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GENETIC TRACEABILITY OF CLAM SPECIES USING MITOCHONDRIAL MARKERS

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Genetic differentiation of the clam species *Meretrix meretrix*, *Meretrix lusoria*, *Ruditapes decussatus*, *Ruditapes philippinarum* and *Chamelea gallina* is very important for the authentication and the correct labelling of sea food products. Morphological criteria such as shell characteristics (shape, color and striation) as well as siphon shape and location allow to distinguish among these species when they are sold alive. Nevertheless, they are usually commercialised frozen and mixed with other ingredients. Since the shell is removed there is a real possibility that these species can be accidentally or fraudulently substituted. Due to their difference in prices, taste and flesh texture, nonmorphological methods are then necessary for the authentication of bivalves in order to assure consumers about the identity and quality of food products purchased. Moreover, it is a legal requirement for their commercialisation to show on the label the scientific and commercial name of the species, the production method and the capture zone. Finally, an additional important requirement is that companies (for instance, import/export companies) in recent years ask to safeguard themselves from potential swindles or improper commercialisation of sea food products.

This study is part of the project 'Study of new analytical and biotechnological methods for species control and traceability of different animal products (bovine, ovine and fish chain)', supported by Regione Veneto and CNR, in collaboration with private companies. The aim in the fish chain is to develop new molecular marker systems and reliable mitochondrial DNA-based assays for the identification of clam species. Clams imported from different sea areas of the world were kindly provided by Rivamar (Taglio di Po, Rovigo). Initially CAPS (Cleaved Amplified Polymorphic Sequence) markers were developed mainly on the 16s mitochondrial gene. New specific primer pairs were designed from the multiple alignment of known mitochondrial sequences of *Ruditapes decussatus* and *Ruditapes philippinarum* as well as other different organisms. Amplicons were sequenced to confirm that the amplified products were from the target 16s rRNA region and to design suitable restriction sites. Different combinations of restriction enzymes were used to distinguish among these species. CAPS markers proved to be effective, cost and time saving, and offered several advantages over protein analysis (i.e. electrophoretic, chromatographic and immunological techniques). DNA is less affected by processing as it is more stable and it is also present in all cells of a given organism, enabling to obtain the same information from any tissue. Single-locus polymorphisms developed through the analysis of genomic DNA with AFLP (Amplified Fragment Length Polymorphism) markers as well as SNP (Single Nucleotide Polymorphism) markers designed on known genic DNA sequences will be also investigated in order to set up a molecular assay suitable to distinguish clam species in a faster and easier way.