J. Limnol., 2014; 73(3): 454-458
DOI: 10.4081/jlimnol.2014.938

ORIGINAL ARTICLE

Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia): origin of the Polish population and GenBank data

Marianna SOROKA,[1] Maria URBAŃSKA,[2*] Wojciech ANDRZEJEWSKI[3]

[1]Department of Genetics, University of Szczecin, Felczaka 3c, 71-412 Szczecin; [2]Department of Zoology, Institute of Zoology, Poznań University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań; [3]Department of Division of Inland Fisheries and Aquaculture, Institute of Zoology, Poznań University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland
*Corresponding author: urbanska@up.poznan.pl

ABSTRACT

DNA sequences of the mitochondrial cox1 *gene were analysed in 4 new local populations of* Sinanodonta woodiana *(Bivalvia) in Poland, in the first reported Polish population, and in a population from Hungary. The gene sequences of Polish specimens were identical to those of specimens from Hungary, Italy and Ukraine, but different from those of Romanian specimens (data from GenBank). According to fish farm documentation,* S. woodiana *had spread in Poland by 2 routes: i) direct import of fish infected by glochidia of* S. woodiana *from Hungary; and ii) indirectly, by the major distributor of thermophilous fish in Poland, Gosławice Fish Farm, which started to import Hungarian fish in the 1960s. The genetic analysis and available documentation unambiguously confirm that Polish populations of* S. woodiana *derive from a source population in Hungary. In addition, we noticed doubtful identification of this species in GenBank data and further research is needed to resolve this problem.*

Key words: Bivalves, freshwater mussels, invasive species, Chinese pond mussel, mitochondrial DNA, cox1.

Received: February 2014. Accepted: April 2014.

INTRODUCTION

Knowledge of the origin of the Chinese pond mussel *Sinanodonta woodiana*, recorded in Poland since the 1990s, is based on import data about exotic fish, which most probably carried its glochidia and in this way introduced this bivalve species to Polish waters, giving rise to numerous local populations (Protasov *et al.*, 1993; Kraszewski and Zdanowski, 2001). This has led to the hypothesis that populations of *S. woodiana* appeared in Polish waters as a result of fish import from Hungary, mostly cyprinid fish species, such as bighead carp (*Aristichthys nobilis,* Richardson, 1845) and silver carp (*Hypophthalmichthys molitrix,* Valenciennes, 1844). However, there was no available published research to confirm this hypothesis.

The first genetic studies of *S. woodiana*, from warm water bodies, show a low genetic variation between specimens of the first Polish population (Soroka and Zdanowski, 2001; Soroka, 2005). In case of some water bodies with natural water temperature, which are used as fish ponds, documentation shows that the imported fry originated from Gosławice Fish Farm, belonging to the Konin heated lakes system. Gosławice Fish Farm bought earlier the fish in Hungary. In other places where the mussel *S. woodiana* is found, populations may be of different origin, as this species could have invaded Poland by many routes.

The aim of this study was to determine if the individuals of *S. woodiana* found in various localities in Poland were derived from a single source population in Hungary, which would be consistent with documentation of fish import to Poland, or there exist some different, unknown and undocumented sources of introduction of this bivalve species to Poland. The genetic analysis of several Polish and Hungarian specimens allowed also an assessment of the mode and routes of its invasion into Poland. Additionally, the obtained mitochondrial DNA sequences were compared with available GenBank data for this species. Moreover, we examined the documentation of fry origin in the fish farms where material for genetic research was collected.

METHODS

Detailed characteristics of the Polish localities, *i.e.* ponds Urocze, Wojnowice, Polna Rzeka, and Oko, as well as Gosławice Fish Farm, were presented in earlier works (Soroka and Zdanowski, 2001; Urbańska *et al*., 2012; Andrzejewski *et al.*, 2013). For this study, specimens of *S. woodiana* were collected from 4 new localities (fish ponds in various parts of Poland), and compared with specimens from Gosławice Fish Farm (near Konin, Poland) and from the River Danube (south of Budapest, Hungary) (Fig. 1).

