



Resolving taxonomic uncertainties in ailiid catfishes (Siluriformes: Ailiidae) using Genome-Scale data

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ABSTRACT

The Ailiidae family, endemic to South and Southeast Asia, comprises diverse freshwater catfish inhabiting major river basins across the region. Evolutionary relationships and taxonomic classification within Ailiidae family remain contentious. Here, we resolve these uncertainties using phylogenomic data from 29 specimens representing 11 species and six of the seven recognized genera, supplemented by two outgroup taxa (*Pachypterus atherinoides* and *P. acutirostris*). Hybridization target enrichment and high-throughput sequencing yielded 4,434 single-copy nuclear coding loci. After trimming loci with more than 50 % missing taxa, 2,102 loci, totaling 505,110 bp, were used for phylogenomic analyses. The results confirm monophyly of all studied genera (*Ailia*, *Ailiichthys*, *Clupisoma*, *Eutropiichthys*, *Silonia* and *Laidies*), after the reclassification of *Clupisoma montanum* and *C. sinense* into the genus *Laidies*, based on concordant molecular, morphological and meristic evidence. Notably, *Ailia coila* and *Ailiichthys punctata* formed an early diverging clade, sister to remaining Ailiidae. Furthermore, our results corroborate that the genera *Eutropiichthys* and *Silonia* represent distinct lineages within Ailiidae, warranting their formal retention in the family. Divergence dating estimated the most recent common ancestor of Ailiidae arose during the Pleistocene, with a median age of 2.1 million years ago (95 % HPD: 3.34–1.0 Ma). Biogeographic reconstructions identified the Ganges–Brahmaputra River basin (South Asia) as the center of origin for Ailiidae, followed by dispersal to the Irrawaddy–Salween, Pahang and Indus River basins. Pleistocene climatic fluctuations, tectonic uplift and changes in sea-level may have influenced the dispersal of Ailiidae catfishes. These geological events restructured river systems and exposed land bridges, allowing movement across previously isolated basins. Paleo-river connections between the major drainages of South and Southeast Asia likely facilitated multiple events of vicariance and dispersal, driving diversification within the Ailiidae.

1. Introduction

The family Ailiidae was first proposed by Bleeker (1858) as Ailichthyoidei for a subfamily containing the genus *Ailia* Gray. With the advent of molecular studies, the number of genera and species within the family has been increased (e.g., Alam et al., 2019; Karinthanyakit and Jondeung, 2012; Laskar et al., 2020; Limbu et al., 2024; Wang et al., 2016). However, the taxonomic placement of genera and species within Ailiidae remains contentious, with persistent taxonomic ambiguities regarding the phylogenetic affinities of certain genera, including *Ailiichthys*, *Eutropiichthys* and *Silonia* (e.g., Kottlet, 2013; Nelson et al., 2016; Wang et al., 2016). These taxonomic conflicts are reflected with

inconsistent classifications found in authoritative databases such as FishBase (Froese and Pauly, 2025) and the Eschmeyer Catalog of Fishes (Fricke et al., 2025) (Table 1). For example, Kottlet (2013) assigned the genera *Clupisoma*, *Eutropiichthys*, *Laidies*, *Silonia* and *Proeutropiichthys* to Schilbeidae. While, Nelson et al. (2016) redefined Ailiidae to include six genera (*Ailia*, *Clupisoma*, *Eutropiichthys*, *Laidies*, *Proeutropiichthys* and *Silonia*), but they notably omitted any discussion of the systematic placement of *Ailiichthys*. In a further phylogenetic inquiry into five schilbeid taxa, Wang et al. (2016) restricted Ailiidae to three genera (*Ailia*, *Laidies* and *Clupisoma*), concurrently reassigning *Eutropiichthys*, *Proeutropiichthys* and *Silonia* to Schilbeidae based on the inferences drawn from Mo (1991), a study that notably did not scrutinize their

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taxonomic status. Fricke et al. (2025) placed the three genera within Ailiidae in alignment with Wang et al. (2016). Subsequent studies (Sarkar et al., 2013; Gupta and Banerjee, 2016; Khatun et al., 2022) further compounded these discrepancies, underscoring the need for a phylogenomic resolution on these controversies.

Resolving the taxonomic boundaries of Ailiidae remains challenging due to persistent disagreements over its generic and species diversity. The Eschmeyer Catalog of Fishes recognizes seven genera and 25 species, whereas FishBase restricts the family to five genera and fewer than 20 species. These discrepancies likely arise from historical sampling gaps and reliance on limited molecular data. For example, earlier studies by Karinthanyakit and Jondeung (2012) and Wang et al. (2016) focused on only three genera (*Clupisoma*, *Lalides*, *Eutropiichthys* and *Ailia*, *Lalides*, *Clupisoma*, respectively). Both studies relied exclusively on mitochondrial markers and a single nuclear marker. Beyond these studies, no further phylogenetic investigation has been published. A number of questions remain unresolved. For instance; i) the precise phylogenetic relationships among *Ailia*, *Ailiichthys*, *Clupisoma*, *Eutropiichthys*, *Lalides*, *Proeutropiichthys* and *Silonia* within Ailiidae; ii) monophyletic status of these genera; iii) the taxonomic status of *Ailiichthys*, the genus consistently overlooked in major taxonomic revisions of Ailiidae (e.g., Kottelat, 2013; Nelson et al., 2016); iv) conflicting familial placements of *Eutropiichthys* and *Silonia* (Ailiidae vs. Schilbeidae) and the composition of Ailiidae.

Estimating evolutionary timescales is a critical aspect of biological research, relevant to both population and macroevolutionary studies (Arbogast et al., 2002). These estimates are typically derived from genetic data using phylogenetic methods rooted in the molecular clock hypothesis. In freshwater fish biogeography, reconstructing historical dispersal patterns, the movement of populations or species across geographic barriers is essential for understanding the colonization of new habitats (Ho et al., 2015). Although such events are infrequent, they can result in isolated populations that experience gradual genetic divergence (Cook and Crisp, 2005). Dispersal capacity varies widely among freshwater fish taxa and these differences are crucial for deciphering the evolutionary and biogeographic history of groups such as the catfish family Ailiidae.

Although specific studies on the dispersal ability of the Ailiidae family are currently lacking, potential patterns can be inferred from closely related catfish families that are primarily distributed in Asia such as Bagridae, Siluridae and Sisoridae. For instance, *Hemibagrus guttatus* (Bagridae) in South China exhibited phylogeographic patterns shaped by sea-level fluctuations during the Pleistocene (Yang and He, 2008). Similarly, sisorid catfishes of the Glyptosternoid group show evidence of diversification from the Miocene to Pleistocene (Guo et al., 2005), while silurid catfishes of the genus *Silurus* are believed to have dispersed from China to Japan during periods of sea-level change (Chen et al., 2024). These families have undergone major diversification events during the Late Miocene and Quaternary, largely driven by regional tectonic activity and climatic oscillations (Kappas et al., 2016; Zhou et al., 2016). Such patterns likely reflect broader biogeographic processes that may have similarly shaped the evolutionary history of the family Ailiidae. By contrast, the biogeographic history and taxonomic classification of

Ailiidae remain poorly resolved, largely due to inadequate phylogenetic sampling and a scarcity of genomic data. Address these uncertainties, we generated phylogenomic data from 4,434 nuclear loci across 29 specimens representing 11 species and six genera, using hybridization-based exon capture (Li et al., 2013; Jiang et al., 2019). Specimens of *Proeutropiichthys*, a genus described in 1937 (Hora) but lacking recent taxonomic data was unavailable, emphasizing the need for renewed systematic efforts. Our study aimed to clarify three objectives: (i) to validate the inclusion of *Ailiichthys*, *Eutropiichthys* and *Silonia* within Ailiidae as proposed by Fricke et al. (2025); (ii) to resolve phylogenetic relationships and estimate divergence times among ailiid lineages; and (iii) to test biogeographic hypotheses underlying their diversification during the Pleistocene.

2. Materials and methods

2.1. Sampling, preservation, DNA extraction and shearing

Each species included 1–5 individuals collected from locations in India, Pakistan, Myanmar, Malaysia and Nepal (Fig. 1, Supplementary File S1). Two species of Horabagridae (*Pachypterus atherinoides* and *P. acutirostris*) were sampled as outgroups. Fish were captured by cast net (0.5, 1 and 2 cm mesh size) and gill net (1, 2, 3 and 4 cm mesh size). Muscle tissue or caudal fin was preserved in 95 % ethanol and was stored at 4 °C until DNA extraction. Permit number and processing protocol followed can be found in Ethical Statement.

Total genomic DNA was extracted from ethanol-preserved tissue using an Ezup Column Animal Genomic DNA Purification Kit (Sangon, Shanghai, China). The purified DNA was quantified with a NanoDrop 3300 Fluorospectrometer (Thermo Fisher Scientific, Wilmington, DE, U.S.A.) and was visualized using 1 % agarose gel electrophoresis. Approximately 50 µL of genomic DNA was sheared to ~ 250 bp range using the Covaris M220 with standard PCR tubes. The size distribution of the sheared DNA was then checked on a mini agarose gel.

2.2. Library preparation, exon capture, pooling and sequencing

Library preparation and exon capture were performed following Meyer and Kircher (2010) with modifications (Li et al., 2013) to accommodate capturing target loci of divergent species. A total of 300 ng sheared DNA was used for library preparation for each sample. Ostariophysan baits available in our lab were used for exon capture, which were 120 bp long and 2x tiling, designed on reference sequences of zebrafish (Li et al., 2013). Two rounds of exon capture were performed on each sample to increase on-target reads of captured loci. Samples were labeled with an 8 bp index on the P7 adapter to facilitate multiplex sequencing. Enriched and indexed products were pooled in equimolar ratios for sequencing.

2.3. Data assembly

We adopted a suite of 4,434 single-copy nuclear coding sequence (CDS) markers identified and validated by Li et al. (2018) for

Table 1
Conflicting family assignments of ailiids genera in ichthyological literature.

