Introducing genetic markers for identification of three fish species *Rutilus frisii kutum*, *Abramis brama* and *Barbus capito* in Caspian Sea using PCR-RFLP

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Abstract

In this research, three species of Cyprinidae (*Rutilus frisii kutum*, *Abramis brama* and *Barbus capito*) were studied on the central coast of the Caspian Sea basin. For extraction of DNA samples from fish fin fins by phenol-chloroform method, a pair of primers were designed according to the sequence of cytochrome b of the gene of *rutilus rutilus caspicus*, resulting in a PCR product of length bp1117. To digest the enzymes of the PCR product, the enzymes *Alu* I, *Bam*H I, *Rsa* I, *Alw*26I, *Dpn* I, *Eco*47 I, *Hha*I, *Hae* III, *Hinc* II, *Hinf* I, *Msp* I, *Taq* I, and *Mbo* I were used. The electrophoresis patterns obtained from PCR enzymes digestion for *Hha* I, *Mbo* I, *Hinf* I, *Rsa* I and *Hinc* II enzymes showed three genotypes and the other enzymes of the same genotype. In this research, REAP and PAUP software were used to analyze the data. Based on the obtained data, evolutionary distance between *Barbus capito* and *Abramis brama*, 0.2523, between *Abramis brama* and *Rutilus frisii kutum* was 17.82 and between *Abramis brama* and *Rutilus frisii kutum* was 0.3500. Comparison of the data shows that the most evolutionary distance is between *Rutilus frisii kutum* and *Barbus capito*, and the minimum distance is between *Abramis brama* and *Rutilus frisii kutum*. The Monte-Carlo simulation test (χ^2 test) showed a significant difference between species based on genetic similarity or genetic disparity of the studied species with 1000-fold simulation ($P < 0.0001$). In addition, the cladograms derived from molecular data analysis were based on the UPGMA model, which was obtained with the same equilibrium of all nucleotide traits and the analysis of 46 traits, four clusters, and a central tree was obtained by combining these cladograms.

Keyword: genetic markers, PCR-RFLP, *Rutilus frisii kutum, Abramis brama*, *Barbus capito*