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Oxana Munjiu
Moldova State University

Toderas Ion
Institute of Zoology

ACTA ZOOLOGICA BULGARICA

Acta Zool. Bulg. 72 (4), December 2020: 531-538

Published online 18 December 2020
http://www.acta-zoologica-bulgarica.eu/00SIO_1_01

Special Issue:
ESENIAS and DIAS Scientific Reports 4
Research Article

Distribution and Characteristics of the Invasive Alien Species *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in the Republic of Moldova

Oxana Munjiu*, Igori Șubernetkii, Nadejda Andreev, Nadejda Railean & Ion Toderaș

Institute of Zoology, 1 Academiei Street, 2028 Chișinău, Republic of Moldova; E-mail: oksana.munjiu@gmail.com

Abstract: The invasive alien bivalve species *Sinanodonta woodiana* has been reported for the first time from the Republic of Moldova in 2003. In the study period 2008–2017, this species was recorded at six sampling stations in the Prut River basin: Leova, Goteşti, Cahul, Lake Manta, Lake Beleu, and Cîşliţa-Prut, which basin part belongs to the Wetland Danube River Area of international importance. The most numerous size group was represented by specimens with a shell length of 8–12 cm. The highest density of *S. woodiana* was 40 ind./m^2, while the highest biomass was 750 g/m^2. The largest measured shell had a length of 240 mm. The smallest mature specimen had a shell length of 55 mm. Our results show that this mussel species has successfully established population in the R. Moldova, spreading upstream in the Prut River basin with estimated rate of 15 km per year. We discuss certain competitive advantages of *S. woodiana*, such as phenotypic plasticity, regeneration capacity, early maturation, high density and biomass, and tolerance to a wide range of environmental factors, which may pose a high risk to the native unionid bivalves in the Lower Prut River basin.

Key words: Chinese pond mussel, distribution, density, biomass, morphology, Moldova.

Introduction

The Eastern Asiatic freshwater species, the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae), with synonyms: *Symphynota woodiana* Lea, 1834, and *Anodonta magnifica* Lea, 1834 (Bogatov & Sayenko 2002), is the largest European representative of the family Unionidae, with a shell width reaching up to 30 cm (Pou-Rovira et al. 2009). The native area of this species is Eastern Asia, including the basins of the Amur River, the Yangtze River, and the Primorye, as well as the territories of China, Taiwan, Cambodia, Thailand, and Japan (Zhadin 1952, Zatravkin & Bogatov 1987, Kraszewski & Zdanowski 2007). *Sinanodonta woodiana* was firstly described in Canton (Guangzhou), in the Pearl River drainage basin (Bespalaya et al. 2017). According to the DNA barcoding analyses, in spite of the strong differences in the shell shape, only one haplotype of the species has been identified in Europe (Guarneri et al. 2014). This mussel populates various habitats from slowly running rivers to eutrophic ponds (Zhadin 1952).

Sinanodonta woodiana has a fragile shell of irregular rhombic shape with clear growth lines and variable colour, from green to brown or blackish, sometimes with a greenish hue. The umbo sculpture has strong concentric wavelike folds (Zhadin 1952, Yurishinets & Korniushin 2001). The species

*Corresponding author

becomes sexually mature in the first year of life, when the shell length reaches 3–4 cm. It grows rapidly and can reach 10 cm in lenght during the 2–4 years of life. The average life span is 12–14 years. Up to 3% of the individuals are hermaphrodite. The males produce mature gonads throughout the year, however, the females come into reproductive stage only during the spring. There is no sexual dimorphism of the shell form (Dudgeon & Morton 1983). The parasite phase of the life cycle is 5–14 days, depending on the water temperature (Kiss 1995).

