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RESEARCH ARTICLE

# Genetic Variation in Catfish Family: Ariidae, Bagridae and Plotosidae of India Using MtDNA Cyt B Gene

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## Abstract

To find the genetic variation and phylogenetic status of Ariidae, Bagridae and Plotosidae catfishes, this study was performed using mitochondrial cytochrome b (*Cyt b*) gene sequence. Totally 155 species were used in phylogenetic reconstructions under maximum parsimony, maximum likelihood and Bayesian inference criteria. The oneway ANOVA in base composition distance in Ariidae F= 184.77, df = 3; 6437, P< 0.0001 in Bagridae F= 19.41, df = 3; 1322, P< 0.0001 and in Plotosidae F= 1.40, df = 2; 32, P < 0.0001. The genetic distances between taxa increase as the taxonomic rank increases. In MP, ML and BI based phylogenetic tree of Ariidae, *Cathyrops dasycephalus* formed the longest branching clade suggesting that it would have accumulated mutations in neutral selection. Most of the other species in this tree showed that they have recently diverged. In Bagridae family, *Olyra longicaudatus* and *Leiocassis argentivittatus* are the two species that formed a long branched attraction affecting the related species branched to its major node. *Batasio* and *Bagrichthys* genera have combined to form a monophyletic clade. *Mystus* genus displayed monophyletic group with higher bootstrap and posterior probability values for all the species. *Plotosus* genus displayed monophyletic group with higher bootstrap and posterior probability values for all the species. The phylogram shows that the branch length of all the taxa except *Plotosus canius* showed branch length more or less 0.05 depicting moderate substitution rates throughout this genus.

**Keywords:** MtDNA, Cytochrome b, Catfish, Phylogenetic Analysis, Geneticvariation.

## 1. Introduction

Genetic variation in any species makes it able to survive and adapt to environmental challenges in the habitat (De Luca et al., 2014). Research on genetic variation provides solution for evolution, conservation, and management of natural resources and genetic improvement programs. Many molecular markers have been developed to detect genetic variation in individual organisms, species, or populations (Mortiz 1994). Mitochondrial DNA (mtDNA) is maternally inherited haploid genome and is found outside the nucleus of the eukaryotic cells (Kumar et al., 2016). Due to rapid evolutionary rate as compared with nuclear DNA, the mtDNA has widely been used to identify both genetic variability and population genetic structure ( Kenthao et al., 2016, Ha et al., 2020). Cytb gene has also been one of the most frequently utilized segments of mtDNA because it is easy to align and it has been characterized in many vertebrates (Thangaraj and Lipton 2011, Kumar et al., 2016, Liu et al., 2020). Certain regions of *Cyt b* gene diverge rapidly while some are conserved therefore

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are valuable for determining the phylogenetic distance among species (Kocher et al., 1989, Parvez et al., 2022). In the case of fish, *Cyt b* gene has been used to address many phylogenetic questions, from relationships among closely related species to deep phylogenetic questions (Liu et al., 2020). So the *Cyt b* gene is considered to be a good marker to research on genetic differentiation and phylogenetic relationship (Kumar et al., 2016). Although many diverse characteristics and methods have been used to analyze the genetic structure of intensively exploited catfishes, researches on the genetics of these species have increased dramatically over the last three decades, largely due to the increased availability of techniques and increased awareness of the value of geneticdata (Megarani et al., 2020, Barathkumar and Thangaraj, 2020).

The major purposes of the present study were to analyze the genetic variation and explore the phylogenetic pattern of three catfish families collected from Tamilnadu using partial sequences of *Cyt b* gene. We construct the phylogenetic evidence to reconstruct the most likely patterns of diversification of the mtDNA lineages that may provide guideline for future molecular systematic studies. The results of this investigation will contribute details to researchers and fishery managers.

## 2. Materials and Methods

### 2.1 Sample Collection

Sixteen catfish species were collected from Mudasalodai (11° 45′19″ N; 79° 47′45″E), Cuddalore (11°42′37″ N; 79° 46′28″ E), Perumal Lake (11° 34′30″ N; 79° 40′18″ E) and Vadavar River (11° 08′03″ N; 79°27′05″ E) of Tamil Nadu state, India. They were identified by standard reference book (Jayaram, 1984). Dorsal fin tissues were taken out and preserved in 95% ethanol for DNA extraction. DNA was isolated from the following species and number of individuals within parenthesis: *Neotropius atherinoides* (3), *Mystus bleekeri* (3), *Mystus cavasius* (4), *Mystus dibrugarensisn* (3), *Mystus armatus* (3), *Mystus gulio* (2), *Arius arius* (3), *Arius jella* (2), *Arius maculatus* (3), *Arius gagora* (2), *Arius subrostratus* (2), *Plicofollis tenuispinis* (2), *Plicofollis platystomus* (2), *Plotosus lineatus* (1), *Plotosus limbetus* (1), *Plotosus canius* (1).

### 2.2. DNA Isolation, PCR and Sequencing

Genomic DNA was isolated by standard phenol/chloroform method (Sambrook et al., 1989) and the concentration of isolated DNA was estimated using a UV spectrophotometer. The DNA was diluted in TAE buffer to a final concentration of 100 ng /µL. Cytochrome c oxidase-1 (CO1) gene was amplified in a 50 µL volume PCR mix with 5 µL of 10X Taq polymerase $MgCl_2$ (50 mM) buffer, 1µL of each dNTP (0.05 mM), 1µL of each primer (0.01 mM), 0.6 U of Taq polymerase, 2 µl ofgenomic DNA and 36 µl of double distilled water. The universal primer, *Glu2*-5'- AACCACCGTTGTTATTCAACTA-3' and *ProR1*-5′ – TAGTTTAGTTTAGAATTCTGGCT

TTGG- 3' (Hardman and Page, 2003) was used for the amplification of the CO1 gene. The thermal regime consisted of an initial step of 2 min at 95 °C followed by 35 cycles of 40 s at 94°C, 45 s at 52 °C and 1in 10 s at 72 °C followed in turn by final extension of 10 min at 72 °C. The PCR products were visualized on 1.5% agarose gels, and the most intense amplicons were elected for sequencing. The cleanedup PCR product was sequenced by a commercial sequencing facility (Eurofins, Bangalore). The CO1 gene partial sequences of 16 individuals were edited using MEGA 5.0 (Tamura et al. 2011) and aligned with Clustal W 1.6, implemented in same software. The haplotype definitions have been submitted to the NCBI GenBank through BankIt.

