Scientific report

Population genetic history of the *Anodonta (Sinanodonta) woodiana* invasion: expansion pattern across Europe

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Objectives:

1. Study the evolutionary history of *Anodonta (Sinanodonta) woodiana* across its invasion range
   
   1.1. Developing an additional set of high polymorphic microsatellite markers for the invasive species *Anodonta (Sinanodonta) woodiana*
   
   1.2. Collecting *Anodonta (Sinanodonta) woodiana* populations samples from Europe and native area.
   
   1.3. Performing laboratory work on the genetic variability of *Anodonta (Sinanodonta) woodiana* populations
1. Study the evolutionary history of *Anodonta (Sinanodonta) woodiana* across its invasion range

1.1. Developing an additional set of high polymorphic microsatellite markers for the invasive species *Anodonta (Sinanodonta) woodiana*

The freshwater mussel *Anodonta (Sinanodonta) woodiana* (Lea, 1834) (Chinese Huge Mussel or Swan Mussel) (Bivalvia: Unionidae) is the largest unionid species present in the European fauna. Its native range is in East Asia (South-Eastern Russia to Malaysia), but it has spread rapidly across Europe over the last few decades and the species is invasive also in other parts of the world (Bogan et al. 2011; Demayo et al. 2012).

The initial establishment of *A. woodiana* in Europe was enabled by introduction of East Asian cyprinids species (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix*) for aquaculture in 1963–1965. Stocked fish possessed glochidia, the parasitic larval stage of the unionids (Figure 1). Further spread of the species is believed to be correlated with translocations of their native hosts across Europe (Sarkany-Kiss 1984). Trade with the host fish species between different countries and regions enabled *A. woodiana* to penetrate to new regions.

![Figure 1. Glochidia Larvae of Freshwater mussels from Unionidae on Fish Gills By Robert Calentine](image)

Physiological, ecological and biological peculiarities of the species offered it some advantages compared with the native unionid species which can explain its invasive success (Corsi et al. 2007).

Studies of the population genetics of *A. woodiana* in Europe used slowly evolving markers such as allozymes and mitochondrial COI DNA sequences for the analysis of several isolated populations (Nagel et al. 1996; Soroka 2005; Soroka et al. 2014). However, for the recent and rapid spread of the species across Europe, fast evolving markers, such as DNA microsatellites, are
needed to understand important aspects of the population genetics of this invasive species: the route(s) of invasion, the time and number of colonization events, and other details. The first eight microsatellite markers for the species have been described in 2011 (Popa et al. 2011). However, this number is low to infer aspects of the evolutionary history of populations and additional microsatellite loci are needed to increase to power of future genetic studies of this species (Koskinen et al. 2004).

In this project, we describe the development of nine new polymorphic microsatellite loci for *A. woodiana*. We also combined new and previously described loci into three multiplex sets allowing reducing the time and money costs of genotyping, as well as decreasing the risk for samples mishandling.

*Anodonta woodiana* is one of the most invasive bivalves in European waters. Phenomena of freshwater biological invasions are becoming increasingly common, but our understanding of the process from biological and genetic point of view, is still largely unknown (Douda et al. 2012). The development of new DNA markers is the first step to study important aspects of the invasion process of invasive species such as the route(s) of invasion and the time and number of colonization events.

We developed a highly enriched DNA library which allowed us to identify nine new microsatellite loci for *A. woodiana*. The number of the polymorphic loci from the total number of loci tested in *A. woodiana* is 9.57% (94 loci tested only 9 polymorphic). The low number of polymorphic loci could be explained by intrinsic factors, such as a particular microsatellite frequency within the genome of these species, the structure of microsatellite sequences and their flanking region and also by extrinsic factors such as the well known difficulties occurring in the development of molecular markers in mollusk species (McInerney et al. 2011). The polymorphic microsatellite loci described in this paper exhibit a number of alleles that ranged from 3 (loci AW 378 and AW28) to 8 (AW 570) in the sample of 27 genotyped individuals (Figure 2). These values are in the same range with the previously published study of this mussel species (Popa et al. 2011).

