Aquatic Ecology
Research Article

ACTA ZOOLOGICA BULGARICA
Acta zool. bulg., 65 (3), 2013: 315-326

Distribution of *Corbicula fluminea* (Mollusca: Corbiculidae) over an Eleven-Year Period of its Invasion in Bulgaria

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Abstract: The first record of the Asian clam *Corbicula fluminea* in Bulgaria was from the Danube River at Vetren in 2001. In 11 years the species has established in the entire Bulgarian stretch of the Danube, from Vrav (rkm 836) to Vetren (rkm 395), where it reached densities up to 16 560 ind./m^2. The species extended its range rapidly upstream of the Danube tributaries reaching 80 km upstream from the confluence with the Danube (Iskar River). Recently, the species also established in standing waters, two reservoirs and one sand-pit lake, located at altitudes of up to 525 m a.s.l. The most probable dispersal vector of *C. fluminea* in the inland waters of Bulgaria is passive upstream transport which is probably facilitated by some human activities, such as fishing, fish stocking, recreational activities, sand and gravel extraction.

Key words: Invasive alien species, Asian clams, occurrence, abundance, Danube River basin, Bulgaria

Introduction

Two invasive freshwater bivalve species *Corbicula fluminea* (O. F. Müller, 1774) and *Corbicula fluminalis* (O. F. Müller, 1774) have largely expanded their range of distribution throughout the world. The native range of the Asian clam *C. fluminea* includes Southeast Asia, but at present it is being introduced across North America, South America and Europe. In North America it was first documented in 1924 based on empty shells (Counts 1981) and in 1938 on living specimens (Burch 1944). Since then the species has rapidly spread, reaching high densities in many major waterways in the eastern and western United States (McMahon 1982, Foster *et al*. 2013). It was also found in Canada, Mexico, Panama, Argentina, Brazil, Uruguay, Venezuela (CABI 2013).

In Europe, *C. fluminea* was first recorded in 1980 in the Dordogne River, southern France, and in the Tagus River estuary in Portugal (Mouthon 1981); while in 1983 it was found in the Weser River in Germany (Kinzelbach 1991). It was suggested that the species was unintentionally brought to Europe from North America through ballast water (Kinzelbach 1991). Since these first findings, the species has been introduced to most of the European river systems and countries, e.g. the water basins in the Iberian Peninsula (Ayres 2008, Pérez-Quintero 2008, Morais *et al*. 2009, Lois 2010); the four main river basins in France: the Garonne, the Rhone, the Loire and the Seine (Vincent, Brancotte 2002, Marescaux *et al*. 2010); the River Po basin in Italy (Fabbri, Landi 1999, Cianfanelli *et al*. 2007, Kamburska *et al*. 2013); the rivers of Central

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Europe (Brauckmann *et al*. 1999); the Elbe basin in Germany (Grabow, Martens 1995) and Czech Republic (Beran 2000, 2006); the Norfolk Broads drainage system, the Thames River and the Great Ouse system in UK (Howlett, Baker 1999, Elliott, zu Ermgassen 2008, Willing 2007); and the rivers Barrow, Nore and Shannon in Ireland (Lucy *et al*. 2012).

The first record of *C. fluminea* in the Rhine River was in the delta area in the Netherlands in 1988 (Bij de Vaate, Greijdanus-Klaas 1990). The species rapidly extended its range in the upstream direction. In 1990 it was found in the middle reaches in Germany (Bij de Vaate 1991), in 1991 in the Upper Rhine near Karlsruhe, and in 1995 it was reported near Basel in Switzerland (Rey *et al*. 2004, Schmidlin, Baur 2007, Schmidlin *et al*. 2012). It was assumed that the rapid spread of the species in the Rhine River was due to transportation by cargo ships (Tittizer 1997, Leuven *et al*. 2009). When the species appeared in the lower reaches of the Main River in 1991 (Kinzelbach 1991), colonization of the Danube River through the Main-Danube canal was expected. This canal was opened for shipping in 1992 (Tittizer *et al*. 1994), connecting the Rhine and Danube basins.

