Utility of Citochrome Oxidase I (COI) DNA Barcode in generating robust molecular criteria for fish species and stocks identification

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Motivation

Mitochondrial sequence diversity has been used to distinguish closely allied species for more than 20 years. More recently, 'DNA barcoding', the survey of sequence diversity in a 648 bp segment of the mtDNA gene cytochrome c oxidase subunit I (COI) has been proposed as a standard tool for species-level identifications of all animals. Aside from the benefits of creating a DNA-based identification system, DNA barcoding is an effective tool for gaining an initial sense of the patterning of genetic divergences. Because of this, several authors have suggested that DNA barcoding will aid rapid progress in traditional taxonomic work by speeding the discovery of new species and in the recognition of synonymies. The present study builds on this work, assembling a DNA barcode library for some fish of commercial interest. The global trade of seafood can originate intentional product mislabeling. A very common fraud in Sicily is the substitution of fresh swordfish (Xiphia gladius) from Mediterranean area with frozen fishes, usually imported from the North Atlantic or the Indian Ocean. The fraud is particularly easy since this kind of fish is sold sliced. The aim of the present study was test whether the mitochondrial DNA gene, COI, could be used not only as marker for specie identification but also for distinguishing different stocks of swordfish from various origin.

Methods

We applied the DNA barcoding technologies, upon a 686 bp segment of COI, to compare swordfishes from different sources. We obtained about 60 samples from import/export companies that could certify the fish origins. In addition 10 fish slices, claimed to be fresh swordfish of mediterranean origin, were used as "unknown" samples. The fish was used for extracting genomic DNA, amplifying and sequencing a 686 bp fragment of the cytochrome oxidase I. The sequences obtained were aligned using ClustalX. Ambiguous regions of the alignment were systematically identified and removed using the programme GBlocks. Phylogenetic relationships among the 16 sequence haplotypes obtained were examined using Neighbour-joining (NJ) and Bayesian analyses. The NJ tree was constructed using

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pairwise distances calculated following the application of Kimura's two-parameter (K2P) correction for multiple substitutions in MEGA v. 4.0. The robustness of internal branches of distance was estimated by bootstrapping with 1000 replicates. Modeltest v. 3.06 was used to select the most appropriate models of sequence evolution for the Bayesian analysis, under the Akaike Information Criterion. This was the general time reversible model (GTR) taking into account the shape of the gamma distribution (G) and the number of invariable sites (I). The Bayesian analysis was implemented in MrBayes v. 3.0, using a Metropolis-coupled, Markov Chain Monte Carlo (MCMC) sampling approach. Parameters were estimated with four Markov chains incrementally heated with the default heating values. All analyses starter with randomly generated trees and ran for 1x106 generations, saving one tree in every 100 generations.

Results

The phylogenetic trees constructed with the NJ (K2P model) and the Bayesian methods were superimposable and revealed that the 60 swordfish sequences clusterized in three main groups, related to the geographical origins. When in the analysis were added the 10 unknown origin samples (but labeled as mediterranean), the sequence comparison showed that 2 of them come from Indian Ocean, 4 from Atlantic Ocean and only 4 samples were caught in the Mediterranean Sea. This means that in 6/10 cases the declared origin was false. Our preliminary data predict the potential of the 686 bp segment of citochrome oxidase I as an efficient specie and stock-specific molecular marker in swordfish Xiphias gladius.

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