

Supplementary Materials

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

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Supplementary Materials and Methods

Data collection

In total, 82 species from 18 genera of Nemacheilidae were examined as ingroups. Sample information is provided in Table S2 and Fig. 1A and overall sample distribution is shown in Table S3 and Fig. 1A. The selection of outgroup species, including *Vaillantella maassi*, *Botia lohachata*, *Jinshaia abbreviata*, *Sinogastromyzon sichangensis*, *Beaufortia szechuanensis*, and *Gyrinocheilus aymonieri*, was based on the molecular phylogeny of Cobitoidea in Liu et al. (2012) and Chen et al. (2019) (Table S3).

After fixing the "voucher specimens" in a solution of 10% formalin or 95% alcohol, they were preserved in 75% alcohol and stored at the Kunming Institute of Zoology (KIZ). The left pelvic fin was cut and stored in 99% alcohol to allow for future molecular laboratory analysis.

DNA extraction, amplification, sequencing, and alignment

We studied five molecular markers, including two mitochondrial genes (cytochrome oxidase subunit 1 (COI) and cytochrome *b* (*cyt b*)) and three nuclear genes (recombination-activating gene 1 (RAG1), inverted repeat binding protein 2 (IRBP2), and rhodopsin (RH)). DNA was extracted from fin tissues preserved in absolute ethanol using standard phenol-chloroform extraction (Sambrook et al., 1989). Polymerase chain reaction (PCR) (50- μ l volume) was completed using the following protocols: initial denaturation step at 95 °C for 5 min, 35 cycles at 94 °C for 30 s, 52–56 °C (depending on primer, Table S4) for 45 s, and 72 °C for 1 min, with a single final extension at 72 °C for 10 min. Fragments were sequenced by the Shanghai DNA Biotechnologies Company (China). DNA sequences of the five loci were aligned individually using default settings in MAFFT v7 (<http://mafft.cbrc.jp/alignment/server/>) (Kato & Standley, 2013), and, if necessary, adjusted by eye. All protein-coding gene sequences were translated into amino acids to check for stop codons.

Molecular phylogenetic analysis

Bayesian inference (BI) analysis was first performed on single-gene datasets to test conflicting positions of major lineages. As no such case was observed, the concatenated dataset was analyzed. The five gene fragments were concatenated and partitioned based on codon position using SequenceMatrix v1.7.8 (Vaidya et al., 2011) and PartitionFinder v1.1.1 (Lanfear et al., 2012). BI analysis was performed in MrBayes v3.2.6 (Ronquist et al., 2012). Four incrementally heated Markov chains (default heating value of 0.1) were run for 10×10^7 million generations, with the chains sampled at intervals of 1 000 generations. Two independent runs were carried out and the first 25% of samples were discarded as burn-in. We used the best substitution model for each partition based on the partitioning strategy selected using PartitionFinder.

Divergence time estimation

To estimate the ages of the major clades, we employed three calibration points in our analysis. The calibration points utilized in our analysis were identical to those described by Bohlen et al. (2020): (1) we set the age of Nemacheilidae in analysis with uniform distribution to 60–0 Mya (Bagley et al., 2018; Cavender, 1991); (2) we set the isolation of the Indian freshwater fauna from the Southeast Asian fauna in analysis with uniform distribution to 45–24 Mya (Ali and Aitchison, 2008; Clark et al., 2004; Klaus et al., 2010; Wang, 2004); (3) we set the minimum age of the genus *Triplophysa* in analysis to 5.3 Mya (Böhme and Ilg, 2003; Prokofiev, 2007). This approach ensured consistency and allowed us to compare our results with those of previous studies.

The dataset with one specimen of each species was analyzed to estimate divergence times using Beast v2.6.2 (Bouckaert et al., 2014). The species-tree prior assumed a Yule process. Markov chain Monte Carlo (MCMC) analyses (20×10^7 generations) were run independently twice, with sampling every 2 000 generations. LogCombiner v2.6.2 was used to combine the two results. Chain convergence was determined using Tracer v1.6 (Rambaut et al., 2014), with target effective sample size (ESS) >200 for all parameters. A maximum clade credibility (MCC) tree was built in TreeAnnotator v.2.6.2 (Zhang et al., 2020) after discarding the first 30% of trees as burn-in.

Ancestral range estimations

We used the pruned Beast tree to estimate ancestral geographical ranges in BioGeoBEARS package (Matzke, 2013). Because the program uses the species tree to infer biogeographical history and cannot consider multiple samples from the same species (Matzke, 2014). Distribution units were set based on freshwater ecoregions (Abell et al., 2008), with several ecoregions (e.g., different parts of the same basin, neighboring basins, regions far from the main study areas) linked to avoid overpopulation of units. In detail, distribution units ‘A’, ‘C’, ‘D’, ‘I’, ‘J’, ‘L’, and ‘P’ each contained two or three ecoregions from Abell et al. (2008). In total, 18 biogeographic units were obtained: A. Middle & Lower Yangtze; B. Upper Yangtze; C. Balkash-Alakul, Dzungaria & Western Mongolia; D. Sitang-Irrawaddy; E. Ganges Delta & Plain, Middle Brahmaputra, Chin Hills-Srakan Coast; F. Lower & Middle Salween; G. Upper Salween; H. Upper Lancang (Mekong); I. Honshu-Shikoku-Kyushu, Eastern Yellow Sea Drainages; J. Western Ghats, Sri Lanka Dry Zone; K. Song Hong; L. Lower Lancang (Mekong), Dratie-Stung Treng; M. Xi Yiang; N. Hainan; O. Upper Huang He, Upper Huang He Corridor; P. Central & Western Europe, Middle Amur; Q. Chao Phraya; R. Southern Anatolia (Fig. S6c). These biogeographic units are marked by colours according to their position in India (yellow), western of Indochina (blue), eastern of Indochina (red), Southern China (green) and norther areas of China and Asia (purple).

Since Ree and Sanmartin (2018) questioned the validity of biogeographic models

that rely on jump dispersal events to explain the distribution of organisms, we tested three biogeographic models (DEC, DIVALIKE, and BAYAREALIKE) in the R package of BioGeoBEARS (Matzke 2014). We set the maximum area to two and pruned the outgroup from the tree prior to analysis. Likelihood values of the three BioGeoBEARS models were compared using Akaike information criterion (AIC).

Diversification rates of Nemacheilini

To understand the evolution of the two Nemacheilini lineages, we investigated their diversification and accounted for incomplete taxon sampling. As there is no evidence to clarify species composition in each Nemacheilini lineage, we calculated incomplete taxon sampling based on the percentage of study species to all known species in Nemacheilidae. We generated BIs of the diversification rates of date-phylogeny with posterior mean branch lengths using BAMM v2.5 (Rabosky, 2014) and the BAMMtools R package (Rabosky et al., 2014). Priors for speciation and extinction were set empirically using the setBAMMpriors function. The geometric prior on the expected number of regime shifts was set to 1, as our group contained fewer than 500 species. We ran MCMC analysis for 10 million generations at a sampling frequency of 1/1 000, and checked MCMC convergence by visually inspecting the likelihood trace plots and calculating ESS after discarding the first 10% of the run as burn-in. We calculated the 95% credible set of distinct shift configurations and the best shift configuration. We also plotted the net diversification rates over time for Nemacheilidae, Nemacheilini I, and Nemacheilini II, respectively.

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Supplementary Table S1 Model comparisons of DEC, DIVALIKE, BAYARELIKE using BioGeoBears.

