnoides* sp. 2 *sensu* Stierandová *et al.* (2016).

We used a molecular approach to identify the newly recorded *Alburnoides* population from the Neretva river. Comprehensive comparative dataset is available for the mitochondrial marker *cytochrome b* for the European *Alburnoides* (Stierandová *et al.*, 2016; Barbieri *et al.*, 2017). Our analysis identified the population from the Neretva river as *Alburnoides* sp. 1 (Fig. 2). This still undescribed species inhabits the Sava river basin, which belongs to the Danube basin. It was reported, based on molecular data, from Slovenia, Croatia, Bosnia and Herzegovina and Serbia (Stierandová *et al.*, 2016).

The ichthyofauna of the Neretva river basin is very specific, comprising many species endemic only to this basin. Only two species are considered to be shared with the adjacent rivers belonging to the Danube river basin. Molecular data provided an evidence on the conspecificity of *Phoxinus* from the Neretva river and some karstic basins connected underground to it, with the populations from the Danubian rivers (Palandačić *et al.*, 2015). The other case is *Cottus gobio*; however, the relationships of populations of *Cottus* from the Neretva River and the Danube basin are awaiting clarification. Both *Phoxinus* and *Cottus* are considered native to the Neretva river basin (Karaman, 1928; Glamuzina *et al.*, 2013) and were known from here since the first report about the ichthyofauna of the Neretva river basin (Karaman, 1928), unlike *Alburnoides*, which has never been recorded before from this river (see the discussion below). No contemporary connection

between the Neretva river basin and Sava tributaries is known, neither surface nor underground (Palandačić *et al.*, 2015, for details see their Supplementary material), thus natural dispersion of *Alburnoides* from the Sava to the Neretva river basin is unlikely.

Although it is currently abundant, there are no previous records of *Alburnoides* from the Neretva river basin in any of the numerous faunistic papers (*i.e.* Karaman, 1928; Kosorić and Vuković, 1966; Kosorić, 1974, 1977, 1978; Vuković, 1977; Kosorić *et al.*, 1989; Šanda *et al.*, 2008b; Glamuzina *et al.*, 2013). This indicates that it is an introduced species. There are no data on the possible time of introduction, but as it was not mentioned by Glamuzina *et al.* (2013, 2017), it is presumably a case of rather recent introduction. The *cytochrome b* haplotypes of the specimens from the Neretva river basin are identical or very similar to those from the Vrbas and Bosna river basins in Bosnia and Herzegovina (Fig. 3). This fact suggests the introduction from the nearby located area of the Danube river basin, most probably directly within the country. Both juveniles and adults were present, and the species is quite common at both places from which we have obtained samples, thus we consider the population to be well established.

Alburnoides sp. is the 32nd alien species recorded in the Neretva river basin (see Tab. S2) (Glamuzina *et al.*, 2013, 2017; Dulčić *et al.*, 2017; Tutman *et al.*, 2017b; Šukalo *et al.*, 2018). Although not all alien species adapted successfully (Glamuzina *et al.*, 2017), the presence of such a high number of alien species, which approaches the number of native freshwater species ever recorded in the basin (34–see Tab. S1) and almost doubles the number of endemic species (17–see Tab. S1), is alarming. Very high proportions of the alien versus native species were observed also in other rivers of the Adriatic Sea slope (Jelić *et al.*, 2016). A high proportion of the alien species in the Neretva river basin had been most probably translocated from the geographically very closely located Danube basin (Black Sea slope) – 14 species (see Tab. S2), which is almost a half of the total number of the introduced species in this river basin. Translocations were also documented in other Mediterranean countries, such as Greece or Italy, and even concern several endemic species (Bianco and Ketmaier, 2001; Koutsikos *et al.*, 2012; Bianco, 2014; Barbieri *et al.*, 2015). In the case of the Neretva river basin, translocations can be particularly dangerous because ecologically and morphologically similar species of the same genera inhabit both the Neretva and the Danube basins (*e.g.* *Alburnus*, *Chondrostoma*, *Squalius*, *Rutilus*, *Scardinius*, etc.) and can replace the native species. As a deterrent example, extinction of the native *Protochondostoma genei* in the Soča river in Slovenia was observed after the introduction of *Chondrostoma nasus* from the Danube basin (Povž, 1995).

We assume an important negative role of the sport fishery management in the spreading of non-native species in the Neretva river basin, as a result of intentional introductions of commercially important species (like *Cyprinus carpio*, *Sander lucioperca*, etc.), which are obviously accompanied by unintentional introductions of other, commercially unimportant, species (*Gymnocephalus cernuus*, *Pseudorasbora parva*, etc.) (Glamuzina *et al.*, 2017). *Alburnoides* sp., a species without any economic importance, was probably introduced unintentionally or as a bait fish.

The native fish assemblage of the Neretva river basin is undoubtfully threatened by introductions of alien fish species. It is indispensable to raise public awareness especially among the local fishermen to prevent further increase of the number of the alien fish species.

Supplementary Material

Table S1 List of native fish species recorded from the Neretva river basin (including the karstic fields) based on the published data.
Table S2. List of introduced fish species recorded from the Neretva river basin (including karstic fields) based on the published data.
Table S3 List of samples and sequences used in the study. Information about the lineage according to Stierandová *et al.* (2016), locality, coordinates, haplotype, GenBank accession number and source included.
The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2019007/olm.

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