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Spreading of *Dreissena rostriformis bugensis* (Andrusov, 1897) in the Danube River (Serbia)

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Abstract: Detailed limnological investigations of the Serbian Danube stretch at 11 sampling sites in a 396 km long sector of the Danube River (from 1260 r-km to 863.4 r-km) were performed from April 2002 to November 2010. Samples were collected seasonally (April, June, September and November). A Ponto-Caspian element, quagga mussel – *Dreissena rostriformis bugensis* (Andrusov 1897), was found for the first time in the Serbian Danube stretch in April 2010 at sampling site Veliko Gradiste (1059 r-km). Our furthest upstream finding of this species was at site Ram (1071 r-km). Species identification was done by the morphology of its shells. In addition, a comparison with another dreissenid species *Dreissena polymorpha* (Pallas 1771) was made based on shell morphology. Population density of quagga mussel was low, with the ratio of *D. polymorpha* to *D. rostriformis bugensis* being around 5:1. The finding of *D. rostriformis bugensis* in the Serbian Danube stretch is evidence that this invasive Ponto-Caspian species moved the limit of its distribution upstream in the Danube River which is one of the main corridors used for its spread from Eastern (Romania, Bulgaria) to Western Europe.

Key words: Dreissenidae; Ponto-Caspian species; Invasive species; Spreading; Serbia; Danube

Introduction

The freshwater bivalve, zebra mussel *Dreissena polymorpha* (Pallas 1771) native to the Ponto-Caspian region, quickly spread from Eastern to Western Europe in the 19th and 20th century through a corridor of river basins interconnected by man-made canals (Kinzelbach 1992). Over the last two centuries, zebra mussel has continued its expansion into large rivers throughout much of Western Europe. In contrast, *Dreissena rostriformis bugensis* (Andrusov 1897), another Ponto-Caspian species, has been far slower in its range expansion (Molloy *et al*. 2007). European dispersal of Ponto-Caspian species has resulted from international stocking programs and unintentional transport facilitated by human activities including commercial shipping and canal net development (Tittizer 1997, Tittizer, Banning 2000, Orlova *et al*. 2004). The phenomenon of penetration of European and North American continental waters (rivers and lakes) by invasive and introduced species that originated mainly from Asia was noticed some 40 years ago (Nedeljkovic 1967, 1979, Ricciardi, MacIsaac 2000, Ojaveer et al. 2002, Reid, Orlova 2002, Bij de Vaate *et al*. 2002, Lyashenko *et al*. 2004, Dumont *et al*. 2004, Karatayev *et al*. 2008, Gergs *et al*. 2008, Kipp *et al*. 2010). We registered the same process in our 25-year bottom fauna investigation of the Danube River basin in Serbia (Martinovic-Vitanovic, Kalafatic 2002, Martinovic-Vitanovic *et al*. 2006, 2008, 2009, 2010), and in our present investigations. The various aspects of two dreissenid species expansions from the Ponto-Caspian region to freshwater habitats of North America and Europe were the object

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of the investigations of the following authors: Pathy, Mackie 1993, Mills *et al*. 1999, Vanderploeg *et al*. 2002, Orlova *et al*. 2004, Zhulidov *et al*. 2004, 2005, 2010, Son 2007, Van der Velde, Platvoet 2007, Molloy *et al*. 2007, Grigorovich *et al*. 2008, Imo *et al*. 2010. Detailed limnological studies of the Danube in the Serbian sector of the river initiated in 1958 have continued to the present time (Nedeljkovic 1967, Martinovic-Vitanovic, Kalafatic 1990, 1995, 2002). The communities of zoobenthos are represented by forms typical of lowland rivers, such as the Danube River in Serbia, with oligochaetes and molluscs – bivalves and gastropods followed by insects (especially chironomids) as principal components of the community in regard to species richness (Nedeljkovic 1967, Martinovic-Vitanovic, Kalafatic 2002, Martinovic-Vitanovic *et al*. 2006, 2009, and in our present investigations). Micu, Telembici (2004) followed by Popa, Popa (2006) first recorded the spreading of quagga mussel in the Romanian Danube stretch. In 2007 Hubenov, Trichkova (2007) published the first record of the quagga mussel in the Bulgarian Danube stretch. Our study represents the first record of the quagga mussel in the Serbian Danube stretch, as well as the present distribution of quagga and zebra mussels in Serbia, and describes the spreading of their European and global ranges.

