FIRST DATA ON GENETIC DIVERSITY OF THE SMALL PEARL OYSTER *PINCTADA RADIATA* (LEACH, 1814) IN MEDITERRANEAN POPULATIONS BY MEANS OF THE MITOCHONDRIAL *COX1* MARKER

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Abstract

This study gives a first molecular insight into the genetic structure of *Pinctada radiata* by analysing a mitochondrial DNA marker in four South-Central Mediterranean populations of this lessepsian bivalve. Specimens were collected from four central Mediterranean locations. Six polymorphic sites were found, defining seven haplotypes. Haplotype and nucleotide diversity values were low to moderate. The low levels of within-population genetic diversity detected in the present study are consistent with a scenario of founder effect, as expected for an invasive species. The shallow genetic structuring among the sampled populations may result from two factors – long larval duration which promotes the homogenization of the genetic pool through an effective gene flow or the current populations may not have had enough time to differentiate.

Keywords: Genetics, Bivalves, Alien species, South-Central Mediterranean

Introduction: The small pearl oyster *Pinctada radiata* was the first lessepsian bivalve reported in the Mediterranean. Successively its introduction for aquaculture purposes has been also documented (Serbetis, 1963; Zenetos *et al.*, 2004). Zibrowius (1992) proposed vessel-mediated dispersal as the most likely modality of introduction, either within fouling communities or as larvae in ballast waters. The three-week larval stage provides a high dispersal potential, as a consequence, species' geographical distribution within the Mediterranean Sea is expected expand progressively. With the present study a first molecular insight is provided into species genetic structure by analysing four Mediterranean populations of *P. radiata* by means of a mitochondrial DNA marker.

Materials & Methods: A fragment of the *COX1* gene was amplified and sequenced in a total of 47 individuals. 10-15 specimens were collected from four south-central Mediterranean locations – Qalet Marku and Bahar ic-Caghaq (Malta), Agios Nikolaos (Crete, Greece) and Gulf of Gabes (Tunisia). Within population genetic diversity was estimated by haplotype (h) and nucleotide (π) diversity indices. A median-joining network was constructed to depict relationships among haplotypes. Analysis of molecular variance (AMOVA Excoffier *et al.*, 1992) was carried out to partition genetic diversity in the within- and among-population genetic diversity.

Results: Overall, six polymorphic sites were found, defining seven haplotypes. Haplotype and nucleotide diversity values were low to moderate, ranging from h = 0.200 to 0.694 and $\pi = 0.0005$ to 0.0020, respectively. Highest and lowest values for both estimators were obtained for the Greek and Tunisian populations, respectively. Thirty-five individuals (74%) shared the haplotype H_1, and each of the other six haplotypes differed from H_1 by one mutational step. AMOVA partitioned the largest part of molecular variance (95%) in the within-populations level; the remaining 5% was relative to the among-populations component, that however was significant ($\Phi_{\rm ST} = 0.054$, P = 0.043).

Discussion: The low levels of within-population genetic diversity detected in the present study are consistent with a scenario of founder effect, which is expected for an alien invasive species (Nei *et al.*, 1975). However, the comparison with estimates of samples collected in the species' native range is needed to validate this hypothesis. Nonetheless, the estimates calculated in the present study were lower than those found in the Mediterranean for the alien bivalve *Brachidontes pharaonis*, using the same molecular marker (Sirna Terranova *et al.*, 2007). Moreover, population bottlenecks in Mediterranean *P. radiata* cannot be excluded; at this regard, dramatic demographic collapses has been documented in Tunisia ((Zaouali & Beaten, 1985). The shallow genetic structuring among the sampled populations may result from two main factors. On one hand, the long larval duration may promote the homogenization of the genetic pool through the action of an effective gene flow; on the other hand, current populations may have had not enough time to differentiate since the founder event. The present study represents a first insight into the genetic diversity of the invasive bivalve *P. radiata* in the Mediterranean Sea. In order to disentangle the relative contributions of current and historical factors, the analysis of a larger number of individuals and sampling locations is needed. Moreover, it is fundamental to include populations from the native species' range.

References

1 - Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes. Application to human mitochondrial DNA restriction data. *Genetics*, 131: 479–491

2 - Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in natural populations.*Evolution*, 29: 1-10.

3 - Serbetis CD (1963). L'acclimatation de la *Meleagrina (Pinctada)* margaritifera (Lam.) en Greece. CIESM, 17: 271-272.

4 - Sirna Terranova M, Lo Brutto S, Arculeo M, Mitton JB (2007) A mitochondrial phylogeography of *Brachidontes variabilis* (Bivalvia: Mytilidae) reveals three cryptic species. *Journal of Zoological Systematics and Evolutionary Research*, 45: 289-298

5 - Sirna Terranova M, Lo Brutto S, Arculeo M, Mitton JB (2007) A mitochondrial phylogeography of *Brachidontes variabilis* (Bivalvia: Mytilidae) reveals three cryptic species. *Journal of Zoological Systematics and Evolutionary Research*, 45: 289-298

6 - Zaouali J, Beaten S (1985) Etude des peuplements macrobentiques de la zone centrale et du bassin oriental de la mer des Bibans (Tunisie meridionale) par la methode de l'analyse factorielle des correspondances.*Rapport de la Commission Internationale de la Mer Méditerranée*, 29: 199–203

7 - Zenetos, A, Dosi, A., Abatzopoulos, T.J., Triantafyllidis, A., Bejaoui, N., Soufi, E., Ammar, I. A. & Ibrahim, A. (2004). Study to investigate an invading bioindicator in the Mediterranean, *Pinctada radiata* (Leach, 1814). Study group on Ballast and other ship vectors ICES/IOC/IMO, Cesenatico, Italy, p 376.

8 - Zibrowius H. (1992) Ongoing modifications on the Mediterranean marine fauna and flora by the establishment of exotic species. *Mesogée*, 51: 83–107