Genus	FishBase	Eschmeyer Catalog of Fishes	Kottelat (2013)	Nelson et al., (2016)	Wang et al. (2016)
<i>Ailia</i>	Ailiidae	Ailiidae	Ailiidae	Ailiidae	Ailiidae
<i>Ailiichthys</i>	Ailiidae	Ailiidae	–	–	–
<i>Clupisoma</i>	Ailiidae	Ailiidae	Schilbeidae	Ailiidae	Ailiidae
<i>Eutropiichthys</i>	Schilbeidae	Ailiidae	Schilbeidae	Ailiidae	Schilbeidae
<i>Lalides</i>	Ailiidae	Ailiidae	Schilbeidae	Ailiidae	Ailiidae
<i>Silonia</i>	Schilbeidae	Ailiidae	Schilbeidae	Ailiidae	Schilbeidae
* <i>Proeutropiichthys</i>	Ailiidae	Ailiidae	Schilbeidae	Ailiidae	Schilbeidae

* Not included in this study; – not discussed/included.

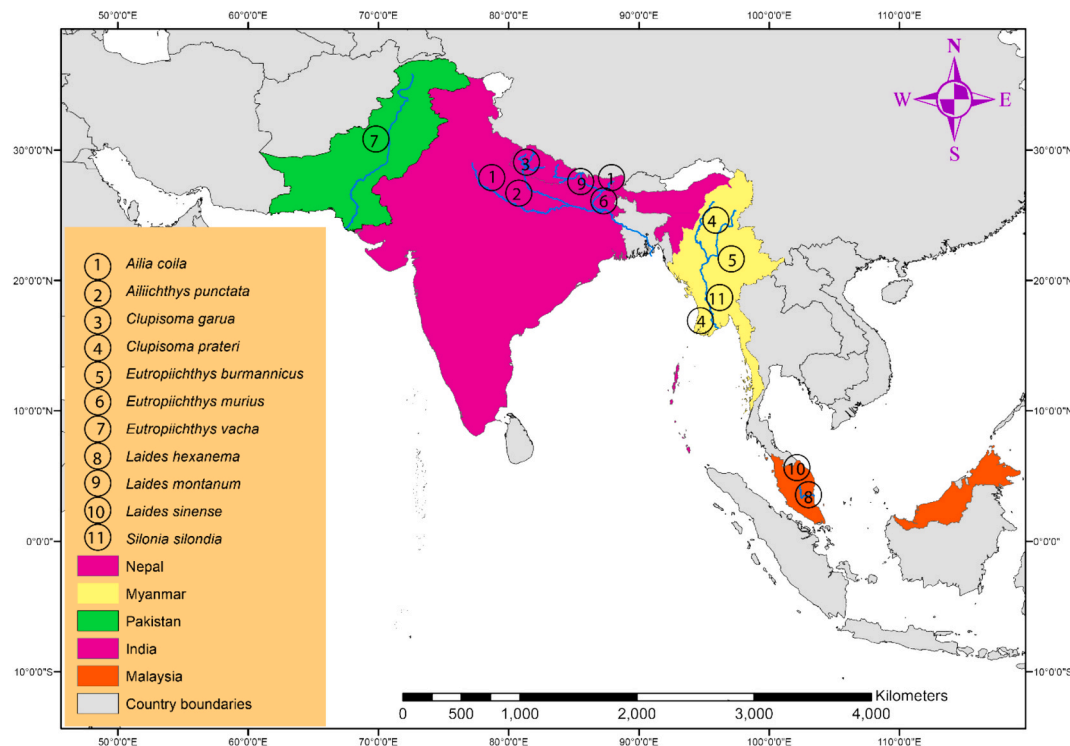


Fig. 1. Map showing sampling sites.

phylogenomic analysis. Data preparation and assembly was done according to Yuan et al. (2016) and Jiang et al. (2019). Briefly, Trim.Galore Version 0.6.7 (Krueger, 2021) and Cutadapt v1.2.1 (Martin, 2011) with default parameters were used to trim the adapter sequences and low quality sites ($Q < 20\%$) from the reads. The resulting trimmed reads were then assembled into contigs using a Perl script wrapper developed by Yuan et al. (2019) and subsequently filtered. Locus-based output was obtained using a Perl script wrapper developed by Yuan et al. (2019), which also included a paralog removal protocol to eliminate potential paralogs. In the next step, loci with poor enriched data ($<50\%$) were excluded, while the remaining loci were retained for alignment. A filtered dataset consisting of 2102 loci was used for subsequent alignment. All sequences were aligned in batch using MAFFT (Katohh and Standley, 2013). Pairwise distance was calculated for all loci using Biopython library in Python 3.8.3 and sequences with abnormal high distance values were checked by eye and abandoned if they could not be rectified manually. The aligned sequences have been made available in [Supplemental Materials](#) (Supplementary File S2).

Due to the unresolved taxonomic status of *Eutropiichthys* and *Silonia* and the lack of tissue samples from African schilbids, we obtained available sequences from GenBank (<https://www.ncbi.nlm.nih.gov/>, accessed on 20 April 2025). Among African schilbids, only four species had nuclear recombination-activating gene 1 (RAG1) sequences, while a larger number had mitochondrial cytochrome oxidase subunit 1 (COI) sequences. To ensure consistency across markers, we selected four species with both RAG1 and COI sequences. For ailiid taxa, although COI sequences were available, RAG1 sequences were absent. We extracted RAG1 sequences from our Illumina-trimmed reads using a custom Python script (Supplementary File S3), with the *Ailia coila* RAG1 gene (GenBank Acc. No. DQ492541) as the reference for alignment and extraction. Including both COI and RAG1 genes was essential because *Eutropiichthys* and *Silonia* remain classified under Schilbeidae in FishBase (Froese and Pauly, 2025), and omitting African schilbids could compromise the phylogenetic assessment of these genera. Additionally, we retrieved COI and RAG1 sequences of two Horabagridae species (*Horabagrus brachysoma* and *Pseudeutropius brachyopterus*) and

outgroup taxa (*Glyptothorax lampris* and *G. macrocephalus*) from GenBank for phylogenetic analysis. All sequences of the COI and RAG1 genes, along with their associated accession numbers, are provided in Supplementary File (Supplementary File S4, S1A, S1B).

2.4. Phylogenetic analyses

The aligned DNA sequences (506,742 bp) were used for phylogenetic reconstruction. Maximum likelihood (ML) analyses were performed on concatenated sequences, with partitioning initially based on codon position and further optimized using PartitionFinder v2.1.1 (Lanfear et al., 2016). Codon-based partitioning was applied with the GTR + G model and the rcluster algorithm, and the optimal scheme was selected using the Bayesian Information Criterion (BIC).

The ML tree was inferred using RAxML v8.2.8 (Stamatakis, 2014) under the GTRGAMMA model, with 1000 bootstrap replicates for branch support. For additional validation, we also conducted analyses in IQ-TREE v2.1.4-beta (Minh et al., 2020), where ModelFinder (Kalyaanamoorthy et al., 2017) was used to identify the best-fit model (selected via BIC). Although PartitionFinder was initially employed, ModelFinder ensured compatibility within IQ-TREE's framework and cross-validated model selection. The best-fit model (GTR + F + R3) was applied with 1000 SH-aLRT replicates (Anisimova et al., 2011) to assess branch support.

In addition, a separate analysis was conducted using the COI and RAG1 sequences to include African schilbid representatives, which were unavailable in the targeted enrichment dataset. The two gene alignments were first assessed for incongruence using the incongruence length difference (ILD) test (Farris et al., 1995), which yielded a non-significant result ($P > 0.05$), permitting concatenation. Phylogenetic reconstruction of the combined dataset was conducted in IQ-TREE v2.1.4-beta under the TIM2 + F + I + R2 model (selected by ModelFinder), with 1,000 ultrafast bootstrap replicates for branch support. Pairwise genetic distances among 11 ailiid and four schilbid species were computed under the Kimura 2-parameter (K2P) model with 1,000 bootstrap replicates in MEGA 11 (Tamura et al., 2021) and visualized as

a heatmap using the “heatmaply” R package (Galili et al., 2018).

Individual gene trees were inferred in RAxML v8.2.8 under the GTRGAMMA model (Supplementary File S5). A coalescent-based species tree was then reconstructed using ASTRAL v5.6.3 (Mirarab et al., 2014) with all gene trees as input. The analysis employed the full annotation option ($-t\ 2$) to display quartet support and local posterior probabilities for each branch (Hu et al., 2023). The $-q$ parameter was used to compute the quartet score, branch lengths, and ASTRAL support values. To further evaluate branch support, we calculated the Gene Concordance Factor (gCF) and Site Concordance Factor (sCF) in IQ-TREE v2.1.4-beta (Minh et al., 2020). The gCF represents the percentage of decisive gene trees containing a given branch (accounting for taxon coverage differences), while the sCF reflects the percentage of decisive alignment sites supporting that branch. These metrics were derived from individual gene trees (gCF) and the concatenated alignment (sCF) (Roa-Varón et al., 2021).

2.5. Estimation of divergence time

The preliminary data set, incorporating 11 ingroups and three outgroups (Sisoridae, Bagridae and Horabagridae), was assembled following the protocol described in section 2.3 to facilitate fossil calibration. A total of 4,434 loci with complete data across 17 species were initially recovered. After quality filtering and alignment trimming, 492,627 base pairs (bp) across 2,110 loci were retained. For divergence time estimation (Supplementary File S6). Input files written in XML were created using BEAUTi v2.3.2 (Drummond and Rambaut, 2007). The data were divided into three codon positions (1 + 2 + 3). Site models were unlinked across partitions, whereas clock models and trees were linked. The substitution model used was GTRGAMMA, with a substitution rate of 1.0 and four gamma categories. A relaxed log-normal clock with a birth–death tree model was employed as the clock model. The weights of four parameters Birth Death Subtree Slide, Narrow, Wide and Wilson-Blading were set to zero to fix the tree topology, as described in Li et al. (2018).