Materials and Methods

In the 10-year period of investigations from 2002 to 2011, samples were collected seasonally (four times per year), and during 2010 *Dreissena* species, along with the quagga mussel for the first time, were recorded at the investigated Danube stretch in Serbia (eleven standard sampling sites in a 396 km long sector of the Danube River from 1260 r-km to 863.4 r-km).

In this study, the investigated Serbian Danube stretch was divided into three parts according to Martinovic-Vitanovic *et al*. (2006):

I – Riverine zone: from r-km 1260 (Ledinci) to r-km 1250 (Novi Sad)

II – Flow-through reservoir: from r-km 1192 (Stari Banovci) to r-km 1072 (Ram); and

III – Lake ecosystems – Iron Gate I and II (Djerdap I and II) reservoirs: from r-km 1059 (Veliko Gradiste) to dam Iron Gate I at r-km 942.9, and from r-km 934 (Kladovo) to dam Iron Gate II at r-km 863.4, respectively.

Limnological studies were performed using standard methods and techniques (APHA-AWWA-WEF 1995). Sediment samples with benthic organisms were taken with Van-Veen type of grab (270 cm^2; three grabs per site/4 times per year), benthological dredge, and by hand collecting. In 2010, Van-Veen samples sites totalling 35 sites (8 in each season – April, June and November equalling 24, plus 11 in September). Samples were collected from a depth of 1-33 m. Material for the analysis of meio- and macrozoobenthos came from the different bottom facies. Substrate classification was performed by visual evaluation in situ, and in the laboratory (using stereo zoom microscope with binocular magnifier-magnification 5-50x, Krüss, Germany), based on the diameter of sediment particles (Wentworth 1922) and according to the national classification (Martinovic-Vitanovic, Kalafatic 1995; Lakusic *et al*. 2005). All samples were preserved in situ with 4% formaldehyde and transported to the laboratory, where they were further processed. In the laboratory, mussels were separated from other material under a dissection microscope, identified to species when feasible, and enumerated. Specimens were assigned to either *Dreissena polymorpha* or *D. rostriformis bugensis*. Species identification was done according to traditional dreissenid taxonomy based principally on shell morphological attributes (Pathy, Mackie 1993, Grigorovich *et al.* 2008): 1-Presence/absence of the ventro-lateral carina; 2-Degree of flatness of the shell at ventral region; 3-Overlap of valves and 4-Curvature and asymmetry of the ventral margin of valves. The ages of quagga mussels were determined by shell sectioning (Hebert *et al*. 1989). Appearance frequencies (F = 0-1) of Ponto-Caspian and introduced (Neozoa) species per site were calculated as $\mathbf{F = m \times M^{-1}}$, where **m** stands for the number of samples in which the particular species was found, and **M** stands for the total number of samples. Eleven quagga mussel's specimens were randomly chosen and deposited in the repository (code 11/2010) of the Department of Hydroecology and Water Protection of the Institute for Biological Research "Sinisa Stankovic" (University of Belgrade, Serbia).

Results

Our study represents the first record of the quagga mussel in the Serbian Danube stretch. Out of a 396 km long sector of the investigated Danube River in