### 2.3. Phylogenetic Analysis

The probability of rejecting the null hypothesis that sequences have evolved with the same pattern of substitution, as judged from the extent of differences in base composition biases between sequences (Disparity Index test). A Monte Carlo test (500 replicates) was used to estimate the *P*-values (Kumar and Gadagkar, 2001). The estimates of the disparity index per site are shown for each sequence pair. The final alignment of the mitochondrial 5′ region of *Cytb* partial gene had 1095 bp (Ariidae), 504 bp (Plotosidae) and 863 bp (Bagridae) and analysed in MEGA 5.0 (Tamura et al., 2011).

Along with 36 sequence of our studied 16 catfish species, additional *Cyt b* sequences of Ariidae, Bagridae and Plotosidae were retrieved from the GenBank for the phylogenetic tree construction and the tree was constructed by MP, ML, and BI criteria. The initial conditions used for different analyses are summarized in Table 1. The MP reconstructions were conducted in PAUP v. 4.0b10 (Swofford 2002) via heuristic searches with random addition (RA) of sequences and tree- bisection-reconnection (TBR); clade support was evaluated using non-parametric bootstrapping with RA and TBR.

For ML and BI, the best-fit models of sequence evolution were estimated using the Bayesian information criterion (BIC) in ModelTest v. 3.7 (Posada and Crandall, 1998). All analyses were run as unpartitioned. The ML analyses were performed in program RAxML v.7.04 (Stamatakis, 2006). ML nodal support was evaluated in RAxML using the rapid bootstrapping algorithm with automatic estimation of runs. For RAxML searches, several runs from random starting seeds were performed to check convergence of likelihood scores. Model parameters were estimated simultaneously (*i.e.,* unfixed). Remaining settings were left at their default values.

The BI analyses were performed in MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003) *via* Markov chain Monte Carlo (MCMC) iterations. The MCMC analyses were conducted in triplicate using four chains and sampling trees every 100 generations. Conservatively, 25% of the first trees sampled in each MCMC run were discarded as burn-in. Marginal probabilities of summary parameters, consensus phylograms, and posterior probabilities of nodes were estimated from the postburn-in samples of the three independent runs are given in Table 2.

To confirm that post-burn-in trees were sampled from the actual MCMC posterior distribution, marginal parameters (MrBayes log file) were analyzed using the Effective Sample Size (ESS) statistic in program Tracer (Drummond et al., 2007); ESS greater than 200 suggests that MCMC searches were run long enough to accurately represent the posterior distribution (Drummond et al., 2007).

### 2.4. Statistical Analysis

One-way ANOVA was performed to test the significance of taxa groupings in different subsets for taxonomic hierarchy (species, genus and family level) followed by Duncan multiple range test in SPSS software. The limit of statistical significance was set at $P < 0.05$. From this package, the basic module was employed for calculating mean and variance parameters, as well as those for one way of parametric analysis of variance (ANOVA). Statistical output were then made graphically to display on means plot and box plot.

## 3. Results

Mitochondrial *Cytb* gene was successfully amplified using the primers *Glu2* and *ProR1* in 16 catfish species. All haplotypes were deposited in GenBank, with the accession numbers KF862946 - KF862982. The difference in base composition bias per site in family, genus and species level (Kumar and Gadagkar, 2001) is shown in Figure 1-3. The evolutionary analyses results were plotted for the significant values (Figure 4).

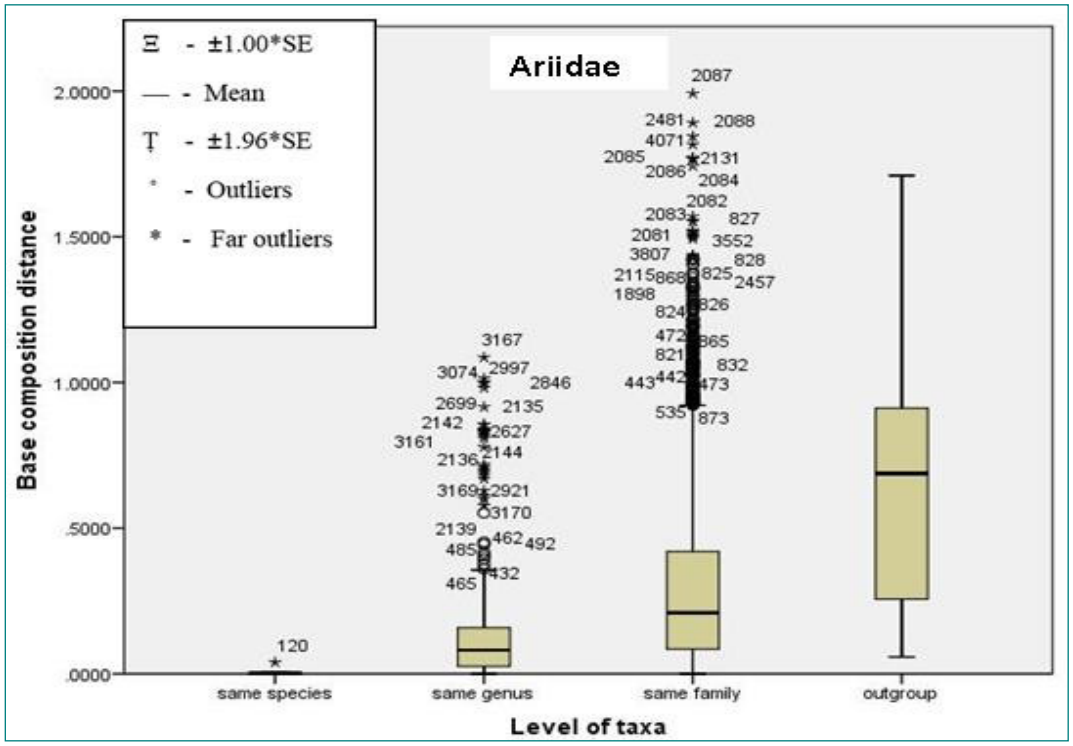



**Figure 1.** *Categorized distributions and mean of base composition distance of Cyt b genein family Arridae (F= 184.77, df = 3; 6437, P< 0.0001).*

