

A Molecular Phylogenetic Perspective on Less Diversification and Speciation Pattern in The Population of Indian Cichlids

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Abstract- Cichlid fishes are excellent model system to study the basic dynamics of evolution due to adaptive radiation and unparalleled species richness. Despite of the efforts to establish relationship between cichlid fishes using different makers the phylogeny of these fishes remain unresolved due to rapid diversifications in the lineage. In the present study an evolutionary hypothesis of Indian and an exotic cichlids based on sequence information inferred from mitochondrial 16S rRNA gene sequences was proposed. The tree constructed using minimum evolution method shows high bootstrap value for the placement of Indian/Malagasy cichlids as most basal lineage with sister group relationship with monophyletic African and Neotropical assemblages. The extensive speciation surveyed within African and Neotropical cichlids may be attributed to the diverse habitats and varied climatic conditions. Comparatively, both the Neotropical and African lineages have experienced sequential episodes of diversification with few species limited to Indian subcontinent. A detailed discussion to figure out the reasons underlying less diversification of Indian cichlids fishes in contrast to rapid rate of adaptive radiation observed in other lineages was carried through.

Keywords- cichlids, phylogeny, 16SrRNA gene, less diversification, speciation

I. INTRODUCTION

The family Cichlidae is comprised of over 2000 species of fishes found in fresh as well as brackish water habitats in Africa, South America, North America and Southern parts of India. Native Cichlids are largely absent in Asia, except for nine species in Israel, Lebanon and Syria, two in Iran, and three in India and Sri Lanka (Nelson, 2006). Cichlids have always been attracted researchers mainly due to their great adaptive radiation, species richness and their importance as food fish (Barluenga *et al.*, 2006). To understand the process of speciation, adaptation and coevolution detailed interspecific molecular phylogenies are often necessary (Avice, 1994; Harvey, 1996; Cotton and Page, 2004; Schluter, 2000). Unfortunately, as taxa become more closely related,

more difficult to reconstruct because of the lack of variation necessary to retrace their evolutionary relationships (Whithall *et al.*, 2006). The characteristic single parental inheritance allows geneticists to use data from mitochondrial DNA sequences to draw conclusions about evolution. The smaller size, simplicity, faster rate of evolution than the nuclear genes have attracted many researchers to use mitochondrial genome as a model to study molecular evolution in animals. The phylogenetic utility of the 16S mitochondrial gene has been established at several taxonomic levels among vertebrate taxa (Mindell and Honeycutt 1990; Hillis and Dixon 1991; Mindell and Thacker 1996) and freshwater fish (Alves-Gomes *et al.*, 1995; Ortí *et al.*, 1996; Murphy and Collier, 1996; Ortí and Meyer, 1997). In spite of considerable homoplasy, the 16S rRNA gene data set contains reliable phylogenetic signal to establish relationships among major lineages of cichlids.

Cichlids are most diverse in Africa and South America. Africa alone is estimated to host at least 1,600 species (Nelson, 2006). Central America and Mexico have about 120 species. Contrary to the extensive speciation events in other lineages cichlids from Indian Malagasy regions stands varied homing for less number of indigenous cichlids. The paucity of diversification and retention of ancestral characters in Indian cichlids remain a question under discussion.

Hence, the present study combines findings on molecular phylogeny using mitochondrial 16S rRNA gene sequence of Indian cichlids *Etroplus suratensis* (Bloch, 1790), *Etroplus maculatus* (Bloch, 1795) and an exotic species *Pterophyllum sclarae* (Schultze, 1823) with close consideration on less diversification events in Indian cichlids.