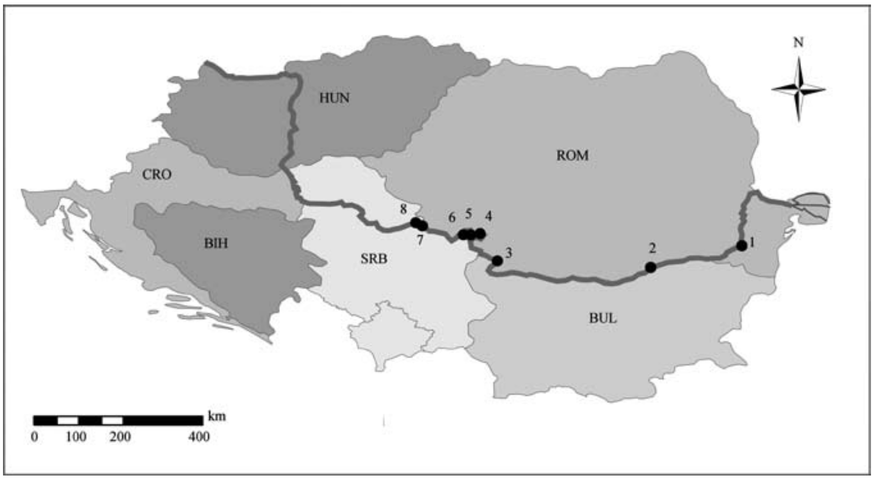

Fig. 1. The distribution of quagga mussels in the Danube River: (1) Cernavoda, Romania in 2004 (Micu, Telembici 2004); (2) Koshava, Bulgaria in 2006 (Hubenov, Trichkova 2007); (3) Sandrovo, Bulgaria in 2007 (Hubenov, Trichkova 2007); (4) Drobeta Turnu Severin, Romania in 2005 (Popa, Popa 2006); (5) Kladovo; (6) Tekija; (7) Veliko Gradiste and (8) Ram, Serbia in 2010 (present study)

Serbia, in 137 km long sector of the Danube River, the quagga mussel was found for the first time: on the 12th of April 2010 at Veliko Gradiste sampling site in the Djerdap I accumulation (N 44°46'16.4", E 021°31'20.8"; 1059 r-km), and for the second time on the 13th of April 2010 at Kladovo sampling site in the Djerdap II accumulation (N 44°37'02.7", E 022°36'34.8"; 934 r-km). For the third time, on the 21st of June 2010, it was found furthest upstream near the old town of Ram sampling site in Flow-through reservoir (N 44°48'42.0", E 021°22'16.1"; 1072 r-km). After that, the quagga mussel was found, for the fourth and final time, on the 9th of September 2010 at site Tekija in the Djerdap I accumulation (N 44°41'11.6", E 022°24'11.1"; 956 r-km). The map presents combined data from literature and our own results about the distribution of quagga mussels in the Danube River in Romania and Bulgaria, and Serbia, respectively (Fig. 1 and Table 1). In total, 858 specimens of dreissenid species were found, among which 802 were identified as *D. polymorpha* and 56 as *D. rostriformis bugensis* (Table 1). Both dreissenid species were found in nine out of 35 samples collected in 2010 at 11 investigated sampling sites. In June, at the Ram sampling site, quagga mussel was found at the bottom, water depth being 11 m. In April and September, at Veliko Gradiste, quagga mussel specimens were gathered from the bottom, water depth being 5 m and 15 m. In September and November, at the Tekija site, quagga specimens were collected from the bottom, water depth being 1 m and 33 m. At the Kladovo site, in April, June, September and November, quagga specimens were collected from the bottom, water depth varying between 1 and 9 m. Quagga mussels were found at four sampling sites with hard and soft substrates (cobble, pebble, sand and silt) while zebra mussels were found at nine sampling sites with similar substrate types (Table 1). Of the total number of found dreissenid (n=340) at the four sampling sites in the Serbian Danube River during 2010, the number of quagga mussels (n=56) in relation to zebra mussels (n=284) was 1:5. Specimens were assigned to either *D. rostriformis bugensis* or *D. polymorpha* based upon the examination of shell characters (Fig. 2): – Shell shape, actual size and colour: rounder (26 mm) and triangular (16 mm), respectively. Zebra mussels ranged in size from 1-5 mm (for juveniles) to greater than 15 mm (for adults). Their minimum shell length was 20.25 mm; mean length was 22.96 mm, while the maximum length was 24.90 mm. Another distinguishing characteristic for its identification is the shell shape. Quagga mussels have a pointed umbone, are rounded ventroposteriorly, have a much more rounded ventral surface with little or no ventrolateral shoulder or ridge, and may have a more rounded and higher dorsoanterior slope or winglike extension. In addition, there may be a difference in the colour pattern or marks between the ventrolateral and dorsolateral sides (Fig. 2A). An often useful distinguishing feature of the quagga mussel is the appearance of a white stripe or line across the middle of the shell from the umbone toward the posterior end. As with *D. polymorpha*, the quagga mussel has

Table 1. Sampling sites in three river parts (I-III), geographical coordinates, r-km, dates, substrate types, and quagga and zebra mussel abundances – **a** as number of individuals per sample) collected seasonally (April – **A**, June – **J**, September – **S** and November – **N**) during the year 2010 in the Serbian Danube stretch.

