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STANDARDIZATION OF INTER SIMPLE SEQUENCE REPEAT TECHNIQUE TO ESTIMATE GENETIC VARIABILITY OF *RUDITAPES DECUSSATUS*

STANDARDIZZAZIONE DELLA TECNICA INTER SIMPLE SEQUENCE REPEAT PER STUDIARE LA VARIABILITÀ GENETICA DI RUDITAPES DECUSSATUS

Abstract - Ruditapes decussatus *(L., 1758) is a bivalve autochthonous of the Mediterranean. We focused on the possible use of the ISSR technique to investigate its genetic variability. We tested four primers on 15 specimens from three northern Sardinian sites. We evidenced that i) ISSRs can detect satisfactory levels of genetic variability and ii) produce replicable and easily scorable results.*

Key-words: *brackish-water molluscs, population genetics, resource conservation.*

Introduction - Nowadays, although with a reduced biological impact when compared to Eritrean invasion, encroachment of alien species involved in both sea transports and mariculture activities in the Mediterranean has a negative influence on native species from harbors and brackish-waters. Among them the carpet shell clam, *Ruditapes decussatus* (L., 1758) is a bivalve autochthonous in the Mediterranean, which could be one of the next victims of the above described 'modern invasions'. In the last two decades, its ecological niche was occupied by the congeneric, allochthonous *R. philippinarum*, which is endemic of Indo-Pacific region. Furthermore, the possible genetic impact of the introduced species *R. philippinarum* on the native *R. decussatus* through hybridization and introgression is an issue that needs to be considered (Hurtado *et al.*, 2011). The aim of this pilot study was to evaluate the possibility to use the nuclear ISSR markers (Inter Simple Sequence Repeat) in order to acquire new insights on the genetic variability of populations of *R. decussatus*. ISSR technique is a fingerprinting method which produces highly reproducible bands and results with low statistical errors, leading to multilocus and highly polymorphic banding patterns without prior DNA sequences knowledge (Zietckiewicz *et al.*, 1994). In the last years, ISSRs have proved to be an effective tool to gain helpful genetic information from natural populations (Casu *et al.*, 2009 and references therein), able to provide important insights on population structure and gene flow. This method shows high levels of resolution especially when combined with recently introduced powerful statistical approaches, such as Bayesian statistics (Vekemans, 2002). The choice of using ISSR lies on the avoidance of i) problems related to the mtDNA doubly uniparental inheritance (DUI), and ii) the expensive, time-consuming microsatellite isolation.

Materials and methods - We analyzed 15 specimens from the North-East of Sardinia, five from Santa Teresa di Gallura – Porto Pozzo (RDPP), five from Olbia (RDOM) and five from San Teodoro – Punta Aldia (RDST). Small portions of adductor muscle were used to collect DNA. Total DNA was extracted and amplified according to Casu *et al.* (2005). We tested four primers (see Tab. 1): two anchored at 3' (UBC811 and SAS3, T_a 51 °C), and two anchored at 5' (+GACA and +CA,

T_a 56 °C). For each primer, negative controls and replicates were included in the amplifications in order to both check the occurrence of PCR artifacts, and verify the repeatability of results. The PCR products were analyzed by electrophoresis on a 2% agarose gel stained with EtBr in 1× SBA (Sodium Boric Acid) buffer. Gels were run at 90 V for 110 minutes. One hundred base pair ladders were run for size band reference with each primer. We assumed that each ISSR fragment (those constantly detected after three different PCRs) represents a different locus.

Results and conclusions - Overall primers, we found clearly reproducible banding patterns that yielded a total of 24 bands, nine of which in +GACA, five in +CA, five in UBC811, and five in SAS3. Their size ranged from 300 to 900 *bp* (base pair) for the primer +GACA, from 350 to 900 for the primer +CA, from 300 to 1100 for the primer SAS3 and from 300 to 800 for the primer UBC811 (Tab. 1). Overall populations, we detect 19 fragments in RDPP, 15 in RDOM, and 11 in RDST, which were all polymorphic. Results obtained demonstrate that ISSRs can be successfully used to carry out future studies on population genetics of *R. decussatus*, with a considerable spare of time and costs thus excluding troubles involved in the occurrence of DUI in the mitochondrial DNA of this species (Passamonti and Scali, 2001).

Tab. 1 - Primer names and primer sequences, annealing temperature (T_a), range of molecular weight in base pairs (*bp*) and number of bands per primer.
*Nome dei primer, sequenze dei primer, temperature di annealing (T_a), intervallo del peso molecolare in paia di basi (*bp*) e numero di bande per primer.*

Primer	Sequence (5'-3')	T_a (°C)	Size range of bands (*bp*)	# of bands
SAS3	GAGGAGGAGGAGGC	51	300-1100	5
UBC811	GAGAGAGAGAGAGAGAC	51	300-800	5
+GACA	WBGACAGACAGACAGACA	56	300-900	9
+CA	RYCACACACACACACA	56	350-900	5

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