	LnL	numparams	d	e	AIC	AIC_wt
DEC	-278.331	2	0.004541	0.024226	560.6626	1.46E-22
DIVALIKE	-274.72	2	0.00426	0.015112	553.4398	5.39E-21
BAYAREALIKE	-299.716	2	0.007444	0.070008	603.4324	7.52E-32

Supplementary Table S2 Information about study samples, including species, voucher number and Genbank accession number. Underline represents that the sequences are downloaded from GenBank.

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Barbatula</i>		<u>KP715096</u>	<u>KP738604</u>	<u>KP738565</u>	<u>FJ650476</u>	<u>KP738524</u>
<i>barbatula</i> (n=1)						
<i>Barbatula toni</i>		<u>AB242162</u>	<u>AB242162</u>	<u>EU711133</u>	<u>FJ197030</u>	<u>FJ197079</u>
(n=1)						
<i>Claea dabryi</i>	KIZ	MG23811	MG23821	MG23792	MG23801	MG23831
(n=3)	2009003103	8	4	2	5	2
	KIZ	MG23811	MG23821	MG23792	MG23801	MG23831
	2009002750	9	5	3	6	3
	KIZ	MG23812	MG23821	MG23792	MG23801	MG23831
	2009003611	0	6	4	7	4
<i>Homatula</i>	KIZ	<u>MF95318</u>	<u>HM01058</u>	<u>HM01066</u>	MG23801	MG23831
<i>anguillioides</i>	20080304	<u>2</u>	<u>3</u>	<u>9</u>	8	5
(n=3)						
	KIZ	<u>MF95318</u>	<u>HM01058</u>	<u>HM01067</u>	MG23801	MG23831
	20080306	<u>4</u>	<u>4</u>	<u>5</u>	9	6
	KIZ	MG23812	<u>HM01052</u>	<u>HM01067</u>	MG23802	MG23831
	2008005993	1	<u>7</u>	<u>2</u>	0	7
<i>Homatula</i>	KIZ	<u>MF95318</u>			MG23802	MG23831
<i>change</i> (n=2)	2015005110	<u>5</u>			1	8
	KIZ	<u>MF95318</u>			MG23802	MG23831
	2015005111	<u>6</u>			2	9
<i>Homatula</i>	KIZ 07308	MG23812	<u>HM01056</u>	<u>HM01066</u>		MG23833
<i>cryptoclathrata</i>		9	<u>9</u>	<u>3</u>		2
(n=2)						
	KIZ 07310	MG23813	<u>HM01056</u>	<u>HM01068</u>		MG23833
		0	<u>6</u>	<u>3</u>		3
<i>Homatula</i>	KIZ	MF95319	MG23821	MG23792		MG23832
<i>disparizona</i>	2012000622	0	7	5		0
(n=2)						

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Homatula guanheensis</i> (n=2)	KIZ	<u>MF95319</u>	MG23821	MG23792	MG23802	MG23832
	2012000626	<u>4</u>	8	6	3	1
	KIZ	MG23812	MG23821	MG23792	MG23802	MG23832
	2005014382	2	9	7	4	2
<i>Homatula longidorsalis</i> (n=4)	KIZ	MG23812	MG23822	MG23792	MG23802	MG23832
	2005014383	3	0	8	5	3
	KIZ	<u>MF95319</u>	<u>HM01052</u>	<u>HM01061</u>	MG23802	MG23832
	20060274	<u>6</u>	<u>2</u>	<u>8</u>	6	4
<i>Homatula potanini</i> (n=2)	KIZ	<u>MF95319</u>	<u>HM01055</u>	<u>HM01061</u>	MG23802	MG23832
	20060276	<u>7</u>	<u>0</u>	<u>6</u>	7	5
	KIZ	MG23812	<u>HM01050</u>	<u>HM01061</u>	MG23802	MG23832
	2008005906	4	<u>0</u>	<u>5</u>	8	6
<i>Homatula pycnolepis</i> (n=2)	KIZ	MG23812	<u>HM01056</u>	<u>HM01061</u>	MG23802	MG23832
	2008005908	5	<u>8</u>	<u>4</u>	9	7
	KIZ	MG23812	<u>JF340399</u>	<u>JF340420</u>	MG23803	MG23832
	2010000233	6			0	8
<i>Homatula variegata</i> (n=2)	KIZ	<u>MF95320</u>	<u>JF340385</u>	<u>JF340426</u>	MG23803	MG23832
	2010000280	<u>1</u>			1	9
	KIZ	MG23812	MG23822	MG23792	MG23803	MG23833
	2010002524	7	1	9	2	0
<i>Homatula wuliangensis</i> (n=2)	KIZ	MG23812	MG23822	MG23793	MG23803	MG23833
	2010002525	8	2	0	3	1
	KIZ	MG23813	<u>HM01058</u>	<u>HM01062</u>	MG23803	MG23833
	200406173	1	<u>7</u>	<u>2</u>	4	4
<i>Homatula wuliangensis</i> (n=2)	KIZ	MG23813	MG23822	MG23793	MG23803	MG23833
	2009002725	2	3	1	5	5
	KIZ	<u>MF95322</u>	<u>HM01051</u>	<u>HM01060</u>	MG23803	MG23833
	2008008159	<u>0</u>	<u>7</u>	<u>9</u>	6	6
<i>Lefua costata</i> (n=1)	KIZ	<u>MF95322</u>	<u>HM01049</u>	<u>HM01061</u>		MG23833
	2008008160	<u>1</u>	<u>6</u>	<u>2</u>		7
<i>Lefua echigonia</i> (n=1)		<u>EU670788</u>	<u>EU670788</u>	<u>KP738551</u>		<u>KP738511</u>
<i>Mesonoemacheilus guentheri</i> (n=1)		<u>AB054126</u>	<u>AB054126</u>	<u>EF458305</u>	<u>FJ197028</u>	<u>FJ197077</u>
		<u>KX28931</u>	<u>KP738588</u>	<u>KP738548</u>		<u>KP738508</u>
		<u>2</u>				

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Nemacheilus binotatus</i> (n=2)			<u>KP738586</u>	<u>KP738546</u>		<u>KP738506</u>
			<u>KP738587</u>	<u>KP738547</u>		<u>KP738507</u>
<i>Nemacheilus corica</i> (n=2)		<u>AP011445</u>	<u>KP738592</u>	<u>KP738552</u>		<u>KP738512</u>
		<u>NC031640</u>	<u>KP738593</u>	<u>KP738553</u>		<u>KP738513</u>
<i>Nemacheilus longistriatus</i> (n=3)	KIZ 2015001411	MG23813 3	MG23822 8	MG23793 6	MG23804 1	MG23834 2
	KIZ 2015001412	MG23813 4	MG23822 9	MG23793 7	MG23804 2	MG23834 3
	KIZ 2015001414	MG23813 5	MG23823 0	MG23793 8	MG23804 3	MG23834 4
<i>Nemachilichthys ruppelli</i> (n=2)		<u>NC031583</u>	<u>NC031583</u>	<u>KP738533</u>		<u>KP738493</u>
		<u>AP011305</u>	<u>AP011305</u>	<u>KP738534</u>		<u>KP738494</u>
<i>Oreonectes platycephalus</i> (n=2)	KIZ 2005101504 2	<u>NC031579</u>	MG23823 1	MG23793 9	MG23804 4	MG23834 5
	KIZ 2005101504 3	<u>AP011296</u>	MG23823 2	MG23794 0	MG23804 5	MG23834 6
<i>Paracanthocobitis botia</i> (n=2)		<u>AP012138</u>	<u>KP738589</u>	<u>KP738549</u>		<u>KP738509</u>
		<u>AP012139</u>	<u>KP738590</u>	<u>KP738550</u>		<u>KP738510</u>
<i>Paracanthocobitis mandalayensis</i> (n=2)	KIZ 2014005900	MG23811 6	MG23821 2			MG23831 0
	KIZ 2014005901	MG23811 7	MG23821 3	MG23792 1		MG23831 1
<i>Paracanthocobitis zonalternans</i> (n=2)		<u>AP012140</u>	<u>AP012140</u>	<u>KP738542</u>		<u>KP738502</u>
		<u>NC033959</u>	<u>NC033959</u>	<u>KP738543</u>		<u>KP738503</u>