Most probably, *S. woodiana* has appeared accidentally in Europe, with the herbivorous cyprinid fish, which hosts the glochidia of this species (Yurishinets & Korniushin 2001). The first specimens of *S. woodiana* in Europe were collected in 1979 in Romanian fish farms (Sárkány-Kiss 1986). Later, this species has spread rapidly in many other European water bodies, thus, being recorded in Hungary in 1980 (Petró 1984), in France in 1989 (Girardi & Ledoux 1989), in Slovakia in 1995 (Košel 1995), in the Czech Republic in 1997 (Beran 1997), in Italy, Austria, and Poland in 1998 (Manganelli et al. 1998, Reischütz 1998, Böhme 1998), in Ukraine in 2001 (Yurishinets & Korniushin 2001), in Germany (Glöer & Zeittler 2005) and Bulgaria (Hubenov 2006) in 2005, in Serbia, Croatia and Sweden in 2006 (Paunovic et al. 2006, von Proschwitz 2006, Lajtner & Crnčan 2011), in Spain in 2009 (Pou-Rovira et al. 2009), and in Montenegro in 2013 (Tomović et al. 2013). The species has appearred also in other parts of the world in such countries as Indonesia, the Dominican Republic, Costa Rica (Watters 1997), as well as in North America (Bogan et al. 2011). It is important to note, that specimens from different countries have been found for the first time in anthropogenically transformed water bodies, such as: fish farms in Romania and Hungary (Sárkány-Kiss 1986), hydropower station cooling ponds, e.g. warm lakes and canals of the Konin Lakes system (Afanasjev et al. 2001), and other heavily modified and artificial habitats (Paunovic et al. 2006).

In the Republic of Moldova, the first two empty shells of *S. woodiana* (with a length of 10.4–14.3 cm and age of 4–7 years) were recorded in the partly anthropogenically transformed water bodies of Lake Manta in 2003 (Munjiu & Shubernetski 2008), while the first living individuals of the same species were recorded in Lake Beleu in 2008 (Munjiu & Shubernetski 2008). Both Manta and Beleu lakes are part of the Prut River basin. Part of the Moldavian water bodies such as the Lower Prut and its lake system belong to the Wetland Danube River Area – the lower part of the so called Southern Invasion Corridor (Galil et al. 2008). One of the factors which has contributed to the spread of this species is the construction of the Giurgiulesti International Free Port in the Danube part of R. Moldova in 2006 (Munjiu & Shubernetski 2010).

In this study we aimed to investigate the distribution and some qualitative and quantitative characteristics of *S. woodiana* on the territory of the Republic of Moldova.

Materials and Methods

Study area

The Prut River is the second largest river in the Republic of Moldova and the most eastern largest left tributary of the Danube River, its confluence being at 174 rkm from the Black Sea. The total length of the Prut River is 963 km, 695 km of which crosses the territory of R. Moldova; its catchment represents about 24% of the territory of the country. The Danube River flows through the territory of R. Moldova over an area of 480 m only.

Lake Beleu is situated in Southern Moldova near the Danube Delta on the territory of the 'Prutul de Jos' Protected Wetland Area (since 1991) and the Lower Prut Lakes Ramsar Site (No 1029; 19,152 ha; N 45°42', E 028°11'). Lake Beleu is a shallow relict lake, approximately 5,000–6,000 years old. It is the largest natural lake of R. Moldova, connected with the Prut River by four natural arms. The lake has high quantity of suspended matter and a muddy bottom (Munjiu et al. 2016). It has a surface area of 6.26–9.50 km^2, an average length of 5 km, a width of 2 km, and a depth range from 0.5 to 3 m depending on hydrological situation in the Danube and Prut rivers (Bejan et al. 2016).

The complex lake system of Manta is situated in the southern part of the R. Moldova near the Danube Delta and consists of three lakes: Badelnic (1,443 km^2), Dracele (2,774 km^2), and Rotunda (2,329 km^2).

Collection and processing of samples

During the investigation period 2008–2017, the sampling was regularly performed on the territory of the Republic of Moldova, in the main rivers Dniester and Prut, with annual, seasonal and monthly frequency.

The sampling was done at 14 sampling stations in the Dniester River basin and at 17 sampling stations in the Prut River basin.

Since *S. woodiana* can burrow deeply into the substrate at depths up to 30–40 cm (Bastin et al. 2014), the sampling was done by hand and other standard methods for collection of benthic invertebrates, such as the Petersen and Ekman grabs with a capture area of 1/40 m^2, nets, bottom scrapers, and a dredge with an area of capture of 8 m^2 (Abakumov 1983, AQEM Consortium 2002). The mussels were gathered also by hand from an area of 1–5 m^2. Near the river banks the samples were collected from a depth of 0.3–1.5 m, while in the lakes from a depth of 0.3–1.0 m. All specimens were collected in the riverbed only in places in which there is slow (0.2–0.7 m/s) or no current, in backwaters and in the lakes with clay soft sediments.

