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Large-scale mitochondrial COI gene sequence variability reflects the complex colonization history of the invasive soft-shell clam, *Mya arenaria* (L.) (Bivalvia)

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ABSTRACT

The aim of the study was to determine genetic diversity in the soft-shell clam *Mya arenaria* on a wide geographical scale using mtDNA COI gene sequences. Low levels of genetic diversity was found, which can most likely be explained by a bottleneck effect during Pleistocene glaciations and/or selection. The geographical genetic structuring of the studied populations was also very low. The star-like phylogeny of the haplotypes indicates a relatively recent, rapid population expansion following the glaciation period and repeated expansion following the founder effect(s) after the initial introduction of the soft-shell clam to Europe. North American populations are characterized by the largest number of haplotypes, including rare ones, as expected for native populations. Because of the founder effect connected with initial and repeated expansion events, European populations have significantly lower numbers of haplotypes in comparison with those of North America. We also observed subtle differentiations among populations from the North and Baltic seas. The recently founded soft-shell clam population in the Black Sea exhibited the highest genetic similarity to Baltic populations, which confirmed the hypothesis that *M. arenaria* was introduced to the Gulf of Odessa from the Baltic Sea. The most enigmatic results were obtained for populations from the White Sea, which were characterized by high genetic affinity with American populations.

1. Introduction

The soft-shell clam, *Mya arenaria*, stands out among other marine invasive species because of its extensive scale of expansion that has been caused by repeated human-mediated and natural introductions. The species originated on the Pacific coasts in the Miocene and extended its range to the west coasts of the Atlantic. In Europe, the soft-shell clam appeared in the late Pliocene; thus, it was native at that time. At the beginning of the Pleistocene, the soft-shell clam became extinct in Europe and the Pacific and remained only in eastern America (Hessland, 1946). The recent dating of Holocene fossil shells found in the Baltic and North seas places the reappearance of *M. arenaria* in Europe in the thirteenth century (Petersen et al., 1992; Beets et al., 2003; Behrends et al., 2005). Because of a relatively short pelagic stage of about three weeks, it seems unlikely that the larvae were transported to Europe by surface ocean currents. Therefore, it is hypothesized that the soft-shell clam might have been introduced from North America by the Vikings (Petersen et al., 1992). The first introduction most likely took place in Jutland; from here the species expanded into the Baltic and North seas and the Atlantic. The present-day distribution range in Europe extends from the White Sea through the British Isles and the North Sea to the Bay of Biscay. *Mya arenaria* also appeared in Iceland and Spitsbergen (Óskarson, 1958, 1961; Gulliksen et al., 1985; Pempkowiak et al., 1999; Ólafsson and Þórarinsdóttir, 2004). The soft-shell clam has also extended its distribution range to southern Europe, and the species was reported recently in several locations in Spain and Portugal (Conde et al., 2010, 2012 and references therein). In 1966, *M. arenaria* was reported near Odessa in the Black Sea and became a dominant

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species in numerous regions of the Black and Azov seas several years later (Gomoiu, 1981). The first findings of the clam in the Mediterranean Sea date from 1976. Specimens were collected in Saronikos Bay, Greece (Zenetos et al., 2005). In the 1990s, high density populations of *M. arenaria* were recorded in two French lagoons—de Berre and de Vaine (Stora et al., 1995; Porcheddu et al., 1998). In addition, the soft-shell clam has expanded from its native Atlantic west coast to the eastern Pacific. Prior to 1874, the bivalve invaded the San Francisco Bay presumably with the introduction of oysters for mariculture. As a consequence of natural dispersion and farming, its present distribution in the eastern Pacific extends from Alaska to Monterey Bay (Hanna, 1966). *Mya arenaria* is also found in the western Pacific from the Kamchatka Peninsula to southern Japan and China; however, its occurrence in this region remains ambiguous because of possible taxonomic misidentification with the endemic *Mya japonica*, Jay 1856 (Strasser, 1999). The taxonomic status of *M. japonica* itself is also ambiguous since some authors consider it to be a separate species, while according to others it is a synonym of *M. arenaria* (Ponurovskii and Kolotukhina, 2000). Although the soft-shell clam is a common species in a number of coastal systems world wide, compared to other bivalves little is known about its population genetics. The first genetic analyses of the soft-shell clam using isoenzyme electrophoresis were carried out by Morgan et al. (1978) on two populations from the Atlantic coasts of North America. The results indicated a low level of genetic polymorphism. The first genetic analyses of European soft-shell clam populations also examined the electrophoretic variation of enzymes in populations from the North and Baltic seas, and these results also confirmed a low level of intra- and inter-population genetic variation in *M. arenaria* (Lasota et al., 2004). Similarly, ITS-1 ribosomal DNA sequencing of populations from New England demonstrated low genetic diversity in this species (Caporale et al., 1997). Strasser and Barber (2009) and Cross et al. (2016) published results of mitochondrial cytochrome oxidase I sequencing in populations from the northwest Atlantic, the northeast Pacific, and several sites in Europe restricted to the North Sea and the British Isles. The studies showed a very low within and between populations genetic variation. New *M. arenaria* microsatellite markers were developed and characterized recently, which indicate that, in contrast to results obtained using coding markers and ITS-1, there are high levels of genetic polymorphism and geographical structuring in the soft-shell clam (Krapal et al., 2012; St-Onge et al., 2013; Cross et al., 2016). The studies mentioned above focused mainly on American populations, whereas little remains known about the genetic structure of European populations. The aim of the present study was to determine the genetic diversity in the soft-shell clam, *Mya arenaria*, on a large geographical scale using mtDNA COI gene sequences with special emphasis on European populations, including marginal populations and those established recently through both natural and human-mediated expansions. We hypothesize that different types of introduction and expansion, natural and human mediated, will show the different impacts on the genetic diversity of the studied species.