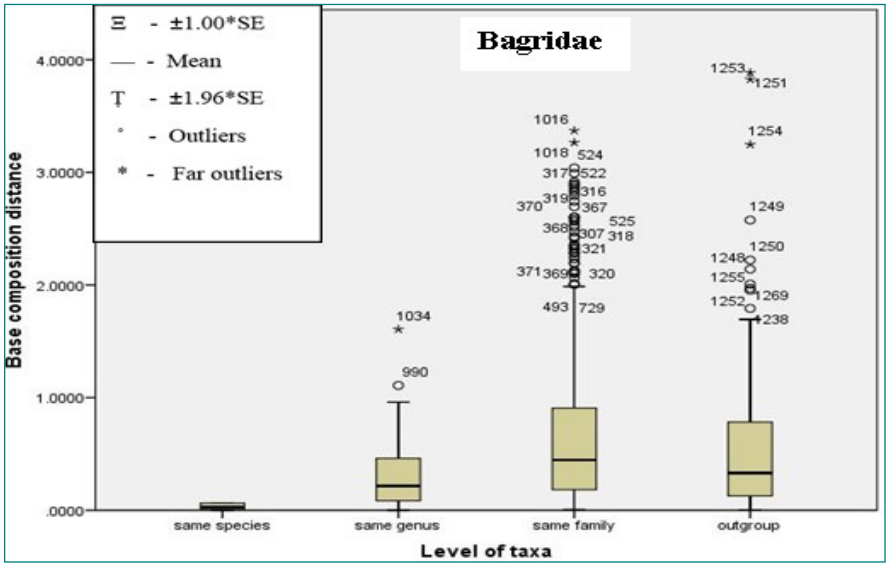



**Figure 2.** *Categorized distributions and mean of base composition distance of Cyt b genein family Bagridae (F= 19.41, df = 3; 1322, P< 0.0001)*

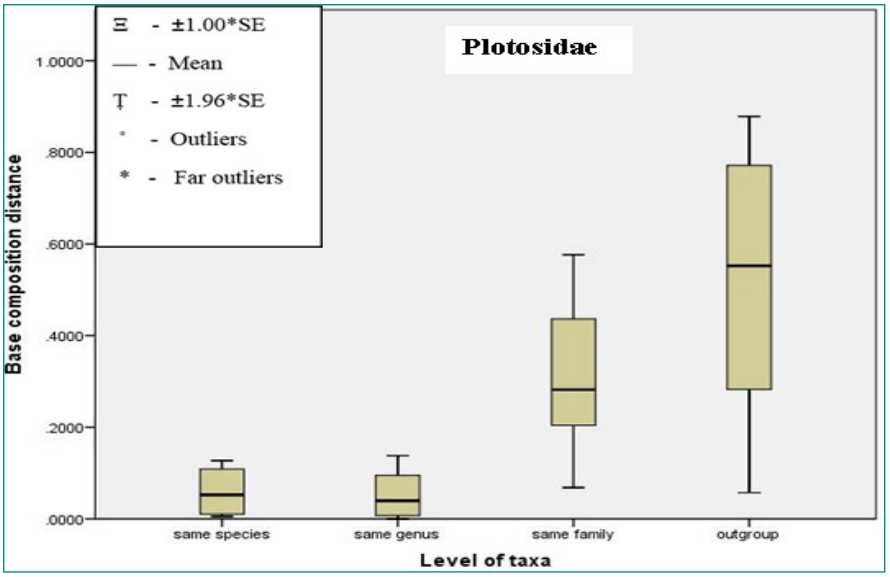



**Figure 3.** *Categorized distributions and mean of base composition distance of Cyt b genein family Plotosidae (F= 1.40, df = 2; 32, P < 0.0001)*

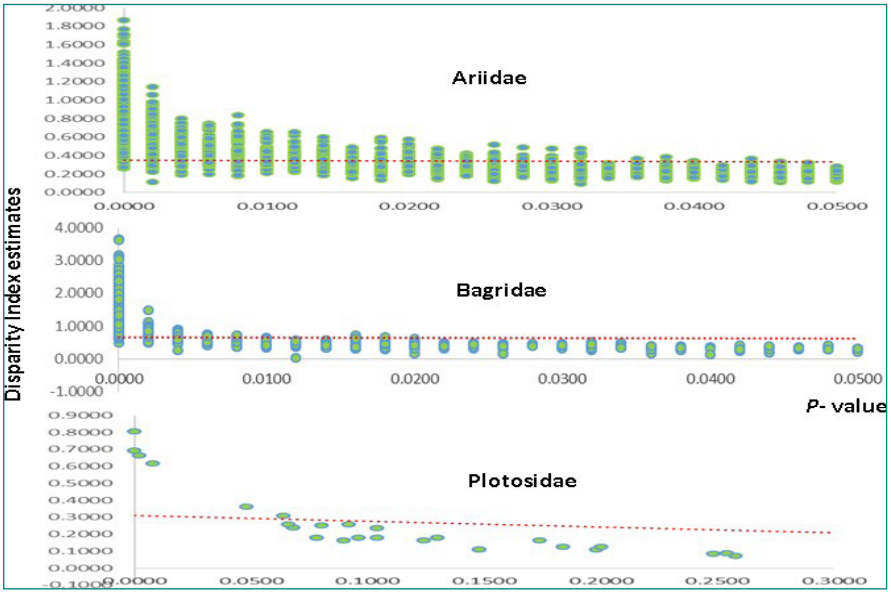



**Figure 4.** *Correlation of disparity index in three catfish families based on Cyt b gene sequence*

In order to obtain best estimate of the underlying pattern of substitution, this study confined its analysis to singletons, parsimony informative sites (Pi), twofold, fourfold degenerate sites, transition/transversion bias and interspecific to family level comparisons at $1^{st}+2^{nd}+3^{rd}$ codon positions as shown in Table 1. The results were plotted for the significant values (Figure 4). Correlation of disparity index estimates between all species pairs relative to homogeneity in substitution pattern with statistical significance (*P*-value) were plotted in given Figure 4. Comparisons between groups (populations, species within genera, genera and family) with gradual increase in *P*-values are indicated by dotted circles plotted with trendline (Figure 4).