Genetic analyses were performed for the 5 Polish populations, each represented by 3 randomly selected individuals, and for 5 individuals from Hungary. Total DNA

was isolated from gills of 20 specimens. The standard phenol/chloroform extraction and ethanol precipitation were used (Skibinski *et al.*, 1994). The PCR reaction was performed with primers LCO1490 and HCO2198 (Folmer *et al*., 1994) for the mitochondrial cytochrome *c* oxidase subunit I (*cox1*) gene. The PCR conditions were as follows: initial denaturation for 2 min at 96°C, followed by 5 cycles of 30 s at 95°C, 1 min at 45°C, 1.5 min at 72°C, followed by 28 cycles of 30 s at 95°C, 45 s at 55°C, 1 min at 72°C, and a final extension for 7 min at 72°C. The total volume of each PCR reaction mixture was 20 µL. After 1% agarose gel electrophoresis, the amplification products were viewed under UV light. The results were saved and the size of the PCR products was analysed with Vision Works®LS Analysis Software (UVP, Cambridge, UK). PCR products were purified using Clean-up columns (A&A Biotechnology, Gdynia, Poland) and cycle-sequenced in both directions (by Macrogen, Seoul, South Korea, http://dna.macrogen.com/eng/). Both DNA strands were assembled into consensus using DNAMAN 5.2.9 software (Lynnon, Quebec, Canada). The haplotypes for the *cox1* gene of the specimens were submitted to GenBank and their accession numbers are: KJ125078 and KJ125079 for Polish and Hungarian speciments, respectively. The *cox1* sequences were aligned using ClustalW program, being a part of MEGA4 software (Tamura *et al*., 2007). This program was also used to calculate sequence divergence (Kimura 2-parameter, K2P, and Maximum Composite Likelihood, MCL, distances). MEGA4 was applied to phylogeny reconstruction with minimum evolution approach (Swofford *et al*., 1996, Nei and Kumar 2000) with K2P and MCL distances.

We also analysed the documentation of the fish farms from which the material for analyses was collected, to identify the sources of introduced fish fry and of the parasitic glochidia of *S. woodiana* encysted on the fry, which gave rise to populations of this accidentally introduced bivalve species.

RESULTS

For 15 individuals of *S. woodiana* from 5 Polish populations, identical haplotypes were obtained for the mitochondrial *cox1* gene fragment, with a mean length of 661 bp. For 5 individuals of this species from the Danube in Hungary, also identical haplotypes were obtained for the analysed *cox1* fragment, with a mean length of 662 bp. Haplotypes from Poland and Hungary showed 100% similarity to sequences of *S. woodiana* from Italy and Ukraine, whereas significant differences were observed (about 6%) in comparison with individuals from Romania (Tab. 1). Blast analyses in GenBank confirmed our observations and showed that the obtained sequences have only 95% sequence identity with *S. woodiana* from Romania and 100% with specimens from Italy and Ukraine.

Fish farm documentation indicates that *S. woodiana* had spread in Poland by 2 routes. In ponds Urocze and Wojnowice, the mussels arrived with fish directly from Hungary. By contrast, in ponds Polna Rzeka and Oko, the mussels arrived with fish indirectly, from Gosławice Fish Farm, which in the 1960s introduced large amounts of thermophilous fish from Hungary (Fig. 1).

DISCUSSION

The Chinese pond mussel, distributed also in Europe, was initially named *Anodonta woodiana* (Petró, 1984; Kiss and Pekli, 1988; Nagel *et al*., 1996; Piechocki and