Site Code/Sampling Site	Geographical coordinates		R-km	Date	Substrate types	Zebra mussel (a)				Quagga mussel (a)			
						Months				Months			
						A	J	S	N	A	J	S	N
Part I – Riverine zone													
1. Ledinci	N 45° 13' 4.7"	E 019° 48' 22.6"	1260	06.09.2010	sand			1					
2. Novi Sad	N 45° 15' 34.2"	E 019° 53' 22.3"	1252	06.09.2010	boulder			61					
Part II – Flow-through reservoir													
3. Stari Banovci	N 44° 59' 10.7"	E 020° 17' 38.9"	1192	06.09.2010	cobble, sand			214					
4. Oresac	N 44° 39' 20.8"	E 020° 49' 29.7"	1124	06.09.2010	silt			1					
5. Smederevo	N 44° 41' 36.7"	E 020° 57' 22.6"	1112	21.06.2010; 07.09.2010	cobble, sand, silt		31	199					
6. Ram	N 44° 48' 42.0"	E 021° 22' 16.1"	1072	21.06. 2010; 08.11.2010	sand, Mollusca shell		5		4		1		
Part III – Lake ecosystems													
Iron Gate I – Djerdap I													
7. Veliko Gradiste	N 44° 46' 16.4"	E 021° 31' 20.8"	1059	12.04.2010; 08.09.2010; 08.11.2010	cobble, pebble	4		22	39	1		1	
8. Donji Milanovac	N 44° 28' 12.4"	E 022° 08' 39.6"	991	22.06.2010	silt		10						
9. Tekija	N 44° 41' 11.6"	E 022° 24' 11.1"	956	22.06.2010; 09.09.2010; 09.11.2010	silt		1	86	10			23	2
Iron Gate II – Djerdap II													
10. Kladovo	N 44° 37' 02.7"	E 022° 36' 34.8"	934	13.04.2010; 23.06.2010; 09.09.2010; 10.11.2010	pebble, sand	24	1	40	48	4	3	2	19
11. Kusjak	N 44° 18' 40.9"	E 022° 33' 06.9"	864	14.04.2010; 23.06.2010; 09.09.2010; 10.11.2010	silt			1					
Total number of individuals Σ=858						**Σ = 802**				**Σ = 56**			