II. MATERIALS AND METHOD

Taxon samples and DNA methods

Genomic DNA was isolated from white muscle tissues of the cichlids using NucleoSpin® Tissue Kit following manufacturer's instructions and the quality of isolated DNA was checked by agarose Gel Electrophoresis. The PCR amplification was carried out in a PCR thermal

cycler (GeneAmp PCR System 9700, Applied Biosystems) by using the following primers; forward 5'CGCCTGTTTATCAAAAACAT3' and reverse 5'CCGGTCTGAACTCAGATCACGT3'. Double stranded PCR products were sequenced by using BigDye Trmimator v3.1 Cycle sequencing Kit(Applied Biosystems, USA) on an automated sequencer (ABI 3500 DNA Analyzer, Applied Biosystems) following manufacturer's instructions. The nucleotide data determined for the present paper are deposited in GenBank (accession numbers: MF662800, MF662801, MF662802).

BLAST analysis

The 16S rRNA gene sequences BLAST analysis and the sequences that showed close identity were chosen for the phylogenetic analysis.

Phylogenetic analysis

Phylogenetic tree based on distance method was constructed using MEGA (Version 7.0) (Kumar *et al.*, 2016). The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2 parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm (Nei and Kumar, 2000) at a search level of 1. The Neighbor-joining algorithm (Saitou and Nei, 1987) was used to generate the initial tree. The analysis involved 42 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 515 positions in the final dataset.

III. RESULTS AND DISCUSSION

The ME phylogenetic tree constructed by using the 16S rRNA gene sequences in the present study shows three main groups of cichlids namely African, Neotropical, Indian/Malagasy cichlids and an outgroup constituting of members from Lutjanidae family (Fig.1).The tree shows high bootstrap value for the placement of Indian/Malagasy cichlids as most basal lineage with sister group relationship with monophyletic African and Neotropical assemblages. The tree is drawn to scale with branch length in the same unit as those

of evolutionary distances, which are in the units of number of base substitutions per site(Fig.1). The analysis involved 42 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 515 positions in the final data set. Bootstrap value is proportional to reliability of each node in the phylogram.

The representatives shown in the tree belongs to 4 subfamilies of the family Cichlidae, namely, Pseudocrenilabrinae (which constitutes the African cichlids), Etroplinae (which constitutes the Malagasy cichlids), Cichlasomatinae and cichlinae (that constitutes a part of South American cichlids). The indigenous cichlids under study belong to subfamily Etroplinae and the exotic cichlid belongs to the subfamily Cichlasomatinae. It can be inferred from the tree that *Etroplus maculatus* shares a common ancestor with the recent ancestor lineage that constitute *E. suratensis* and *E. canarensis*. On the other hand, the exotic cichlid *P. scalare* belongs to a different lineage from that of the indigenous cichlids. Another exciting result that can be inferred from the tree is the high species richness found in Neotropical and African region with few species limited to Indian/Malagasy region.

ME phylogenetic tree indicates that the Indian/Malagasy cichlids occupies a basal position. It can also be seen that the Malagasy cichlids are a sister group to the African and the Neotropical cichlids. This finding coincides with the findings of Farias *et al.* (1999), which consisted of a data set of 64 taxa and the phylogram being constructed using the 16SrRNA gene sequence. A similar result is obtained by Stiassny (1991), in the study using several nuclear genes to establish the phylogenetic relationships. *Etroplus* and *Paretroplus* have been found as sister lineages in every well-sampled phylogenetic analysis that has included them. Only one phylogeny, a 16S parsimony analysis of Sparks (2004) recovered a paraphyletic *Etroplus*. When Sparks (2004) adds CO1 to this 16S dataset, *Etroplus* is recovered as monophyletic and sister to *Paretroplus*.

Regional changes in diversification of fishes within a taxonomic group has bewildered ichthyological research. The East African lakes and the Neotropical region show extensive diversification and greater species richness of cichlids when compared to Indian cichlids. The predominantly intralacustrine speciation mode in these regions might have been shaped mainly by sexual selection and trophic specialization. It can be also be presumed that a great diversity of cichlids is harboured by the Neotropics followed by African land mass. When fresh water fish diversity is considered, Neotropics host more than three quarters of the world(76.6%)(Leveque *et al.*, 2008) whereas the two other

tropical realms, namely the Oriental and Afrotropical realms, each hosts only one fifth (17.8% and 20.8% respectively (Toussaint *et al.*,2016). This coincides with the hypothesis that the number of species within a taxonomic group tends to increase with decreasing latitude (Fischer, 1960; Pianka, 1966).