Due to the scarcity of well-documented ailiid fossils, the earliest divergence of Siluriformes was constrained using fossil evidence from the Late Cretaceous. The oldest fossils confidently assignable to Siluriformes are isolated dorsal and pectoral fin spines recovered from the Adamantina formation of the Bauru Group in Brazil (Alves et al., 2016; 2019). These remains date to the Turonian–Santonian stages (93.9–83.6 Ma) based on stratigraphic correlations with the regional geological framework (Dias-Brito et al., 2001). A log-normal distribution (mean = 3.24, SD = 0.4, offset = 83.6 Ma) was applied, with a hard minimum age of 83.6 Ma (Turonian–Santonian stages evidence) and a 95 % soft maximum age of 145.5 Ma, reflecting the oldest fossil of the crown group Ostariophysi, †*Rubiesichthys gregalis* (stem Gonorynchiformes), from the Berriasian of Spain (Fara et al., 2010; Benton et al., 2015). This approach follows the framework of previous studies integrating molecular and fossil evidence to estimate Siluriformes divergence (Kappas et al., 2016), though here the soft maximum age is calibrated using the oldest crown Ostariophysi fossil (†*Rubiesichthys gregalis*) rather than molecular estimates. For the second calibration, the earliest definitive Bagridae fossil, *Bagrus* sp. from the late Eocene of Egypt (33.9–37 Ma; El-Sayed et al., 2020), was used. A log-normal distribution (mean = 3.35, SD = 0.45, offset = 33.9 Ma) was implemented, with a hard minimum of 33.9 Ma and a 95 % soft maximum of 93.9 Ma, based on the oldest confirmed Siluriformes fossil record from the Turonian stage (Alves et al., 2016; 2019). The third calibration incorporated the oldest known Sisoridae fossil, *Bagarius* sp., from the Pliocene (5.3–1.8 Ma; Lydekker, 1886). A log-normal distribution (mean = 3.33, SD = 0.4, offset = 1.8 Ma) was applied, with a hard minimum of 1.8 Ma and a 95 % soft maximum of 37 Ma, aligned with the late Eocene *Bagrus* sp. Fossil (El-Sayed et al., 2020).

Two independent BEAST analyses were conducted, each comprising 60 million Markov chain Monte Carlo (MCMC) generations, with

parameters sampled every 1,000 generations. Convergence of chains and effective sample sizes (ESS > 200 for all parameters) were verified using Tracer v1.6 (Rambaut et al., 2018). Post-analysis, tree files from both runs were combined with LogCombiner v2.3.2 (Drummond and Rambaut, 2007), discarding the first 20 % of generations as burn-in. A maximum clade credibility (MCC) tree, annotated with mean node ages and 95 % highest posterior density (HPD) intervals, was generated using TreeAnnotator v1.8.1 (Drummond and Rambaut, 2007). Final trees were visualized and edited in FigTree v1.4.0.

2.6. Reconstruction of ancestral distribution of Ailiidae

The RASP v4.3 (Yu et al., 2020) was used to reconstruct ancestral distribution of the family Ailiidae based on three models: Statistical dispersal-variance analysis (S-DIVA), Lagrange (DEC) and Bayes-Lagrange (S-DEC) (Ree and Smith, 2008; Yu et al., 2015). A calibrated Bayesian time tree, pruned of outgroups using R v4.4.2 (R Core Team, 2024) with the ape package (Paradis and Schliep, 2019), served as the input (Supplementary File S7). Four biogeographic regions were defined based on major river basins and historical freshwater connectivity. (A) Ganges–Brahmaputra River basin (India and Nepal), encompassing interconnected Nepalese and Indian river systems; (B) Irrawaddy–Salween River basin (Myanmar); (C) Indus River basin (Pakistan); and (D) Pahang River basin (Malaysia). Species distributions were assigned using the “Load States (Distribution)” option in RASP. Three models were tested on the trees independently. For comparative analysis, six additional models implemented in the BioGeoBEARS package (DEC, DEC + J, DIVALIKE, DIVALIKE + J, BAYAREALIKE, BAYAREALIKE + J; Matzke, 2018) were tested under identical constraints (maximum range size = 4 regions; Supplementary Table S1). Models were ranked using Akaike Information Criterion (AIC) scores (Supplementary Table S1), with results from the best-supported model presented.

3. Results

3.1. Phylogenetic relationship

The concatenated dataset (Supplementary File S1) comprised 2,102 loci (505,110 bp), containing 44,517 parsimony-informative sites (8.81 %) and 92,686 distinct patterns. The alignment included 36,988 singleton sites and 423,605 constant sites (83.86 %), with the high proportion of parsimony-informative sites providing strong phylogenetic resolution. Maximum likelihood (ML) analysis under the optimal partitioning scheme (selected by PartitionFinder) yielded a topology congruent with codon-based partitioning (Fig. 2; Supplementary File S8). The ML tree exhibited robust nodal support, with 98 % of branches receiving ≥ 95 % bootstrap values. Only two nodes showed lower support, 64–78 % and 94–97 %, respectively (Fig. 2). All ailiid genera were recovered as monophyletic with maximum bootstrap support (100 %) except for *Clupisoma*. Within *Clupisoma*, *C. prateri* and *C. garua* clustered closely, while *C. montanum* (reclassified as *Laidex montanum* hereafter) and *C. sinense* (reclassified as *Laidex sinense* hereafter) resolved a separate lineages and grouped with *Laidex hexanema*. *Ailia coila* and *Ailiichthys punctata* formed distinct, well-supported clades. Notably, *Laidex hexanema* grouped sister to *C. sinense*, suggesting closer evolutionary ties than previously recognized, though it retained distinctiveness. In *Eutropiichthys*, *E. vacha* and *E. burmannicus* were closely related, whereas *E. murius* diverged as a distinct lineage. *Silonia silondia* formed a monophyletic clade, clearly separated from other genera.

The phylogenetic analysis of concatenated COI and RAG1 sequences revealed a clear separation between African Schilbeidae and Asian Ailiidae, with each forming distinct clades supported by strong nodal values (Supplementary File S9). All Asian ailiid species clustered together in a single, well-supported monophyletic clade, while African schilbid species formed a separate lineage, confirming a deep

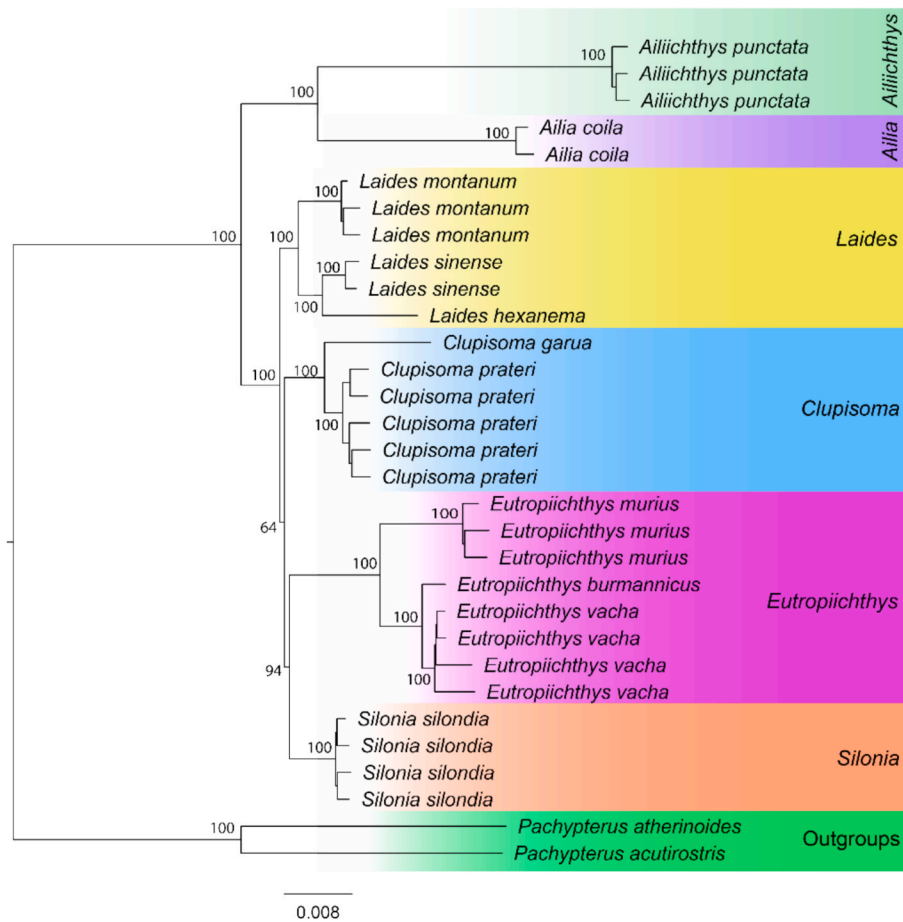


Fig. 2. Maximum likelihood tree of 11 ailiid species based on 2,102 nuclear loci (505,110 bp), partitioned by the best partitioning scheme suggested by PartitionFinder. Numbers at the nodes show bootstrap supports from RAXML analysis and SH-aLRT test values from IQ-TREE are not shown.

evolutionary divergence between the two families. Pairwise genetic distance analysis further supported this distinction, showing interspecific divergence between ailiids and schilbids ranging from 20 % to 57 % (Supplementary File S10). Notably, *Parailia congica* exhibited substantially lower divergence from ailiid species (11 %–14 %), suggesting a potentially closer evolutionary relationship or an unresolved taxonomic placement within Schilbeidae.