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Petruichthys brevis</i> (n=2)		<u>AP011442</u>	<u>AP011442</u>	<u>KP738531</u>		<u>KP738491</u>
		<u>NC031637</u>	<u>NC031637</u>	<u>KP738532</u>		<u>KP738492</u>
<i>Physoschistura elongata</i> (n=2)			<u>KP738608</u>	<u>KP738568</u>		<u>KP738528</u>
			<u>KP738609</u>	<u>KP738569</u>		<u>KP738529</u>
<i>Physoschistura shuangjiangensis</i> (n=2)	KIZ 2005012418	MG23819 0	MG23828 4	MG23799 0	MG23809 6	MG23840 1
	KIZ 2005004744	MG23819 1	MG23828 5	MG23799 1	MG23809 7	MG23840 2
<i>Physoschistura sp.</i> (n=2)			<u>KP738600</u>	<u>KP738560</u>		<u>KP738520</u>
			<u>KP738601</u>	<u>KP738561</u>		<u>KP738521</u>
<i>Pteronemacheilus lucidorsum</i> (n=2)			<u>KP738606</u>	<u>KP738566</u>		<u>KP738526</u>
			<u>KP738607</u>	<u>KP738567</u>		<u>KP738527</u>
<i>Pteronemacheilus meridionalis</i> (n=4)	KIZ 2010001570	MG23813 9	MG23823 6	MG23794 4	MG23804 9	MG23835 0
	KIZ 2006010387	MG23813 7	MG23823 4	MG23794 2	MG23804 7	MG23834 8
	KIZ 2010001443	MG23813 8	MG23823 5	MG23794 3	MG23804 8	MG23834 9
	KIZ 2006010379	MG23813 6	MG23823 3	MG23794 1	MG23804 6	MG23834 7
	KIZ 2014001499	MG23814 5	MG23824 1			MG23835 6
<i>Schistura albirostris</i> (n=2)	KIZ 2014001502	MG23814 6	MG23824 2		MG23805 5	
	KIZ 2012004196	MG23817 8	MG23827 0	MG23797 7	MG23808 4	MG23838 7
<i>Schistura amplizona</i> (n=2)	KIZ 2015002065	MG23814 7	MG23824 3	MG23794 9	MG23805 6	MG23835 7

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Schistura balteata</i> (n=1)		<u>AB242172</u>	<u>AB242172</u>	<u>EU711131</u>	<u>FJ197029</u>	<u>FJ197078</u>
<i>Schistura bolavenensis</i> (n=2)			<u>KP738575</u>	<u>KP738535</u>		<u>KP738495</u>
			<u>KP738576</u>	<u>KP738536</u>		<u>KP738496</u>
<i>Schistura callichroma</i> (n=2)	KIZ 200401056	MG23814 8	<u>JN837652</u>	<u>JN837664</u>	MG23805 7	MG23835 8
	KIZ 2013004935	MG23814 9	MG23822 4	MG23795 0	MG23805 8	MG23835 9
<i>Schistura caudofurca</i> (n=2)	KIZ 2015030701	MG23815 1	MG23824 6	MG23795 2	MG23806 0	MG23836 1
	5 KIZ 2007002415	MG23815 0	MG23824 5	MG23795 1	MG23805 9	MG23836 0
<i>Schistura cf. fasciolata</i> (n=4)	KIZ 2005000858	MG23816 0	MG23825 4	MG23796 0	MG23806 7	MG23837 0
	KIZ 2010000023	MG23816 1	MG23825 5	MG23796 1	MG23806 8	MG23837 1
	KIZ 2010000366	MG23816 2	MG23825 6	MG23796 2	MG23806 9	MG23837 2
	KIZ 2010001729	MG23816 7	MG23826 0	MG23796 6	MG23807 4	MG23837 7
<i>Schistura conirostris</i> (n=3)	KIZ 2010001442	MG23815 2	MG23824 7	MG23795 3	MG23806 1	MG23836 2
	KIZ 2010001444	MG23815 3	MG23824 8	MG23795 4		MG23836 3
	KIZ 2010001445	MG23815 4	MG23824 9	MG23795 5		MG23836 4
<i>Schistura cryptofasciata</i> (n=3)	KIZ 2005014232	MG23815 5	<u>JF340401</u>	<u>JF340418</u>	MG23806 2	MG23836 5
	KIZ 2005041700	MG23814 3	MG23824 0	MG23794 8	MG23805 3	MG23835 4

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
	KIZ 2015002175	MG23815 7	MG23825 1	MG23795 7	MG23806 4	MG23836 7
<i>Schistura disparizona</i> (n=3)	KIZ 20150231	MG23820 1	MG23829 5	MG23800 0	MG23810 4	MG23841 2
	KIZ 2005005189	MG23815 8	MG23825 2	MG23795 8	MG23806 5	MG23836 8
	KIZ DAN08.12.1	MG23815 9	MG23825 3	MG23795 9	MG23806 6	MG23836 9
<i>Schistura fasciolata</i> (n=4)	KIZ 2010000795	MG23816 3	MG23825 7	MG23796 3	MG23807 0	MG23837 3
	KIZ 2010000796	MG23816 4	<u>JN837655</u>	<u>JN837667</u>	MG23807 1	MG23837 4
	KIZ 2012003704	MG23816 5	MG23825 8	MG23796 4	MG23807 2	MG23837 5
	KIZ 2012003701	MG23816 6	MG23825 9	MG23796 5	MG23807 3	MG23837 6
<i>Schistura hypsiura</i> (n=2)			<u>KP738584</u>	<u>KP738544</u>		<u>KP738504</u>
			<u>KP738585</u>	<u>KP738545</u>		<u>KP738505</u>
<i>Schistura implicata</i> (n=2)	KIZ 20130294	MG23819 5	MG23828 9	MG23799 5		MG23840 6
	KIZ 20130288	MG23819 6	MG23829 0	MG23799 6		MG23840 7
<i>Schistura kengtungensis</i> (n=1)	KIZ 20090106	MG23816 8	MG23826 1	MG23796 7	MG23807 5	
<i>Schistura kloetzliae</i> (n=3)	KIZ 2009001413	MG23816 9	MG23826 2	MG23796 8	MG23807 6	MG23837 8

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
	KIZ 2010001134	MG23817 1	MG23826 3	MG23797 0	MG23807 8	MG23838 0
	KIZ 2010003114	MG23814 0	MG23823 7	MG23794 5	MG23805 0	MG23835 1
<i>Schistura kongphengi</i> (n=2)	KIZ 20150129	MG23817 2	MG23826 4	MG23797 1	MG23807 9	MG23838 1
	KIZ 20150130	MG23817 3	MG23826 5	MG23797 2	MG23808 0	MG23838 2
<i>Schistura latidens</i> (n=2)	KIZ 20150133	MG23817 4	MG23826 6	MG23797 3	MG23808 1	MG23838 3
	KIZ 20150134	MG23817 5	MG23826 7	MG23797 4	MG23808 2	MG23838 4
<i>Schistura latifasciata</i> (n=3)	KIZ 2008000127	MG23817 6	MG23826 8	MG23797 5	MG23808 3	MG23838 5
	KIZ 2012001465	MG23817 7	MG23826 9	MG23797 6		MG23838 6
	KIZ 20150096	<u>MF95322</u> 3	MG23827 1	MG23797 8	MG23808 5	MG23838 8
<i>Schistura longa</i> (n=2)	KIZ 05178	MG23818 0	MG23827 3	MG23798 0	MG23808 7	MG23839 0
	KIZ 2010002364	MG23817 9	MG23827 2	MG23797 9	MG23808 6	MG23838 9
<i>Schistura macrocephalus</i> (n=2)	KIZ 2010001595	MG23818 1	MG23827 4	MG23798 1	MG23808 8	MG23839 1
	KIZ 2010003136	MG23818 2	MG23827 5	MG23798 2	MG23808 9	MG23839 2

