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Genetic diversity of introduced Manila clam *Ruditapes philippinarum* populations inferred by *16S* rDNA

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ABSTRACT

Manila clam *Ruditapes philippinarum* – synonym *Venerupis philippinarum* (Adams and Reeve, 1850) – is one of the most successful marine invaders, being introduced worldwide for aquaculture and fisheries. Genetic diversity and structure of its populations are largely unknown, especially in the invaded environments. Herein we present molecular genetic data on 12 introduced Manila clam populations in Italy, Spain, and Portugal from both Mediterranean (Adriatic) and Atlantic sampling sites. The phylogenetic information was investigated by the direct sequencing of *16S* mitochondrial DNA. Results of mtDNA analyses showed the occurrence of 11 haplotypes for European introduced populations, as a consequence of multiple founder effects from different source populations. Rp hap1 was the most frequent, shared among all populations. The other 10 haplotypes were rare and distributed at local scale, in agreement with what was observed in other invasive bivalves. Biogeographic and phylogenetic analyses based on *16S* rDNA of introduced Manila clam populations showed a complex scenario, dominated by multiple pools of individuals coming from different sources, as a consequence of multiple introductions.

1. Introduction

Among the variety of worldwide commercial seafood products, cultivation of molluscs, mainly bivalves, has a long history and it has become largely established in many countries (Bostock et al., 2010). Moreover, many bivalves species have been introduced outside their native habitats for aquaculture and fisheries purpose (Grigorakis and Rigos, 2011); they became successful invaders, occurring at high densities and accounting for the major proportion of the benthic faunal biomass (Sousa et al., 2009). In particular, species belonging to the family Veneridae (Rafinesque, 1815) are actually exploited for human consumption (Edwards, 2005), mostly due to the fishing and farming of Manila clam *Ruditapes philippinarum* – synonym *Venerupis philippinarum* (Adams and Reeve, 1850).

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R. philippinarum represents itself almost 20% of worldwide mollusc total production (Edwards, 2005), being one of the most successful invaders (FAO, 2013). Originally from the Indo-Pacific region of Japan, Korea and China (Gosling, 2003), it was introduced into the west coast of North America, Atlantic (Portugal, France, Spain, Ireland, England) and Mediterranean European coasts (France, Italy) (Gosling, 2003). Moreover, aquaculture trials resulted in seed being imported into French Polynesia, the US Virgin Islands, Norway, Germany, Belgium, Tunisia, Morocco, and Israel (FAO, 2013). In Europe, the species was introduced for commercial cultivation (Bald et al., 2009); since its introduction, production of Manila clam improved, and Europe became the leading area in the world after China (Guo et al., 1999).

Although commercially exploited and deeply manipulated by human activities, Manila clam is still poorly known under many biological points of view, especially genetics. Surprisingly, few papers have been published concerning genetic diversity of native and introduced populations of Manila clam. In China, Korea and Japan, less than a dozen of manuscripts have been published using allozyme markers (see Vargas et al., 2008), mtDNA sequencing (see Kitada et al., 2013), AFLP (Liu et al., 2007); RAPDs and microsatellites (see An et al., 2012; Kitada et al., 2013). In Europe, we can actually account only two papers on Italian populations (Chiesa et al., 2011; Mura et al., 2012).

Genetic data in invasion biology are fundamental from many points of view (- Frankham et al., 2009; Estoup and Guillmaud, 2010): to correctly identify an invasive species (Scalici et al., 2009); to understand whether or not the introduced species could become invasive, to construct predictive models of future spread; to evaluate the evolutionary changes in invasive species and their impact on native species.

Moreover, molecular genetics could provide valuable data for the tracing and tracking of seafood products, as already requested by the European Union law EC No. 2065/2001 (Filonzi et al., 2010; Caldelli et al., 2014), on the basis of their geographic distribution. The identification of informative genetic markers and their application to Manila clam populations is particularly important for the tracing of harvested clams. Clams cultivation and fisheries in both Mediterranean and Atlantic coastal lagoons frequently occurs in low or moderated polluted areas (Sfriso et al., 2008; Freitas et al., 2012) posing risks for human's safety, especially in coastal communities. The genetic profiles of cultivated and harvested bivalves populations can certify their geographic origin, to guarantee consumer's safety and product's quality control. These informations will improve the value of bivalves products, both for consumers and producers.

We herein present data on 12 introduced Manila clam productive populations belonging to Italy, Spain, and Portugal to assess their genetic diversity on a larger scale, on both Mediterranean and Iberian Atlantic populations. We investigated the biogeographic and the phylogenetic information throught the direct sequencing of *16S* mitochondrial DNA, which has been proven to be a useful marker for intraspecific diversity in invasive bivalves (Stepien et al., 1999), and in Veneridae family (Canapa et al., 2003; Kappner and Bieler, 2006; Mikkelsen et al., 2006).

2. Materials and methods

2.1. Sampling procedures

Twenty adult individuals of Manila clam were collected from 12 introduced harvested populations from both Mediterranean and Atlantic coasts of Europe. Sampling sites were distributed in Northern Adriatic Sea from the Marano Lagoon (two sites), the Venice Lagoon (three sites) to the River Po Delta (two sites) and Sacca Scardovari (one site). Moreover, one population was collected in North Western Spain (Galician coast) and three from Northen Portugal (Ria de Aveiro Lagoon) (Fig. 1 and Table 1).

2.2. DNA extraction and purification

High molecular weight genomic DNAs were extracted and purified from ethanol-fixed mantle and foot tissue stored at −20 °C. The DNA was extracted from these tissues to avoid the interferences due to the "Doubly Uniparental Inheritance" (DUI) of mtDNA (Plazzi and Passamonti, 2010). Genomic DNA was extracted by Wizard genomic DNA Purification kit (PROMEGA, Madison, WI, USA). DNA quality and concentration were tested by 1% agarose gel electrophoresis in 1 × TAE buffer, by visual comparison with a DNA ladder mix and by spectrophotometry at 260–280 nm. The extraction procedure typically yielded not less than 40 ng/μl of HMW (high molecular weight) DNA.

2.3. Mitochondrial DNA analyses

Amplification of a *16S* rRNA gene fragment about 600 bp length was achieved using primers *16Sar-ALT* (5′ GCCTGTTTATCAAAAACATSG 3') and *16Sbr-ALT* (5′ CCGGTCTGAACTCAGATCATGT 3') specifically designed for family Veneridae (Mikkelsen et al., 2006). A reaction volume of 50 μl containing 1 U of GoTaq Polymerase (PROMEGA, Madison, WI, USA), Mg^{2+} 1.5 mM and dNTPs 0.2 mM, and 10 pmol of each primer was used. PCR – touch down profile was set as follows: 2 cycles of 15 s at 94 °C, 15 s at 52–48–46 °C, and 30 s at 70 °C; then 30 cycles of 15 s at 94 °C, 15 s at 50 °C, and 30 s at 70 °C, after an initial 1 min denaturation step at 94 °C and a final extension at 70 °C for 10 min (Chiesa et al., 2011). Fragments sequencing was performed by MACROGEN Europe service (Amsterdam, the Netherlands). The obtained sequences were compared with those available in genomic databases using Blast and multiple alignments of sense and antisense sequences were conducted using software as Clustal X (Thompson et al., 1997) and Sequencer 4.2 (Gene Code Corporation). When downloading sequences