

ABSTRACT

Barcoding is an emerging reliable and authentic technique for species identification. This technique involves the conserved region (COX I) of mitochondrial genome for identification of species. In this work we identify type specimen fishes taken from Stephenson natural history museum GC University, Lahore. Direct PCR was carried out by gill homogenate of each sample and COX I gene was amplified using reported set of primers obtained sequence data was analyzed by using bioinformatics tools. The neighbor joining (NJ) tree analysis was able to establish the phylogenetic relationships among species. The peaks in K2P distance-based NJ trees were supported with high bootstrap values (100%) in case of all species specimens *Clupisoma naziri*, *Barilius vagra pakistanicus*, *Noemacheilus naziri* and *Noemacheilus griffithi hazarensis*. Congeneric species clustered together and so did the confamilial species. Our DNA barcoding approach separated different species belonging to same family with 0.608%, 0.44%, 0.42% and 0.608% distance among individuals of congeneric species respectively showing that the average nucleotide divergence among conspecific individuals is less than 30-fold.