The coalescent species tree reconstructed using ASTRAL was consistent with the ML trees (Fig. 3). All branches in the ASTRAL species tree received full nodal support, with quartet support values (q1) of 1.0 and an effective number (EN) of 1.0, suggesting that all gene trees provided fully informative support for the resolved topologies. The normalized quartet score obtained from the ASTRAL analysis was 0.82, indicating strong concordance among the gene trees. However, we observed inconsistencies in branch support estimates between quartet support and gene and site concordance factors (gCF, sCF). Despite the presence of numerous short internodes, all relationships received unequivocal support from the quartet-based analysis (normalized quartet score = 0.82), whereas gCF (6.3–98.1 %; Supplementary File S10, S2A) and sCF (31.9–99.3 %; Supplementary File S10, S2B), values were comparatively lower for several nodes, indicating hidden conflict across loci.

3.2. Divergence time estimates

Both independent BEAST runs achieved convergence, with all parameters exhibiting Effective Sample Size (ESS) values exceeding 413. Mean divergence times and 95 % highest posterior density (HPD) intervals are summarized in Fig. 4. The stem age of Ailiidae, marking its

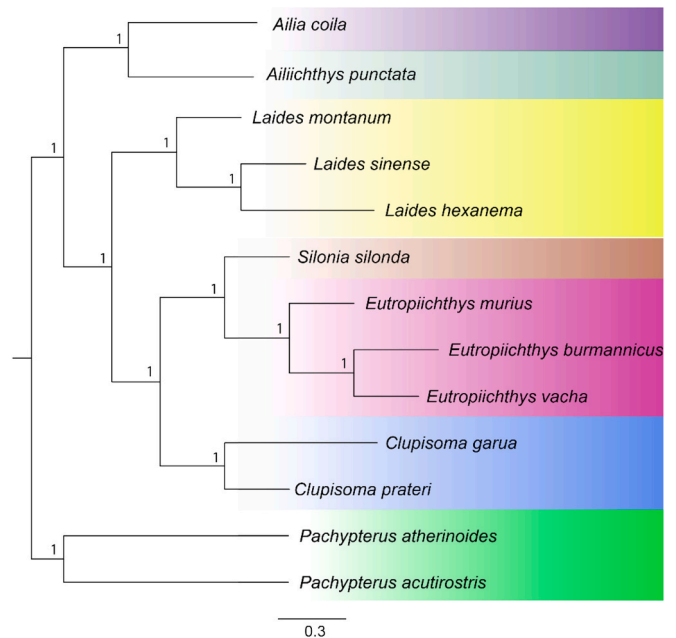


Fig. 3. A coalescent tree of the ailiids resulted from ASTRAL analysis on 2,102 nuclear loci (505,110 bp). Number at nodes show the branch support values.

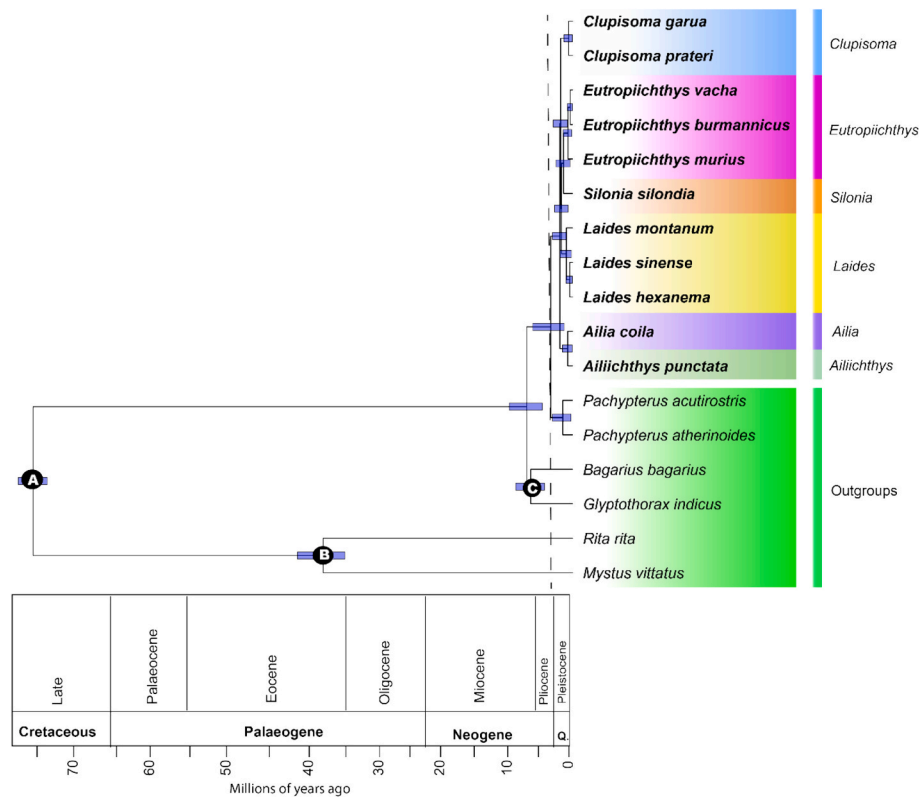


Fig. 4. Divergence time estimation of ailiids using BEAST analysis based on the 2,110 loci (492,627 bp). Six closely related outgroups representing two species each (from Bagridae, Sisoridae and Horabagridae) were included for fossil calibration. Shaded bars on nodes represent 95 % highest posterior credible age intervals for each.

divergence from the closely related family Horabagridae, was estimated at 3.6 Ma (95 % HPD: 6.5–1.38 Ma). The crown age or the most recent common ancestor (MRCA) of all sampled Ailiidae lineages, was estimated at 2.1 Ma (95 % HPD: 3.34–0.96 Ma). The first major split within Ailiidae separated *Ailia* and *Ailiichthys* from the rest of the family, indicating their basal position. Around 1.96 Ma (95 % HPD: 3.17–0.80 Ma), the *Clupisoma* lineage emerged, marking the next major split, followed by a diversification event at 1.8 Ma (95 % HPD: 2.90–0.70 Ma), which led to the separation of *Eutropiichthys* and *Laides*. Further divergence occurred between *Eutropiichthys* and *Silonia* around 1.5 Ma (95 % HPD: 2.70–0.40 Ma), reflecting continued lineage diversification during the Pleistocene. Divergence between *Ailia coila* and *Ailiichthys punctata* was estimated at 0.8 Ma (95 % HPD: 1.87–0.06 Ma), *Eutropiichthys*

murius from *E. vacha*/*E. burmannicus* at 0.8 Ma (95 % HPD: 1.51–0.14 Ma) and *E. vacha* from *E. burmannicus* at 0.4 Ma (95 % HPD: 0.9–0.009 Ma). Divergence between *Laides montanum* from *L. sinense*/*L. hexanema* at 1.03 Ma (95 % HPD: 2.08–0.19 Ma) and *L. sinense* and *L. hexanema* diverged at 0.5 Ma (95 % HPD: 1.08–0.03 Ma), while *Clupisoma garua* and *C. prateri* diverged at 0.62 Ma (95 % HPD: 1.43–0.03 Ma).

3.3. Historical biogeography of the ailiids

The Dispersal-Extinction-Cladogenesis (DEC) model was selected as the best-fit for biogeographic reconstruction (lowest AIC; [Supplementary Table S1](#)). Results from S-DIVA analysis in RASP were largely consistent with output generated from the DEC model using

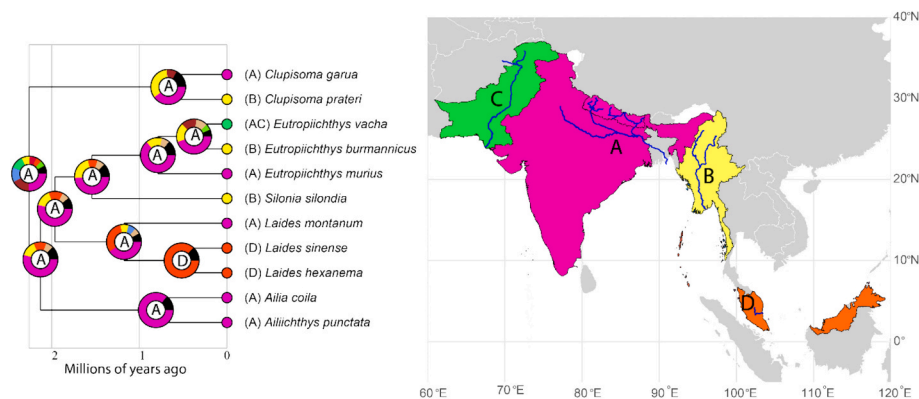


Fig. 5. Time calibrated tree of ailiids with estimated distribution range of nodes under DEC model implemented in BioGeoBEARS. Outgroups were discarded from analysis. Four river basins were assigned. A: Ganges–Brahmaputra basin (India and Nepal), B: Irrawaddy–Salween basin (Myanmar), C: Indus basin (Pakistan) and D: Pahang basin (Malaysia). The pie chart on each node represents different possibilities of distribution, with the letter in the middle of the pie chart indicating the distribution with highest probability.

BioGeoBEARS (Fig. 5, Supplementary File S11). Ancestral range reconstruction identified the Ganges–Brahmaputra River basin (South Asia) as the ancestral origin for Ailiidae (Fig. 4), followed by dispersals into the Irrawaddy–Salween, Pahang and Indus River basins. Basal lineages *Ailia coila* and *Ailiichthys punctata* retained their ancestral distribution in the Ganges–Brahmaputra basin without secondary dispersal. The *Laides montanum*/*Laides sinense*/*Laides hexanema* clade exhibited an interesting biogeographic split: *L. montanum* remained in the in the Ganges–Brahmaputra basin, while *L. sinense* and *L. hexanema* colonized the Pahang (Southeast Asia). Similarly, the clade containing *Eutropiichthys burmannicus*, *E. vacha*, *E. murius* and *Silonia silondia* also originated in the Ganges–Brahmaputra basin before dispersing into the Irrawaddy–Salween basin further supporting multiple independent colonization events in this region. *Clupisoma garua* and *C. prateri* share a common ancestor in the Ganges–Brahmaputra River basin but later *C. prateri* dispersed into the Irrawaddy–Salween basin. These patterns collectively highlight multiple independent colonization events shaping the biogeography of Ailiidae.