The samples were preserved in 37% formalin or 96% ethanol solutions. For more detailed shell analysis and measurements live mussels were transported to the laboratory and placed in containers with water. The shell dimensions (length, width, and height) were determined by using an optic micrometer for mature glochidia and vernier calipers for large specimens. The weight was measured with an analytical balance ABS 80-4 Kern to 0.0001 g and an ISOLAB balance to 0.01 g precision.

The mussels were determined by standard identification keys for freshwater invertebrates (Zhadin 1952, Tsalolihin 2004). The identification was done using stereomicroscopes МБС-9, upright Jenaval (Zeiss), SteREO Discovery.V8 (Zeiss), and an upright microscope Axio Imager A.2 (Zeiss).

The density and biomass of S. woodiana was estimated and converted into ind./m^2 and g/m^2, respectively. The age of mussels was determined by counting the annual growth rings. Part of the collected mussels, namely 43 live specimens, was used for the morphometric measurements of the shells. For morphometric purposes, the mussels were divided into length classes according to Afanasjev et al. (2001).

The statistical analysis was performed using Statistica V.10, Excel 2007 and 2010 (Microsoft Office) software.

Results

Distribution, density and biomass

The results of our investigation during 2008–2017 revealed 210 live specimens of *S. woodiana* from a total of 296 quantitative and qualitative samples of benthic macroinvertebrates at six sampling stations of the Prut River basin: Leova, Goteşti, Cahul, Lake Manta, Lake Beleu, and Cîşliţa-Prut (Fig. 1, Table 1). Of them 143 specimens were used in the analyses (Table 1).

The highest density and biomass by hand sampling were recorded at Cîşliţa-Prut at the amount of 5 ind./m^2 and 750 g/m^2, respectively. The highest density and biomass by grabs sampling were recorded at Lake Beleu, 40 ind./m^2 and 720 g/m^2, respectively. The highest mean density and biomass were detected at the sampling point of Cîşliţa-Prut, 1.92±1.24 ind./m^2 and 214.20 ±70.96 g/m^2, respectively (Table 1).

Fig. 1. Map of water basins of the Republic of Moldova and sampling stations where *Sinanodonta woodiana* was recorded during 2008–2017 in the Prut River basin (marked). From North to South: L: Leova, G: Goteşti, C: Cahul, M: Lake Manta, B: Lake Beleu, and C-P: C*î*şliţa-Prut.

Considering the first registered live specimen of *S. woodiana* in Lake Beleu (Munjiu & Shubernetski 2008) in 2008 and the found empty shells in 2015 and living specimens in 2016 at the Leova sampling station, as well as knowing the distance of 100 km from Lake Beleu to Leova, it was estimated that the rate of spreading of this invasive alien species on the territory of R. Moldova is approximately 15 km per year. However, additional field data are required to prove this estimation.

Shell size variation

According to the results obtained, the length of the shells ranged from 3.60 cm to 18.70 for the living specimens and up to 24.00 cm for the largest empty shell. The most numerous groups were IInd and IIIrd classes (of small and medium size), consisting of 12 and 24 specimens, respectively. The IVth class had

Table 1. Locations of *Sinanodonta woodiana* with mean density and biomass in the Prut River basin during 2008–2017 on the territory of the Republic of Moldova.

Location	Latitude/ Longitude	Date of the first record	Number of specimens	Number of samples	Density Mean ± std ind./m²	Biomass Mean±std g/m²
Leova	46˚28'13"/ 28˚14'44"	23.03.2016	1	64	–	–
Goteşti	46˚10'37"/ 28˚8'39"	30.05.2014	1	31	–	–
Cahul	45˚55'16"/ 28˚7'33"	10.08.2011	16	82	1.55 ± 1.30	104.45 ± 41.51
Cîşliţa-Prut	45˚32'57"/ 28˚9'60"	5.11.2010	52	82	1.92 ± 1.24	214.20 ±70.96
Lake Manta	45°49'52"/ 28°10'12"	2008	25	15	0.74 ± 0.58	101.83 ± 71.94
Lake Beleu	45˚35'9"/ 28˚8'53"	2008	48	22	0.83 ± 0.50	124.53 ± 88.25

Table 2. Morphometric measurements of the shells (cm) of *Sinanodonta woodiana* collected from the different sampling stations of the Prut River basin during 2008–2017 on the territory of the Republic of Moldova. N – number of specimens measured; L – total length; H – wing height (the line of the most distant points); W – width; Min – minimal value; Max – maximal value; Mean – mean value; and SD – standard deviation.