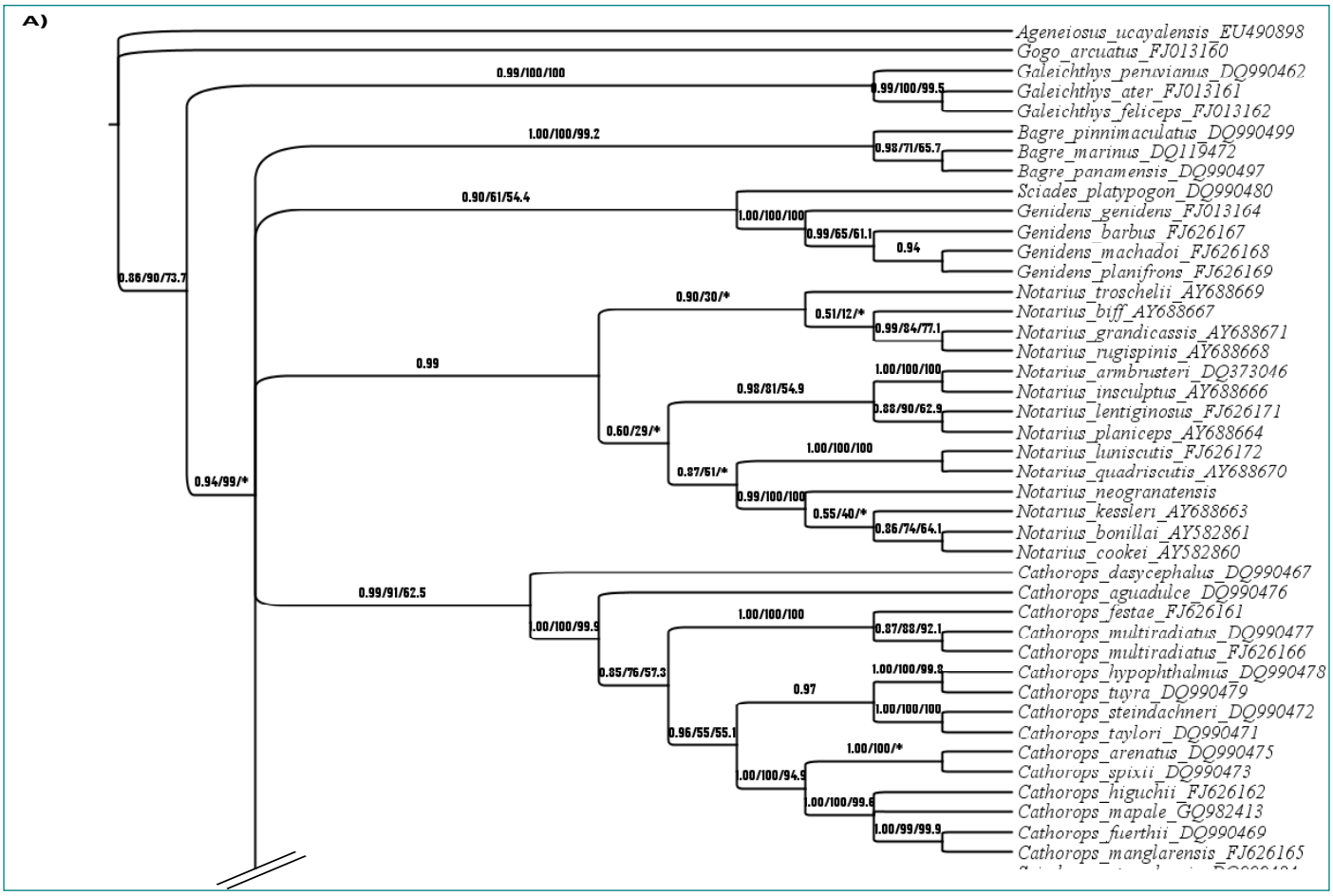
**Table 1.** *Codon-wise nucleotide substitution patterns. All frequencies are averages over all taxa*

| Position | A+T | G+C | Singleton | 2-fold | 4-fold | Pi sites |
|---|---|---|---|---|---|---|
| **Ariidae** | | | | | | |
| 1st | 49.0 | 51.0 | 9 | 3 | 0 | 0 |
| 2nd | 62.2 | 37.8 | 3 | 0 | 0 | 0 |
| 3rd | 53.4 | 46.6 | 9 | 108 | 127 | 0 |
| For 1095bp | 54.9 | 45.1 | 21 | 111 | 127 | 506 |
| *Arius* genus | | | | | | |
| 1st | 47.3 | 52.7 | 16 | 24 | 0 | 17 |
| 2nd | 63.3 | 36.7 | 2 | 0 | 0 | 4 |
| 3rd | 51.2 | 48.8 | 48 | 143 | 176 | 130 |
| For 1095bp | 53.6 | 46.4 | 66 | 167 | 176 | 88 |
| *Plicofollis* genus | | | | | | |
| 1st | 48.8 | 51.2 | 5 | 31 | 0 | 14 |
| 2nd | 62.1 | 37.9 | 0 | 0 | 0 | 7 |
| 3rd | 52.9 | 47.1 | 54 | 149 | 183 | 36 |
| For 1095bp | 54.7 | 45.3 | 59 | 180 | 183 | 57 |
| **Plotosidae** | | | | | | |
| 1st | 51.2 | 48.8 | 57 | 21 | 0 | 35 |
| 2nd | 61.2 | 38.8 | 12 | 0 | 0 | 9 |
| 3rd | 55.6 | 44.4 | 171 | 133 | 163 | 113 |
| For 504bp | 56.0 | 44.0 | 245 | 154 | 163 | 157 |
| *Plotosus* genus | | | | | | |
| 1st | 52.3 | 47.7 | 63 | 30 | 0 | 0 |
| 2nd | 61.6 | 38.4 | 15 | 0 | 0 | 0 |
| 3rd | 53.3 | 46.7 | 216 | 141 | 178 | 0 |
| For 1155bp | 55.5 | 45.5 | 294 | 171 | 178 | 0 |
| **Bagridae** | | | | | | |
| 1st | 49.8 | 50.2 | 29 | 2 | 0 | 0 |
| 2nd | 62.7 | 37.3 | 25 | 0 | 0 | 0 |
| 3rd | 59.2 | 40.8 | 21 | 108 | 0 | 0 |
| For 863bp | 57.2 | 42.8 | 75 | 110 | 130 | 481 |
| *Mystus* genus | | | | | | |
| 1st | 49.4 | 50.6 | 31 | 28 | 0 | 0 |
| 2nd | 63.8 | 36.2 | 26 | 0 | 0 | 0 |
| 3rd | 67.2 | 32.8 | 22 | 124 | 159 | 0 |
| For 863bp | 56.7 | 43.3 | 79 | 152 | 159 | 342 |

Start codon ATG was present in all the *Cyt b* datasets in first site. Optimality results obtained under different analyses and model testing on the three data partitions are summarized in Table 2. Among the three reconstruction methods conducted (MP, ML-RAxML, BI) on the *Cyt b* dataset, BI analysis resulted in least resolved tree. The consensus tree of three methods is shown in Figure 5-7. Bootstrap values and posterior probability values are labelled and indicated with gradient colour scheme for each of the respective node in BI/ML/MP format.

