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## SURVEY OF THE GENETIC VARIABILITY OF POPULATIONS OF *RUDITAPES PHILIPPINARUM* FROM THE GULF OF OLBIA (N-E SARDINIA) BY MICROSATELLITES

### INDAGINE SULLA VARIABILITÀ GENETICA DI POPOLAZIONI DI *RUDITAPES PHILIPPINARUM* PROVENIENTI DAL GOLFO DI OLBIA (N-E SARDEGNA)

**Abstract** - Genetic variability was investigated at six microsatellite loci of the Manila clam *Ruditapes philippinarum* (Adams & Reeve, 1850) (*Bivalvia*) from the Gulf of Olbia (N-E Sardinia) and Sacca di Goro (N Adriatic Sea). We found no significant differentiation among Sardinian samples and between those and the Adriatic one, which suggests the absence of a founder effect in Sardinian population.

**Key-words:** *Ruditapes philippinarum*, introduced species, genetic structure, microsatellites.

**Introduction** - The Manila clam *Ruditapes philippinarum*, which is a bivalve native to the Pacific coast of Asia, was deliberately introduced in the N Adriatic in the 1983 for commercial purposes. This species found favourable environmental conditions, occupying the ecological niche previously dominated by the indigenous *R. decussatus* (Paesanti and Pellizzato, 2000). Recently, several specimens of *R. philippinarum* were found in the Gulf of Olbia (Cannas *et al.*, 2009). In this context, the aim of this study was to analyse the genetic diversity of *R. philippinarum* populations collected in the Gulf of Olbia (N-E Sardinia), comparing their genetic variability with a population from the Sacca di Goro (N Adriatic).

**Materials and methods** - A total of 195 specimens, collected from six sites (size of each sample from 22 to 50) within the Gulf of Olbia, and 99 specimens from the Sacca di Goro (as geographic outgroup) were genotyped for six loci (*Asari16*, 24, 43, 55, 62, 64) (Yasuda *et al.*, 2007). Genomic DNA was isolated from muscle or mantle by an alkaline and CTAB/NaCl method, adjusted by the Authors, and a silicon dioxide treatment. PCR was carried out in 25  $\mu$ l volumes containing: 20-40 ng of genomic DNA, 1 $\times$  buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 1U of Taq, multiplexing primers to different concentration. A primer of each pair was end-labelled with a fluorescent dye. After amplification, microsatellite loci were visualised with 3130 $\times$ 1 Genetic Analyzer Capillary (Applied Biosystem). Within population genetic diversity was estimated by observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ) for each sample, and the Hardy-Weinberg Equilibrium (HWE) for each locus. The genetic structure was investigated by a Bayesian individual-based clustering approach (BA), using STRUCTURE (Pritchard *et al.*, 2000). The analysis of molecular variance (AMOVA), performed by GENALEX (Peakall and Smouse, 2006), was used to search for the occurrence of genetic divergence between Sardinian and Adriatic population.

**Results** - Values of  $H_o$  and  $H_e$  were very similar across the six populations from the Gulf of Olbia, ranging from 0.509 $\pm$ 0.088 to 0.666 $\pm$ 0.060, and from 0.631 $\pm$ 0.106 to 0.681 $\pm$ 0.079, respectively. Pooling the Sardinian samples, we found  $H_o$ =0.621 $\pm$ 0.077, and  $H_e$ =0.662 $\pm$ 0.091. Comparable values were obtained in the Adriatic site ( $H_o$ =0.626 $\pm$ 0.090;  $H_e$ =0.680 $\pm$ 0.087). With the Sardinian samples pooled, significant departures from HW were found at five loci (*Asari16*<sup>\*\*\*</sup>, 24<sup>\*\*\*</sup>, 55<sup>\*\*\*</sup>, 62<sup>\*\*\*</sup>, 64<sup>\*\*\*</sup>); whereas

in the Adriatic sample three loci (*Asari24*<sup>\*\*\*</sup>, *55*<sup>\*\*</sup>, *64*<sup>\*\*\*</sup>) were not at equilibrium. In both cases, the deviations from HWE were due to a heterozygotes deficiency, except for *Asari24*. BA retrieved  $K=1$  as the best solution. Accordingly, AMOVA revealed not significant differences between the Gulf of Olbia and the Adriatic populations ( $R_{ST}=0.003^{NS}$ ).

**Conclusions** - The estimates of genetic variability evidenced a high similarity among the six Sardinian samples, and between those and the Adriatic one. However, the overall Sardinian population showed a number of loci departing from HWE higher than the Adriatic population (5 vs. 3). This finding is probably due to the recent introduction of *R. philippinarum* in the Gulf of Olbia, whose population may have not yet reached the equilibrium. Results of BA and AMOVA evidenced that no genetic structure is present between Sardinian and Adriatic populations. Furthermore, BA did not evidenced additional genetic sub-structuring in Sardinia. The absence of genetic differentiation found between the two areas suggests the two populations belong to the same gene pool; furthermore, the lack of a significant founder effect from Adriatic populations, indicates that the number of individuals of *R. philippinarum* released in the Gulf of Olbia may have been high enough to prevent genetic bottlenecks. Results of our work also indicated that *R. philippinarum* is rapidly colonising the Gulf of Olbia and the near areas, probably favoured by its high potential of dispersal. This may raise concern on the conservation and management of the autochthonous *R. decussatus*, whose populations may suffer for the competition with *R. philippinarum* (Chessa *et al.*, 2005), and by the onset of phenomena due to maladaptation, as hybridization between the two species may occur (Hurtado *et al.*, 2011).

## References

- CANNAS A., TRENTADUE M., MANCA S., MURA L., FOIS N. (2009) - Inseadimento di *Ruditapes philippinarum* (Adams & Reeve) nel golfo di Olbia (Sardegna, Italia) e distribuzione in rapporto a *Ruditapes decussatus* (L.). *Biol. Mar. Mediterr.*, **17** (1): 320-321.
- CHESSA L., PAESANTI F., PAIS A., SCARDI M., SERRA S., VITALE L. (2005) - Perspectives for development of low impact aquaculture in a Western Mediterranean lagoon: the case of the carpet clam *T. decussatus*. *Aquacult. Int.*, **13**: 147-155.
- HURTADO N.S., PÉREZ-GARCÍA C., MORÁN P., PASANTES J.J. (2011) - Genetic and cytological evidence of hybridization between native *Ruditapes decussatus* and introduced *Ruditapes philippinarum* (Mollusca, Bivalvia, Veneridae) in NW Spain. *Aquaculture*, **311**: 123-128.
- PAESANTI F., PELLIZZATO M. (2000) - *Manuale di allevamento della vongola verace Tapes philippinarum*. Veneto Innovazione 2° Ed.: 74 pp.
- PEAKALL R., SMOUSE P.E. (2006) - Genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*, **6**: 288-295.
- PRITCHARD J.K., STEPHENS M., DONNELLY P. (2000) - Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945-959.
- YASUDA N., NAGAI S., YAMAGUCHI S., LIAN C.L., HAMAGUCHI (2007) - Development of microsatellite markers for the Manila clam *Ruditapes philippinarum*. *Mol. Ecol. Notes*, **7**: 43-45.