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Schistura magnifluvis</i> (n=1)	KIZ 2008008301	MG23814 4	<u>JN837654</u>	<u>JN837666</u>	MG23805 4	MG23835 5
<i>Schistura malaisei</i> (n=3)	KIZ 2014005902	MG23818 3	MG23827 6	MG23798 3		MG23839 3
	KIZ 2014005903	MG23818 4	MG23827 7	MG23798 4	MG23809 0	MG23839 4
	KIZ 2014005904	MG23818 5	MG23827 8		MG23809 1	MG23839 5
<i>Schistura notasileum</i> (n=2)	KIZ 2016000362	OQ94695 6	OQ94505 0	OQ97329 8	OQ97330 0	OQ97330 2
	KIZ 2016000356	OQ94695 7	OQ94505 1	OQ97329 9	OQ97330 1	OQ97330 3
<i>Schistura notostigma</i> (n=2)		<u>AP011308</u>	<u>AP011308</u>	<u>KP738556</u>		<u>KP738516</u>
		<u>NC031585</u>	<u>NC031585</u>	<u>KP738557</u>		<u>KP738517</u>
<i>Schistura poculi</i> (n=1)	KIZ 2005041700 7	MG23814 2	MG23823 9	MG23794 7	MG23805 2	MG23835 3
<i>Schistura polytaenia</i> (n=2)	KIZ 2006010328	MG23818 6	MG23828 0	MG23798 6	MG23809 2	MG23839 7
	KIZ 2006010350	MG23818 7	MG23828 1	MG23798 7	MG23809 3	MG23839 8
<i>Schistura porthos</i> (n=2)	KIZ 2010003121	MG23818 8	MG23828 2	MG23798 8	MG23809 4	MG23839 9
	KIZ 2010003122	MG23818 9	MG23828 3	MG23798 9	MG23809 5	MG23840 0
<i>Schistura pridii</i> (n=2)		<u>AP011443</u>	<u>AP011443</u>	<u>KP738562</u>		<u>KP738522</u>

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
		<u>NC031638</u>	<u>NC031638</u>	<u>KP738563</u>		<u>KP738523</u>
<i>Schistura savona</i> (n=2)		<u>KJ542585</u>	<u>KP738598</u>	<u>KP738558</u>		<u>KP738518</u>
		<u>KJ542586</u>	<u>KP738599</u>	<u>KP738559</u>		<u>KP738519</u>
<i>Schistura scaturigina</i> (n=3)	KIZ 20110027	MG23819 7	MG23829 1	MG23799 7	MG23810 0	MG23840 8
	KIZ 20110028	MG23819 8	MG23829 2		MG23810 1	MG23840 9
	KIZ 20110030	MG23819 9	MG23829 3	MG23799 8	MG23810 2	MG23841 0
<i>Schistura susannae</i> (n=1)	KIZ 20130314	MG23819 4	MG23828 8	MG23799 4		MG23840 5
<i>Schistura vinciguerrae</i> (n=2)	KIZ 2006004060	MG23819 2	MG23828 6	MG23799 2	MG23809 8	MG23840 3
	KIZ 2006009576	MG23819 3	MG23828 7	MG23799 3	MG23809 9	MG23840 4
<i>Schistura yingjiangensis</i> (n=1)	KIZ 2014002137	MG23820 0	MG23829 4	MG23799 9	MG23810 3	MG23841 1
<i>Sectoria heterognathos</i> (n=2)	KIZ 20150177	MG23820 3	MG23829 7	MG23800 2	MG23810 6	MG23841 4
	KIZ 2012001420	MG23820 2	MG23829 6	MG23800 1	MG23810 5	MG23841 3
<i>Seminemacheilus lendlii</i> (n=2)		<u>MT07700</u> 8	<u>KP738577</u>	<u>KP738537</u>		<u>KP738497</u>
		<u>MT22167</u> 2	<u>KP738578</u>	<u>KP738538</u>		<u>KP738498</u>

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Traccaticthys pulcher</i> (n=2)	KIZ 2005101704 2	<u>NC031581</u>	MG23822 5	MG23793 3	MG23803 8	MG23833 9
	KIZ 2005101704 3	<u>AP011301</u>	MG23822 6	MG23793 4	MG23803 9	MG23834 0
<i>Triplophysa anterodorsalis</i> (n=1)		<u>NC024597</u>	<u>NC024597</u>	<u>MG72556</u> <u>4</u>	<u>MG72560</u> <u>3</u>	<u>MG72548</u> <u>5</u>
<i>Triplophysa bleekeri</i> (n=2)	KIZ 20130069	MG23820 4	MG23829 8	MG23800 3		MG23841 5
	KIZ 20130070	MG23820 5	MG23829 9	MG23800 4		MG23841 6
<i>Triplophysa brevicauda</i> (n=2)	KIZ 050422004	MG23820 6	MG23830 0	MG23800 5	MG23810 7	MG23841 7
	KIZ 050422005	MG23820 7	MG23830 1	MG23800 6	MG23810 8	MG23841 8
<i>Triplophysa dorsalis</i> (n=1)		<u>NC029423</u>	<u>NC029423</u>	<u>MG72556</u> <u>9</u>	<u>MG72560</u> <u>5</u>	<u>MG72548</u> <u>7</u>
<i>Triplophysa gundriseri</i> (n=1)		<u>KX03965</u> <u>6</u>		<u>FJ650420</u>	<u>FJ650480</u>	<u>FJ650492</u>
<i>Triplophysa markehenensis</i> (n=1)		<u>KT213594</u>	<u>KT213594</u>	<u>MG72556</u> <u>3</u>	<u>MG72560</u> <u>2</u>	<u>MG72548</u> <u>4</u>
<i>Triplophysa nanpanjiangensis</i> (n=1)	KIZ 20080361	MG23820 8	MG23830 2	MG23800 7	MG23810 9	MG23841 9
<i>Triplophysa obscura</i> (n=2)	KIZ 2010000273	MG23820 9	MG23830 4	MG23800 9	MG23811 1	MG23842 1