In April 1997, as expected, live *Corbicula* specimens (*C. fluminea/ C. fluminalis*) were recorded in the Upper Danube – downstream of the mouth of the Isar River, Germany (Tittizer, Taxacher 1997), and in winter of the same year *C. fluminea* was found in the Lower Danube, Romania, at Berzasca and Moldova Nouã (Skolka, Gomoiu 2001). However, in 1995 juvenile *Corbicula* specimens were already recorded in the Ukrainian sector of the Danube Delta (Alexandrov *et al.* 2007, Son 2007). The authors assumed that the introduction of the species in the Danube River basin happened independently in different sectors and at different times, with at least two main introductions: one in the Lower Danube, and one starting in 1997 in the Upper Danube, from where the species spread quickly downstream (Son 2007). *C. fluminea* was further reported in the Danube and its backwaters from Austria (Fischer, Schultz 1999), Hungary (Csányi 1998-1999), Romania (Bij de Vaate, Hulea 2000, Popa, Popa 2006, Popa, Murariu 2009), Bulgaria (Hubenov 2001), Slovakia (Vrabec *et al.* 2003), Ukraine (Lyashenko *et al.* 2005, Son 2007, Lyashenko, Makovskii 2011) and Serbia (Paunović *et al.* 2007). It was also reported in some of the Danube tributaries, such as Sava and Tisza rivers in Serbia (Paunović 2004, Paunović *et al.* 2007), Prut River in Moldova (Munjiu, Shubernetski 2010) and side arms in Hungary (Bódis *et al.* 2011, 2012).

The native range of *C. fluminalis* is considered to be Central Asia, a small European territory along the southwestern coast of the Caspian Sea and the northeastern parts of Caucasus, Middle East and North Africa (Zhadin 1952, Kinzelbach 1992, Korniushin 2004, CABI 2013). During the Pleistocene interglacials, the species was described to occur in North-West Europe (Meijer, Preece 2000). The presence of subfossil specimens of *C. fluminalis* together with *C. fluminea* was reported in the Romanian sector of the Danube Delta by Grossu (1962). At present the species has been introduced in a number of river basins in Europe, such as: Meuse, Rhine, Mosel and Weser rivers in Germany, Netherlands, France, Luxembourg, and Belgium (CABI 2013); Rhine River in Switzerland (Schmidlin *et al*. 2012); Loire and Seine rivers and canals in France (Mouthon, Parghentanian 2004, Marescaux *et al*. 2010); Oder River in Poland (Łabecka *et al*. 2005); Lakes in northern Italy (Ciutti, Cappelletti 2009). Along the Danube River, the species was recorded in Hungary (Csányi 1998-1999), in Ukraine (Voloshkevitch, Son 2002, Son 2007) and in Serbia (Paunović *et al.* 2007). It was also recorded in the tributary Sava River in Serbia (Paunović *et al.* 2007).

The taxonomic status of *C. fluminea* and *C. fluminalis* is still under discussion. Some authors (e.g. Zhadin 1952, Renard *et al.* 2000, Mouthon, Parghentanian 2004) accepted them as separate species with specific distribution ranges, while other authors considered them as synonyms or morphotypes (Pfenninger *et al*. 2002, Paunović *et al*. 2007, Marescaux *et al*. 2010, Bódis *et al***.** 2011, Pigneur et al. 2011).

The rapid spread and the invasion success of *C. fluminea* throughout Europe, North and South America is firstly related to its biological characteristics (rapid growth, early sexual maturity, high fecundity, variety of reproduction strategies, planktonic larvae, and potential of reaching high population density), and secondly to its physiological tolerance (McMahon 2002, Sousa *et al*. 2008a). The species is found in freshwaters as well as in brackish waters (Alexandrov *et al.* 2007, Sousa *et al*. 2008b). It inhabits different types of water bodies (rivers, lakes,

reservoirs, canals), but prefers larger water bodies and reaches higher densities in lotic waters (*e.g.* reviewed in Lucy *et al*. 2012, Schmidlin *et al*. 2012). When in high abundance, the species has the potential to displace or reduce available habitats of other benthic species, to compete for benthic food resources, to limit planktonic food for other species and to ingest large numbers of unionid sperm, glochidia and juveniles due to its high filtration rate, as well as to transfer parasites and pathogens (Sousa *et al.* 2008a). Impact on surface water use (power plants, drinking and industrial water systems, irrigation systems and canals) due to bio-fouling was reported in the United States (Ingram 1959, Isom *et al*. 1986, Prokopovich, Hebert 1965) and Europe (Rosa *et al*. 2011).

The goal of the present study was to present the recent distribution of *C. fluminea* in the water basins in Bulgaria after 11 years of invasion.

Material and Methods

The study was based on material collected by the authors in the period 2001-2012. Specimens provided by other researchers were also used. The sampling sites are presented with geographical names and coordinates, altitude, UTM-codes and river kilometers.