The diversity of Neotropical and African region can be attributed to the finding of Connor and McCoy (1979) and Nguyen and De Silva (2006) that larger land masses provide greater diversity of fish habitats resulting in higher species diversity. River size (surface area of drainage basin and mean annual river discharge) and energy availability (net primary production) are the most important factors influencing patterns of fish species richness according to Oberdorff *et al.*, (1995) and Guegan *et al.*, (1998). Presence of the Amazon River basin and the lush vegetation associated with it may act as a factor that promotes cichlid diversity in the Neotropics.

The phylogenetic pattern observed in these cichlids is completely congruent with the fragmentation of Gondwana and suggests drift-vicariance explanations (Stiassny, 1991). A study by Ivory *et al.* (2016) states that tectonic activities which took place about 1.2 Ma have created diverse habitats in the great lakes of Africa which lead to explosive diversification of cichlids. From examining the phylogeography of Cichlids, it could be assumed that during the Early Cenozoic, the ancestral cichlids were widespread in Africa and dispersed to Madagascar and India. Posterior radiations of modern cichlid fishes in Africa displaced these basal lineages, causing their extinction except for those in the Malagasy and Indian regions. The highly endangered status of most Malagasy cichlid species caused by the introduction of Tilapia and mouth-brooding *Oreochromis* species from Africa (Stiassny and Raminosoa, 1994) shows how representatives of the more modern cichlid lineages can displace the more basal cichlids in a very short time span (probably <200 years). The dispersal hypothesis further implies that Neotropical Cichlids reached South America long after its assumed separation from Africa

at 106 ± 84 Mya. They' may have reached South America by colonizing the Caribbean plate directly from Africa or via North America, and then dispersing to South America along island chains for which geological evidence exists (Marshall & Sempere, 1993).

Cichlids from Madagascar and India are the most basal group to diverge from ancestral African-Neotropical cichlids, which coincides with the drifting of the India-Madagascar subcontinent during the late Jurassic, ca. 150 Ma (Smith *et al.*, 1994). Farias *et al.* (1999) propose that more than 65 Ma ensuing since the separation of Gondwana fragments, Neotropical and African cichlids followed independent evolutionary pathways. Africa and South America have undergone radically different climatic histories since their separation in their Cretaceous. The well documented paucity of the Tropical African flora has been linked to an extensive spread of aridity in that continent following mid Oligocene global cooling trends (Goldblatt, 1993). Biotic factors also may have determined significantly different selective pressures for the two continental ichthyofauna. The combination of biotic and abiotic factors may have triggered high extension rates in Africa (Lundberg, 1993; Orti and Meyer, 1997). Surviving ancestral lineages of African cichlids have recently undergone explosive radiation in east African lakes accounting for the high number of extant cichlid species and for their low level of genetic differentiation.

Kocher (2004) identifies ecological selection, sexual selection and genetic conflicts as the major selective forces that lead to diversification of cichlids in African lakes. The small land area, absence of large river basins like the Amazon and the East African lakes and lush forests associated with them and low tectonic activities of the Indian plate (Khullar, 1999) may be some reasons for the low diversity exhibited by the Indian cichlids.