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
	KIZ 2010000274	MG23821 0	MG23830 5	MG23801 0	MG23811 2	MG23842 2
<i>Triplophysa orientalis</i> (n=1)		<u>KJ631323</u>	<u>KJ631323</u>	<u>MG72556</u> <u>6</u>	<u>MG72560</u> <u>4</u>	<u>MG72548</u> <u>6</u>
<i>Triplophysa rosa</i> (n=1)		<u>JF268621</u>	<u>JF268621</u>	<u>MG72556</u> <u>5</u>	<u>JF317227</u>	<u>JF317224</u>
<i>Triplophysa scleroptera</i> (n=2)	KIZ 20100076	MG23821 1	MG23830 7	MG23801 2	MG23811 3	MG23842 4
	KIZ 20100075		MG23830 6	MG23801 1		MG23842 3
<i>Triplophysa stenura</i> (n=1)		<u>KX35497</u> <u>5</u>	<u>KX35497</u> <u>5</u>	<u>JN837669</u>	<u>MG69759</u> <u>2</u>	<u>MG69882</u> <u>4</u>
<i>Triplophysa stoliczkai</i> (n=1)		<u>JQ663847</u>	<u>JQ663847</u>	<u>MG72557</u> <u>6</u>	<u>KP695752</u>	<u>KP695090</u>
<i>Triplophysa strauchii</i> (n=1)		<u>KP297875</u>	<u>KP297875</u>	<u>MG72556</u> <u>7</u>		<u>MT53672</u> <u>4</u>
<i>Yunnanilus pleurotaenia</i> (n=2)	KIZ 2004102300 1		MG23830 8	MG23801 3	MG23811 4	MG23842 5
	KIZ 2004102300 2		MG23830 9	MG23801 4	MG23811 5	MG23842 6
Outgroup						
<i>Beaufortia szechuanensis</i> (n=1)		<u>JN177231</u>	<u>AY62572</u> <u>6</u>	<u>JN177179</u>	<u>JN177205</u>	<u>JN177281</u>
<i>Botia dario</i> (n=1)		<u>MK57207</u> <u>0</u>	<u>AY88779</u> <u>8</u>	<u>KP695636</u>	<u>EU409641</u>	<u>EU409673</u>
<i>Cobitis striata</i> (n=1)		<u>AB054125</u>	<u>AB054125</u>	<u>EF458303</u>	<u>HM22393</u> <u>8</u>	<u>AB531282</u>

Species name	Voucher Number	Genbank Accession Number				
		COI	Cytb	RAG1	RH	IRBP
<i>Gyrinocheilus</i> <i>aymonieri</i> (n=1)		<u>KY30784</u> 4	<u>KY30784</u> 4	<u>EU292682</u>	<u>FJ197071</u>	<u>EU409695</u>
<i>Jinshaia</i> <i>abbreviata</i> (n=1)		<u>JN177228</u>		<u>JN177042</u>		JN177274
<i>Sinogastromyzon</i> <i>sichangensis</i> (n=1)		<u>NC024534</u>	<u>NC024534</u>	<u>KP695620</u>	<u>KP695734</u>	<u>JN177275</u>
<i>Vaillantella</i> <i>maassi</i> (n=1)		<u>AB242173</u>	<u>AB242173</u>	<u>EU711132</u>	<u>FJ197031</u>	<u>FJ197080</u>

Supplementary Table S3 Geographic information about the analysed specimens.

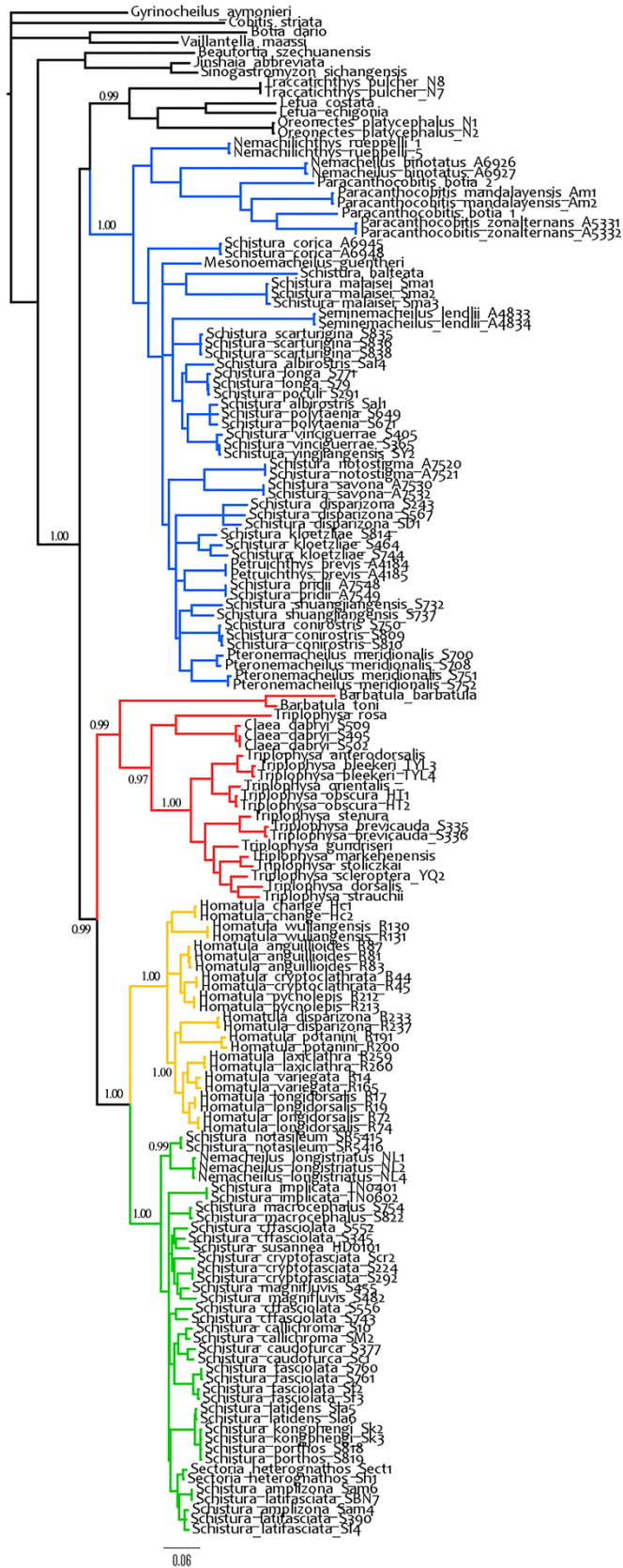
Species	Biogeogr. unit	Locality
<i>Barbatula barbatula</i>	P	Czech Republic
<i>Barbatula toni</i>	P	Russia; Amur
<i>Claea dabryi</i>	AB	China, Sichuan; Jinshajiang, Yangtze
<i>Homatula anguillioides</i>	H	China, Yunnan; Mekong
		China, Yunnan; HaixiLake, Mekong
<i>Homatula guanheensis</i>	A	China, Shanxi; Yangtze
<i>Homatula change</i>	K	China, Yunnan; Mekong
<i>Homatula cryptoclathrata</i>	G	China, Yunnan; Salween
<i>Homatula disparizona</i>	K	China, Yunnan; Red
<i>Homatula longidorsalis</i>	AM	China, Yunnan; Jinshajiang, Yangtze
		China, Yunnan; Nanpanjiang, Pearl
<i>Homatula potanini</i>	A	China, Sichuan; Yangtz
		China, Sichuan; Yangtze
<i>Homatula pycnolepis</i>	H	China, Yunnan; Mekong
<i>Homatula variegata</i>	A	China, Yunnan; Jinshajiang, Yangtze
		China, Sichuan; Yangtz
<i>Homatula wuliangensis</i>	H	China, Yunnan; Mekong
<i>Lefua costata</i>	I	China, Liaoning
<i>Lefua echigonia</i>	I	Japan
<i>Mesonoemacheilus guentheri</i>	J	Southern India
<i>Nemacheilus binotatus</i>	Q	Thailand
<i>Nemacheilus corica</i>	E	Himalaya Foothills
<i>Nemacheilus longistriatus</i>	L	China, Yunnan; Mekong
<i>Nemachilichthys ruppelli</i>	J	India
<i>Oreonectes platycephalus</i>	M	China, Guangxi; Pearl.
<i>Paracanthocobitis botia</i>	E	India
<i>Paracanthocobitis mandalayensis</i>	D	Myanma; Irrawaddy
<i>Paracanthocobitis zonalternans</i>	D	Brahmaputra
<i>Petruichthys brevis</i>	F	Myanmar; Salween
<i>Physoschistura elongata</i>	E	India
<i>Physoschistura shuangjiangensis</i>	H	China, Yunnan; Mekong
<i>Physoschistura sp.</i>	F	Salween
<i>Pteronemacheilus lucidorsum</i>	D	Myanmar
<i>Pteronemacheilus meridionalis</i>	GL	China, Yunnan; Salween
		China, Yunnan; Mekong
<i>Schistura albirostris</i>	D	China, Yunnan; Irrawaddy
<i>Schistura amplizona</i>	L	China, Yunnan; LuosuoJiang, Mekong
		China, Yunnan; ManlaoJiang Mekong
<i>Schistura balteata</i>	F	Salween
<i>Schistura bolavenensis</i>	L	Mekong
<i>Schistura callichroma</i>	K	China, Yunnan; LixianJiang, Red

