Phylogenetic Relationship of *Notopterusnotopterus* (Pallas) and *Chitalachitala* (Hamilton-Buchanan) Inferred from Mitochondrial 12S rRNA Gene Sequences

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Abstract—Identification and classification of animal species is a key pre-requisite for many biological studies. Species identification strictly on the basis of morphological characters alone is quite unreliable on account of the confusing and overlapping characters. Study by Herbert et al., (2003) indicated four main significant limitations for species identification based on morphological characters. There are some significant limitations for species identification based only on morphological characters, such as phenotypic plasticity, genetic variability, and the presence of morphological keys in some particular life stages and gender selective keys.

Application of techniques based on molecular markers especially mitochondrial markers is the current trend in resolving phylogenetic and taxonomic issues. N. notopterus (Pallas, 1769) and C. chitala (Hamilton-Buchanan) belongs to class Actinopterygii, Order Osteoglossiformes and family Notopteridae. The status of the N. notopterus(Pallas) and Chitalachitala (Hamilton-Buchanan) belonging to family Notopteridae has been controversial on account very few morphological characters which have been used to differentiate these two fish species. In the present study, an attempt has been made to study the phylogenetic relationship of thesetwofishspecies from Harike wetland (A Ramsar site in Punjab) using mitochondrial 12S rRNA gene. The fish muscle samples were collected from Harike wetland in the month of November 2016. DNA was isolated using standard procedures and Isolated DNA was checked for its quality and quantity by spectrophotometric analysis and Agarose Gel Electrophoresis respectively. They were then amplified using Forward primer (5'-CAA ACT GGG ATT AGA TAC CCC ACT AT-3) and Reverse primer (5'- GAG GGT GAC GGG CGG TGT GT-3). The samples were then sequenced from Chromas Biotech, Bangalore, and analysed using MEGA6 software. In phylogenetic analyses using 12S rRNA, the outgroup species were well separated from the other species. One of the two obtained clusters showed species of Genus Chitala while the other cluster showed N. notopterus. The present study thus reinforces the belief that these two fish species i.e. Notopterus notopterus and Chitala chitala have been rightfully separated by Jayaram (2010) to be belonging to different genera and not just to one genus Notopterus as earlier described the Talwar and Jhingran (1991).

Keywords: Notopteridae, molecular markers, 12S rRNA, Phylogenetic Relationship.