**Table 2.** *Summary of initial conditions and results obtained in phylogenetic reconstructions and model testing*

| Analysis | Ariidae | Bagridae | Plotosidae |
|---|---|---|---|
| **MP** | | | |
| RA replicates | 10 | 10 | 100 |
| Bootstrap replicates | 1000 | 1000 | 1000 |
| Optimal trees retained | 93 | 32 | 3 |
| Optimal tree score (steps) | 1567 | 897 | 343 |
| Consistency index | 0.4234 | 0.5214 | 0.3487 |
| Consensus type | 50% majority rule | 50% majority rule | 50% majority rule |
| *Initial Model* | | | |
| Bayesian information criterion | TrN+G+I | HKY+G | GTR+G |
| Number of substitution rate parameters | 2 | 2 | 2 |
| **ML** | | | |
| Search replicates | 10 | 10 | 100 |
| Optimal tree score (InL) | -19734.1298 | -9010.1954 | - 4725.2197 |
| Bootstrap replicates | 1000 | 1000 | 1000 |
| **BI** | | | |
| Search replicates | 3 | 4 | 3 |
| Generations | 7 $x10^6$ | 3 $x10^6$ | 1.8$x10^6$ |
| Burn-in | 7 $x10^5$ | 3 $x10^5$ | 1$x10^5$ |
| Mean InL | -16479.165 | -9842.354 | -4738.001 |
| Effective Sample size (all parameters) | >200 | >200 | >200 |
| Consensus type | 50% majority rule | 50% majority rule | 50% majority rule |



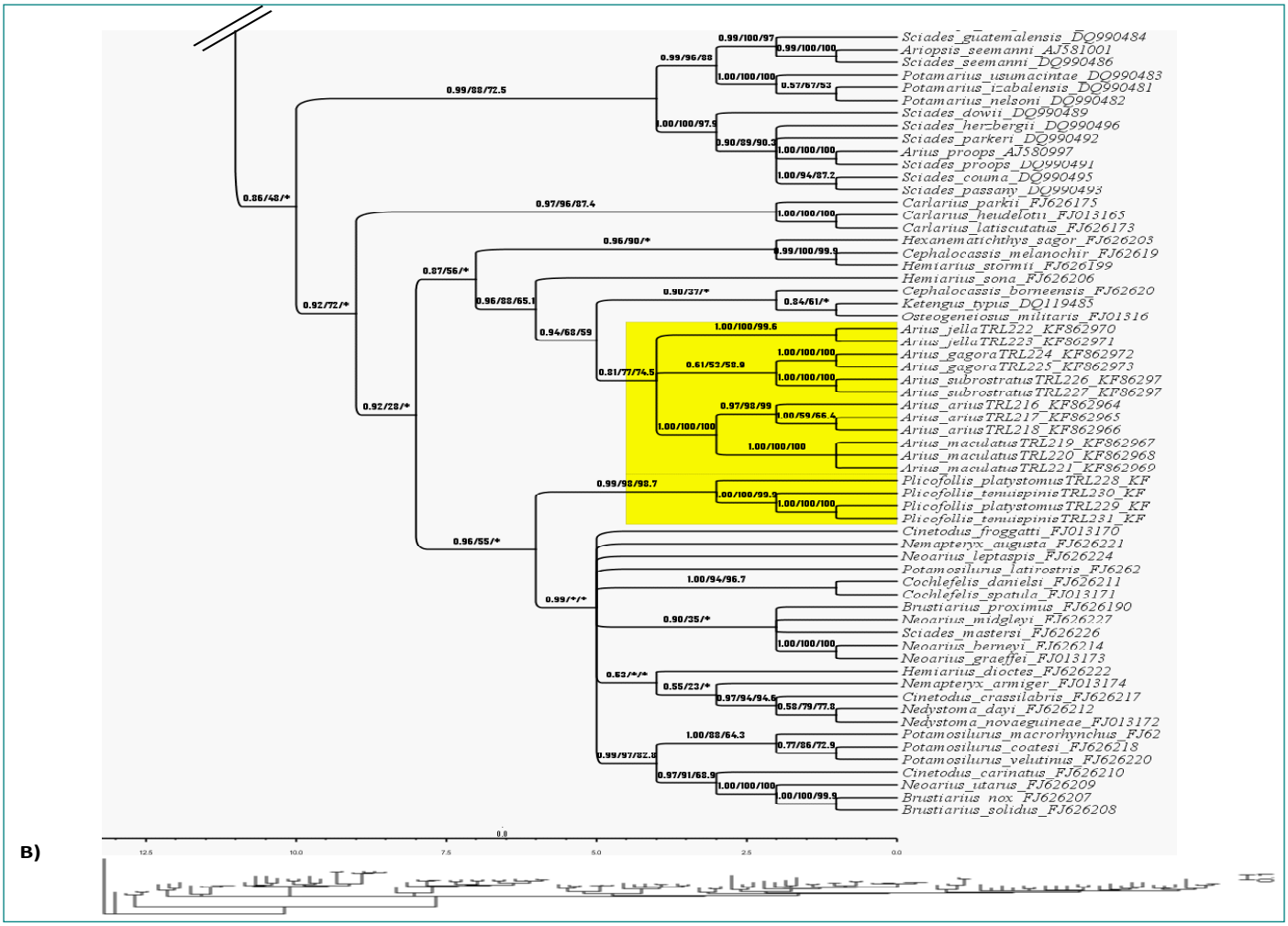
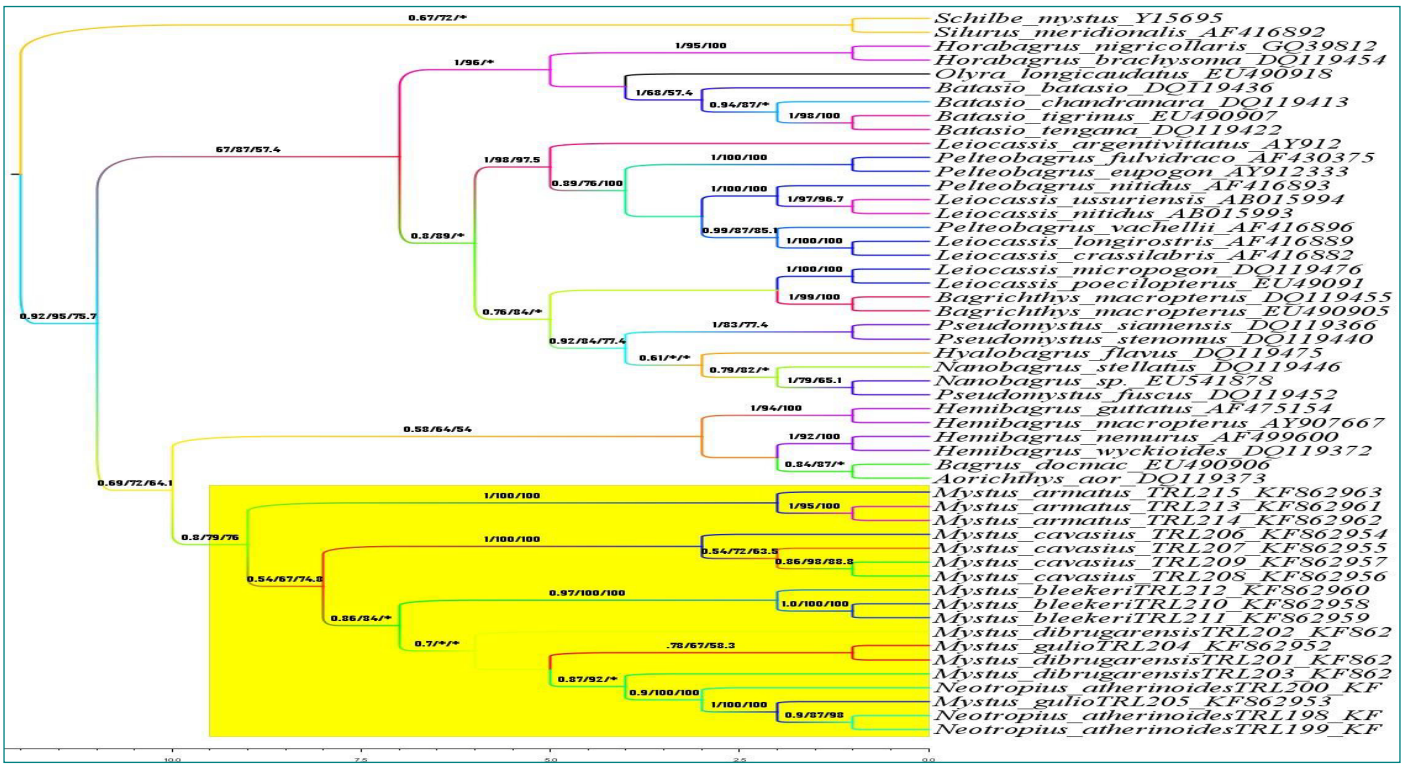