Groups	N=43	L (cm)	H (cm)	W (cm)	Ratio L/H	Ratio L/W
Class I Min – Max	2	3.60–4.60	3.00–3.60	1.50–1.60	1.20–1.28	2.40–2.88
Class II Mean±SD	12	8.02±1.45	5.44±0.93	3.07±0.65	1.49±0.24	2.65±0.26
Min – Max		5.70–9.80	4.20–6.90	1.80–3.80	1.23–1.96	2.22–3.17
Class III Mean±SD	24	12.08±1.37	7.99±0.96	4.49±0.68	1.52±0.09	2.72±0.33
Min – Max		10.40–14.60	7.00–10.50	3.10–6.40	1.33–1.66	2.08–3.55
Class IV Mean±SD	5	17.68±1.23	11.10±1.13	6.94±1.67	1.60±0.09	2.63±0.47
Min – Max		16.60–19.30	9.60–12.20	5.20–9.20	1.50–1.73	2.03–3.19

five specimens, while the Ist class was represented only by two specimens (Table 2). The largest empty shell we found, which also is the largest shell ever found in the R. Moldova, had a length of 24.00 cm, height of 16.20 cm, and a width of 9.20 cm. The largest living specimen recorded had a weight of 610 g and shell dimensions of 18.70 x 12.20 x 9.20 cm, while the smallest one was only 14 g, and had dimensions of 3.60 x 3.00 x 1.50 cm, respectively; both specimens were found in Lake Beleu.

The analysis of the morphometric measurements showed that the wing height (H) fluctuated between 3.00 cm and 16.20 cm. The ratio of the total length and wing height L/H reflects the more rounded or more elongated shape of the shells. In our study this ratio varied between 1.20 and 1.96, while the L/W ratio varied from 2.03 to 3.55 (Table 2).

All young specimens of *S. woodiana* had rounded shells (Fig. 2a), while the adult specimens had both rounded and elongated shells, with prevalence of the latter (Fig. 2b). Despite of the shell shape or length classes, the specific umbo sculpture was kept in all specimens. The young specimens with flattened, fragile shells also had a specific clear umbo sculpture (Fig 2c).

Two specimens of different phenotypic form were found in Lake Beleu. They had very strong shells, in contrast with the fragile shells of the majority of *S. woodiana.*

Two mature individuals with glochidia in gills (Fig. 3a) were recorded in Lake Beleu on 27 April 2011, including the smallest mature specimen with a length of 5.5 cm and a weight of 18 g. Later, in May 2014, a mature specimen (length of 13.5 cm, weight of 228 g) with glochidia in gills was found also at the Goteşti sampling station.

We observed a high capacity of *S. woodiana* to regenerate its damaged shells. The species not only

