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ANALYSIS OF THE GENETIC VARIABILITY OF *PATELLA*
FERRUGINEA GMELIN, 1791 (GASTROPODA: PATELLIDAE)
POPULATIONS FROM THE NORTH-EAST SARDINIA

ANALISI DELLA VARIABILITÀ GENETICA IN POPOLAZIONI
DI *PATELLA FERRUGINEA GMELIN, 1791*
(GASTROPODA: PATELLIDAE) PROVENIENTI
DALLA SARDEGNA NORD-ORIENTALE

Abstract - The mollusc *Patella ferruginea* (Gastropoda, Patellidae), endemic to the Mediterranean, is the most endangered marine species on the list of the European Council Directive 92/43/EEC and it is presently under serious risk of extinction. This research was aimed to unravel the genetic variability of some Sardinian populations sampled on the North-Eastern coast, in order to shed light on their status of conservation.

Key-words: rare species, exploitation, population genetics.

Introduction - Human impact on coastal habitats have led to the extinction of local populations of invertebrates (Little and Kitching, 1996). The intertidal zone is being progressively squeezed between encroaching onshore developments (Raffaelli and Hawkins, 1996) and consequently many sedentary species inhabiting rocky shores may disappear. Among them, the limpet, *Patella ferruginea*, endemic to the Mediterranean Sea, is the most endangered marine species on the list of the European Council Directive 92/43/EEC on the conservation of Natural Habitats and of Wild Fauna and Flora, 1992 (Ramos, 1998), and it is presently under serious risk of extinction (Templado and Moreno, 1997). This work, performed by Inter-Simple Sequence Repeat (ISSR) markers, was aimed to shed some light on the genetic variability and level of gene flow among samples from different protected and non-protected sites, subjected to high level of onshore development from the Sardinian North-Eastern coasts.

Materials and methods - 37 specimens were collected from North-East Sardinia, in Cala Greca (CGR, n.8), Pittulongu (PIT, n.6) and Punta li Francesi (PLF, n.10), and from two sites located in the National Park of Arcipelago di La Maddalena (Madonnetta, MAD, n.10, and Nido d'Aquila, NAQ, n.3). The non-lethal protocol of tissue sampling, sequence of the 7 ISSR primers used (IT1, IT2, IT3, SAS1, SAS3, UBC811, UBC827), PCR reaction mixtures, amplification program, electrophoresis conditions and gel staining are reported in Casu *et al.* (2006). Genetic relationships at interpopulation level were investigated using the software Splitstree (available on-line), performing a maximum parsimony analysis with a heuristic search with TBR (Tree bisection-reconnection) branch swapping and random addition of sequences, and building a consensus network from the equally parsimonious trees. The software Genalex (available on-line) was used to perform the analysis of molecular variance (AMOVA, 1,000 permutation) and calculate the value of Φ_{PT} . The partitioning of genetic variation (ΔK) was estimated applying the Bayesian method implemented in the software BAPS (available on-line).

Results - Network analysis based on 32 polymorphic loci, evidenced two main clusters without any evident geographical structuring (NAQ+PLF and CGR+MAD+PIT). Consistently, i) AMOVA pointed out that the differentiation among these clusters is significant, with a value of $\Phi_{PT}=0.287$ ($P<0.001$), and ii) Bayesian analysis assumed the best partition with $\Delta K=2$, with two groups comprising NAQ and PLF, and CGR, MAD and PIT, respectively.

Conclusions - Results obtained evidenced that - notwithstanding the closeness of the samples analysed - the *P. ferruginea* populations from North-East Sardinia cannot be regarded as a genetically homogeneous unit. Although two out of the five sites sampled are located within a protected area (Arcipelago of La Maddalena), the high onshore developments and the consequent high human pressure - mainly due to summer recreation activities on the coastline - probably make the *P. ferruginea* populations vulnerable to effects of disturbance, which reflect on the genetic drift observed in that area. If the genetic divergence will not be stopped throughout an active recovering plan for this species, the future of these populations may be the progressive loss of genetic variability (the so-called "genetic erosion"), which may culminate with local extinction (see Casu *et al.*, 2006; Lai *et al.*, 2010).

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