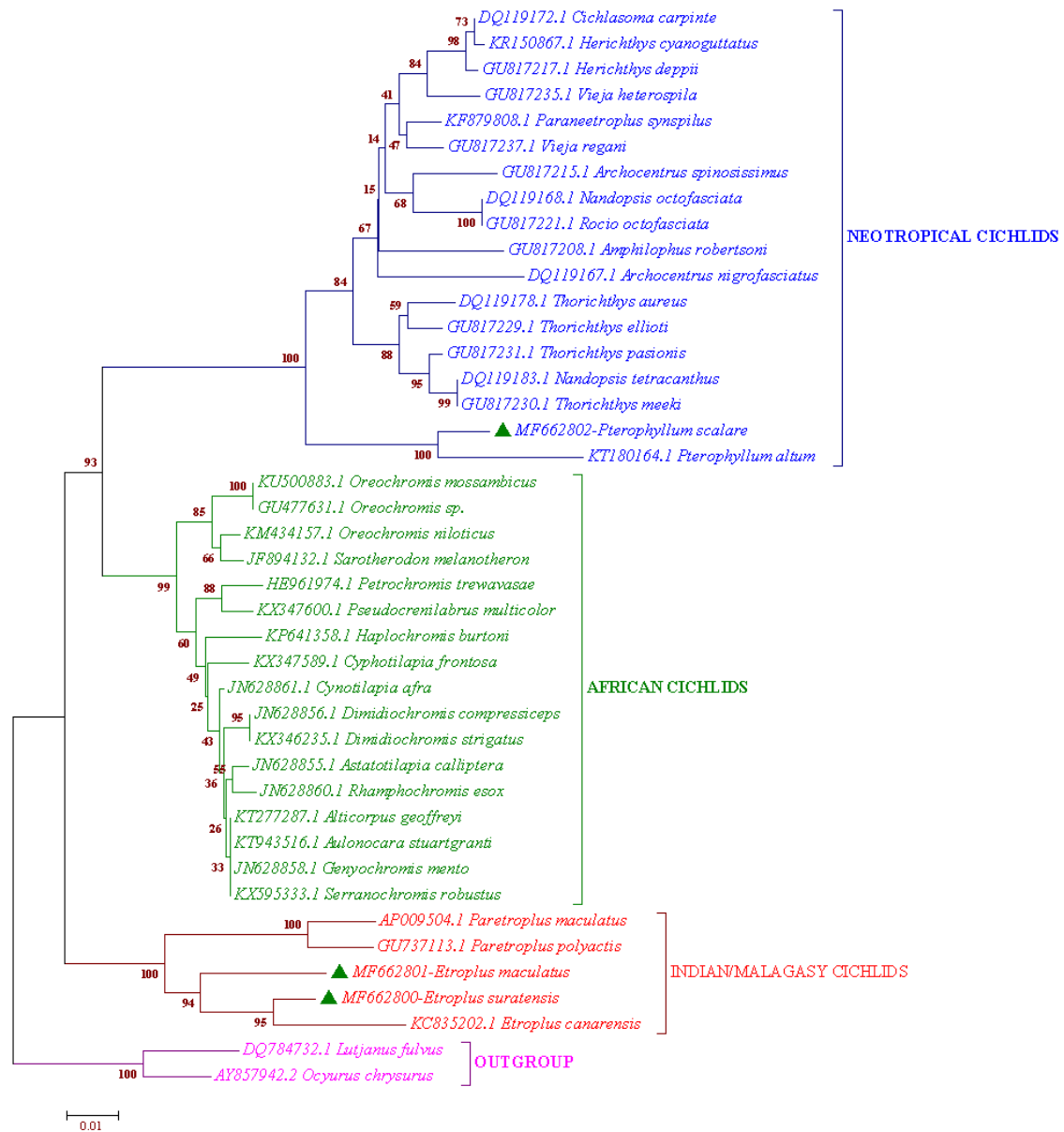


Figure legend

Figure 1. ME phylogram representing relationship between different subfamilies of Cichlidae (▲ indicates 3 cichlids under study, the numbers on the branches indicate bootstrap values for each node) The evolutionary history was inferred using the Minimum Evolution method (Rzhetsky and Nei, 1992). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2 parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. The

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