

Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) in determination of phylogenetic relationships of *Puntius* spp.: A pilot study

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Genus *Puntius* comprises a major component of freshwater fish fauna. Among 16 species of *Puntius* recorded in Sri Lanka, eight are endemic and nine are identified as threatened species. Importance of conservation of these species has been highlighted. Correct identification of the species and their phylogenetic relationships are important in adopting conservation strategies.

Present study focused on identification of molecular markers to differentiate selected six *Puntius* species, namely *P. titteya*, *P. nigrofasciatus*, *P. dorsalis*, *P. filamentosus*, *P. bimaculatus* and *P. chola* to study their genetic and morphological relationships and to ascertain that morphological and molecular relationships are in agreement.

Ten individuals of each *Puntius* spp. were collected from different locations of Nilwala river basins, their morphological data (morphometric and meristic) were recorded and analyzed by cluster analysis using Euclidian Distances using Primer (version 5) software package. DNA was extracted from muscle tissues of two selected individuals representing each morphological cluster. A segment of 12s rRNA was amplified and the resultant fragment was restricted using seven restriction enzymes. Restriction fragment length polymorphism was recorded and analysed by the same method used in morphological data analysis.

P. titteya and *P. nigrofasciatus* grouped into a single cluster in both analyses showing that they are closely related with each other morphologically as well as genetically. In morphological data analysis, they formed a separate cluster from other four species indicating that they are morphologically different from other species. This outcome was not evident in molecular data analysis. In contrast, *P. bimaculatus* and *P. dorsalis* separated into two different clusters in molecular data analysis which were not apparent in morphological analysis. Results of the two analyses show that morphological and molecular relationships of six species are not in fully agreement and the possibility of using PCR-RFLP markers in future to determine the phylogenetic relationships of all *Puntius* species in Sri Lanka.