

Vicariance and monsoon as drivers of diversification of nemacheilid loaches (Teleostei: Cypriniformes) around the Hengduan Mountains of China

DEAR EDITOR,

High mountainous terrains facilitate the diversification of biota by providing a variety of opportunities for isolation (Favre et al., 2015). Within the Nemacheilidae family of loaches, the highly diverse Nemacheilini tribe is a polyphyletic group, with extensive distribution across the Indian and Eurasian plates (Chen et al., 2019; Sgouros et al., 2019). Here, to examine the potential effects of mountain uplift on Nemacheilini polyphyly, we analyzed two mitochondrial (cyt *b* and *COI*) and three nuclear DNA loci (RAG1, IRBP2, and RH) using Bayesian inference (BI) to generate a dated phylogeny of the four major morphological tribes within Nemacheilidae (Yunnanilini, Lefuini, Triplophysini, and Nemacheilini) distributed around the Hengduan Mountains of Southwest China. We further reconstructed their ancestral regions and estimated their diversification rates. The ancestral region reconstruction indicated that nemacheilid loaches originated on the Eurasian plate, with subsequent dispersion to the Indian plate in the late Eocene. Dated phylogeny suggested that the various phases of Hengduan Mountain uplift contributed to the separation of the major Nemacheilidae lineages. Additionally, Asian desertification and monsoon in the Miocene hindered species gene flow between northern and southern China. The diversification estimates revealed that the Nemacheilini II lineage diverged at a faster rate than the Nemacheilini I lineage. We postulate that uplift of the Hengduan Mountains played a significant role in the diversification of Nemacheilidae, particularly the accelerated differentiation of Nemacheilini II, with minimal direct influence on Nemacheilini I.

Our study focused on two specific clades, Nemacheilini I and II, identified through single-gene tree analysis (Supplementary Figures S1–S5). For BI phylogeny, we analyzed a combined dataset of 4 844 bp from 166 individual specimens. Phylogenetic analysis divided the nemacheilids into four main clades (Figure 1B). The Yunnanilini-Lefuini clade diverged first, while Nemacheilini formed a polyphyletic rather than monophyletic clade. Using BI analysis, the divergence times of 89 operational taxonomic units (OTUs) were determined, dating the Nemacheilidae origin to approximately 49.5 million years ago (Ma) (60.0–40.9 Ma)

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(Supplementary Figure S6). Divergence between the Yunnanilini-Lefuini and Triplophysini-Nemacheilini clades occurred approximately 39 Ma (47.3–31.5 Ma), while divergence between Nemacheilini I and Triplophysini-Nemacheilini II occurred about 36.4 Ma (44.3–29.6 Ma) and divergence between Triplophysini and Nemacheilini II occurred around 32 Ma (40–26 Ma). The optimal DIVALIKE model (Supplementary Table S1) of the combined data indicated that the Yangtze and Pearl river basins (AM) were the most likely ancestral regions of Nemacheilidae (Supplementary Figure S7). Furthermore, Nemacheilini I and II potentially originated from the Irrawaddy, Salween, and Chao Phraya basin (DQ) and Lancang River basin (HL), respectively. To explore scenarios of two Nemacheilini lineages turnover through time in relation to the geological movement, we estimated the net diversification rate of Nemacheilidae, Nemacheilini I and II. After discarding 10% as burn-in, Markov Chain Monte Carlo (MCMC) chain convergence was confirmed based on Bayesian analysis of macroevolutionary mixtures (BAMM), with effective sample size (ESS) values of 2 196.174 and 3 577.846 for log-likelihood and rate shifts, respectively. The background net diversification rate for the Nemacheilidae family was 0.31 myr⁻¹, which was higher in the Nemacheilini II clade (0.61 myr⁻¹) but lower in the Nemacheilini I clade (0.26 myr⁻¹). Four shift configurations were identified in the 95% credible shift set with the highest posterior probabilities (Supplementary Figure S6). The net diversification rate of Nemacheilidae increased slowly until approximately 23 Ma, then increased significantly from about 10 Ma to the present day (Figure 1C). A time-series plot of the diversification rates of the two Nemacheilini clades revealed distinct diversification patterns. Notably, the Nemacheilini II showed accelerated diversification over the last 16 Ma, while Nemacheilini I showed a lower and constant diversification rate (Figure 1C).