4. Discussion

4.1. Phylogenetic relationships among the Ailiidae species

The phylogeny strongly supported (100 % bootstrap) the monophyly of Ailiidae, encompassing *Ailia*, *Ailiichthys*, *Clupisoma*, *Eutropiichthys*, *Silonia* and *Laides* (genus *Proeutropiichthys* not analyzed). The high normalized quartet score (0.82) and full quartet support across all nodes in the ASTRAL tree indicated strong topological concordance among gene trees. However, the comparatively lower gCF (6.3–98.1 %; Supplementary File S10, S2B) and sCF (31.9–99.3 %; Supplementary File S10, S2A) values observed at some short internodes imply underlying gene tree discordance. Notably, the separation of the genera *Clupisoma*, *Eutropiichthys* and *Silonia* was supported by moderate sCF values of 31.8 and 31.9, indicating some level of discordance at this node. Furthermore, the separation of *Laides*, *Eutropiichthys* and *Silonia* exhibited significantly lower gCF values of 6.32 and 7.79, suggesting even greater gene tree conflict at this node. These low gCF values reflect potential challenges in resolving the evolutionary relationships among these genera, likely due to incomplete lineage sorting (ILS) or historical introgression events. Our findings are consistent with those of Roa-Varón et al. (2021), who reported gCF values ranging from 2.5–24.4 % and sCF values between 33.2–41.6 %, indicating moderate to low support for several nodes. These discrepancies across loci underscore the complexity of the evolutionary processes within the Ailiidae family and emphasized the need for phylogenomic analyses.

The taxonomy of the Ailiidae family, based on limited morphological traits and molecular markers (Ferraris and Vari, 2007; Van der Laan et al., 2014), remains inclusive until now. Traditional classifications grouped *Ailia*, *Clupisoma*, *Eutropiichthys*, *Proeutropiichthys*, *Silonia* and *Laides* into Ailiidae based on characters like the absence of a dorsal fin and elongate anal fin (Talwar and Jhingran, 1991; Nelson et al., 2016). However, these traits are now recognized as homoplastic, overlapping with those of Schilbeidae and Pangasiidae, leading to taxonomic instability (Stewart et al., 2014). Molecular studies have further complicated classifications; for example, Wang et al. (2016) reclassified *Eutropiichthys*, *Proeutropiichthys* and *Silonia* into Schilbeidae, while retaining *Ailia*, *Clupisoma* and *Laides* in Ailiidae. Schilbeidae itself is non-monophyletic, with African and Asian lineages resolved as distinct clades (Sullivan et al., 2006; Wang et al., 2016). This deep divergence supports that the African and Asian lineages are not closely related and the African Schilbeidae may share a closer relationship with other African catfish families than with their Asian counterparts (Ferraris and Vari, 2007). Consequently, the name Ailiidae has been proposed as the valid family-group name for the Asian lineage (Li and Zhou, 2018). Our phylogenomic analysis of 4,434 nuclear loci, robustly validates Ailiidae as a distinct family, comprising *Ailia*, *Ailiichthys*, *Eutropiichthys*, *Laides*,

Clupisoma and *Silonia*. Notably, *Ailia coila* and *Ailiichthys punctata* form a basal lineage reinforcing monophyly of this group and resolving long-standing taxonomic ambiguities. Our results establish a clear framework for the classification of Ailiidae, distinct from the African Schilbeidae.

The genus *Clupisoma* was recovered as paraphyletic, indicating potential taxonomic inconsistencies within its current classification. In contrast, in all other ailiid genera exhibit robust monophyly, reinforcing their systematic validity. These findings compel a critical re-evaluation of *Clupisoma* to resolve its phylogenetic discordance and establish a more taxonomically coherent framework. Molecular studies of *Clupisoma* are limited and its phylogenetic relationships remain poorly understood (Wang et al., 2016). While some studies based on mitochondrial cytochrome c (e.g., Saraswat et al., 2014; Laskar et al., 2020) have been conducted on a few species, the morphological characters defining this genus including nasal barbels extending beyond the anterior margin of the eye, maxillary barbels reaching midway along the pelvic fins and an anal fin with 40–44 rays (Hora, 1937) do not consistently hold across all species. For example, *C. garua*, and *C. prateri* form a well-supported sister clade, aligning with Hora's (1937) morphological distinctions. However, *Laides montanum* forms a distinct clade, showing closer phylogenetic affinity to *Laides sinense* and *Laides hexanema*. The morphological and meristic characters of *C. montanum* described by Ng (1999) and Shrestha (2019) including body shape, fin-ray counts, caudal fin structure and pectoral spine length exhibit strong similarities to those of *Laides*. These synapomorphies, combined with robust phylogenetic evidence, support the reclassification of *C. montanum* as *Laides montanum*. Key diagnostic characters for *Laides* include a more elongated body, a distinct abdominal keel and melanophore patterns that differ from *Clupisoma*. Furthermore, *Laides* species typically exhibit shorter maxillary barbels and a more anterior placement of the dorsal fin relative to the pelvic fins. The close phylogenetic relationship between *Clupisoma sinense* and *Laides hexanema*, supported by genomic data, contradicts prior morphological classifications that synonymized *L. sinense* with *C. sinense* based on barbel counts (Vidthayanon and Roongthongbaisuree, 1993; Ng, 1999; Ferraris, 2004; Chen et al. 2005). However, this aligns with morphological revisions by Kottelat (1989) and Rainboth (1996), who emphasized traits like nostril structure and fin morphology to reclassify *C. sinense* as *L. sinense*. Despite current recognition of *C. sinense* in FishBase (Froese and Pauly, 2025) and Eschmeyer's Catalog of Fishes (Fricke et al., 2025), our phylogenomic and morphological evidence necessitates its formal transfer to *Laides*.

Moreover, the phylogenetic analysis supports *Ailia coila* and *Ailiichthys punctata* as closely related species within Ailiidae and forming a sister clade to other ailiids. *Ailiichthys punctata* is a less studied taxon with very limited available information. Menon (1999) reclassified it as a synonym of *A. coila* but Ferraris and Vari (2007) resurrected it as a distinct, valid species. However, morphological and molecular data remain lacking, except for partial Cytochrome c sequence (Genbank Acc. No. PQ389427). The body appearance of *A. coila* and *A. punctata* appears strikingly similar; however, a significant morphological distinction lies in the pelvic fin, since it is absent in *A. punctata*. This peculiar and defining morphological trait, coupled with our robust phylogenetic analysis, supports the conclusion that these two taxa are distinct, valid species within Ailiidae.

The *Eutropiichthys* species *E. murius*, *E. burmannicus* and *E. vacha* exhibit close phylogenetic affinities, as evidenced by robust bootstrap values that strongly support their monophy within the family. Moreover, *E. burmannicus* was recovered as the sister species to *E. vacha*. Taxonomic placement of *Eutropiichthys* remains highly contentious. While FishBase (Froese and Pauly, 2025) retains it within Schilbeidae, Eschmeyer's Catalog of Fishes (Fricke et al., 2025) and Limbu et al. (2024) placed it under Ailiidae. This discrepancy persists in contemporary literature, as exemplified by Sarkar et al. (2013), Gupta and Banerjee (2016) and Khatun et al. (2022), all of which continue to assign *Eutropiichthys* to Schilbeidae, further complicating the understanding of

its true familial affiliation. However, our analysis suggests that *Eutropiichthys* belongs in Ailiidae, therefore, we recommend its reclassification. Similarly, the genus *Silonia*, forms a distinct clade separate from *Eutropiichthys*, highlighting the evolutionary divergence within the family. This pattern mirrors that observed in *Eutropiichthys*, reinforcing our phylogenetic findings, which strongly support the placement of *Silonia* within Ailiidae. Therefore, we recommend the classification of *Silonia* under Ailiidae. The genetic distance analyses further support the placement of *Eutropiichthys* and *Silonia* within the family Ailiidae. The pairwise genetic variation between species: *Eutropiichthys burmannicus*, *E. murius*, *E. vacha* and *Silonia silondia* and African schilbeid species (*Schilbe intermedius*, *Pareutropius debauwi*, *Parailia congica* and *Parailia pellucida*) ranged from 20 % to 57 %, reflecting substantial evolutionary divergence (Supplementary File S9). In contrast, their genetic distances from other Asian ailiid congeners were markedly lower (0.5–5.9 %). This pronounced contrast in genetic differentiation underscores the closer phylogenetic affinity of *Eutropiichthys* and *Silonia* to Asian ailiids than to African schilbeids. These results, congruent with our phylogenetic findings, provide compelling molecular evidence for reclassifying both genera under Ailiidae.

4.2. Divergence time and historical biogeography

Our study further estimated divergence times of the species within Ailiidae family, a group of catfishes distributed across South and Southeast Asia. The stem age of Ailiidae, marking its divergence from the closely related family Horabagridae, was estimated at 3.6 Ma. These relatively young estimates compared to other catfish families may reflect limited taxon sampling and the uncertain phylogenetic position of *Pachypterus* sp., which was traditionally placed in Schilbeidae but more recently assigned to Horabagridae, although some evidence suggests it may instead belong to Ailiidae. If *Pachypterus* sp. (or another “horabagrid”) is indeed an ailiid, the true MRCA of Ailiidae could be older than estimated here. Future studies with denser sampling of ‘Big Asia’ taxa are needed to clarify these relationships and refine divergence time estimates. Our molecular clock analyses estimate the most recent common ancestor (MRCA) of the sampled Ailiidae lineages to have originated during the Pleistocene at 2.1 Ma (3.34–0.96 Ma), with the uncertainty interval spanning the late Pliocene to the early Pleistocene (Fig. 3). Karinthanyakit and Jondeung (2012) investigated the phylogenetic relationships of pangasiid and schilbeid catfishes in Thailand, including four species now recognized as members of Ailiidae: *Clupisoma sinense* (syn. *Laidis sinense*), *L. longibarbis*, *C. prateri*, and *Eutropiichthys salweenensis*. Their analysis, based on mitochondrial DNA and a single nuclear marker with one fossil calibration point, estimated divergence times for these species between 4.5 and 2.5 million years ago (Ma). However, our divergence time estimation suggested that the most recent common ancestor of Ailiidae diverged at 2.1 Ma during the Pleistocene. These differences might be because results from Karinthanyakit and Jondeung (2012) were based on few taxa, a single fossil record used for calibration and analysis of mitochondrial genes sequences along with a single nuclear marker, which usually generate older age estimates (Dornburg et al., 2014).

