Order Perciformes is the largest and the most diversified of all fish orders. Indeed, it is the largest order of vertebrates containing about 9300 teleost species and occupies an interesting phylogenetic position within Teleostei. Most of the perciform fishes are commercially important as food fishes or ornamental varieties. About 23% of all perciforms (approximately, 2335 species) use freshwater during their lifespan, and 2040 species normally occur only in freshwater and there are about 50 species belonging to 11 families, recorded from freshwaters of Indian region. Despite the abundance of perciforms and their commercial and scientific importance, the classification and phylogeny of this group remain confused/controversial, since most families in many suborders are basically similar and are not easily definable in terms of common shared derived characters. There have been several recent attempts to alleviate this confusion especially in the marine perciforms, using species-specific molecular (DNA) signatures. However, no serious attempt has been paid on Indian freshwater perciform fishes and they are poor in terms of available genomic information and tools. Hence, the present study was undertaken to (i) generate reference molecular signatures of the commercially important perciform fish species found in Indian freshwater bodies using partial sequences of three mitochondrial genes — 16SrRNA, COI and Cyt b; and (ii) analyse the genetic divergence within and between species to resolve taxonomic ambiguity, if any, and establish a phylogenetic tree of freshwater perches of Indian waters.

Altogether 32 perciform species (five specimens each) from 11 families as described above were collected for the study from different agro-climatic zones of India. Field identification and nomenclature of the species collected were followed based on the morphological, meristic and anatomical characters described by Talwar and Jhingran (1991) and Jayaram (1999).
Fresh fin clips and muscle tissue samples (10mm approximately) were collected from the live fish using non-invasive methods and were used for DNA extraction following the standard salt extraction protocol. Three DNA fragments were amplified, representing three partial gene regions such as 16S rRNA, COI and Cyt b by employing specific universal primers.

A total of 998 bidirectional partial sequences of mitochondrial 16S rRNA (520-570 bp), COI (630-678 bp) and Cyt b (800-1142 bp) genes were generated from a total of 165 individuals belonging to 32 species and 16 genera (viz., Ambassis, Chanda, Parambassis, Anabas, Colisa, Pseudosphromenus, Etroplus, Gerres, Badis, Dario, Nandus, Pristolepis, Sillago, Terapon, Scatophagus and Channa) (n=5 each species) collected from different drainages of India in the present study. The edited sequences (different haplotypes only), after their confirmation were submitted to NCBI GenBank under the accession numbers KC774633-KC774731; KC774734-KC774763; KC835200-KC835208; KC858282-KC858292; KC880525 for public access.

A Kimura-2-parameter (K2P), pair-wise sequence divergence levels within families were generated using Kimura’s two-parameter method. A cluster analysis within and between families was done with the Neighbour-Joining algorithm (NJ) as implemented in software MEGA.

The number of haplotypes ranged from a minimum of one to maximum of four in all the genes; but in most cases it was two. The estimates of genetic divergence with all the three genes were sufficient enough to discriminate individuals of different species under study. The mean pair-wise K2P genetic distance values among species based on 16S rRNA ranged from 1.8% to a maximum of 24%. With COI, the maximum inter-specific K2P distance was 32% and minimum 3%, whereas with Cyt b, the values were 43% (maximum) and a minimum of 9%. For all the three genes, the K2P values within the species (among haplotypes), ranged between 0.1% and 0.5%.

Transitions outnumbered transversions in all the species and the observed transition versus transversion ratios in the present study are also comparable to those of many teleosts. For both COI and Cyt b, most of the transitional and transversional substitution changes in the taxa within the perciforms were third codon position (77.0 – 85.6%) followed by first
codon position (12.8 – 18.0%), and with least change in second codon position (1.6 – 6.0%).

★ The neighbour-joining (NJ) tree revealed distinct clusters in concurrence with the taxonomic status of the species. A total of six major clades were obtained with each clade supported by bootstrap values ranging between 50% and 99%. The phylogenetic relationship among the species was clearly established, and similar species were clustered under same nodes while dissimilar species were clustered under separate nodes. Congeneric species always clustered together and in most cases, so did the confamilial species.

★ The low within-species and high between species divergence values and the identical tree topologies indicate the usefulness of partial sequences of the three genes (16SrRNA, COI and Cyt b) employed in the study in accurately delineating various freshwater perciform species of Indian waters.