Species	Biogeogr. unit	Locality
<i>Schistura caudofurca</i>	K	China, Yunnan; LixianJiang Red
		China, Yunnan; SinanJiang, Red
<i>Schistura cf. fasciolata</i>	KM	China, Yunnan; GulaHe, Pearl
		China, Yunnan; YuanJiang, Red
		China, Guangdong; MoyangJiang
		China, Guangxi; Pearl
<i>Schistura conirostris</i>	L	China, Yunnan; Mekong
<i>Schistura cryptofasciata</i>	GL	China, Yunnan; NantingHe, Salween
		China, Yunnan; ManlaoJiang Mekong
<i>Schistura disparizona</i>	F	China, Yunnan; NangunHe, Salween
	G	China, Yunnan; Salween
<i>Schistura fasciolata</i>	KN	China, Yunnan; Red
		China, Hainan;
<i>Schistura hypsiura</i>	D	Myanmar
<i>Schistura implicata</i>	L	Vietnam, Lam Dong; Mekong
<i>Schistura kengtungensis</i>	H	China, Yunnan; Mekong
<i>Schistura kloetziiae</i>	H	China, Yunnan; Mekong
	L	China, Yunnan; Mekong
		China, Yunnan; LuosuoJiang, Mekong
<i>Schistura kongphengi</i>	L	China, Yunnan; ManlaoJiang Mekong
<i>Schistura latidens</i>	H	China, Yunnan; Mekong
<i>Schistura latifasciata</i>	L	China, Yunnan; NanlaHe, Mekong
		China, Yunnan; LuosuoJiang, Mekong
		China, Yunnan; Mekong
<i>Schistura longa</i>	G	China, Yunnan; Salween
<i>Schistura macrocephalus</i>	L	China, Yunnan; LuosuoJiang, Mekong
<i>Schistura magnifluvis</i>	H	China, Yunnan; Mekong
<i>Schistura malaisei</i>	D	Myanmar; Irrawaddy
<i>Schistura notasileum</i>	H	China, Yunnan; Mekong
<i>Schistura notostigma</i>	J	Sri Lanka
<i>Schistura poculi</i>	G	China, Yunnan; Salween
<i>Schistura polytaenia</i>	D	China, Yunnan; Dayinjiang, Irrawaddy
		China, Yunnan; Irrawaddy
<i>Schistura porthos</i>	L	China, Yunnan; LuosuoJiang, Mekong
<i>Schistura pridii</i>	F	Thailand
<i>Schistura savona</i>	E	Brahmaputra
<i>Schistura scaturigina</i>	D	China, Xizang; Yarlung Zangbo River
<i>Schistura susannae</i>	K	Vietnam, Hai Duong; Red
<i>Schistura vinciguerrae</i>	D	China, Yunnan; Irrawaddy
		China, Yunnan; LongchuanJiang, Irrawaddy
<i>Schistura yingjiangensis</i>	D	China, Yunnan; Irrawaddy
<i>Sectoria heterognathos</i>	L	China, Yunnan; LuosuoJiang, Mekong

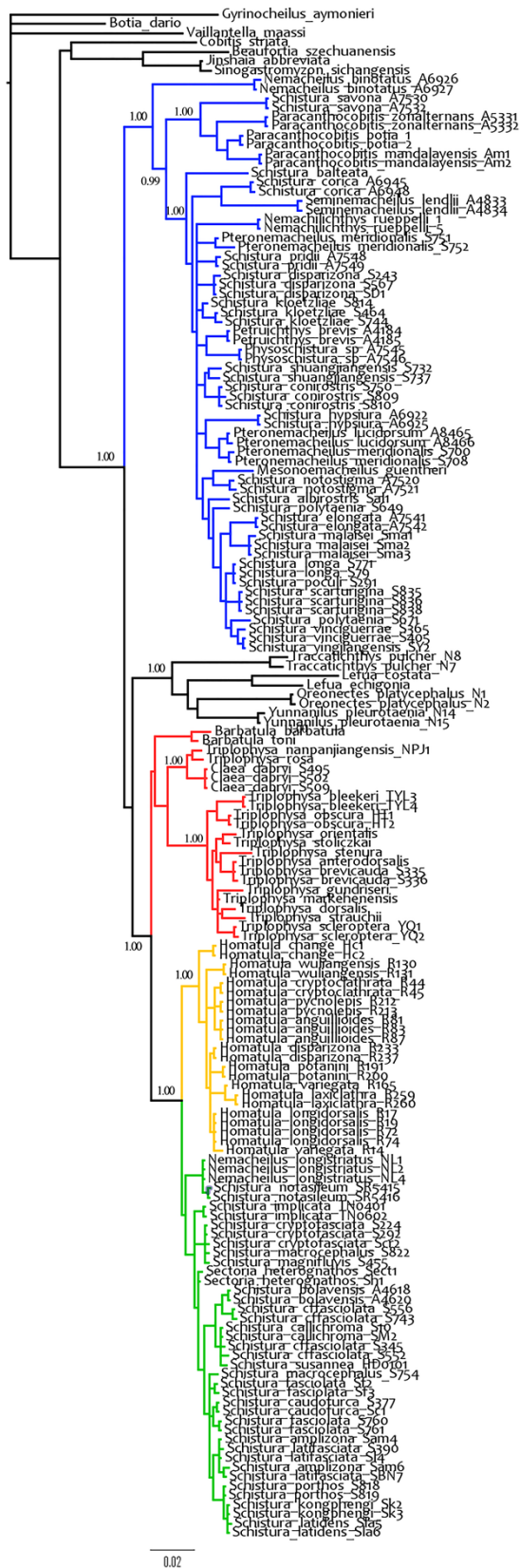
Species	Biogeogr. unit	Locality
		China, Yunnan; ManlaoJiang Mekong
<i>Seminemacheilus lendlii</i>	R	Southern Anatolia
<i>Traccatichthys pulcher</i>	M	China, Guangxi; Pearl
<i>Triplophysa anterodorsalis</i>	A	China, Sichuan; Yangtze
<i>Triplophysa bleekeri</i>	A	China, Sichuan; Yangtze
<i>Triplophysa brevicauda</i>	H	China, Yunnan; Mekong
<i>Triplophysa dorsalis</i>	C	China, Xinjiang
<i>Triplophysa gundriseri</i>	C	Russia
<i>Triplophysa markehenensis</i>	B	China, Qinghai
<i>Triplophysa nanpanjiangensis</i>	M	China, Yunnan; Pearl
<i>Triplophysa obscura</i>	O	China; Yellow
<i>Triplophysa orientalis</i>	O	China, Sichuan; Yellow
<i>Triplophysa rosa</i>	A	China; Yangtze
<i>Triplophysa scleroptera</i>	O	China, Qinghai; Yellow
<i>Triplophysa stenura</i>	B	China, Yunnan; Yangtze
<i>Triplophysa stolickai</i>	O	China, Gansu
<i>Triplophysa strauchii</i>	C	China, Xinjiang
<i>Yunnanilus pleurotaenia</i>	A	China, Yunnan; Pearl

Supplementary Table S4 Primers used in this study. Positions with mixed bases are labeled with their IUB codes: R = A/G; Y = C/T; S=G/C; N=A/T/C/G.