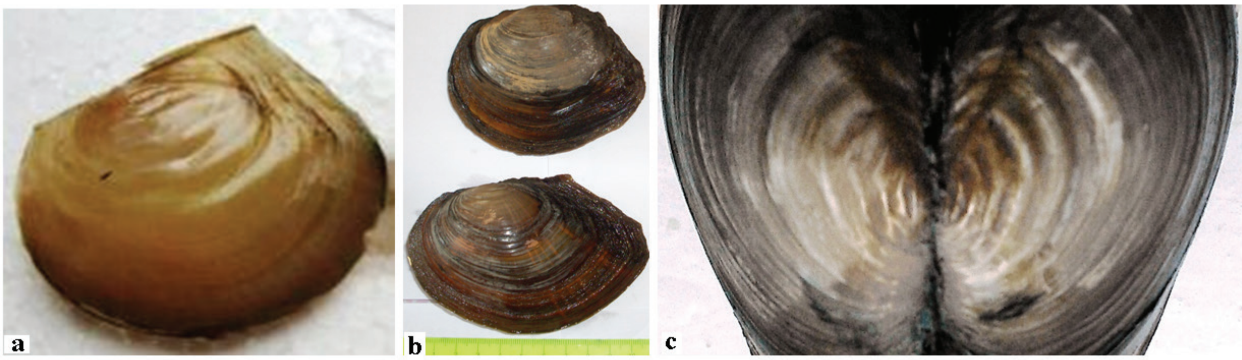

Fig. 2. Morphological characteristics of *Sinanodonta woodiana*: 2a) the smallest specimen recorded (L=3.60 cm); 2b) elongated and rounded shell forms of adult specimens; 2c) umbo sculpture with concentric wavelike folds (Photos: Oxana Munjiu).

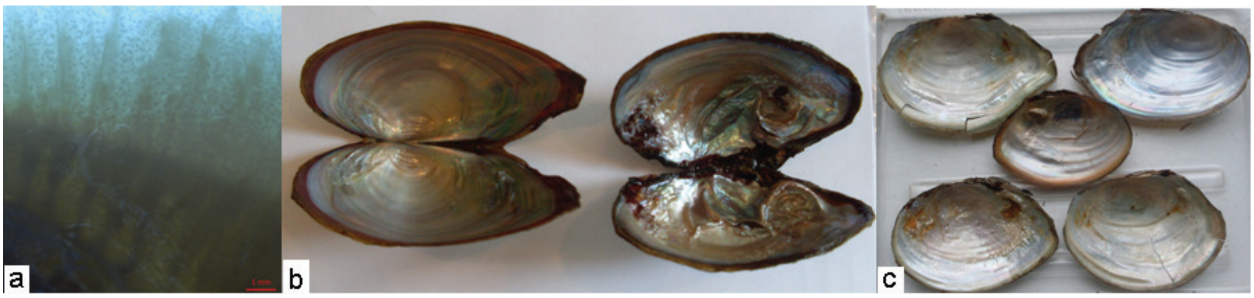

Fig. 3. *Sinanodonta woodiana* findings in the Prut River basin: 3a) a specimen with glochidia in gills; 3b) normal shell and regenerated damaged shell; 3c) shells with teratogenic features from Lake Beleu (Photos: Oxana Munjiu).

recovered its shells but also increased its weight with approximately 50% or up to 25.57 g in healthy shells and 59.12 g in damaged shells (Fig. 3b). More than 40% of the *S. woodiana* specimens collected near the shore in Lake Beleu had teratogenic features (Fig. 3c).

Discussion

Invasive species have many competitive advantages that contribute to their establishment and active spread in a new region (Alimov & Bogutskaya 2004). Some of the advantages of *S. woodiana* are their phenotypic plasticity and regeneration capacity. The large phenotypic variability of *S. woodiana,* such as rounded and elongated shells and shells with teratogenic features collected in the Prut River basin, reflects the capacity of this mollusc to adapt to various environmental conditions in its habitats. Some researchers report that river specimens have more rounded shells than lake specimens (Guarneri et al. 2014). However, in our study such relation was not observed, probably due to the fact that Lake Manta and Lake Beleu have areas with slow-flowing current, with conditions similar to the Prut River. Furthermore, our results showed that over 40% of the *S. woodiana* specimens collected near the lake shore in Lake Beleu had teratogenic features. One of the reasons may be that local people use the lake shore as a pasture for cows. The animals are damaging the shells of *S. woodiana* but the species can recover the damaged shell and survive. The high regeneration capacity of S. *woodiana* in its native area in China is used for economic purposes to produce pearls (Rahayu et al. 2013). In areas as the lower sector of the Prut River, *S. woodiana* may threaten the native population of bivalves where there is a big native molluscan diversity (Munjiu et al. 2016) without a high regeneration capacity.

Another competitive advantages of *S.woodiana* are its capacity to reproduce 2–3 times per year, while compared to the native species which reproduce only once per year, and also to reach its sexual maturity already in the first year of life (Dudgeon & Morton 1983). The early maturation or reproducing females with small sizes was observed in the smallest mature specimens with length of 5.5 cm from Lake Beleu, and it can be comparable with the growth rate in its native region (Dudgeon & Morton 1983) and even in the European cooling water (Labecka & Domagala 2018). However, it is important to mention that Lake Beleu is a natural lake without any influence of a hydropower station.