Qualitative and quantitative samples of clams were collected with a triangular bottom dredge, hand dredge, Petersen bottom sampler and by hand (at low water level). In September 2012, 14 sites were sampled along the Danube River by dredging from the shore or from a boat and by a Surber's net (35x30cm) at different substrate types in the littoral zone.

In order to identify *C. fluminea* and differentiate it from its closely related species *C. fluminalis* during our study, we used different shell characters, such as pattern of shell sculpture, shape, color, size, etc. Differences in shell characters between the two taxa from literature (e.g. Zhadin 1952, Araujo *et al*. 1993, Korniushin 2004, Skuza *et al*. 2009) are summarized in Table 1. Additionally, specimens of both taxa collected on June 16, 1998 in the Hollandsch Diep, a river stretch in the Rhine delta, The Netherlands, were used for comparison. Pictures of the specimens are presented in Figs. 1 (A, B) and 2 (A, B) for illustration.

Density was calculated as individuals per square meter (ind./m^2). The standard morphomet-

Table 1. Shell characters of *Corbicula fluminea* and *Corbicula fluminalis*

Shell Characters	***Corbicula fluminea***	***Corbicula fluminalis***
Form	Round and broad form	Saddle and narrow form
Concentric ridges	More widely spaced and coarser	More closely spaced and finer
Length	Average maximum values of 30-40 mm	Average maximum values of 20-30 mm
Height	Always lower than the length	Equal to or higher than the length
Asymmetry	Asymmetrical shell	Almost symmetrical shell
Umbo	Slightly protruded; does not match the middle vertical axis	Largely protruded; matches the middle vertical axis
Lateral teeth	Reach mid height	Exceed past mid height
Teeth	Clearly serrated lateral teeth	Slightly serrated lateral teeth
Color	With whitish inner surface	With a violet inner surface

Fig. 1. *Corbicula fluminalis* – **A.** right valve interior. **B.** right valve exterior. Specimen collected in the Hollandsch Diep, Rhine delta, The Netherlands, June 16, 1998, by A. Bij de Vaate. (Photo: A. Ignatov)

Fig. 2. *Corbicula fluminea* – **A.** right valve interior. **B.** right valve exterior. Specimen collected in the Hollandsch Diep, Rhine delta, The Netherlands, June 16, 1998, by A. Bij de Vaate. (Photo: A. Ignatov)

ric shell parameters were measured – length, height and width (in mm). The material has been stored in the collections of the Institute of Biodiversity and Ecosystem Research BAS and the National Museum of Natural History BAS.

Results

During the period 2001-2012, the species *C. fluminea* was recorded at a total of 74 sites in the Danube basin in Bulgaria (Table 2, Fig. 3). These include: 38 localities in the Danube River from Vrav village (rkm 836) to Malak Preslavets (rkm 414); 33 localities in the Danube tributaries Vidbol, Tsibritsa, Ogosta, Skat (tributary of Ogosta River), Iskar, Vit, Osam, Yantra, Rusenski Lom and some of their tributaries; and 3 localities in standing waters, the reservoirs Valchovets and Gorni Dabnik, as well as Negovan Lake (Table 2, Fig. 3). The highest altitude where the species was found was 525 m a.s.l. in Negovan Lake in the Sofia plain.

The population abundance throughout the years and at different sites in the Danube River ranged from less than 1 ind./m^2 to 16 560 ind./m^2. The Danube River at Koshava island (north of Vidin town, rkm 807) was sampled 3 times in the study period (Table 2). In December 2001, a population density from 0.5 to 4 ind./m^2 was estimated. Two years later, in August 2003, at a low water level (less than 100 cm at Vidin), the density of *C. fluminea* was much higher reaching 200-500 ind./m^2. In September 2011, at the same site with water level around 150 cm at Vidin, a population density of 50 ind./m^2 was estimated. In November 2005, at a low water level, a sampling was carried out in the littoral area of the Danube River from rkm 655 to rkm 496 (Gorni Vadin village – Ruse town). The presence of *C. fluminea* was confirmed along the entire stretch with a density of 300-700 ind./m^2. In September 2012, 14 sites were sampled along the Danube River, from Vidin (rkm 796) to Ruse-Prista (rkm 502) (Table 2). The population density ranged from <20 ind./m^2 (Vidin) – 100 ind./m^2 (Dolni Tsibar, Ruse-Prista) to 16 560 ind./m^2 (Zagrazhden).