**Figure 5.** *Phylogeny of family Arridae derived from the Cyt b gene (A). cladogram: Node labels indicate posterior probability and bootstrap values (BI/ML/MP) that are congruent with MP and ML. Asterisks (*) designate insufficient clade support; Highlighted taxa indicate species utilized in this study. (B). phylogram elucidating the branch lengths with scales measuring the rate variation across lineages (taxon arrangement follows the same order as in cladogram)*



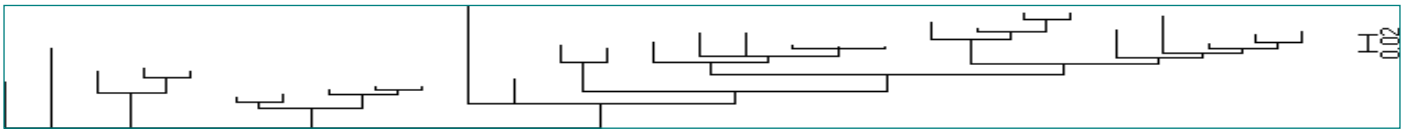


**Figure 6.** *Phylogeny of family Bagridae derived from the Cyt b gene (A). cladogram: colored node labels indicate posterior probability and bootstrap values (BI/ML/MP) that are congruent with MP and ML. Asterisks (*) designate insufficient clade support; Highlighted taxa indicate species utilized in this study. (B). phylogram elucidating the branch lengths with scales measuring the ratevariation across lineages (taxon arrangement follows the same order as in cladogram.*