The divergence of ailiids (3.34–0.96 Ma) aligns with major drainage reorganization in South and Southeast Asia during the Pliocene–Quaternary (Brookfield, 1998). This divergence event may have been influenced by the formation of new river systems or the isolation of populations due to orogenetic activity and changing sea levels (Sartono, 1973). It is unequivocally established that transformations in drainage basin morphology driven by geological processes are fundamental and decisive factors shaping the patterns of biodiversity and the distributions of ichthyofauna (Kottelat, 1989).

The basal divergence between *Ailia coila* and *Ailiichthys punctata* occurred approximately 2.1 million years ago (Ma) during early Pleistocene geological upheavals. This period coincided with Eastern Himalayan uplift and Ganges-Brahmaputra drainage reorganization

(Bracciali et al., 2016), likely driving vicariant speciation. Both species remain endemic to this basin, with no evidence of secondary dispersal. While FishBase (Froese and Pauly, 2025) and Eschmeyer’s Catalog (Fricke et al., 2025) report Pakistani occurrences for these species, we found no supporting primary literature. Without verification, we consider their distribution ancestrally restricted to the Ganges-Brahmaputra system, reflecting the retention of an ancestral distribution pattern.

The diversification of the *Clupisoma* lineage, estimated to have occurred around 1.96 million years ago (Ma), aligns with the onset of major sea-level oscillations during the Pleistocene. These cyclical fluctuations, which repeatedly exposed and inundated extensive landmasses across the Sunda and Sahul shelves (Voris, 2000), likely played a pivotal role in shaping the biogeographic history of freshwater taxa in South and Southeast Asia. The periodic exposure of vast shelf areas led to the formation of extensive ancient river systems, facilitating dispersal across now-fragmented river basins. One notable example is the divergence between *C. garua* and *C. prateri*, estimated to have occurred at approximately 0.62 Ma, during the Middle Pleistocene, a period marked by intensified glacial cycles and dramatic sea-level fluctuations (Sartono, 1973). The genus *Clupisoma* currently comprises seven recognized species with largely allopatric distributions across major South and Southeast Asian river systems, including the Indus (*C. naziri*, *C. garua*), Ganges-Brahmaputra (*C. garua*, *C. bastari*), Irrawaddy (*C. prateri*, *C. roosae*, *C. longianale*) and Salween (*C. yunnanense*) (Fricke et al., 2025). In our study, we included only two species, *C. garua* and *C. prateri*, which limits the scope of a comprehensive biogeographic reconstruction. Nonetheless, the estimated divergence between *C. garua* and *C. prateri* at approximately 0.62 Ma, during the Middle Pleistocene, provides a useful temporal anchor for hypothesizing historical separation processes. The spatial and temporal divergence of *C. garua* (Ganges-Brahmaputra basins) and *C. prateri* (Irrawaddy basin) likely reflects vicariant events linked to Pleistocene climate oscillations, paleodrainage shifts and tectonic activities. During glacial periods, lower sea levels exposed extensive land bridges across the Sunda Shelf, enabling episodic connections between river systems that are currently isolated (Sartono, 1973; Murray-Wallace and Woodroffe 2014; Šlechtová et al., 2021). It is plausible that ancestral populations of *Clupisoma* were once more widely distributed across a connected fluvial network, particularly between the Ganges-Brahmaputra and Irrawaddy systems, facilitated by paleo-river systems like the Brahmaputra-Irrawaddy connection hypothesized for the Late Pleistocene.

The divergence between *Laidis montanum* and the clade containing *L. sinense* and *L. hexanema*, occurred around 1.03 Ma, likely coinciding with significant environmental changes during the late Early Pleistocene. *Laidis montanum* is restricted to the Ganges-Brahmaputra River basin in South Asia, primarily in India and Nepal, while *L. sinense* and *L. hexanema* are found in Southeast Asia, likely within the Irrawaddy–Salween River basin. This geographic separation of these lineages likely indicates a vicariant scenario, in which ancestral populations were once more widely distributed across a hydrologically interconnected landscape during glacial lowstands, potentially linking the Ganges-Brahmaputra and Irrawaddy–Salween river systems. Numerous studies have demonstrated that Asia experienced multiple large-scale glaciation events during the Pleistocene, causing repeated fluctuations in sea level (Voris, 2000; Zachos et al., 2001). Such environmental instability would have contributed to the fragmentation of ancient river systems, suggesting that an ancestral *Laidis* population may have once inhabited a more continuous freshwater network potentially spanning the Indo-Burman region and enhancing connectivity between these river basins.

Biogeographic analysis of *Eutropiichthys* and *Silonia* reveals distinct patterns of historical dispersal and diversification across the Ganges–Brahmaputra and Irrawaddy–Salween river basins. Based on Eschmeyer’s Catalog of Fishes (Fricke et al., 2025), seven species of *Eutropiichthys* are currently recognized: *E. britzi*, *E. burmannicus* and *E.*

salweenensis from the Irrawaddy–Salween system; and *E. cetosus*, *E. goongwaree*, *E. murius* and *E. vacha* from the Ganges–Brahmaputra basin. The monotypic genus *Silonia* (*S. silondia*) occurs in both regions. The results suggest that the genera *Eutropiichthys* and *Silonia* likely originated in the Ganges–Brahmaputra basin and underwent multiple independent colonization events into the Irrawaddy–Salween basin. The most recent divergence was between *E. vacha* and *E. burmannicus* during late Pleistocene (0.4 Ma, 0.94–0.01 Ma, 95 % HPDs). This timeframe coincides with glacial cycles and associated sea level fluctuations of the late Pleistocene, which have been proposed as key drivers of increased speciation (Weins and Donoghue 2004; Mittelbach et al., 2007; Beck et al., 2017).

It is important to acknowledge a key limitation of our study: the limited taxon sampling within the family Ailiidae, covering only 11 out of the 25 currently recognized species. This restricted sampling may influence the robustness of phylogenetic relationships and underestimates the full complexity of the family's biogeographic history. The inferred divergence times and dispersal/vicariant patterns, while informative, should be interpreted with caution, as inclusion of additional taxa could potentially reveal previously undetected lineages or alternative historical scenarios. We emphasize that the proposed scenarios require validation through expanded taxonomic sampling and genomic-scale data. Such comprehensive approaches will be essential to fully resolve the family's complex diversification history and test alternative biogeographic hypotheses.

5. Conclusions

Our phylogenetic analysis confirms the monophyly of Ailiidae inclusive of *Ailia*, *Ailiichthys*, *Clupisoma*, *Eutropiichthys*, *Silonia* and *Laides* (genus *Proeutropiichthys* not analyzed). Robust molecular and morphological evidence supports reclassifying *C. sinense* and *C. montanum* to the genus *Laides*. Furthermore, our study strongly supports the classification of *Eutropiichthys* into Ailiidae, thereby reflecting its true phylogenetic relationship. Additionally, we advocate for the inclusion of *Silonia* within Ailiidae, resolving longstanding conflicts. Our findings support these taxonomic revisions to provide a more accurate reflection of the evolutionary relationships within the diverse and ecologically significant Ailiidae. The Ganges–Brahmaputra River basin in South Asia is likely the ancestral origin of the Ailiidae family. From there, they dispersed into the Irrawaddy–Salween, Pahang and Indus River basins through multiple independent colonization events. These movements were facilitated by paleo-river connections between the major drainages of South and Southeast Asia during the climatic fluctuations of the Pleistocene.

Ethical statement

Department of Forest and Soil Conservation, Nepal (permission ID: 477) permit for data collection of fish species for scientific purposes. All experimental procedures, including specimen handling, followed the Protocol of the Ethics Committee for the Use of Animal Subjects of Shanghai Ocean University (2020), China.

Data availability

All of the data that support the findings of this study are available in the main text and supplementary files. Raw sequence reads are available from the NCBI Sequence Read Archive (SRA), under BioProject PRJNA1230789.

CRediT authorship contribution statement

Jash Hang Limbu: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Dipak Rajbanshi:** Writing – review &

editing, Writing – original draft, Investigation. **Jiantao Hu:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Laxman Khanal:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Chenhong Li:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2025.108384>.