In August 2004, a population density of 225 ind./m^2 was estimated in the Iskar River at Gigen village (about 4 km upstream from its confluence with the Danube).

The species was found at various substrates – sand, coarse sand, gravel and stones (the Danube and Danube tributaries), sand, gravel, clay and mud (Negovan Lake, reservoirs). The populations were most abundant at substrates dominated by coarse sand, and sand and gravel (*e.g.* Zagrazhden) (Table 2).

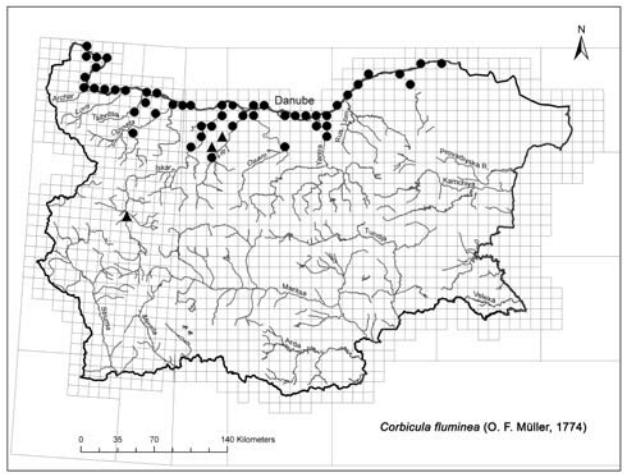

Fig. 3. Distribution of *Corbicula fluminea* in Bulgaria, 2001-2012. Localities in rivers are indicated with filled circles, and localities in lakes with triangles. One circle may correspond to several sites located within one UTM grid zone (10x10 km)

Fig. 4. *Corbicula fluminea*– a large specimen found in the Danube River at Batin in 2011 (length 40.79 mm, height 37.79 mm and width 24.32 mm). (Photo: A. Ignatov)

Specimens of the largest sizes were found during 2010-2012 in the Danube River in the section from Dolno Linevo (rkm 731) to Tutrakan (rkm 432). The largest specimen measured (length 40.79 mm, height 37.79 mm and width 24.32 mm) was collected at Batin (rkm 526) in 2011 (Fig. 4).

Discussion

In Bulgaria, the first specimen of *C. fluminea* was found in June 2001 in the Danube River at Vetren village (rkm 395) (Hubenov 2001). In July of the

Table 2. Localities of *Corbicula fluminea* in Bulgaria, 2001-2012. *Sites where only empty shells were found

№	River, River Basin	Locality; River kilometer (rkm); Altitude (m a.s.l.)	Geographic Coordinates	UTM	Date of finding
1	Danube River	Between Vrav and Novo Selo (rkm 836); 40 m a.s.l.	N 44.186056 E 22.760167	FP49	12.07.2005 27.09.2011
2	Danube River	Novo Selo (rkm 833); 27 m a.s.l.	N 44.163083 E 22.787778	FP49	27.09.2011
3	Danube River	Florentin (rkm 827)		FP48	27.09.2011
4	Danube River	Yasen (rkm 825); 36 m a.s.l.	N 44.122889 E 22.879	FP58	05.10.2005 27.09.2011
5	Danube River	Gomotartsi (rkm 817)	N 44.094736 E 22.971275	FP58	12.07.2005 05.10.2005 13.10.2006
6	Danube River	Koshava (rkm 811); 40 m a.s.l.	N 44.07984 E 23.030391	FP68	12.07.2005 05.10.2005 11.10.2006 03.09.2012
7	Danube River	Koshava Island (rkm 807); 35 m a.s.l.	N 44.058583 E 23.038	FP68	18.12.2001 08.2003 28.09.2011
8	Danube River	Vidin upstream (rkm 796); 38 m a.s.l.	N 44.010556 E 22.949167	FP57	03.09.2012
9	Danube River	Simeonovo upstream, Islands Golyam Bliznak and Malak Bliznak (rkm 779-777)		FP45	28.10.2012
10	Danube River	Simeonovo (rkm 776)	N 43.852084 E 22.8449	FP45	06.10.2005 14.10.2006 26.09.2011
11	Danube River	Botevo (rkm 774)	N 43.836736 E 22.86323	FP55	26.09.2011
12	Danube River	Ratsiaria (rkm 772)	N 43.827453 E 22.890465	FP55	13.11.2010 26.09.2011
13	Danube River	Orsoya (rkm 760-755)		FP65	30-31.07.2012
14	Danube River	Lom upstream (rkm 744); 34 m a.s.l.	N 43.823889 E 23.195	FP75	04.09.2012
15	Danube River	Dolno Linevo (rkm 731-730)		FP85	05.09.2012
16	Danube River	Dolni Tsibar (rkm 717); 31 m a.s.l.	N 43.819722 E 23.518056	GP05	05.09.2012 01.11.2012
17	Danube River	Kozlodui upstream (rkm 704)	N 43.789352 E 23.635175	GP15	05.09.2012
18	Danube River	Oryahovo (rkm 679)	N 43.742449 E 23.952754	KJ54	23.09.2003 06.09.2012
19	Danube River	Ostrov village upstream (rkm 666)	N 43.69734 E 24.10578	KJ64	15.06.2012 (I. Botev)
20	Danube River	Baikal (rkm 641)	N 43.712809 E 24.407013	KJ94	10.08.2006 24.06.2012 (I. Botev)
21	Danube River	Zagrazhden (rkm 630-623); 25-21 m a.s.l.		LJ04	15-16.05.2012 07.09.2012
22	Danube River	Dabovan (rkm 620)	N 43.735011 E 24.624985	LJ04	15.05.2012