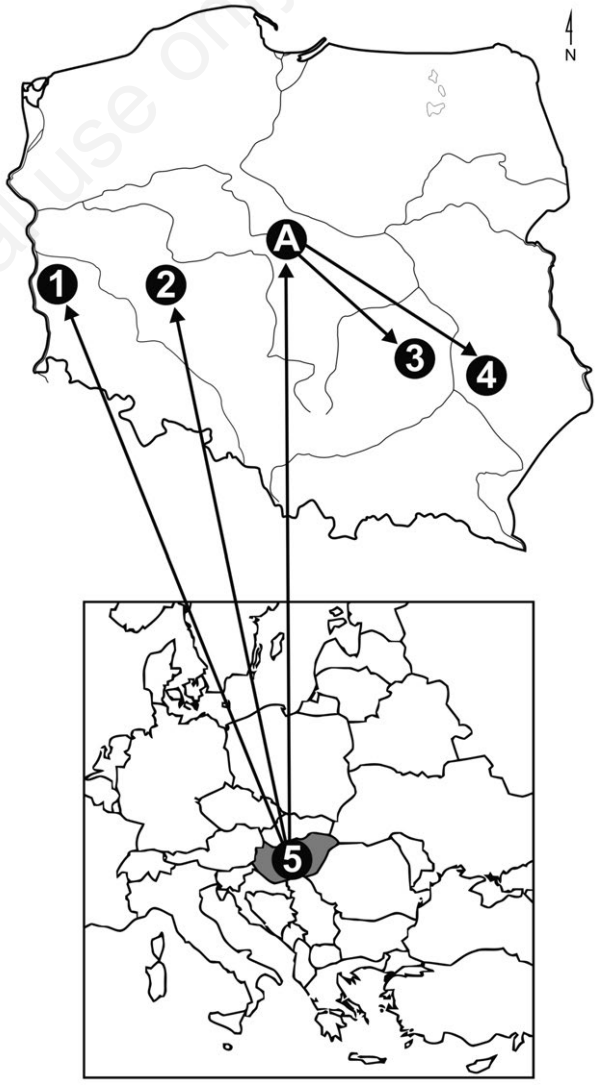

Fig. 1. Directions of spread of *Sinanodonta woodiana* in Poland. Sampling sites in ponds Urocze (1), Wojnowice (2), Polna Rzeka (3), Oko (4), and in the River Danube (5). Gosławice Fish Farm is marked with A.

Riedel, 1997; Watters, 1997; Kraszewski and Zdanowski, 2001; Soroka, 2005, 2008a). Currently it is assigned to the genus *Sinanodonta* Modell, 1945 (Beran, 1997, 2008; Bohme, 1998; Falkner *et al*., 2001; Yurishinets and Korniushin, 2001; Domagała *et al.*, 2007; Gąbka *et al.*, 2007; Popa *et al.*, 2007; Munjiu and Shubernetski, 2008; Cappelletti *et al.*, 2009; Soroka, 2010; Popa *et al.*, 2011). Mitochondrial cytochrome *c* oxidase subunit I gene *cox1* is used most often for species identification and phylogenetic analyses. In over 95% of animal species, characteristic sequences are found within this gene, and its 5'-region, 648 bp long, is used to create the huge Barcode of Life Database (Hebert *et al.*, 2003a, 2003b; Ratnasingham and Hebert, 2007). The international GenBank database includes sequences for the *cox1* gene fragment of mussels identified as *A. woodiana* or *S. woodiana*, showing similarity of 100% to 95%, or even only 91% (using Basic Local Alignment Search Tool, BLAST). Here we use the name *S. woodiana*, and sequences of *A. woodiana* from GenBank should not be treated as a separate species.

So far, studies of European species of Unionidae have revealed a low variation within the *cox1* gene (less than 1%) in *Unio gibbus*, *U. pictorum* and *U. tumidus*, as well as *Anodonta anatina* (Källeresjö *et al*., 2005, Araujo *et al*. 2009, Soroka 2008b, 2010). A higher intraspecific variation of the *cox1* gene was reported for the gastropod *Sadleriana fluminensis* (3%) (Szarowska and Falniowski, 2013) and *Potamilus* species (2.6%) (Roe and Lydeard, 1998). The highest differences (about 7%) were found be-

Tab. 1. Genetic distances computed with K2P (below diagonal) and MCL (above diagonal) between the studied specimens of *S. woodiana* and sequences from GenBank.

		1	2	3	4	5	6	7	8	9
1	Aw EF440349 Poland		0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.183
2	Sw KJ125078 Poland*	0.000		0.000	0.000	0.000	0.000	0.000	0.061	0.183
3	Sw KJ125079 Hungary*	0.000	0.000		0.000	0.000	0.000	0.000	0.061	0.183
4	Sw KF731775 Italy	0.000	0.000	0.000		0.000	0.000	0.000	0.061	0.183
5	Sw KF731776 Italy	0.000	0.000	0.000	0.000		0.000	0.000	0.061	0.183
6	Sw JQ253893 Ukraine	0.000	0.000	0.000	0.000	0.000		0.000	0.061	0.183
7	Sw JQ253894 Ukraine	0.000	0.000	0.000	0.000	0.000	0.000		0.061	0.183
8	Sw JQ435822 Romania	0.060	0.060	0.060	0.060	0.060	0.060	0.060		0.236
9	Aa GU230745	0.182	0.182	0.182	0.182	0.182	0.182	0.182	0.231	

Sw, *Sinanodonta woodiana;* Aw, *Anodonta woodiana;* Aa, *Anodonta anatine;* *novel sequences.

