

Abstract

Evolutionary relationships between *Barbus* species have been already studied in the past, but most of the studies were based only on mitochondrial DNA, there is no detailed study based on nuclear markers. This is mainly due to the fact that *Barbus* species are tetraploid. The western part of Greece has been studied insufficiently, and moreover a greater diversity of freshwater ichthyofauna is expected here. Greece is one of the hotspots of biodiversity. Greek river system is characterized by a significant proportion of endemic species of freshwater fishes. In this work evolutionary relationships between *Barbus* species were studied, with focus on diversity of Greek lineages. The geographical distribution of *Barbus* species has been also revised. In total, 192 individuals were analyzed, 156 individuals were from 40 Greek localities and another 36 *Barbus* species were from other areas of distribution of the genus. Dataset was supplemented with available published sequences. For the phylogenetic analysis mitochondrial gene cytochrom *b*, 1st intron of ribosomal protein gene *S7* and 2nd intron of gene beta-actin were used. For both nuclear markers paralog-specific primers were used. The analysis of cytochrom *b* revealed several well-supported lines: 1. species of southwestern Mediterranean (*B. meridionalis* and *B. haasi*), 2. fluvio-lacustrine species (*B. macedonicus*, *Barbus barbatus*, *B. bergi*, *B. tauricus*, *B. escherichii*, *B. ciscaucasicus*, *B. kubanicus*, *B. tyberinus* and *B. plebejus*), 3. *B. balcanicus* and *B. carpathicus*, 4. Greek and Albanian rheophilic species with which *B. petenyi*, a Danubian species, also clustered. Cytochrom *b* and nuclear gene *S7* revealed existence of 10 well-supported lineages corresponding to seven recognized species in Greece: *Barbus macedonicus*, *B. balcanicus*, *B. cyclolepis*, *B. strumicae*, *B. sperchiensis*, *B. euboicus* and *B. prespensis* and three lineages within presumed distribution area of *Barbus peloponnesius* were discovered, of which one is closely related to *B. prespensis*. Lineage *B. peloponnesius* 1 occurs in the west of Greece, from Peloponnese to the river Acheloos, lineage *B. peloponnesius* 2 occurs from Butrint lagoon to the Acheron river and *B. peloponnesius* 3 was found in the Arachthos river. Nuclear gene beta-actin was rather uninformative, several species clustered together and shared haplotypes; only a few species were separated with high support. In this study a sympatric occurrence of the lineages *B. peloponnesius* 2 and *B. prespensis* was found in the river Kalamas, probably as a consequence of secondary contact. Hybridization between these two taxa was revealed.

Key words: *Barbus*, Greece, freshwater ichthyofauna, cytochrom *b*, *S7*, beta-actin, evolutionary relationships, phylogeography