★ Several authors have considered Parambassis lala as a synonym of P. ranga. But, in my study, complete separation of these two species in the NJ trees with a high bootstrap value and the K2P genetic divergence values that are above the threshold level of intra-species differences reject the above statement. The morphometric characters of these two species were in collections of the present study were also clearly distinct (P. lala: D VII+I 11; A III 13; P I 10; V I 5. Body small and rounded; Lateral line with 90 minute scales. P ranga: D VII+I 11-14; A III 13-15; P I 11-12; V I 5. Body stout and compressed, lateral line with 47-63 minute scales). Hence, both P lala and P. ranga are considered as independent species in the study as reported by Roberts (1994) and Jayaram (2010).

★ In family Sillaginidae, in addition to Sillago sihama and S. vincenti, two putative new species of Sillago (?) were noticed as evidenced by the high genetic divergence (K2P) values and NJ tree branching with all the genes. Further, extensive collection of samples from all over the Indian coast and possibly from the neighbouring countries and an integrated morphological and genetic approach (jointly with taxonomic experts in the group) are needed to resolve this confusion.

★ Nandus nandus under the family Nandidae, collected from southern region (Kerala) and the Gangetic plains (Kolkata), though were
morphologically almost identical, formed sister cades in the NJ trees and exhibited K2P values above the expected within species range with all the three genes. This suggests the possible occurrence of *N. marmoratus* in Peninsular India which is distinct from *N. nandus* as reported by Day (1878) and it also calls for a thorough revision of the taxonomy of the species from the entire Indian subcontinent.

★ *Dario dario* a fish endemic to the northeastern region of India is a member of sub-family Badinae, family Nandidae (or family Badidae according to some taxonomists) along with *Badis badis* and *B. assamensis* (Jayaram, 2010). But, in the present study, it formed a distinct branch with high bootstrap support, away from both *B. badis* and *B. assamensis* with all three genes indicating that *D. dario* cannot be a member of family Badidae/sub-family Badinae. This points out the need for a relook at the systematic status of members of family Nandidae using morphological and genetic tools.

★ The genus *Pristolepis* was earlier considered as a member of subfamily Pristolepidinae under family Nandidae (Talwar and Jhingran, 1991; Nelson, 2006). Based on some distinct characters such as presence of globular teeth on tongue, two (generally bifid) spines on the operculum and normal caudal fin, Britz et al. (2012) upgraded the subfamily Pristolepidinae to family status Pristolepidae (Jayaram, 2010). In the present study, both *Pristolepis marginata* and *Pristolepis rubripinnis* were found far away from the nandid cluster in NJ trees with all the three genes with high bootstrap support indicating the validity of distinct familial status of Pristolepidae.

★ There has been considerable confusion about the taxonomy of the genus *Anabas*. Rao (1968) stated that there are two distinct species, *Anabas testudineus* the first one and *A. oligolepis* to the second species. Other authors have called the latter, *A. cobojius* (Talwar and Jhingran, 1991). However, *A. oligolepis/A. cobojius* was often cited as a junior synonym of *A. testudineus* by others (Bloch, 1795). Two well-separated clusters supported by high bootstrap value in the present study between the individuals of *Anabas* from the eastern (likely, *A. oligolepis* from Kolleru Lake and Bhubaneswar) and Southern India (*A. testudineus* from Kerala) regions indicate occurrence of two distinct species in the family Anabantidae. The K2P genetic divergence varied from 1.8 to 3%, 7.5%
and 9% with 16SrRNA, COI and Cyt b, respectively between A. testudineus and putative A. oligolepis. Further fine scale studies are warranted to confirm this conclusion preferably using nuclear DNA markers along with mtDNA gene regions.

★ The species Pseudosphromenus dayi, which is apparently endemic to Kerala, India, is often considered as a synonym of P. cupanus. Based on the K2P distance values of three genes and the pattern of phylogenetic tree in the present study, the two species are certainly distinct as recognized by aquarists.

★ The information reported in the present study may facilitate further investigations into the molecular phylogeny and evolution of freshwater perciforms of the Indian sub-continent. More rigorous taxonomic sampling and data from morpho-meristics, mtDNA and nuclear genome may provide a better understanding of perciforms, especially to answer some of the queries (as above) raised in the thesis.