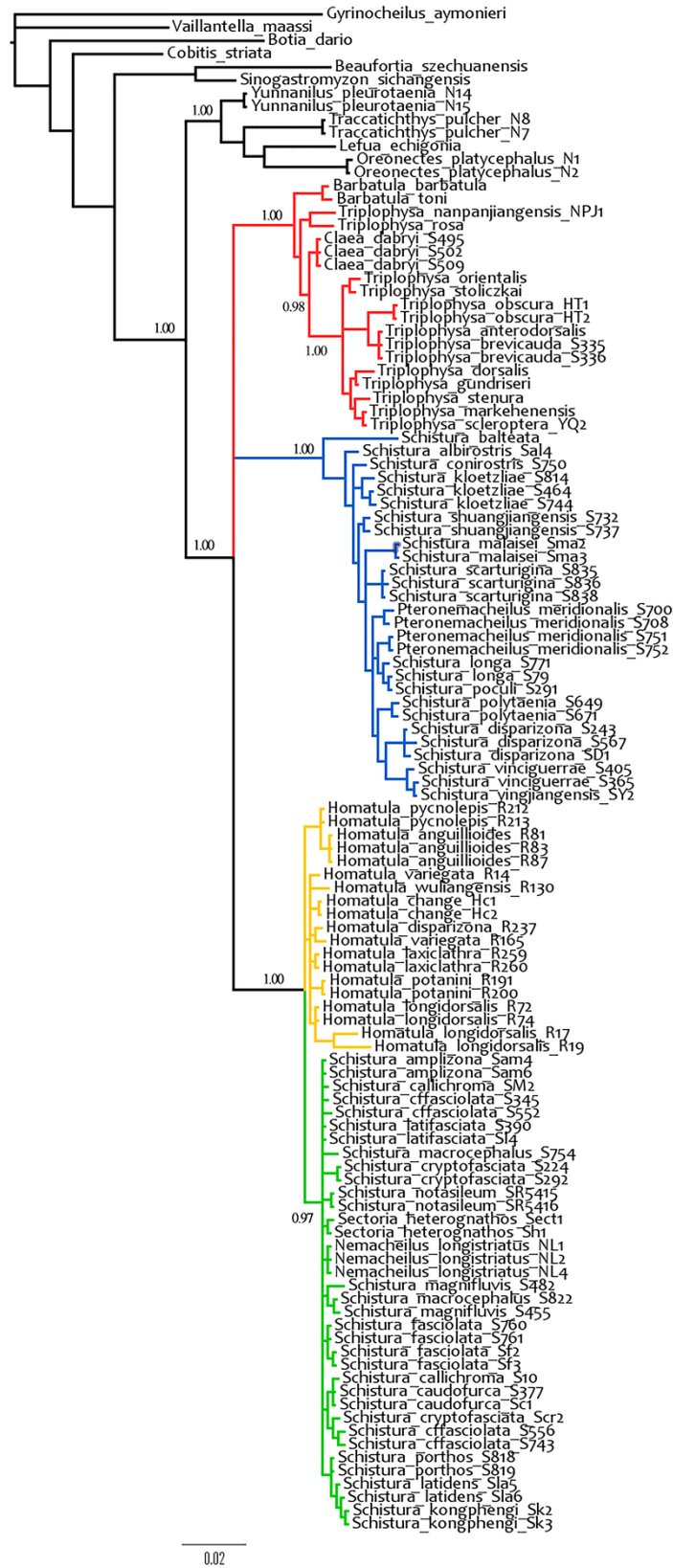
Locus	Primer	Primer sequences (5' - 3')	Annealing Temp.	Reference
Cytb	L14724	GAC TTG AAA AAC CAC CGT TG	52 °C	Xiao, Zhang & Liu, 2001
	H15915	CTC CGA TCT CCG GAT TAC AAG AC		
COI	LCOIa	CCT ACC TGT GGC AAT CAC RCG C	56 °C	Liu et al., 2012
	HCOI	GTG AAT AGG GGG AAT CAG TG		
RAG1	RAG1F	AGC TGT AGT CAG TAY CAC AAR ATG	53 °C	Perdices, Doadrio & Bermingham, 2005
	RAGRV1	TCC TGR AAG ATY TTG TAG AA		
	2533F	CTG AGC TGC AGT CAG TAC CAT AAG ATG T	53 °C	López, Chen & Ortí, 2004
	4090R	CTG AGT CCT TGT GAG CTT CCA TRA AYT T		
IRBP2	109F	AAC TAC TGC TCR CCA GAA AAR C	55 °C	Chen et al., 2008
	1001R	GGA AAT GCA TAG TTG TCT GCA A		
RH	28F	TAC GTG CCT ATG TCC AAY GC	55 °C	Chen et al., 2008
	233F	ATA TGC CTG CCT GGC YGC TTA C		



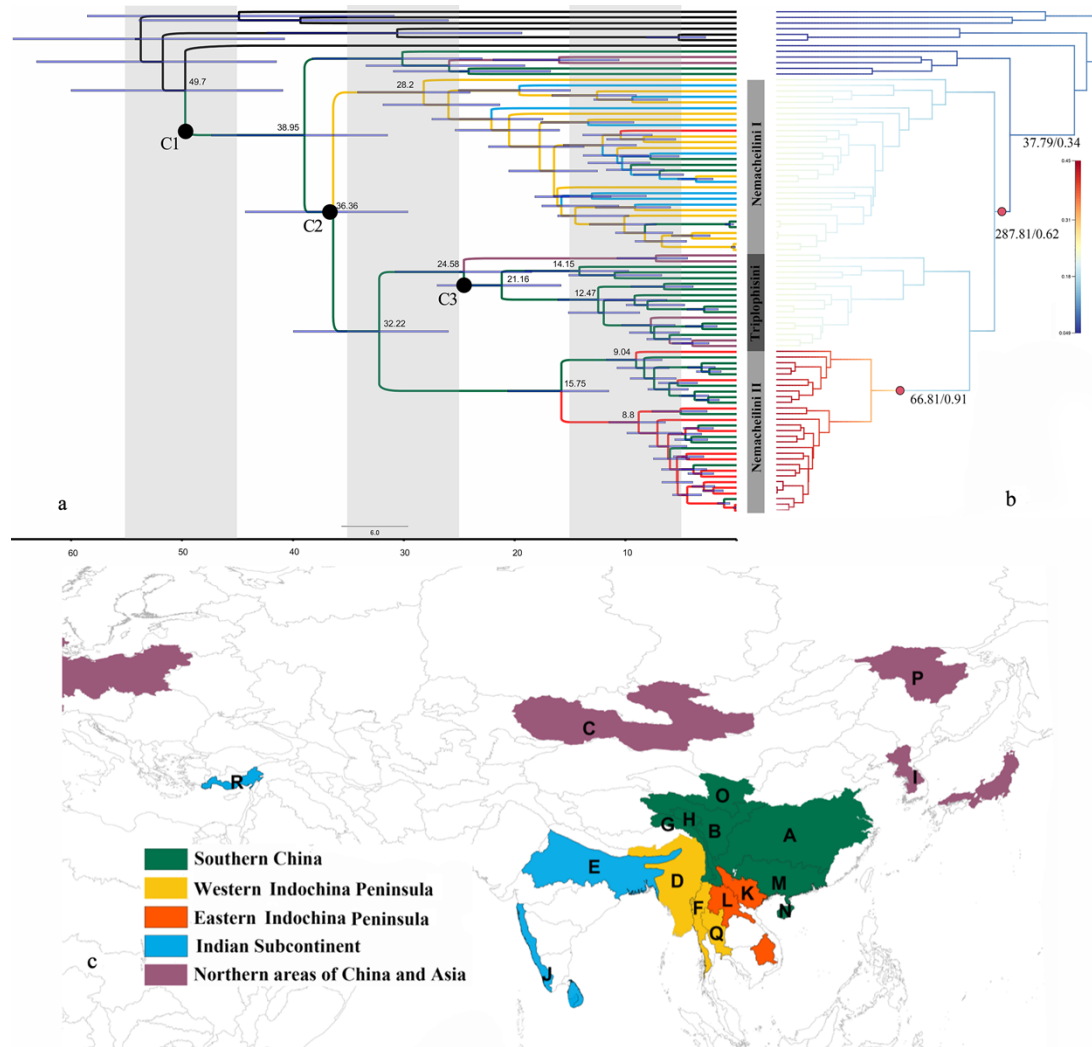
Supplementary Figure S1 Phylogram of 50% majority rule consensus tree derived from Bayesian inference of COI dataset. Nodes with posterior probabilities less than 0.95 are not shown.