![Figure 2. Electroforegrams – genotyping in *A. woodiana*](image-url)
The three PCR multiplex reactions were optimized to facilitate large-scale populations studies for the invasive Chinese huge mussel. Multiplex PCR amplifications often require an extensive optimization in order to minimize the excessive stuttering, the primer dimmer formation which can complicate the genotyping process. In our study, the characteristics of the loci (Na, Ho and He) were not affected by multiplex PCR amplification.

In conclusion, the set of newly isolated markers for *Anodonta (Sinanodonta) woodiana*, combined with those previously described, provides a powerful tool for population genetic studies of this invasive species.

1. 2 Collecting *Anodonta (Sinanodonta) woodiana* populations samples from Europe and native area.

Specimens of *Anodonta woodiana* were collected from 15 European populations and from 2 population from the native area of this species (East Asia – China). (See Figures 3, 4)
1.3. Performing laboratory work on genetic variability on *Anodonta (Sinanodonta) woodiana* populations

Genetic diversity, as a level of biodiversity, refers to the total number of genetic characteristics in the genetic makeup of a species. We refer to genetic diversity for within populations variability and to genetic differentiation as the genetic diversity among populations.

In theory, populations with higher genetic diversity can survive, can cope with environmental change. Populations with low genetic diversity are vulnerable to environmental change, disease or inbreeding depression. After invasion we expect genetic changes in the invading populations as a consequence of the reduced number of founders in the new population.

One such change is genetic admixture, which explains the genetic paradox, i.e. the putative loss of diversity during invasion vs. high fitness observed in invasive species.

In some cases however, the usually accepted hypothesis of founder effects and reduced genetic diversity at introduction seem not to be supported by data. Wares et al. (2005) found invading animal species (29 species reported) retain 80% of the genetic diversity of their native source populations.

**Genotyping**

PCR products were sized by capillary electrophoresis using an ABI Prism® 3130 Genetic Analyzer. Alleles were scored in the software GENEMAPPER v. 5.0 (Applied Biosystems, Foster City, USA) and double-checked manually. GenAlEx 6.501/ Genepop 4.2 was used to test for Hardy-Weinberg equilibrium at each locus and to estimate the number of alleles (NA), observed (HO) and expected heterozygosity (HE) and fixation index (FIS) (Peakall and Smouse 2006). FSTAT was used to test genetic differentiation between population (pairwise Fst). The presence of null alleles, large alleles dropout and scoring errors by stuttering, was tested using MICRO-CHECKER ver. 2.2.3 (Van Oosterhout et al. 2004).

[Diagram of the alleles distribution among our populations]
We tested the population differentiation with the index, Dest (Jost 2008). Dest takes values linearly between 0 (complete identity) and 1 (complete differentiation).

The differentiation between the native Chinese population and the invasive European populations ranges between 0.307-0.525, which we consider also as a moderate level of genetic differentiation between populations. The genetic differentiation between the native and each of the invasive populations was larger than the differentiation between any of the invasive populations.

**CONCLUSIONS AND FUTURE PLANS**

C1. We found the highest genetic diversity (number of private alleles and total number of alleles, observed and expected heterozygosity) in the Chinese population from Nanchang.

**FUTURE PLANS -** study of genetic variation in the native range (5 more populations to analyze)

C2. The genetic data presented here supports the idea that the European (invasive) populations have partially loss the original genetic diversity.

**FUTURE PLANS -** study of genetic variation in the invasive range (15 different populations around Europe to analyze)

C3. The genetic differentiation between the native and each of the invasive populations was larger than the differentiation between any of the invasive populations.

**FUTURE PLANS –** to use other approaches like STRUCTURE and/or an Approximate Bayesian Computation to analyze a complete sample dataset, which will allow the understanding of the genetic mechanism of invasion process of *A. woodiana*. 
REFERENCES:


Director project,