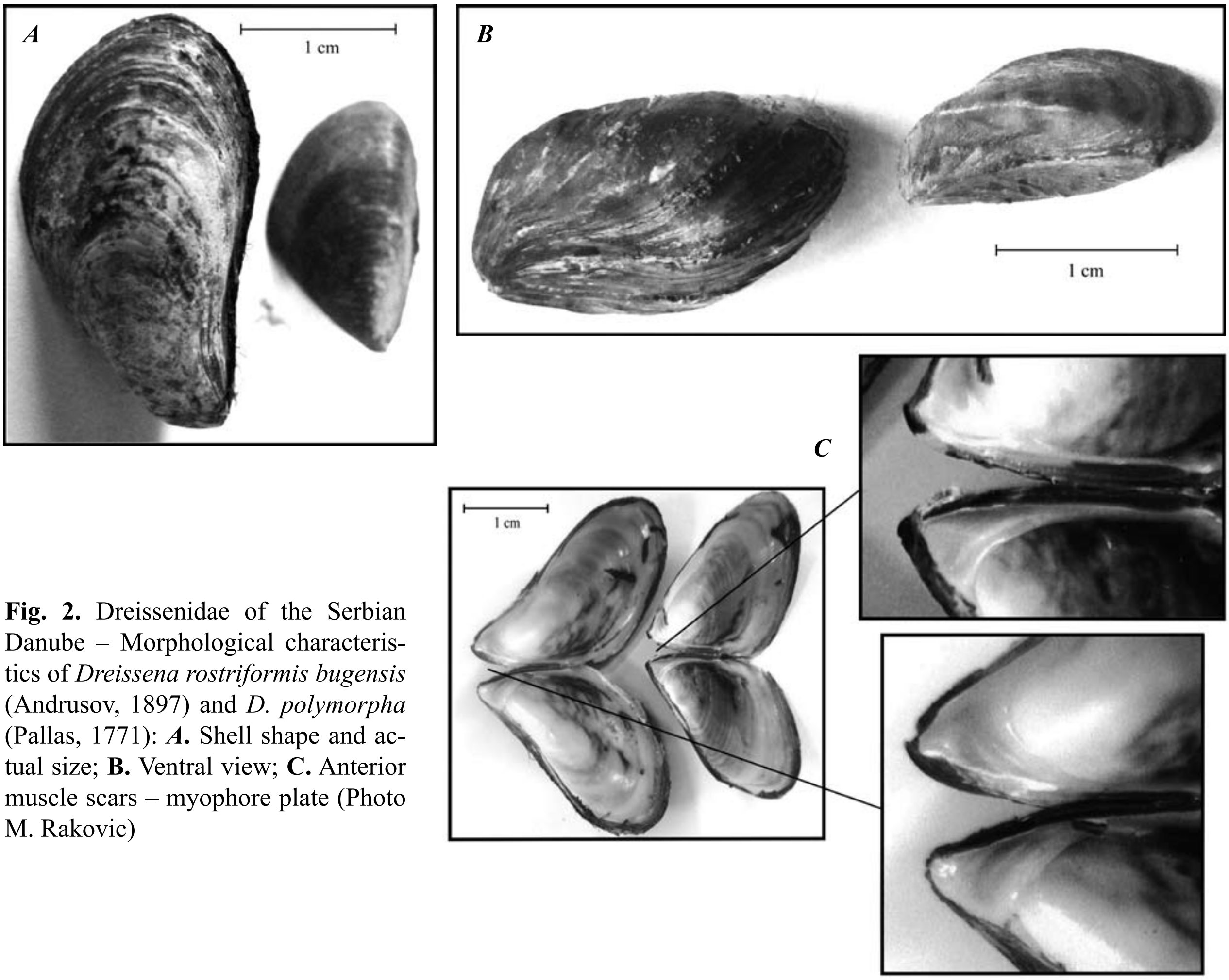

Fig. 2. Dreissenidae of the Serbian Danube – Morphological characteristics of *Dreissena rostriformis bugensis* (Andrusov, 1897) and *D. polymorpha* (Pallas, 1771): ***A.*** Shell shape and actual size; **B.** Ventral view; **C.** Anterior muscle scars – myophore plate (Photo M. Rakovic)

large variability in shell shape, colour, and colour pattern. *D. rostriformis bugensis* had a ventral view with an obvious ridge and ridge absence, the valves are asymmetrical with no straight midventral line, while *D. polymorpha* had the valves bilaterally symmetrical with a straight midventral line (Fig. 2B). In *D. rostriformis bugensis* and *D. polymorpha* both the anterior adductor and anterior byssal retractor muscles attach to the myophore plate. Both external shell features were utilized to investigate the diagnostic usefulness of the anterior adductor and anterior pedal retractor muscle scars on the myophore plate. *D. rostriformis bugensis* was reported to possess an elongated scar outline nearly twice as long as it was wide, whereas that of *D. polymorpha* was characterized by more even dorsal and ventral aspects (Fig. 2C). In the Serbian Danube investigations, from 13 non-native invertebrate species found during 2010, the majority were of Ponto-Caspian origin. Table 2 presents nine Ponto Caspian relict and invasive species together with alien so-called introduced taxa (Neozoa) in the benthofauna of the Danube River in Serbia with their frequency of occurrence. Apart from *D. rostriformis bugensis*, *D. polymorpha*, and Ponto-Caspian relicts, seven other Ponto-Caspian species were recorded: *Hypania invalida* (Grube 1860), *Manayunkia caspica* Annenkova 1928, *Isochaetides michaelseni* (Lastockin 1937), *Jaera sarsi* Valkanov 1936, *Corophium curvispinum* Sars 1895, *Lithoglyphus naticoides* (C. Pfeiffer 1828) and *Theodoxus danubialis* (C. Pfeiffer 1828). In total six of them are denoted as invasive species (Table 2). From Ponto-Caspian species with the highest occurrence, frequencies are as follows: F = 1 – *Dreissena polymorpha* and F = 0.73 – *Corophium curvispinum.* Five species have medium F values (0.31-0.60) being in the range of F = 0.36-0.54. Only two species have low frequencies of occurrence – F = 0.18. Four alien species recorded in our investigations of the Serbian Danube stretch are so-called introduced taxa – Neozoa: *Corbicula fluminea* (Muller, 1774), *C. fluminalis* (Muller, 1774), *Sinanodonta woodiana* (Lea, 1834) and *Theodoxus fluviatilis* (Linnaeus, 1758), the first three being from Eastern Asia. Their