Table 2. Continued

№	River, River Basin	Locality; River kilometer (rkm); Altitude (m a.s.l.)	Geographic Coordinates	UTM	Date of finding
23	Danube River	Gulyantsi (rkm 611); 20 m a.s.l.	N 43.683056 E 24.723056	LJ13	15.05.2012
24	Danube River	Somovit downstream (rkm 605)	N 43.703778 E 24.796336	LJ24	07.09.2012
25	Danube River	Nikopol (rkm 599-597)		LJ34	07.2001 07.09.2012
26	Danube River	Belene - Hisarlaka (rkm 577); 16 m a.s.l.	N 43.681667 E 25.101667	LJ53	08.09.2012
27	Danube River	Svishtov downstream (rkm 550)	N 43.615506 E 25.401447	LJ63	08.09.2012
28	Danube River	Vardim (rkm 546)	N 43.624893 E 25.451632	LJ73	23.11.2005
29	Danube River	Batin (rkm 526); 10 m a.s.l.	N 43.672109 E 25.687237	LJ93	11.11.2011 09.09.2012
30	Danube River	Stalpishte (rkm 516)	N 43.719241 E 25.797465	MJ04	9.10.2005 11.11.2011
31	Danube River	Ruse, Prista (rkm 502), 10 m a.s.l.	N 43.816944 E 25.916111	MJ15	10.09.2012
32	Danube River	Ruse, the Yacht Club (rkm 496); 17 m a.s.l.	N 43.84475 E 25.942806	MJ15	26.11.2008
33	Danube River	Ruse, near Riga hotel (rkm 495)	N 43.853268 E 25.950029	MJ15	9.10.2005
34	Danube River	Sandrovo (rkm 477)	N 43.951703 E 26.108317	MJ26	4.09.2005 9.10.2005 12.10.2005
35	Danube River	Ryahovo (rkm 466)	N 43.993737 E 26.239257	MJ37	17.04.2010
36	Danube River	Tutrakan (rkm 432)	N 44.053552 E 26.618135	MJ67	15.04.2010
37	Danube River	Malak Preslavets (rkm 414)	N 44.10028 E 26.83078	MJ88	13.06.2012 (I. Botev)
38	Danube River	Vetren (rkm 395)	N 44.140557 E 27.031952	MJ08	*15.06.2001 (Hubenov 2001) 10.10.2005 13.10.2012
39	Vidbol River	River mouth, 31 m a.s.l.	N 43.898056 E 22.837222	FP46	16.10.2012
40	Vidbol River	Dunavtsi, 45 m a.s.l.	N 43.902778 E 22.805278	FP46	16.10.2012
41	Tsibritsa River	River mouth	N 43.814239 E 23.526503	GP05	01.11.2012
42	Tsibritsa River	Gorni Tsibar (rkm 3)	N 43.797588 E 23.501923	GP05	01.11.2012
43	Tsibritsa River	Razgrad (rkm 9)	N 43.74937 E 23.487461	GP04	02.11.2012
44	Tsibritsa River	Valchedram (about rkm 15)	N 43.687495 E 23.448713	FP93	10.09.2009 (S. Cheshmedjiev) 02.11.2012