Uplift of the Hengduan Mountains during the Eocene-Oligocene transition led to various speciation events in both animals (Chen et al., 2019; Luo et al., 2017; Šlechtová et al., 2008) and plants (Ding et al., 2020), and further promoted divergence of the western and eastern clades of nemacheilids along the southwest edge of the Hengduan Mountains about 36 Ma (Figure 1B; Supplementary Figure S6).

The India-Asia collision not only promoted mountain uplift,

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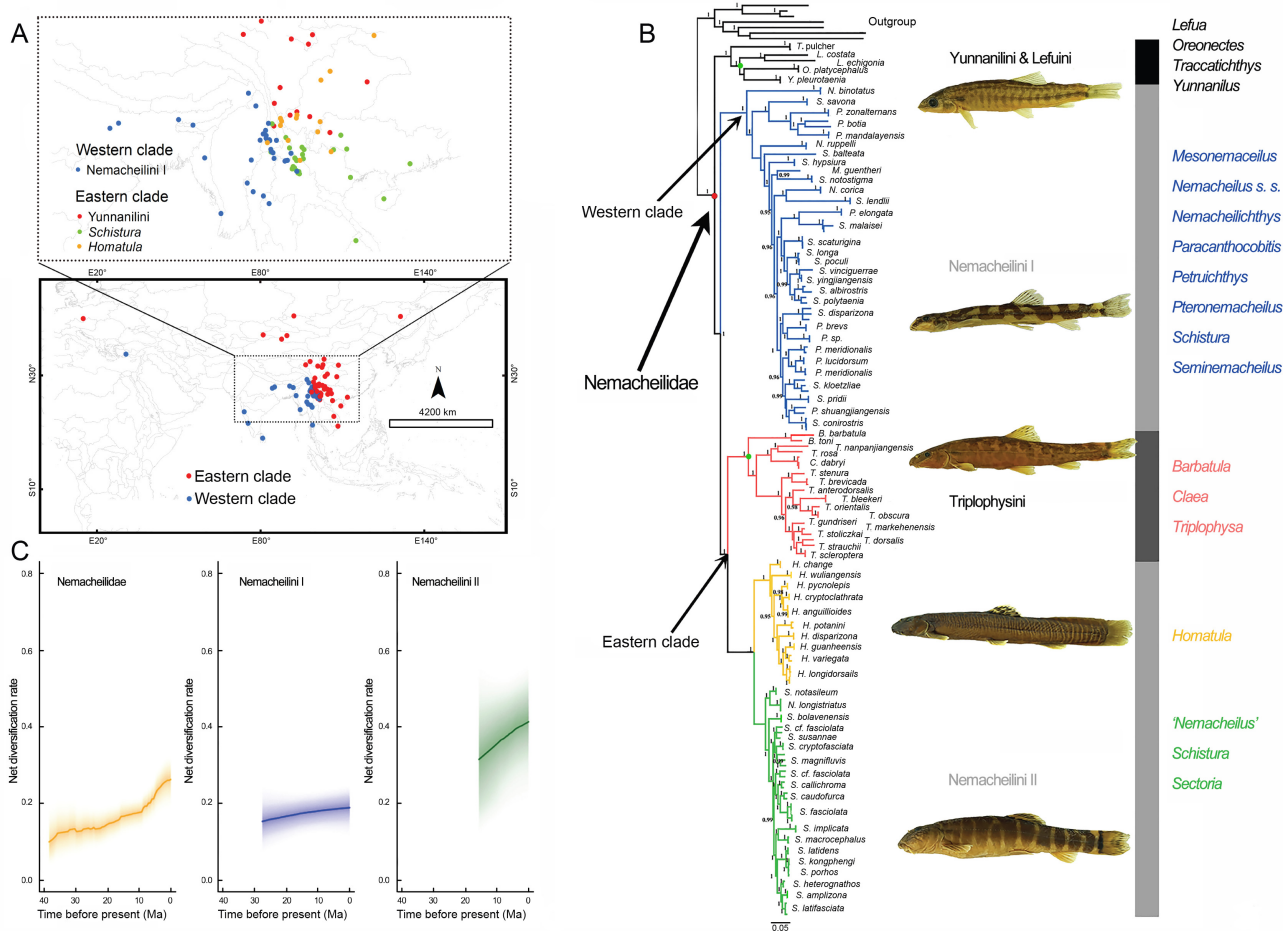