References

- Alam, M.J., Andriyono, S., Lee, S.R., Hossain, M.A.R., Eunus, A.T.M., Hassan, M.T., Kim, H.W., 2019. Characterization of the complete mitochondrial genome of gangetic *ailia*, *Ailia coila* (siluriformes: ailiidae). Mit. DNA b. 4 (2), 2258–2259. <https://doi.org/10.1080/23802359.2019.1627942>.
- Alves, Y.M., Bergqvist, L.P., Brito, P.M., 2016. New occurrences of microvertebrate fossil accumulations in Bauru Group, Late Cretaceous of western São Paulo state, Brazil. J. S. Am. Earth. Sci. 69, 80–90.
- Alves, Y.M., Bergqvist, L.P., Brito, P.M., 2019. The dorsal and pectoral fin spines of catfishes (Ostariophysi: Siluriformes) from the Bauru Group (Late Cretaceous), Brazil: A comparative and critical analysis. J. S. Am. Earth. Sci. 92, 32–40.
- Anisimova, M., Gil, M., Dufayard, J.-F., Dessimoz, C., Gascuel, O., 2011. Survey of branch support methods demonstrates Accuracy, power and robustness of fast likelihood-based approximation schemes. Syst. Biol. 60, 685–699. <https://doi.org/10.1093/sysbio/syr041>.
- Arbogast, B.S., Edwards, S.V., Wakeley, J., Beerli, P., Slowinski, J.B., 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annu. Rev. Ecol. Syst. 33, 707–740.
- Beck, S.V., Carvalho, G.R., Barlow, A., Ruber, L., Hui, T.H., Nugroho, E., Wowor, D., MohdNor, S.A., Herder, F., Muchlisin, Z.A., de Bruyn, M., 2017. Plio-pleistocene phylogeography of the southeast asian blue Panchax killifish. *Aplocheilichthys panchax*. Plos One. 12, e0179557. <https://doi.org/10.1371/journal.pone.0179557>.
- Benton, M.J., Donoghue, P.C.J., Asher, R.J., Friedman, M., Near, T.J., Vinther, J., 2015. Constraints on the timescale of animal evolutionary history. Palaeontol Electron. 18 1.1FC: 1–106.
- Bleeker, P., 1858. De heer Bleeker brengt nog ter tafel het eerste deel van eene ichthyologiae Archipelagi Indici Prodromus Natuurkundig Tijdschrift voor Nederlandsch Indië, 16, 38–41.
- Bracciali, L., Randall, R.P., Yani, N., Andrew, S., Andrew, C., Jan, R.W., 2016. Plio-pleistocene exhumation of the eastern himalayan syntaxis and its domal 'pop-up'. Earth-Sci. Rev 160, 350–385. <https://doi.org/10.1016/j.earscirev.2016.07.010>.

- Brookfield, M.E., 1998. The evolution of the great river systems of southern Asia during the Cenozoic India-Asia collision: rivers draining southwards 22 (3–4), 285–312. [https://doi.org/10.1016/S0169-555X\(97\)00082-2](https://doi.org/10.1016/S0169-555X(97)00082-2).
- Chen, W., Nicolas, H., Yuefei, L., Shuli, Z., Jun, W., Denggao, X., Shang, G., Chunni, K., Jilong, W., Tai, W., Zhiqiang, L., Junjie, W., Xinhui, L., Jie, L., 2024. Mitogenomic phylogeny, biogeography and cryptic divergence of the genus *Silurus* (siluriformes: siluridae). *Zool. Res.* 45 (4), 711–723. <https://doi.org/10.24272/j.issn.2095-8137.2023.311>.
- Chen, X.Y., Ferraris, C.J.J., Yang, J.X., 2005. A new species of catfish of the genus *Clupisoma* (siluriformes: schilbidae) from the Salween River, Yunnan, China. *Copeia* 2005 (3), 566–570. <https://doi.org/10.1643/C1-04-351R>.
- Cook, L.G., Crisp, M.D., 2005. Directional asymmetry of long-distance dispersal and colonization could mislead reconstructions of biogeography. *J. Biog.* 32, 741–754.
- Dias-Brito, D., Musacchio, E.A., de Castro, J.C., Maranhão, M.S.A.S., Suárez, J.M., Rodrigues, R., 2001. The Bauru Group: A continental Cretaceous unit in Brazil - Concepts based on micropaleontological, oxygen isotope and stratigraphical data. *Rev. Paléobiol. Genève* 20 (1), 245–304.
- Dornburg, A., Townsend, J.P., Friedman, M., Near, T.J., 2014. Phylogenetic informativeness reconciles ray-finned fish molecular divergence times. *BMC Evol. Biol.* 14, 169. <https://doi.org/10.1186/s12862-014-0169-0>.
- Drummond, A.J., Rambaut, A., 2007. BEAST: bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7, 214. <https://doi.org/10.1186/1471-2148-7-214>.
- El-Sayed, S., Murray, A.M., Kora, M.A., Abu El-Kheir, G.A., Antar, M.S., Seiffert, E.R., Sallam, H.M., 2020. Oldest record of african bagridae and evidence from catfishes for a Marine influence in the late eocene birket Qarun locality 2 (BQ-2), fayum depression. *Egypt. J. Vert. Pal.* 40 (3). <https://doi.org/10.1080/02724634.2020.1780248>.
- Fara, E., Gayet, M., Taverne, L., 2010. The fossil record of Gonorynchiformes. In: Grande, T., Poyato-Ariza, F.J., Diogo, R. (Eds.), *Gonorynchiformes and ostariophysan relationships: a comprehensive review*. SciencePublishers, Enfield, NH, pp. 173–226.
- Farris, J.S., Källersjö, O.M., Kluge, A.G., Bult, C., 1995. Testing significance of incongruence. *Cladistics* 10, 315–319.
- Ferraris, C.J., 2004. A new species of the asian schilbid catfish genus *Clupisoma* from Myanmar, with a redescription of *Clupisoma prateri* hora (osteichthyes: siluriformes: schilbidae). *Zootaxa* 437, 1–10.
- Ferraris, C.J., Vari, R.P., 2007. Revision of the Catfishes of the genus *Eutropiichthys*, with the description of two new species (Siluriformes: Schilbidae). *Copeia* 4, 866–885.
- Fricke, R., Eschmeyer, W.N., van der Laan, R., 2025. Eschmeyer's catalog of fishes: genera, species, references. (<http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp>). Electronic version accessed dd mmm 2025.
- Froese, R., Pauly, D., 2025. FishBase. World Wide Web electronic publication. www.fishbase.org, version (10/2024).
- Gallili, T., Callaghan, A., Sidi, J., Sievert, C., 2018. Heatmaply: an R package for creating interactive cluster heatmaps for online publishing. *Bioinformatics* 34 (9), 1600–1602. <https://doi.org/10.1093/bioinformatics/btx657>, 2-s2.0-85047061082.
- Guo, X., He, S., Zhang, Y., 2005. Phylogeny and biogeography of chinese sisorid catfishes re-examined using mitochondrial cytochrome b and 16S rRNA gene sequences. *Mol. Phylogenet. Evol.* 35, 344–362.
- Gupta, S., Banerjee, S., 2016. *Eutropiichthys vacha* (Hamilton, 1822), a threatened fish of Indian subcontinent. *J. Fish.* 4 (2), 397–400. <https://doi.org/10.17017/j.fish.120>.
- Ho, S.Y.W., Ton, G.K.J., Foster, C.S.P., Ritchie, A.M., Lo, N., Crisp, M.D., 2015. Biogeographic calibrations for the molecular clock. *Biol. Lett.* 1, 20150194. <https://doi.org/10.1098/rsbl.2015.0194>.
- Hora, S.L., 1937. The game fishes of India-III. *Journal of the B. Nat. Hist. Soc.* 39 (4), 659–678, plus unnumbered pl.
- Hu, J., Li, H., Sakai, H., Mukai, T., Young, S.H., Li, C., 2023. Molecular phylogenetics of the fresh water sleepers Odontobutis (Gobiiformes: Odontobutidae) and its implications on biogeography of freshwater ichthyofauna of East Asia. *Mol. Phylogenet. Evol.* 186, 107871. <https://doi.org/10.1016/j.ympev.2023.107871>.
- Jiang, J.M., Yuan, H., Zheng, X., Wang, Q., Kuang, T., Li, J.Y., Liu, J.N., Song, S.L., Wang, W.C., Cheng, F.Y., Li, H.J., Huang, J.M., Li, C.H., 2019. Gene markers for exon capture and phylogenomics in ray-finned fishes. *Ecol. Evol.* 9, 3973–3983. <https://doi.org/10.1002/ee3.5026>.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermini, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* 14, 587–589. <https://doi.org/10.1038/Nmeth.4285>.
- Kappas, I., Vitas, S., Pantzartzis, C.N., Drosopoulou, E., Scouras, Z.G., 2016. A time-calibrated mitogenome phylogeny of catfish (teleostei: siluriformes). *PLoS One* 11 (12), e0166988. <https://doi.org/10.1371/journal.pone.0166988>.
- Karinthanayak, W., Jondeung, A., 2012. Molecular phylogenetic relationships of pangasid and schilbid catfishes in Thailand. *J. Fish Biol.* 80 (7), 2549–2570. <https://doi.org/10.1111/j.1095-8649.2012.03303.x>.
- Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30 (4), 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Khatun, D., Hossain, M.Y., Rahman, O., Hossain, M.F., 2022. Estimation of life history parameters for river catfish *Eutropiichthys vacha*: insights from multi-models for sustainable management. *Heliyon* 8, e10781. DOI: 10.1016/j.heliyon.2022.e10781.
- Kottelat, M., 1989. Zoogeography of the fishes from indochinese inland waters with an annotated check-list. *Bulletin of the Zoological Museum of the University of Amsterdam* 12, 1–56.
- Kottelat, M., 2013. The fishes of the inland waters of Southeast Asia: a catalogue and Core bibliography of the fishes known to occur in freshwaters, mangroves and Estuaries. *Raffl. Bull. Zool.* 27, 1–663.
- Krueger, F., 2021. Trim_Galore 0.6.7. <https://github.com/FelixKrueger/TrimGalore> (accessed 10th September 2022).
- Lanfear, R., Frandsen, P.B., Wright, A.M., Senfeld, T., Calcott, B., 2016. PartitionFinder 2: new methods for selecting Partitioned models of evolution for Molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* 34, 772–773. <https://doi.org/10.1093/molbev/msw260>.
- Laskar, B.A., Adimalla, H., Kundu, S., Jaiswal, D., Chandra, K., 2020. DNA barcoding of a lesser-known catfish, *Clupisoma bastari* (actinopterygii: ailiidae) from Deccan peninsula. *India. J. Threat. Taxa.* 14 (8), 21605–21611. <https://doi.org/10.11609/jott.6900.14.8.21605-21611>.
- Li, C.H., Hofreiter, M., Straube, N., Corrigan, S., Naylor, G.J.P., 2013. Capturing protein-coding genes across highly divergent species. *Biotechniques* 54, 321–326. <https://doi.org/10.2144/000114039>.
- Li, H.J., He, Y., Jiang, J.M., Liu, Z.Z., Li, C.H., 2018. Molecular systematics and phylogenetic analysis of the asian endemic freshwater sleepers (gobiiformes: odontobutidae). *Mol. Phylogenet. Evol.* 121, 1–11. <https://doi.org/10.1016/j.ympev.2017.12.026>.
- Li, X., Zhou, W., 2018. The species of *Clupisoma* from Yunnan, China (teleostei: siluriformes: ailiidae), with a comment on the validity of the family ailiidae. *Zootaxa* 4476 (1), 77–86. <https://doi.org/10.11646/zootaxa.4476.1.7>.
- Limbu, J.H., Rajbanshi, D., Khanal, L., Hui, W., Adhikari, R.C., Thapa, S., Yang, J.Q., Li, C., 2024. DNA Barcoding of catfishes (order: siluriformes) with new Records of two Species from eastern Nepal. *J. Appl. Ichthy.* 2024, 1–11.
- Lydekker, R., 1886. Indian tertiary and post-tertiary vertebrata: tertiary fishes. *Mem Geol. Surv. India Palaeontol. India Ser.* 10, 241–258.
- Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet J.* 17, 10–12. <https://doi.org/10.14806/ej.17.1.200>.
- Matzke, N.J., 2018. BioGeoBEARS: BioGeography with Bayesian (and likelihood) Evolutionary Analysis with R Scripts. version 1.1.1. <https://github.com/nmatzke/BioGeoBEARS> (accessed 10th September 2022).
- Menon, A.G.K., 1999. Check list - Fresh water fishes of India. Records of the Zoological Survey of India, Occasional Paper No. 175: i-xxix + 1-366.
- Meyer, M., Kircher, M., 2010. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* 2010, pdbprot5448. 10.1101/pdb.prot5448.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* 37, 1530–1534. <https://doi.org/10.1093/molbev/msaa015>.
- Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S., Warnow, T., 2014. ASTRAL: genome-scale coalescent-based species tree estimation. *Bioinformatics* 30, 1541–1548. <https://doi.org/10.1093/bioinformatics/btu462>.
- Mittelbach, G.G., Schemske, D.W., Cornell, H.V., Allen, A.P., Brown, J.M., Bush, M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M., McCune, A.R., McDade, L.A., McPeck, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M., Turelli, M., 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecol. Lett.* 10, 315–331.
- Mo, T.P., 1991. Anatomy, relationships and systematics of the bagridae (teleostei: siluroidei) with a hypothesis of siluroid phylogeny. *Theses Zool.* 17, 1–216.
- Murray-Wallace, C.V., Woodroffe, C.D., 2014. Pleistocene sea-level changes. In: *Quaternary Sea-Level Changes: A Global Perspective*. Cambridge University Press; 2014, 256–319.
- Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. *Fishes of the World*. 5th Edition, John Wiley and Sons, Hoboken. DOI: 10.1002/9781119174844.
- Ng, H.H., 1999. *Lalates longibarbis*, a valid species of schilbid catfish from Indochina (teleostei: siluriformes). *Ichthyol. Expl. Freshw.* 10, 381–385.
- Paradis, E., Schliep, K., 2019. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35 (3), 526–528. <https://doi.org/10.1093/bioinformatics/bty633>.
- R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Rainboth, W.J., 1996. The taxonomy, systematic and zoogeography of *Hypsibarbus*, a new genus of Large Barbs (pisces, cyprinidae) from the Rivers of southeastern Asia. University of California Press, Berkeley, CA.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* 67, 901–904. <https://doi.org/10.1093/sysbio/syy032>.
- Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* 57, 4–14. <https://doi.org/10.1080/10635150701883881>.
- Roa-Varón, A., Dikow, R.B., Carnevale, G., Tornabene, L., Baldwin, C.C., Li, C., Hilton, E. J., 2021. Confronting sources of systematic error to resolve historically contentious relationships: a case study using gadiform fishes (teleostei, Paracanthopterygii, gadiformes). *Syst. Biol.* 70 (4), 739–755. <https://doi.org/10.1093/sysbio/syaa095>.
- Saraswat, D., Lakra, W.S., Nautiyal, P., Goswami, M., Shyamkant, K., Malakar, A., 2014. Genetic characterization of *Clupisoma garua* (Hamilton 1822) from six Indian populations using mtDNA cytochrome b gene. *Mit. DNA* 25 (1), 70–77. <https://doi.org/10.3109/19401736.2013.782014>.
- Sarkar, U.K., Pathak, A.K., Tyagi, L.K., Srivastava, S.M., Singh, S.P., Dubey, V.K., 2013. Biodiversity of freshwater fish of a protected river in India: comparison with unprotected habitat. *Rev. Biol. Trop.* 61, 161–172.
- Sartono, S., 1973. On Pleistocene migration routes of vertebrate fauna in Southeast Asia. In: B.K. Tan (Ed.), *Proc. Reg. Conf. Geology of Southeast Asia*, Kuala Lumpur 1972, Bull. Geol. Soc. Malaysia 6, p. 273–286.