Table 2. Continued

№	River, River Basin	Locality; River kilometer (rkm); Altitude (m a.s.l.)	Geographic Coordinates	UTM	Date of finding
45	Tsibritsa River	Cherni Vrah (rkm 20)	N 43.652876 E 23.416404	FP93	29.07.2012
46	Ogosta River	Kriva Bara (about rkm 20)	N 43.63485 E 23.72365	GP13	17.06.2012 (I. Botev)
47	Ogosta River	Portitovtsi (about rkm 70)	N 43.489109 E 23.357277	FP91	28.07.2012
48	Skat River	Confluence with Ogosta River	N 43.723333 E 23.8625	GP34	25.08.2009 (S. Cheshmedjiev)
49	Skat River	Mizia upstream	N 43.663339 E 23.853035	KJ94	04.11.2011
50	Iskar River	Gigen (about rkm 4); 25 m a.s.l.	N 43.70225 E 24.455444	KJ93	17.08.2004 20.11.2004 19.08.2011 16.05.2012
51	Iskar River	Iskar Village (about rkm 8); 41 m a.s.l.	N 43.668333 E 24.441944	KJ82	16.05.2012
52	Iskar River	Orehovitsa (about rkm 20); 34 m a.s.l.	N 43.585444 E 24.358667	KJ72	20.11.2004 19.08.2011
53	Iskar River	Staroseltsi (about rkm 30); 44 m a.s.l.	N 43.550389 E 24.275667	KJ71	19.08.2011
54	Iskar River	Iskar (Pelovo, about rkm 50); 63 m a.s.l.	N 43.455028 E 24.229667	KJ60	19.08.2011
55	Iskar River	Koinare (about rkm 70); 72 m a.s.l.	N 43.351333 E 24.146861	KJ60	18.08.2011
56	Iskar River	Chomakovtsi (about rkm 80); 92 m a.s.l.	N 43.321333 E 24.068083	FN93	18.08.2011 16.05.2012
57	Iskar River basin	Negovan Lake; 525 m a.s.l.	N 42.760889 E 23.413917	GP34	02.12.2010 20.11.2011
58	Vit River	Gulyantsi (about rkm 5)	N 43.63383 E 24.69727	LJ13	25.06.2012 (I. Botev)
59	Vit River	Kreta (about rkm 10); 24 m a.s.l.	N 43.603056 E 24.673333	LJ13	06.09.2005
60	Vit River	Riben (about rkm 20); 45 m a.s.l.	N 43.533611 E 24.615	LJ02	06.09.2005
61	Vit River	Between villages Sadovets and Krushovitsa (about rkm 55); 107 m a.s.l.	N 43.317222 E 24.397222	KH89	18.09.2012 (A. Popov)
62	Vit River basin	Valchovets Reservoir	N 43.474333 E 24.49463	KJ91	*04.10.2010 (Y. Vidinova)
63	Vit River basin	Gorni Dabnik Reservoir; 167 m a.s.l.	N 43.351028 E 24.338889	KJ80	11.09.2011 04.11.2011 13.05.2012
64	Osam River	Muselievo (about rkm 8); 40 m a.s.l.	N 43.628333 E 24.850278	KJ23	05.09.2005
65	Osam River	Kozar Belene (about rkm 60)	N 43.401667 E 25.153611	LJ50	26.08.2009
66	Yantra River	River mouth	N 43.641138 E 25.573125	LJ83	03.08.2007
67	Yantra River	Krivina (about rkm 6)	N 43.61365 E 25.594003	LJ83	03.08.2007 10.11.2011

Table 2. Continued

№	River, River Basin	Locality; River kilometer (rkm); Altitude (m a.s.l.)	Geographic Coordinates	UTM	Date of finding
68	Yantra River	Beltsov (about rkm 15)	N 43.563368 E 25.642369	LJ92	03.08.2007
69	Yantra River	Dolna Studena (about rkm 20); 31 m a.s.l.	N 43.52451 E 25.70974	LJ92	26.06.2012 (I. Botev)
70	Yantra River	Byala (about rkm 30)	N 43.470191 E 25.725582	LJ91	03.08.2007 01.05.2010 26.06.2012 (I. Botev)
71	Yantra River	Radanovo; (about rkm 50); 39 m a.s.l.	N 43.35463 E 25.63560	LJ80	26.06.2012 (I. Botev)
72	Studena River (Yantra River basin)	Karamanovo downstream; 11 m a.s.l.	N 43.576326 E 25.521026	LJ82	10.11.2011
73	Krapinets River (Yantra River basin)	Stefan Karadzha; 82 m a.s.l.	N 43.903583 E 26.744361	MJ76	*16.04.2010
74	Rusenski Lom River	Sredna Kula District, Ruse (rkm 6)	N 43.806142 E 25.927329	MJ15	10.08.2012