Table 2. Non-indigenous invertebrate species found in Serbian Danube fauna during 2010: Ponto-Caspian relict, invasive and introduced species in the benthofauna of Danube (Serbia) with Frequency of occurrence by Localities (Sampling site numbers grouped in Parts I-III) - **F** = 0-1; number of locations where a taxon was found (for two dreissenid species found in 2010, see also Table 1) - **n** = 1-11; **( )** - **Ponto-Caspian species by its origin**; **(*)** - **Invasive species**; **(**)** - **Introduced species (Neozoa)**.

PONTO-CASPIAN SPECIES	**PARTS I-III / LOCALITIES**
Polychaeta	
* *Hypania invalida* (Grube 1860)	**II-III** / 5, 6, 7, 8, 9, 10 (**F = 0.54**)
**Manayunkia caspica* Annenkova 1928	**III** / 7, 10 (**F = 0.18**)
Oligochaeta	
Isochaetides michaelseni (Lastockin 1937)	**II-III** / 5, 6, 7, 8, 9 (**F = 0.45**)
Isopoda	
Jaera sarsi Valkanov 1936	**II-III** / 5, 6, 7, 10 (**F = 0.36**)
Amphipoda	
* *Corophium curvispinum* Sars 1895	**II-III** / 3, 4, 5, 6, 7, 8, 9, 10 (**F = 0.73**)
Gastropoda	
**Lithoglyphus naticoides* (C. Pfeiffer 1828)	**II-III** / 4, 5, 6, 8, 9, 11 (**F = 0.54**)
Theodoxus danubialis (C. Pfeiffer 1828)	**III** / 9, 10 (**F = 0.18**)
Bivalvia	
* *Dreissena polymorpha* (Pallas 1771)	**I, II and III** / 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 (**F = 1**)
* *Dreissena rostriformis bugensis* (Andrusov, 1897)	**II and III** / 6, 7, 9, 10 (**F = 0.36**)
INTRODUCED SPECIES (NEOZOA)	
Gastropoda	
***Theodoxus fluviatilis* (Linnaeus 1758)	**II and III** / 3, 5, 6, 7, 10 (**F = 0.45**)
Bivalvia	
***Corbicula fluminea* (Muller, 1774)	**I, II and III** / 1, 3, 5, 6, 7, 9, 10 (**F = 0.64**)
***Corbicula fluminalis* (Muller,1774)	**II and III** / 6, 7 (**F = 0.18**)
***Sinanodonta woodiana* (Lea, 1834)	**III** / 7 (**F = 0.09**)

occurrence frequencies were in a wide range from 0.09 to 0.64 (Table 2).

