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PHYLOGENETIC RELATIONSHIPS OF TWENTY- THREE INDIGENOUS CYPRINID SPECIES OF SRI LANKA

BY

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ABSTRACT

An understanding of evolution of animals requires an estimation of phylogeny as well as the analysis of micro evolutionary changes. Estimation of phylogeny of animals requires sources of heritable variations. Until 1970, main source of the heritable variations is the study of morphology of fish. However, during the last two decades molecular investigations such as chromosome analysis, immunological analysis, restriction site analysis, nucleic acid sequencing and protein analysis was used as sources of heritable variations in phylogenetic studies of animals. Protein analysis using elecrophoresis is the most common molecular technique used in the phylogenetic studies of fish. Along with molecular techniques, introduction of computers and especially made software packages in to the field of taxonomy greatly improved the handling of large numbers of characters and the reliability of the analysis of data.

Identification of new species and the study of biology and ecology of fish are very common in the history of cyprinids of Sri Lanka. However, detail studies on osteology morphology, morphometry and protein analysis of cyprinids of Sri Lanka hitherto has not been studied. As a result their interrelationships and phylogeny is not known. Therefore the present study of cyprinid fish included a phylogenetic study based on detail study of osteology, morphology, morphometry and protein analysis of fish.

Twenty-three species of cyprinids were morphologically studied. It included a complete study of osteology of the genus ***Puntius*** and this was compared with osteological

characters of other genera such as ***Danio, Rasbora, Chela, Tor, Garra*** etc. Morphometry and meristics of 23 species of cyprinids were studied. Nineteen body measurements were made and their mean, range and standard deviation were calculated. These body measurements were transformed into 40 ratios and they were statistically analysed to find out the significant deferences among the species. Fourteen meristic characters and two other morphological characters were studied and their mean, range and standard deviations were calculated. ANOVA tests were performed for the characters that vary among the individuals of species. Out of 23 species morphologically studied, 15 were subjected to electrophoretic study. Horizontal starch gel electrophoresis was carried out to test ten enzyme systems of skeletal muscles of fish.

Analyses of data sets were done using computer software packages such as PAUP version 3.0 (Phylogenetic Analysis Using Parsimony) and MacClaid version 3.0 (Analysis of Phylogeny and Character Evolution). Separate matrices were prepared for three different data sets. Initially three separate computer analysis were carried out for three sets of data. Two other computer analysis were carried out for combined morphological and biochemical characters.

Presence of pineal foramen, complete interorbital septum, pharyngeal process, pharyngeal teeth, Webarian apparatus and the absence of basisphenoid and teeth on jaws are characteristic features of cyprinids studied. The Webarian apparatus is formed by the fusion of first four vertebrae of the axial skeleton. The caudal fin skeleton is primitive type and contained 5 or 6 hypurals and two epurals.

Out of 40 body ratios studied 17 were significantly different among the species studied. Analysis of morphometric data produced 100 equally parsimonious cladograms. The majority ruled consensus tree of these 100 cladograms indicates that the 23 species fall into 5 clads.

The ANOVA tests performed on the meristic data of ***Puntius*** species indicated that the counts of gill rakers, vertebrae, lateral line scales and the pectoral fin rays are highly successful in the separation of species into groups. Meristic data analysis of species produced 83 equally parsimonious cladograms with a consistency index of 0.493 and a length of 73 steps. The 50% majority ruled consences tree condensed from 83 cladograms indicated that ***Puntius nigrofasciatus, P. cumingii, P.ticto, P. titteya*** and ***P. vittatus*** are very closely related species and has originated from a common stock. ***P.sarana*** is very closely related to ***P. pleurotaenia***.

Out of ten enzymes tested eight produced clear zymograms with a good resolution of banding patterns. Analysis of the 20-biochemical characters defined by these banding patterns, produced 500 equally parsimonious cladograms each with a consistency index of 0.976 and a length of 83 steps. The 50% majority ruled consences treee of bootstrap analysis indidcated that ***P. nigrofasciatus*** is very closely related to ***P. cumingii*** and ***P. bimaculatus*** is very closely related to ***P. amphibius***. Based on the biochemical data ***Rasbora daniconius*** is very closely related to ***R. vaterifloris***. ***P. sarana*** clustered with ***P.chola*** and ***Esomus danrica***. ***P. dorsalis*** and ***P. filamentosus*** are distantly related to all the other species of the group.

Analysis of 53 characters using combined morphological and molecular data produced four equally parsimonious phylogenetic trees each with a consistency index of 0.702 and a length of 238 steps. The consences trees give clear separation of clusters. The consences tree of combined data indicated that ***Puntius dorsalis, P. amphibius*** and ***P. chola*** are distantly related to all the species studied. ***P.nigrofasciatus, P. cumingii, P. vittatus, P. titteya*** and ***P. bimaculatus*** clustered together and are very closely related to each other. ***P. filamentosus*** is very closely related to ***P. sarana***.

In the comparison of results of analysis of separate data sets that of combine data sets revealed that as the number of characters increased in the data set, the separation of clusters of sub families is clearer. Therefore it is very important to use higher number of characters in

phylogenetic studies. For this purpose, inclusion of biochemical characters in phylogenetic studies is essential. As the number of characters increase, the data analysis is impossible without computers and especially made software packages.