- Shrestha, T.K., 2019. Ichthyology of Nepal: a study of fishes of the Himalayan waters. Prism Color Scanning and. Press Supportive Pvt. Ltd, Kathmandu, Nepal, p. 400.
- Šlechtová, V., Musilova, Z., Tan, H.H., Kottelat, M., Bohnen, J., 2021. One northward, one southward: contrasting biogeographical history in two benthic freshwater fish genera across Southeast Asia (teleostei: cobitoidea: *nemacheilus*, *Pangio*). Mol. Phylogenetics Evol. 161, 107139. <https://doi.org/10.1016/j.ympev.2021.107139>.
- Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>. Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.
- Stewart, T.A., Smith, W.L., Coates, M.I., 2014. The origins of adipose fins: an analysis of homoplasy and the serial homology of vertebrate appendages. Proc. r. Soc. B 281, 20133120. <https://doi.org/10.1098/rspb.2013.3120>.
- Sullivan, J.P., Lundberg, J.G., Hardman, M., 2006. A phylogenetic analysis of the major groups of catfishes (teleostei: siluriformes) using rag1 and rag2 nuclear gene sequences. Mol. Phylogenetics Evol. 41, 636–662. <https://doi.org/10.1016/j.ympev.2006.05.044>.
- Talwar, P.K., Jhingran, A.G., 1991. Inland Fishes of India and Adjacent Countries. Oxford-IBH Publishing Co. Pvt. Ltd, New Delhi, pp. 1158–p.
- Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7), 3022–3027. <https://doi.org/10.1093/molbev/msab120>.
- Van der Laan, R., Eschmeyer, W.N., Fricke, R., 2014. Family-group names of recent fishes. Zootaxa 3882 (2), 1–230. <https://doi.org/10.11646/zootaxa.3882.1.1>.
- Vidthayanon, C., Roongthongbaisuree, S., 1993. Taxonomy of Thai Riverine Catfishes Family Schilbeidae and Pangasiidae. Bangkok: National Inland Fisheries Institute, Department of Fisheries.
- Voris, H.K., 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Bio. 27, 1153–1167. <https://doi.org/10.1046/j.1365-2699.2000.00489.x>.
- Wang, J., Lu, B., Zan, R., Chai, J., Ma, W., Jin, W., Duan, R., Luo, J., Murphy, R.W., Xiao, H., Chen, Z., 2016. Phylogenetic relationships of five asian schilbid genera including Clupisoma (siluriformes: schilbeidae). PLoS One 11 (1), e0145675. <https://doi.org/10.1371/journal.pone.0145675>.
- Wiens, J.J., Donoghue, M.J., 2004. Historical biogeography, ecology and species richness. Trends Ecol. Evol. 19, 639–644.
- Yang, L., He, S., 2008. Phylogeography of the freshwater catfish Hemibagrus guttatus (Siluriformes, Bagridae): implications for South China biogeography and influence of sea-level changes. Mol. Phylogenet. Evol. 49 (1), 393–398. <https://doi.org/10.1016/j.ympev.2008.05.032>.
- Yu, Y., Blair, C., He, X., 2020. RASP 4: ancestral state reconstruction tool for multiple genes and Characters. Mol. Biol. Evol. 37, 604–606. <https://doi.org/10.1093/molbev/msz257>.
- Yu, Y., Harris, A.J., Blair, C., He, X.J., 2015. RASP (reconstruct ancestral state in phylogenies): a tool for historical biogeography. Mol. Phylogenet. Evol. 87, 46–49. <https://doi.org/10.1016/j.ympev.2015.03.008>.
- Yuan, H., Atta, C., Tornabene, L., Li, C.H., 2019. Assexon: assembling exon using gene capture data. Evol. Bioinform. Online 15.
- Yuan, H., Jiang, J.M., Jimenez, F.A., Hoberg, E.P., Cook, J.A., Galbreath, K.E., Li, C.H., 2016. Target gene enrichment in the cyclophyllidean cestodes, the most diverse group of tapeworms. Mol. Ecol. Resour. 16, 1095–1106. <https://doi.org/10.1111/1755-0998.12532>.
- Zachos, J., Pagani, M., Sloan, L., et al., 2001. Trends, rhythms and aberrations in global climate 65 ma to present. Science. 292 (5517), 686–693.
- Zhou, C., Wang, X., Gan, X., Zhang, Y., Irwin, D.M., Mayden, R.L., He, S., 2016. Diversification of Sisorid catfishes (Teleostei: Siluriformes) in relation to the orogeny of the himalayan Plateau. Sci. Bull. 61 (13), 991–1002. <https://doi.org/10.1007/s11434-016-1104-0>.