same year, one specimen was found at Nikopol (rkm 597); while in December, a population density from 0.5 to 4 ind./m^2 was estimated at the Koshava island (rkm 807) (Table 2). Empty shells of the clam were present along the entire coast of the island. The specimens were of small sizes. These first records showed that the species was present in the entire Bulgarian stretch of the Danube River and even though the density was low, it had already established. Most likely the species appeared 3-5 years before this period, as it was already recorded upstream and downstream along the Bulgarian section: in 1997, in the Romanian-Serbian section of the Danube, at Berzasca (rkm 1015) and Moldova Nouã (rkm 1050) (Skolka, Gomoiu 2001), and in 1999, in the Romanian section in the vicinity of Vadu Oii (rkm 238) (Bij de Vaate, Hulea 2000).

The first record of the species in the inland waters of Bulgaria was in August 2004 in the Iskar River at Gigen village (around 4 km from the confluence with the Danube River) with a density of 225 ind./m^2. In November of the same year it was also found at Orehovitsa village (20 km from the confluence with the Danube River). In 2011 the clam was already recorded in the Iskar River at the villages Staroseltsi, Koinare and Chomakovtsi (30, 70 and 80 km from the confluence with the Danube River respectively) (Table 2). If we accept provisionally 2001 (first record in the Bulgarian section of the Danube River) as the beginning of the invasion of the Danube tributaries, it can be estimated that for a 10 year period the clam extended its range in an upstream direction in the Iskar River with an average rate of 8 km per year. For other larger tributaries the average dispersal rate in the upstream direction ranged from 5 km per year (Vit and Yantra rivers) to 7.5 km per year (Osam River). The rate of spread of aquatic exotic species may be accelerated or slowed by various human activities (Karatayev *et al*. 2007). For example, a very high upstream dispersal rate was estimated for *C. fluminea* in the Rhine River, reaching an average of 63 km per year with a maximum of up to 276 km per year, which was probably due to human mediated transport as a result of intensive shipping activities (Leuven *et al*. 2009). A much lower rate of upstream movement of the species was reported in the Elbe River in the Czech Republic – with at least 2.4 km per year (Beran 2006).

In the Bulgarian section of the Danube River, density of *C. fluminea* populations varied within a wide range: from less than 1 ind./m^2 to 16 560 ind./m^2. At Zagrazhden, the high density was formed mostly by juveniles and small individuals (with an average shell length from 2.5 mm to 16 mm). This high abundance was not evenly distributed along the 7 km section sampled (from rkm 630 to rkm 623, Table 2); at some sites within this section the abundance was much lower. Most likely at that particu-

Fig. 5. Massive amounts of *Corbicula fluminea* shell deposits on the shore of the Danube River near Zagrazhden (September 2012). (Photo: T. Trichkova)

lar site at Zagrazhden, the clams were deposited by water current. There were also massive amounts of shell deposits on the shore (Fig. 5). In the Danube River a relatively high density (5000 ind./m^2) was also reported for juveniles in the Vén-Duna side arm in Hungary (rkm 1480-1483) (Csányi 1998-1999). In the Ukrainian section of the river (rkm 39) a maximum density of 1680 ind./m^2 was estimated in 2004 (Lyashenko, Makovskii 2011). In other European rivers, the maximum densities estimated reached: 1500 ind./m^2 in the Upper Rhine River (2003-2005) (Bernauer, Jansen 2006); 2120 ind./m^2 in the Mero River basin (Calicia – Northwest on the Iberian Peninsula) (Lois 2010); up to 2500 ind./m^2 in the Chet River, Norfolk (UK), where the population was also dominated by small individuals (2 to 9 mm in length) (Aldridge, Müller 2001); and 9636 ind./m^2 in the Barrow River in Ireland (Caffrey *et al*. 2011). The highest density reported in the United States was 131 000 ind./m^2, documented in California (in Beran 2006).

