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Molecular Identification, DNA Barcoding and Determination of Genetic Relationship of Killifish (Aplocheilus spp.) Present in Aththanagalu Oya, Sri Lanka

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Abstract

Accurate identification of species is of paramount importance in conserving fish diversity due to practical limitations of recognizing closely related species based on morphological features. DNA barcoding is a rapid and reliable molecular tool widely used to identify organisms at species level. The present study was conducted to identify and determine genetic relationships of the *Aplocheilu*s spp. present in Aththanagalu river basin, Sri Lanka using molecular tools. From 68 sampling sites along the river, *Aplochielus* spp. were collected and identified using morphological characteristics. Meristic characters of each fish sample were subjected to truss analysis by preparing principal component analysis (PCA) using PRIMER 5 software. Genomic DNA extracted from fresh muscle tissues of ten samples of killifish was subjected PCR targeting the COXI gene in mitochondrial genome using a universal primer pair, FishF1/FishR1. Ten PCR amplicons of the expected size (approximately 650 bp) were subjected to DNA sequencing. Eight successfully-sequenced samples were subjected to DNA homology search using BLAST, NCBI and phylogenetic analysis using Geneious 7.1.3 and MEGA 6 software. According to the results of morphological characteristics, 180 *A. dayi* and 143 *A. parvus* species were identified. Truss analysis based on morphological characteristics did not differentiate *A. dayi* and *A. Parvus* into separate clusters. Based on DNA homology search results, two *A. dayi* samples identified morphologically were highly homologous to *A. werneri* (with 83-94% identity and 75-77% query cover). Two other *A. dayi* samples and four *A. parvus* samples, identified morphologically were best matched with *A. blockii* voucher sample (with 90-97% identity and 80-98% query cover). By phylogenetic analysis, the two *A. dayi* samples which showed the highest DNA homology with *A. werneri* formed a separate cluster but indicated a closer relationship with *A. werneri*. The rest of the *A. dayi* and *A. parvus* samples which were highly homologous with *A. blockii* were clustered together and formed a monophyletic clade but showed a closer relationship to *A. blockii*. The findings revealed the ability of identifying *A. parvus* and *A. blockii* which is difficult to be differentiated by morphological characters by DNA barcoding targeting the COXI gene. Exact identification of *A. dayi* is not possible due to unavailability of sequence information of *A. dayi*. Closer genetic relationships of some *A. dayi* with *A. werneri* indicate possible hybrids due to interbreeding.

Keywords: DNA sequencing, Cytochrome c oxidase I gene, Phylogenetic tree, Polymerase chain reaction, Species identification