2. Materials and methods

2.1. Sampling

A total of 522 adult *M. arenaria* specimens were collected from 19 locations in Europe and North America (Fig. 1, Table 1). Four of the locations: Rimouski (Canada – Atlantic), Halifax (Canada – Atlantic), Maine (USA- Atlantic), and New Jersey (USA – Atlantic), are considered to be within the native range of the species. The samples were stored in 96% ethanol before DNA extraction.

2.2. DNA extraction, amplification, and sequencing of mitochondrial cytochrome oxidase subunit I (COI) locus

Total DNA was extracted from fragments of tissue (about 1 mm^3) using the DNeasy Tissue Kit according to the manufacturer's (QIAGEN) protocol. DNA extracts from 19 to 35 individuals per populations were stored at −20 °C. The quality and quantity of DNA extracts were estimated by agarose gel electrophoresis.

Preliminary sequences obtained with COI universal primers (Folmer et al., 1994) were used to define the primer set that is specific to *M. arenaria*:

Mya F 5′-TGTTTTTTCTCTGTGAGCAGG-3′ (forward) and

Mya R 5′CAGTTAAAAGCATTGTTAAAGCC–3′ (reverse). The amplifications were performed in an MJ Research thermal cycler (PTC-200) with a 3 min denaturation step at 95 °C, followed by 35 cycles at 95 °C for 30 s, 58 °C for 60 s (annealing), 72 °C for 1.5 min (extension), and 72 °C for 7 min (final elongation). Each PCR reaction mixture (volume 20 μl) contained: 2 μl of DNA extract solution, 1 μl of each primer (10 mM), 0.4 μl dNTPs (10 mM), 2 μl of 10x PCR buffer, 2 μl MgCl2 (25 mM) and 0.2 μl of GoTaq Flexi DNA Polymerase (5 units/μl). The PCR products were purified using a GENOSCREEN kit and sequenced with a Mya R labeled primer according to the manufacture's protocol.

Sequences for each haplotype were deposited in GenBank (accession numbers: KX576717-KX576736).

2.3. Data analysis

Sequences were aligned using a CLUSTALW version 1.4

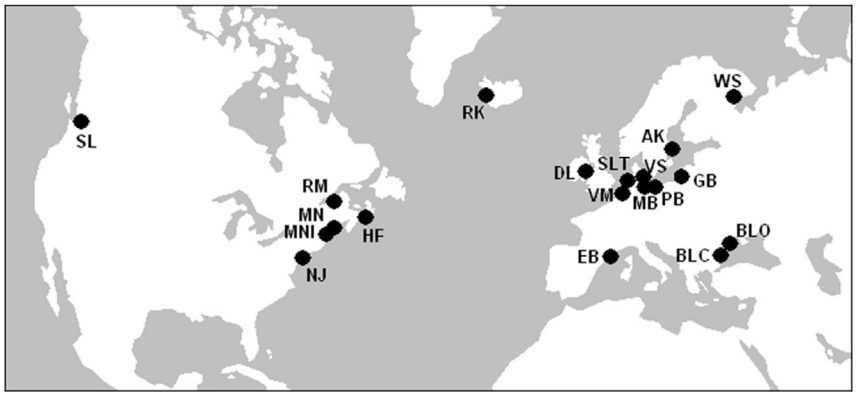

Fig. 1. Locations of the sampling sites.