Some authors reported that the species can achieve high abundance in the first years of an invasion and then generally undergo a decline (Phelps 1994). In our study, this may be the case with the population at the Koshava Island. On a larger scale however, comparatively high abundance was maintained in the Bulgarian Danube section at some sites, especially downstream sites, throughout the 11 year period. The same was reported for the Minho estuary, Portugal, where the *C. fluminea* population persisted in high abundance and biomass for more than 15 years and there was no sign of decline (Sousa *et al*. 2008b,c). Spatial and temporal variations in quantitative parameters can also be influenced by sampling season and methods including different abiotic factors (Sousa *et al*. 2008b). An important factor is the substrate type (Lucy 2012). The preferred substrate of *C. fluminea* is sand mixed with silt and clay (Sousa *et al*. 2008a, Lucy 2012). In our study, at the Danube sites, density was the highest in coarse sand and sand with gravel. At Zagrazhden, where the highest density of juvenile specimens was estimated, the substrate was coarse sand mixed with clam shells (Fig. 5). In the Danube tributaries with a sandy substrate (e.g. Tsibritsa River), the population density was much higher compared to other tributaries. In the lake and reservoirs, where the substrate was sand and gravel mixed with clay and mud, the population density was lower than in the rivers.

Specimens of large sized clams (length >30 mm) were commonly found in the Bulgarian Danube during 2010-2012 in the section from Dolno Linevo (rkm 731) to Tutrakan (rkm 432). The largest specimen measured was collected at Batin in 2011, it has a length of 40.79 mm, height of 37.79 mm and width of 24.32 mm (Fig. 4). Similar maximum shell length (41 mm) was reported for the Tagus River estuary in Portugal at the beginning of the species invasion in Europe (Mouthon 1981). The maximum sizes reported for native populations were: length around 37 mm, height up to 37 mm and width of 25 mm (Zhadin 1952). In the Elbe River, the largest clam found had a length of 36 mm (respectively 36 x 33 x 23 mm) and it was reported as unusual (Beran 2006). Exceptionally large specimens of *C. fluminea* with lengths of 50-65 mm were reported in the United States (Hall 1984 in Beran 2006).

Different vectors facilitate the spread of *C. fluminea* – natural spread (downstream transport) and spread associated with human activities, such as sport fishing, fish socking and aquaculture, transport of sand and gravel, commercial shipping, aquarium trade, introduction as a food item, etc. (reviewed in Lucy *et al*. 2012). Passive upstream movement was also reported as a possible dispersal mechanism in the case of *C. fluminea – e.g.* in the Elbe River in Czech R. (Beran 2006), the Rhine River in Switzerland (Schmidlin, Baur 2007), streams in United States (Voelz *et al*. 1998). It was supposed that the introduction and spread of Asian clams along the Danube River basin was connected with shipping, primarily via exchange of ballast water,

as well as with the introduction of fish species from Asia (Paunović *et al*. 2007). However, the Bulgarian tributaries of the Danube are non-navigable, so the most probable dispersal vector of *C. fluminea* is the passive upstream transport which is probably facilitated by some human activities, such as fishing, recreational activities, sand and gravel extraction and transportation. Similar human activities are most probably responsible for the introduction of the species into the standing water basins – fish stocking in the reservoirs, recreational fishing and use of live bait as well as other recreational activities.

During our study *C. fluminalis* was not found in the Bulgarian section of the Danube River or its tributaries. It can be expected that the species may appear in the Danube, because it has been already reported upstream in Serbia (Paunović *et al.* 2007) and downstream in the Danube Delta in Ukraine (Voloshkevitch, Son 2002, Alexandrov *et al.* 2007, Son 2007). A regular monitoring program for *Corbicula* species is necessary to be included in the river basin management plan for the Danube River basin in Bulgaria. Differences in shell characters in *C. fluminea* and *C. fluminalis* summarized during our study (Table 1, Figs. 1-2) can be used for the identification of the two taxa.

Acknowledgments: The authors would like to thank Eng. Ivan Botev, Dr Alexi Popov, Mr Svetoslav Cheshmedjiev and MsYanka Vidinova for the collected specimens. Special thanks are due to Dr. Abraham Bij de Vaate for the sent material of *C. fluminea* and *C. fluminalis* from the Hollandsch Diep, The Netherlands, and for valuable comments on the manuscript. The authors are also grateful to Dr Asen Ignatov for the preparation of the photos. The study was funded by the Bulgarian Science Fund at the Ministry of Education, Youth and Science within the Project DO-02-283/2008, and co-funded by the International Association for Danube Research (IAD).

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Received: 02.07.2012
Accepted: 05.02.2013