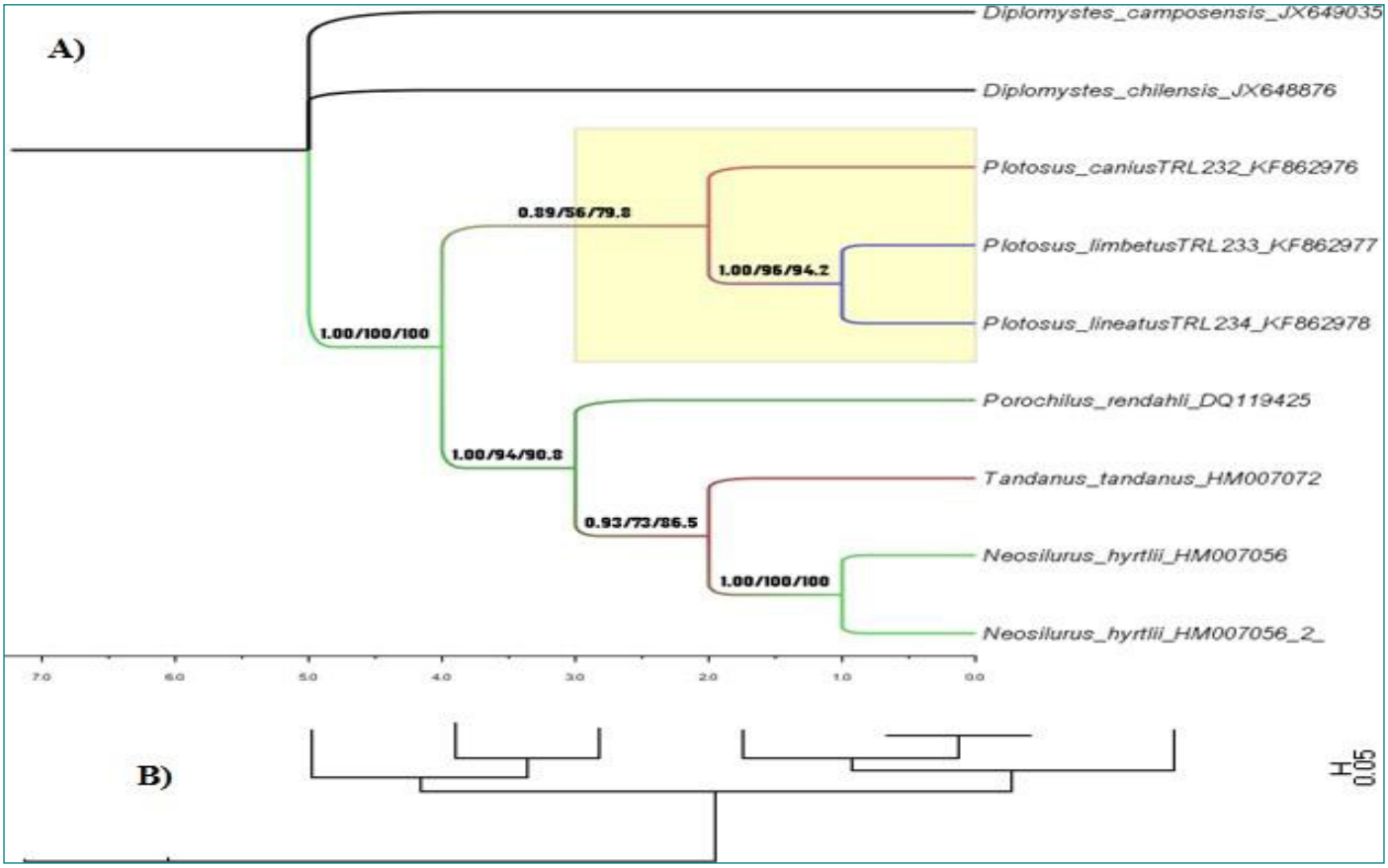



**Figure 7.** *Phylogeny of family Plotosidae derived from the Cyt b gene(A). cladogram: colored node labels indicate posterior probability and bootstrap values (BI/ML/MP) that are congruent with MP and ML. Asterisks (*) designate insufficient clade support; Highlighted taxa indicate species utilized in this study.*

*(B) phylogram elucidating the branch lengths with scales measuring the rate variation across lineages (taxon arrangement follows the same order as in cladogram)*

## 4. Discussion

Using this *Cyt b* marker, this study expands previous molecular phylogenies on Ariid catfishes (Betancur

2009b). In the case of Bagrid and Plotosid catfishes, there were no previous studies recorded regarding phylogenetic analysis using *Cytb* gene. The sequence length of all the 16 studied catfish species exceeds 1000bp. It was observed in previous studies more or less similar the length of *Cyt b* sequence have been used (Betancur, 2009b, Song et al., 1998, Hardman, 2005).

The aligned sequences of the partial *Cytb* gene (1095bp) from 102 Ariid and two outgroup species exhibited 765 variable sites, 506 of which were phylogenetically informative for maximum parsimony analysis. The distribution of phylogenetically informative sites by codon position in all 102 species is similar to patterns observed in other actinopterygian fishes (Lydeard and Roe, 1997). The base composition and nucleotide bias indices of compositional differences for *Cytb* is given in Table 1. The difference in base composition bias per site maintains sparse differences across within species and even within genus with base composition distances not more than 0.1. This shows the relative conserved nature of the *Cyt b* gene regions in sea catfishes. Across same and different genus of the Ariid family, the high base composition distances in outlier regions specify the inconsistent classification of the widely controversial

sea catfishes and inconsistent molecular evidence (Betancur et al., 2007). Several of them within same genus such as; *Cathorops* (0.35 – 0.45), *Notarius* (0.44 – 0.80), particularly between *Arius proops* (0.55 - 1.09) and other *Arius sp*., and between *Sciades mastersi* and *Sciades guatemalensis* (0.98). Between *Notarius rugispinis* and *Arius sp.* (1.55 – 1.99) the base composition distances more than the distance against outgroup species. Simultaneously, the genetic distances between taxa increase as the taxonomic rank increases; there is more genetic distance for confamilial genera than for congenericspecies and for congeneric species than for sister species (Johns and Avise 1998). This is to be expected if the *Cyt b* genetic distances is not overly truncated by saturation effects at these levels (Johns and Avise, 1998). These differences in genetic variation between classes must be taken into account when interpreting *Cytb* data in order to arrive at accurate conclusions.

These contribute 21 singletons, 111 (2-fold) and 127 (4-fold) degenerative sites from the whole Ariidae family. Compared to *COI* marker results in earlier report by Barathkumar and Thangaraj (2020), singletons and 2-fold degenerate sites values are very low which shows the consistent conservedness of the same nearby located *Cyt b* marker in circular mitochondrial genome (Table 1). Ti/tv bias was three-fold higher in 1$^{st}$ codon position (7.5) than the 3$^{rd}$ codon position (2.75). In *Arius* genus, this becomes inverse with ti/tv bias which was six-fold higher in3$^{rd}$ codon position (3.38) than the 1$^{st}$ codon position (2.96). Disparity index estimates showed several genus of this Ariidae to follow different heterogeneity patterns of substitution (Figure 4). *Cathorops, Cinetodus, Nedystoma*, *Hexanematichthys, Sciades* and *Neoarius* genus show difference in homogeneity against the studied Ariidae family and few of them showed higher base composition distances as mentioned just before. The outgroup species, *Ageneiosus ucayalensis* also showed similar and significant homogeneity with all the ingroup species of Ariidae.

Phylogenetic tree of Ariidae family using *Cyt b* is shown in Figure 5. Allthe clades that were displayed were congruent in three different phylogenetic reconstruction methods. The phylogenetic position of few taxa such as *Galeichthys, Bagre* and *Notarius* were consistent with *COI* marker utilized in earlier study (Betancur, 2009b, Barathkumar and Thangaraj, 2020). *Cathyrops dasycephalus* formed the longest branching clade in whole Ariidae family of Bayesian inference phylogeny in this study suggesting that it would have accumulated mutations in neutral selection. Most of the other species in this Ariidae phylogenetic tree showed that they have recently diverged because of their small branch length and node bifurcation. The studies of Betancur et al. (2004), Marceniuk (2007), Acero and Betancur (2007), Marceniuk and Betancur (2008), and Barathkumar and Thangaraj (2020) coincided in results obtained so far, that support and confirm the monophyly of the Ariids. Nevertheless, the monophyly that was validated through previous morphological studies is not congruent with molecular phylogenies and has been challenged by earlier research (Betancur, 2009a).

Okazaki (1999) has analyzed mitochondrial *Cyt b* gene and evaluated the phylogenetic relationships of Russian, Japanese and Korean Bagrids. Another study reported *Cyt b* sequence variations and phylogeny of the East Asian Bagrid catfishes (Peng et al*.,* 2002). Their molecular phylogenetic tree suggested that bagrid catfishes form a monophyletic group and the genus *Mystus* is the earliest divergent in East Asian Bagrids. They have also reported that the genus *Pseudobagrus* is a monophyletic group but the genus *Pelteobagrus* and *Leiocassis* are complicated. Other than this, there is no extensive molecular phylogeny reports regarding Bagridae catfish using *Cyt b*. The aligned sequences of the partial *Cyt b* gene (1138bp) from 52 Bagrid and 2 outgroup species exhibited 863 variable sites, 481 of which were phylogenetically informative for maximum parsimony analysis. The distribution of phylogenetically informative sites by codon position in all 52 species is similar to patterns observed in other actinopterygian fishes (Lydeard and Roe, 1997). Peng research group obtained identical *Cyt b* sequence with the length of 1138bp. They found only 307 variable and 242 potentially informative sites due to the reason of few sample size of 23 species of Bagridae family (Peng et al., 2002). Analogously, this study obtained low G content (13.7%), almost equal contents of A, T and C (28.6, 28.6 and 29.1%), content of A+T is higher (57.2%) and slight raise in transition/transversion ratio (2.14) that gave the concordant results identical to their observations in East Asian Bagrids (Peng et al. 2002).

