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Species characterization using mitochondrial DNA sequences: a preliminary study using fishes of family Myctophidae

Mitochondrial DNA polymorphism is a widely used tool in population differentiation, species identification, and phylogenetic studies. Development of molecular markers for characterixzation of morphologically close fish species is immensely important, especially for identification of early life stages. In the present study, the suitability of DNA sequences of a conserved using three morphologically close, widely distributed Myctophid species, namely *Benthosema glaciale*, glaciale, *Notoscopelus kroeyerii* and *Diaphus dumerilii* collected from the Atlantic Ocean.

About 516 base long segment (approximately one third0 of 16S rRNA gene from fish was amplified by polymerase chain reaction (PCR) and DNA sequences were obtained by cycle sequencing using dye-labelled dideoxynucleotide terminators. The sequence comparison displayed considerable sequence variation among three species and the

pair-wise interspecific sequence divergence varied between 5.1% and 10.5%. This suggests that the mt-16S rRNA gene is a useful marker to distinguish these three species. The sequences were further compared with those from six other Myctophid species (morphologically close). It revealed interspecific sequence divergence between 4.5% and 18.9%. These results show that despite the mt-16S rRAN gene is an evolutionarily conserved gene even among distantly related species, it provides a useful tool in species identification among closely related fishes of the family Myctophidae.

The present analyses also found several sites variable among the species, at which sequences consisted of recognition sites for nine restriction enzymes. Prior knowledge of available restriction sites in the selected gene is a prerequisite for a indirect, yet less expensive method of studying genetic differences, i.e. PCR-based restriction fragment length polymorphism (PCR-RELP) analysis. Restriction site information revealed in this study can be deployed to develop the above method for Myctophid fishes so that a targeted restriction may generate species-specific fragment profiles making the species characterization much easier.