Fig. 2. Minimum-evolution tree computed with Kimura 2-parameter distances. Values above branches represent bootstrap support (2000 replicates). Sw, *Sinanodonta woodiana;* Aw, *Anodonta woodiana;* Aa, *Anodonta anatine;* *novel sequences.

tween syntopic *S. fluminensis* and *S. robici* specimens, which confirms species distinctness of these 2 lineages (Szarowska and Falniowski, 2013). Specimens of *S. woodiana* from 5 analysed Polish populations had identical sequences of the mitochondrial *cox1* gene fragment. They did not differ from those of individuals from other populations in Poland (Soroka, 2010), Hungary, Italy, and Ukraine (Tab. 1, Fig. 2). All the dendrograms showed the same topology of the analysed sequences, so only one of them with higher bootstrap values is presented in Fig. 2. This provides strong evidence that these European local populations may derive from one source population and for specimens in Poland it was Hungarian population, as indicated by both fish farming records and molecular data. By contrast, specimens of *S. woodiana* from Romania show a higher genetic variation (about 6%) as compared to other European populations. Such a high variation may attest to 2 distinct routes of migration of founder individuals of these European populations, and show a high intraspecific variation of this species.

CONCLUSIONS

Earlier research on Polish populations of *S. woodiana* shows significant differences in biometric parameters of individuals from various locations (Soroka and Zdanowski, 2001; Urbańska *et al*., 2012; Andrzejewski *et al*., 2013). The observed phenotypic variation of individuals of *S. woodiana* coming from different Polish ponds seemed to indicate varied provenance of these mussel populations, but molecular data do not confirm that assumption. Their *cox1* gene sequences suggest that all the analyzed Polish specimens derive from Hungarian specimens. The phenotypic variation is probably linked with environmental factors, while the lack of genetic variation of the *cox1* gene sequence between the Hungarian and Polish individuals of *S. woodiana* seems to be a result of the founder effect.

ACKNOWLEDGMENTS

We thank Dr Viktoria Takacs for help in collection of individuals of *S. woodiana* from Hungary.

REFERENCES

Andrzejewski W, Urbańska M, Mazurkiewicz J, Gierszal H, Golski J, 2013. The current invasion status of *Anodonta woodiana* (Lea, 1934) in Poland - study of habitat parameters. Oceanol. Hydrobiol. St. 42:173-180.

Araujo R, Toledo C, Machordom A, 2009. Redescription of *Unio gibbus* Spengler, 1793, a west Palaearctic freshwater mussel with hookless glochidia. Malacologia 51:131-141.

Beran L, 1997. First record of *Sinanodonta woodiana* (Mollusca: Bivalvia) in the Czech Republic. Acta Societatis Zoologicae Bohemicae 61:1-2.

Beran L, 2008. Expansion of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in the Czech Republic. Aquatic Invasions 3:91-94.

Bohme M, 1998. [Ein neuer Fundort der Chinesischen Teichmuschel (*Sinanodonta woodiana*) in Mitteleuropa].[Article in German]. Heldia 2:166.

Cappelletti C, Cianfanelli S, Beltrami ME, Ciutti F, 2009. *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae): a new non-indigenous species in Lake Garda (Italy). Aquatic Invasions 4:685-688.

Domagała J, Łabęcka AM, Migdalska B, Pilecka-Rapacz M, 2007. Colonisation of the channels of Międzyodrze (North-Western Poland) by *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae). Pol. J. Nat. Sci. 22:679-690.

Falkner G, Bank RA, Proschwitz TV, 2001. Check-list of the non-marine Molluscan species-group taxa of the States of Northern, Atlantic and Central Europe (CELECOM I). Heldia 4:1-76.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3:294-299.

Gąbka M, Dolata PT, Antonowicz R, 2007. New localities of the Chinese clam *Sinanodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae) in the Barycz River Valley (Wielkopolska Region). Folia Malacologica 15:71-74.

Hebert PDN, Cywinska A, Ball SL, Dewaard JR, 2003a. Biological identifications through DNA barcodes. P. Roy. Soc. Lond. B Bio 270:313-321.

Hebert PDN, Ratnasingham S, Dewaard JR, 2003b. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. P. Roy. Soc. Lond. B Bio 270:96-99.

Källersjö M, Von Proschwitz T, Lundberg S, Eldenäs P, Erséus C, 2005. Evaluation of ITS rDNA as a complement to mitochondrial gene sequences for phylogenetic studies in freshwater mussels: an example using Unionidae from north-western Europe. Zoologica Scripta 34:415-424.

Kiss Á, Pekli J, 1988. On the growth rate of *Anotonta woodiana* (Lea 1834) (Bivalvia: Unionacea). Bull. Univ. Agric. Sci. Gödöllö 1:119-124.