Supplementary Figure S3 Phylogram of 50% majority rule consensus tree derived from Bayesian inference of IRBP dataset. Nodes with posterior probabilities less than 0.95 are not shown.

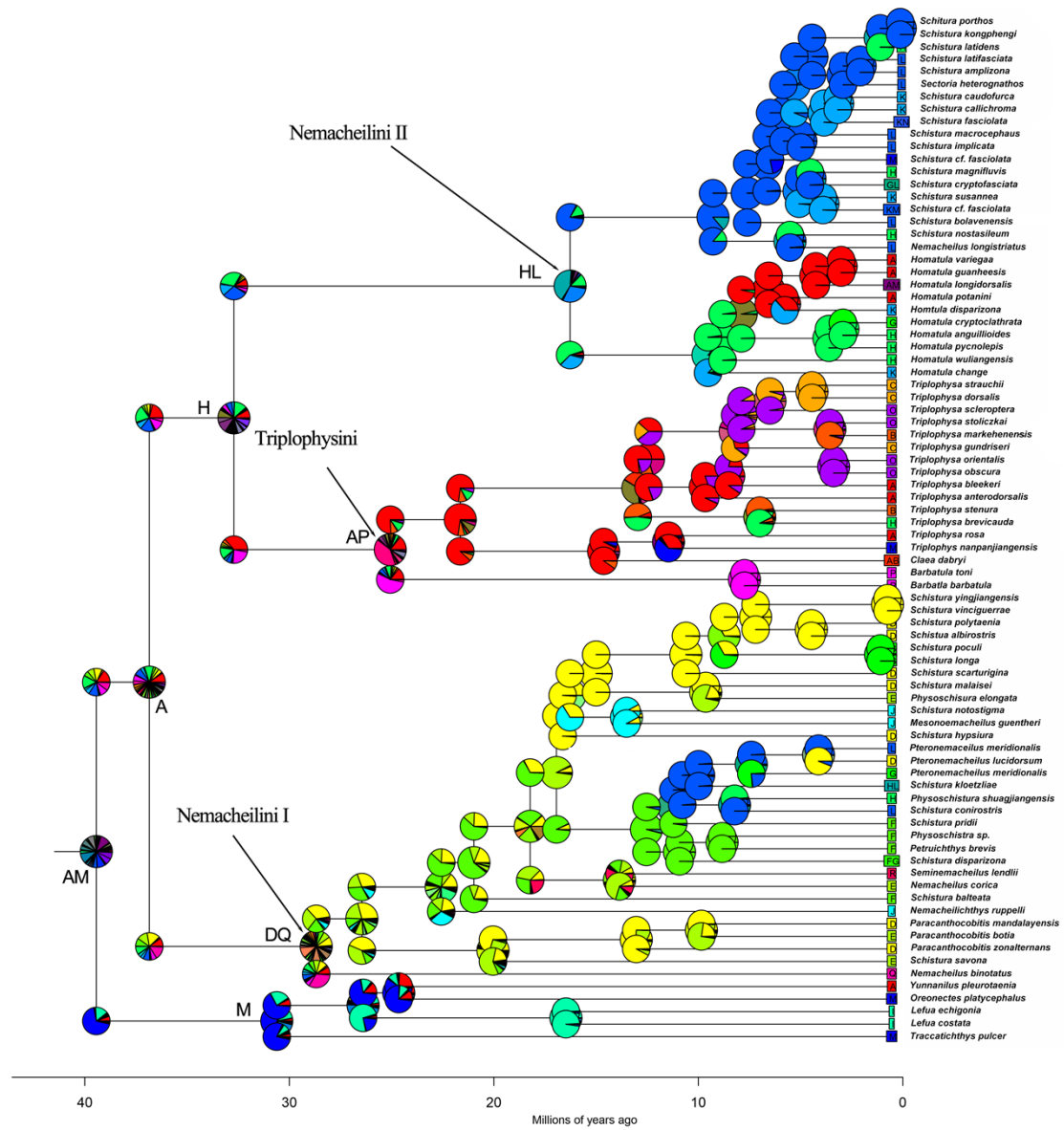


Supplementary Figure S5 Phylogram of 50% majority rule consensus tree derived from Bayesian inference of RH dataset. Nodes with posterior probabilities less than 0.95 are not shown.



Supplementary Figure S6 Ancestral regions of Nemacheilidae (a), the divergence time tree with 95% confidence intervals nodes and the divergence time on nodes; C1,C2,C3 are three calibration points which have been explained in Materials and Methods. Net diversification rates (b), with branch lengths set to posterior means for the date-phylogeny. **Red dots** represent rate shifts addressed from best shift configuration, values under branch are marginal odds/marginal shift probabilities. Area map (c), A- Middle & Lower Yangtze; B- Upper Yangtze; C- Balkash-Alakul , Dzungaria & Western Mongolia; D- Sitang-Irrawaddy; E- Ganges Delta & Plain, Middle Brahmaputra, Chin Hills-Srakan Coast; F- Lower & Middle Salween; G- Upper Salween; H- Upper Lancang (Mekong); I- Honshu-Shikoku-Kyushu, Eastern Yellow Sea Drainages; J- Western Ghats , Sri Lanka Dry Zone; K- Song Hong; L- Lower Lancang (Mekong), Dratie-Stung Treng; M- Xi Yiang; N- Hainan; O- Upper Huang He, Upper Huang He Corridor; P- Central & Western Europe, Middle Amur; Q- Chao Phraya; R- Southern Anatolia. These biogeographic units are marked by colors according to their position in India (**yellow**), western of Indochina (**blue**), eastern of Indochina (**red**), Southern China (**green**) and norther areas of China and Asia (**purple**).

BioGeoBEARS DIVALIKE on speciestree18 M0_unconstrained
 ancstates: global optim, 2 areas max. d=0.0043; e=0.0151; j=0; LnL=-274.72



Supplementary Figure S7 Pie chart of biogeographic analysis for Nemacheilidae under DIVALIKE model.