Sinanodonta woodiana reaches large density and biomass, being more competitive as resources, than the native species. In a previous study (2012–2013), the invasive mussels *Corbicula fluminea* (Muller, 1774) and *S. woodiana* have constituted a significant proportion of macrobenthos in terms of density and biomass at the Cîşliţa-Prut station (Munjiu et al. 2014). Thus, *C. fluminea* has formed about 40% (200 ind./m^2) of the total density of molluscs, while *S. woodiana* has formed more than 70% (260 g/m^2) of the total biomass of malacofauna (Munjiu et al. 2014). In the Cris River in Romania, *S. woodiana* represents 75% of the unionid biomass (Popa & Popa 2006). The density and biomass of *S. woodiana* at the adjacent territoty of the Ukrainian Danube Delta are 0.5–5 ind./m^2 and 61–910 g/m^2, respectively (Pavlyuchenko et al. 2007), and 20–30 ind./m^2 (Son 2007). These results are similar to the outcomes of our study at the territory of the R. Moldova: density of 1–40 ind./m^2 and biomass of 130–750 g/m^2.

Along with the competitive advantages mentioned above, it shall be also taken into consideration that *S.woodiana* is more tolerant to pollution than the native Unionidae (Sîrbu et al. 2005), as well as to a wide range of environmental factors, such as eutrophication or low trophicity, and temperature disturbance (Corsi et al. 2007, Beran 2008, Demayo et al. 2012, Benko-Kiss et al. 2013). The ecosystem of the Low Prut River is affected by the aridisation; the average annual temperature of the water has increased up to 11.1°C, compared to 10.2°C during the period 1989–1999 (Bejan et al. 2016). Such climate conditions could be favourable for the spread of *S. woodiana*, which prefers the temperature range of 10–30°C (Kraszewski & Zdanowski 2007). In Poland, the maximum density and biomass of *S. woodiana* population has reached 60 ind./m^2 and 25 kg/m^2, respectively, in the heated lakes and channels of the Konin Lake system (Kraszewski & Zdanowski 2007).

Based on the analysis of data on the spread of *S. woodiana*, we assume two possible pathways of introduction of this species in the R. Moldova. The first one is an introduction of glochidia with fingerlings of herbivorous fish imported directly from the native region in the 1960–1970s. About one hundred thousand fingerlings of herbivorous fish were imported in the R. Moldova from China in 1961, and from the Amur River basin in 1972–1974. These fingerlings were released in the Dniester and Prut river basins, including Lake Manta and Costeşti-Stînca Reservoir, with the aim to improve fisheries productivity and regulate the aquatic vegetation (Lobchenko 1999). The second possible pathway is an introduction of glochidia of *S. woodiana* with fish hosts from the Danube Delta originating from the Romanian and/or Ukrainian territory. This invasive mussel was recorded in 1998 in Romania (Popa & Popa 2006) and one year later in Ukraine (Yurishinets & Korniushin 2001).

Although the herbivorous fish, possible hosts of glochidia, were introduced in the past from the Eastern Asia to the Dniester and Prut river basins (Lobchenko 1999), our results show that a stable population of *S. woodiana* has been formed only in the lower Prut River basin. This is most likely due to the specific hydrological regime of the Prut River basin, namely the water intrusion from the Danube River, and the hydromorphological conditions as the shallow lakes with silty-clay substrate and water temperatures up to 32^{0}C, which are suitable habitats of *S. woodiana*.

Acknowledgements: This study was conducted within the following projects: 09.832.08.07A,11.817.08.13F, 11.817.08.15A, MIS ETC 1150, MIS ETC 1676, 15.817.02.27 A. The authors would like to thank Bulat Dumitru and Bulat Denis from the Institute of Zoology for participation in the sampling process. The authors also acknowledge Buşmachiu Galina for the scientific support, and Jechiu I. from the Institute of Ecology and Geography of ASM for preparation of the map. Many thanks to Teodora Trichkova for editions and suggestions given to improve the manuscript.

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