Discussion

During our previous 16-year and up-to-date investigations of the Danube River zoobenthic community, along with 33 Mollusca species we found all together 142 potamo-benthic taxa, representatives of 23 faunistic groups (Martinovic-Vitanovic, Kalafatic 2002, Martinovic-Vitanovic *et al*. 2006, 2009, 2010, 2013). The following 14 groups were identified in 2010 with 59 taxa in all: Nematoda (1), Polychaeta (2), Oligochaeta (18), Hirudinea (2), Bryozoa (1), Amphipoda (2), Isopoda (1), Mysidacea (1), Diptera-Chironomidae (14) and Ceratopogonidae (1), Odonata (1), Trichoptera (2), Gastropoda (5) and Bivalvia (9). Respective faunistic lists did not include *D. rostriformis bugensis* as invasive species originated from the Ponto-Caspian region (Martinovic-Vitanovic *et al*. 2006, 2009). Therefore its finding in the present study may be considered its first record in the Serbian Danube River and a new species for the invertebrate fauna of Serbia. Besides zebra mussels and quagga mussels in the benthocenoses of investigated Danube sections we also found the Ponto-Caspian freshwater snails *Lithoglyphus naticoides* and *Theodoxus danubialis*. Within the sediment, *Unio tumidus* was present together with numerous Asian clams *Corbicula fluminea* and *C. fluminalis*. Material for the analysis of meio- and macrozoobenthos came from the different bottom facies. The samples were gathered from the bottom, water depth varying between 1 and 33 m. Both dreissenid species were found in nine out of 35 samples collected in 2010 at 11 investigated sampling sites, and larger numbers were recorded on hard substrates (cobble, pebble, sand) and water depth between 11 and 33 m. The high concentrations of zebra mussels on sandy substrates on the eastern side of Lake Michigan between 15 m and 30 m and expanding populations between 30 m and 50 m (Nalepa *et al*. 2001) benefit from low wave energy and the deep chlorophyll layer that intersects the bottom at these depths. With zebra and quagga mussels clumping together, the chance of misidentification between these two *Dreissena* species or forms is great (Pathy, Mackie 1993). Even though confirmation of the quagga mussel's species identity requires further genetic analysis (May, Marsden 1992), as well as ecological studies (Vanderploeg *et al*. 2002), some external diagnostic shell features such as shell size and shape, as well as the colour pattern or marks between the ventrolateral and dorsolateral sides may be used to distinguish a zebra mussel from a quagga mussel (Pathy, Mackie 1993). As with *D. polymorpha*, the quagga mussel has large variability in shell shape, color, and color pattern. Quagga mussels can be all black, brown, or white and have various striped patterns (Pathy, Mackie 1993). Complementing traditional dreissenid taxonomy based principally on morphological attributes, presented in our study, we accepted what Therriault *et al*. (2004) suggested, based on molecular analyses, that *D. bugensis* and *D. rostriformis* may represent a single species with two distinct races. Therefore, in our paper we accepted that *D. bugensis* has the status of *D. rostriformis bugensis*. Human activities are rapidly changing aquatic ecosystems. The most notable are activities related to canal construction and transoceanic shipping, both of which link water bodies and allow transfer of non-indigenous species between previously isolated aquatic ecosystems. The dreissenids have undergone considerable global redistribution as a result of shipping activities (Nuttall 1990). The spreading of quagga mussel in the Danube River was first recorded by Micu, Telembici (2004) at Cernavoda in 2004, and Popa, Popa (2006) recorded it two years later further westwards (500 r-km upriver) at Drobeta near Turnu Severin. In 2007 Hubenov, Trichkova (2007) published the first record of the quagga mussel in the Bulgarian Danube stretch near the villages Koshava (811 r-km) and Sandrovo (477 r-km). Quagga mussel was found for the first time in the Serbian Danube stretch in April 2010 at sampling site Veliko Gradiste (1059 r-km). Our furthest upstream finding of this species was at the site Ram (1072 r-km). Since its penetration in the Danube River, practically all of Western Europe has been accessible for the quagga mussels, because of the Danube-Main-Rhine canal, which removed the natural barriers for this species. Molloy *et al.* (2007) found this species for the first time on the 19th of April 2006 in the Hollands Diep near Willemstad in the Netherlands, where it occurred in relatively low numbers in between the zebra mussels (1% were quagga mussels). Van der Velde, Platvoet (2007) findings of the quagga mussel in Germany, River Main on the 19th of May 2007 is also confirmation that the quagga mussel has entered via the Main-Danube canal. Zebra mussel, the first dreissenid mussel introduced in North America, rapidly spread throughout many river systems and the Great Lakes causing substantial ecological and environmental impact (Mills *et al*. 1999). The quagga mussel was first observed in North America in September 1989 when it was discovered in Lake Erie near Port Colborne, Ontario (Mills *et al*. 1999). The introduction of both dreissenid species into the Great Lakes appears to be the result of ballast water discharge from transoceanic ships containing veliger, juveniles, or adult mussels. The genus Dreissena is highly polymorphic and prolific with high potential for rapid adaptation attributing to its rapid expansion and colonization. Our finding together with the previously mentioned ones (Micu, Telembici 2004, Popa, Popa 2006, Hubenov, Trichkova 2007, Van der Velde, Platvoet 2007 and Molloy *et al.* 2007), shows that the Danube River is most likely the main pathway of spread of quagga mussel throughout Europe.

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