Figure 1 Collection map, phylogenetic relationships, and net diversification rates of nemacheilids

A: Sample localities of Nemacheilini and Triplophysini. B: Phylogram of 50% majority rule consensus tree derived from BI analysis based on concatenated dataset of nemacheilids; photos by Rui Min. C: Net diversification rates with time. Green dots show the separation of *Barbatula* from *Triplophysa* and the isolation of *Lefua* from *Yunnanilus*.

but also initiated changes in climatic conditions across Asia (An et al., 2006; Guo et al., 2002). Previous studies have highlighted the role of Asian desertification and monsoon in driving salamander species isolation and alpine flora richness in the Hengduan Mountains (Ding et al., 2020; Zhang et al., 2006). Concurrent with these climatic changes, two notable speciation occurrences emerged within the nemacheilids: the separation of *Barbatula* from *Triplophysa* and the isolation of *Lefua* from *Yunnanilus* (Figure 1B; Supplementary Figure S6). These events were also influenced by the onset of Asian desertification, further isolating the distributions of *Barbatula-Lefua* and *Yunnanilus-Triplophysa* in northern Asia and Europe, southwestern China, and mountainous areas around the Qinghai-Xizang (Tibet) Plateau (QTP) (purple and green areas in Supplementary Figure S6c). Between the late Eocene and mid-Miocene, the western and eastern clades evolved independently and spread within their respective ranges until approximately 10 Ma (Figure 1; Supplementary Figure S6). Subsequently, an encounter between the two clades occurred, resulting in the formation of a small-scale overlapping distribution in the middle and lower reaches of the Mekong and Salween rivers (Figure 1A) probably due to diversion of the paleo-Red River. Geological studies have shown that the paleo-Red River, formed by the confluence of the upper Mekong and upper Salween in the early Miocene, was disconnected by the clockwise rotation of the Eastern

Himalayas about 15–10 Ma (Todrani et al., 2022), thus allowing the upper Mekong and upper Salween to connect with the lower Mekong and lower Salween, respectively.

Studies have shown that the occurrence of Hengduan Mountain uplift during the Oligocene and Miocene led to a significant increase in the rate of alpine plant speciation around 8 Ma (Ding et al., 2020). Therefore, the significant increase in the evolutionary rate of Nemacheilini II may be the result of freshwater fish adaptation to the dramatic uplift of these mountains.

Our results suggest that Nemacheilidae originated on the Eurasian Plate and subsequently spread to the Indian Plate via the Indochina Peninsula during the early stages of continental collision. The emergence of geographical barriers due to the collision and extrusion led to the isolation and differentiation of populations on the Eurasian and Indian plates, resulting in independent eastern and western clades. With the geological uplift, rivers radiated to the surrounding areas from the QTP, Himalayas, and Hengduan Mountains. The western and eastern clades evolved along the rivers of the Indian and Eurasian plates, separated by the southern edge of the Himalayas and lower Mekong River (Chen et al., 2019), encompassing most of Eurasia and establishing the distinct geographical distribution of the two Nemacheilidae clades. Climate changes caused by geological events also played a role in the speciation of Nemacheilidae. The

distribution range of Nemacheilini II species was directly influenced by mountain elevation, leading to a higher rate of differentiation. In contrast, Nemacheilini I species were less affected by mountain uplift, resulting in a more uniform rate of differentiation. Therefore, we hypothesize that geological uplift played a significant role in the diversification of Nemacheilidae species, particularly in the accelerated differentiation of Nemacheilini II, but had no direct impact on the differentiation of Nemacheilini I.

DATA AVAILABILITY

The sequencing data of loaches used in the present study are available from the Science Data Bank database (Data DOI 10.57760/sciencedb.j00139.00053) and GenBank (accession numbers MG237921–MG238426, OQ946956–OQ946957, OQ945050–OQ945051, and OQ973298–OQ973303).

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X.Y.C. and J.X.Y. conceived the idea; R.M. and X.Y.C. collected the samples with additional material from collaborators; R.M. collected and analyzed the data; R.M. led the writing with assistance from Y.H.Z., B.K., X.Y.C., and J.X.Y. All authors read and approved the final version of the manuscript.

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