Kraszewski A, Zdanowski B, 2001. The distribution and abundance of the Chinese mussel *Anodonta woodiana* (Lea, 1834) in the heated Konin lakes. Arch. Pol. Fish. 9:253-265.

Munjiu O, Shubernetski I, 2008. First record of *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) in Moldova. Aquatic Invasions 3:441-442.

Nagel K, Badino G, Alessandria B, 1996. Population genetics of European Anodontinae (Bivalvia: Unionidae). Journal of Mollusca Studies 62:343-357.

Nei M, Kumar S, 2000. Molecular evolution and phylogenetics. Oxford University Press: 333 pp.

Petró E, 1984. The occurrence of *Anodonta woodiana woodiana* in Hungary. Állattanu Közl. 71:189-191.

Piechocki A, Riedel A, 1997. Mollusca. In: J. Razowski (ed.), Checklist of the animals of Poland. Instytut Systematyki i Ewolucji Zwierząt PAN, Kraków 4:104-121.

Popa OP, Kelemen BS, Murariu D, Popa LO, 2007. New records of *Sinanodonta woodiana* (Lea, 1834) (Mollusca: Bivalvia: Unionidae) from Eastern Romania. Aquatic Invasions 2:265-267.

Popa OP, Popa LO, Krapal A-M, Murariu D, Iorgu EI, Costache M, 2011. *Sinanodonta woodiana* (Mollusca: Bivalvia: Unionidae): isolation and characterization of the first microsatellite Markers. Int. J. Mol. Sci. 12:5255-5260.

Protasov AA, Afanasjev SA, Zdanowski B, 1993. [Natural self-purification system of the Konin lakes].[Article in Polish]. Komun. Ryb. 6:6-9.

Ratnasingham S, Hebert PDN, 2007. bold: The barcode of Life Data System (http://www.barcodinglife.org). Mol. Ecol. Notes 7:355-364.

Roe KJ, Lydeard C, 1998. Molecular systematics of the freshwater mussel genus *Potamilus* (Bivalvia: Unionidae). Malacologia 39:195-205.

Skibinski DOF, Gallagher C, Beynon CM, 1994. Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. Genetics 138:801-809.

Soroka M, 2005. Genetic variability among freshwater mussel *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) populations recently introduced in Poland. Zool. Sci. 22:1137-1144.

Soroka M, 2008a. Doubly uniparental inheritance of mitochondrial DNA in the freshwater bivalve *Anodonta woodiana* (Bivalvia: Unionidae). Folia Biologica (Krakow) 56:91-95.

Soroka M, 2008b. Identification of gender-associated mitochondrial haplotypes in *Anodonta anatina* (Bivalvia: Unionidae). Folia Malacologica 16:21-26.

Soroka M, 2010. Characteristics of mitochondrial DNA of unionid bivalves (Mollusca: Bivalvia: Unionidae). I. Detection and characteristic of double uniparental inheritance (DUI) of unionid mitochondrial DNA. Folia Malacologica 18:147-188.

Soroka M, Zdanowski B, 2001. Morphological and genetic variability of the population of the population of *Anodonta woodiana* (Lea, 1834) occurring in the heated Konin lakes system. Arch. Pol. Fish. 9:239-252.

Swofford DL, Olsen GJ, Waddell PJ, Hillis DM, 1996. Phylogenetic inference, p. 407-514. In: D.M. Hillis, C. Moritz and B.K. Mable (eds.), Molecular systematics. Sinauer Ass., Inc.

Szarowska M, Falniowski A, 2013. Species distinctness of *Sadleriana robici* (Clessin, 1890) (Gastropoda: Rissooidea). Folia Malacologica 21:127-133.

Tamura K, Dudley J, Nei, M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. 24:1596-1599.

Urbańska M, Łakomy A, Andrzejewski W, Mazurkiewicz J, 2012. The story of one clam. Probably the oldest location of the Chinese pond mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia, Unionidae) in Poland. Int. J. Oceanogr. Hydrobiol. 41:41-45.

Yurishinets VI, Korniushin, AV, 2001. The new species in the fauna of Ukraine *Sinanodonta woodiana* (Bivalvia: Unionidae), its diagnostics and possible ways of introduction. Vestnik Zoologii 35:79-84.

Watters GT, 1997. A synthesis and review of the expanding range of the Asian freshwater mussel *Anodonta woodiana* (Bivalvia: Unionidae). Veliger 40:152-156.