Base composition distance estimated within genus separation with the inter-quartile range below 0.5 units apart. There are two exceptions between the *Pelteobagrus vachellii* with *Pelteobagrus eupogon* (1.1089) and *Pelteobagrus fulvidraco* (1.6072).

Very low values were obtained between *Batasio tengana* and *Batasio tigrinus* (0.0046), *Hemibagrus nemurus* and *Hemibagrus* (0.0104) in *Leiocassis nitidus* and *Leiocassis ussuriensis* (0.0014). The between genus base composition distances were nearly with the inter-quartile range of 0.5 units apart. Between *Batasio*, *Hemibagrus*, *Hemibagrus*, *Horabagrus, Hyalobagrus, Leiocassis*, *Nanobagrus, Pelteobagrus* and *Pseudomystus* against certain species that were across different genus have base composition distances (2.00 – 3.00) that are in near and far outliers (Figure 2). *Mystus* genus follows homogenous patterns of substitution rates with low ID values (0.34 – 0.66) at 5% significant level. ID values showed several species of this Bagridae to follow different heterogeneity patterns of substitution (Table 1). *Pelteobagrus fulvidraco* against *Batasio chandramara* and *Hemibagrus guttatus* displayed highest ID values (3.16 and3.0927) at 5% significant level. All the species that are abovementioned for the highest base composition distances also have high ID values (2 -3) which reflects their evolutionary adaptations that are different at the molecular level that follows heterogeneous pattern of substitution rates in evolution of Bagridae family.

Molecular phylogeny of Bagridae using *Cyt b* gene shows, most of the species that have been diverged very recently (Figure 6). There is no polytomy as observed from the phylogenetic tree using Bayesian method and well resolved with the appropriate HKY+G+I substitution model. *Olyra longicaudatus* and *Leiocassis argentivittatus* are the two species that formed a long branched attraction affecting the related species branched to its major node. *Batasio* and *Bagrichthys* genera have combined to form a monophyletic clade that was same as observed from the molecular phylogeny using *COI* gene in previous report (Barathkumar and Thangaraj, 2020). Species of *Pelteobagrus* and *Leiocassis* genera were intermingled that did not form a generic distinct monophyletic clade patterns.

This observation supports with the earlier study as these species were sampled in East Asian inland waters (Peng et al., 2002). The cladogram through MP, ML and BI phylogenetic methods from Figure 6 for *Mystus* genus displayed monophyletic group with higher bootstrap and posterior probability values for all the species which were similar with earlier study by Barathkumar and Thangaraj, (2020) and Peng et al. (2002). The range at which *Cyt b* is phylogenetically informative encompasses the levels of genetic divergence typically associated with confamiliar genera, congeners and sister species because the gene is not likely to be severely compromised by saturation effects involving superimposed nucleotide substitutions (Johns and Avise, 1998).

This systematic study on Plotosids is related to Hardman's (2005) analysis of *Cyt b* sequences have proposed that relationships with Cetopsids, or Akysids and Rita, a genus traditionally placed among Bagrids (Jayaram 1966, Mo 1991). The A+T content at 1$^{st}$ and 3$^{rd}$ codon position was around 53% and the ti/tv bias was 4.00 for the whole Plotosidae family and 2.16 for the *Plotosus* genus. Other than this, abnormal extremities were not observed on singletons, Pi sites, two-fold and four-fold degeneracy sites at all the 1+2+3 coding sites observed for all the species of *Plotosus* genus that may maintain balancing selection at the nucleotide level without any drastic change (Table 1). There are 157 Pi sites in whole Plotosidae family but there is no Pi site in *Plotosus* genus. This might show the conserved nature of this gene in this particular genus. The base composition distance for all the species of *Plotosus* genus fell within limits of same species distance (< 0.002). Higher values of base composition distance (~ 0.30) across all the genus within Plotosidae family below the values of outgroup comparisons (~0.56) (Figure 3). Heterogeneity pattern of substitution has been exhibited rejecting the null hypothesis between the *Plotosus* genus with *Porochilus rendahli* and *Tandanus tandanus* (ID = 0.62 - 0.43). In Figure 7, *Plotosus* genus displayed monophyletic group with higher bootstrap and posterior probability values for all the species. The phylogram shows that the branch length of all the taxa except *Plotosus canius* showed branch length more or less 0.05 depicting moderate substitution rates throughout this genus. Earlier report on phylogeny of Chinese catfish inferred from *Cyt b* sequences described that the position of the family Plotosidae was not resolved by Bayesian analysis and maximum parsimony inference (Peng et al. 2005). Despite the representation of distant taxa in this study,it did not have a long-branch attraction problem that has been described in some other diverse groups (Wilcox et al., 2004). The lack of phylogenetic resolution at deep levels within Plotosidae may be attributed to several factors, including saturation effects at the third position of *Cyt b* gene, rate heterogeneity and rapid cladogenesis within a relatively short time period.

Even though *Cyt b* gene evolves slowly in terms of non-synonymous substitutions which cause different amino acids to be coded in protein, this gene is

excellent for phylogenetic work because the rate of evolutions in silent positions which do not change the amino acid sequence of the translated protein is relatively fast (Kvist, 2000). The *Cyt b* gene also contains large regions of interspecies sequence diversity with little or no intraspecific variation, as well as several regions that are conserved, allowing for short fragments along its entire length to be amplified using trans-vertebrate primers (Telechea et al., 2008).

## 5. Conclusion

The findings of this study proved that the *Cyt b* gene is a potential molecular marker for analyzing genetic variations at family, genus and species level. The phylogenetic pattern clearly indicated the differences that formed three different clusters among the families. This study will be useful in differentiating inter and intra-specific genetic diversities in the catfish families and also help to develop strategies for genetic conservation, and adaptation to the environmental changes. This phylogenetic analysis is a first step toward this objective, although it still needs more comparative ecological data for a comprehensive analysis of the evolution of feeding and breeding habits. By this study we could establish a phylogenetic hypothesis for all the 36 catfish families in the world and examine themonophyly status of the subfamilies and genera.

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**Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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