

Phylogeny of the Labeoninae (Teleostei, Cypriniformes) based on nuclear DNA sequences and implications on character evolution and biogeography

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Abstract The Labeoninae is a subfamily of the family Cyprinidae, Order Cypriniformes. Oromandibular morphology within the Labeoninae is the greatest among cyprinid fishes. Although several phylogenetic studies about labeonines have been undertaken the results have been inconsistent and a comprehensive phylogeny is needed. Further, an incongruence between morphological and molecular phylogeny requires a systematic exploration of the significance of morphological characters on the basis of the molecular phylogeny. In this study, a total of 292 nucleotide sequences from 73 individuals (representing 24 genera and 73 species) of Labeoninae were analyzed. The results of the phylogenetic analysis indicate that there are four major clades within Labeoninae and three monophyletic lineages within the fourth clade. Results of the character evolution show that all oromandibular morphological characters are homoplastically distributed on the molecular phylogenetic tree and suggests that these characters evolved several times during the history of labeonines. In particular, the labeonine , a specific disc on the lower lip, has been acquired three times and reversed twice. These morphological characters do not have systematic significance but can be useful for taxonomy. The results of biogeography suggest that the Labeoninae originated from Southeast Asia and separately dispersed to Africa, East Asia and South Asia [*Current Zoology* 58 (6): 837–850, 2012].

Keywords Phylogeny, Character evolution, Biogeography, Labeoninae

The Labeoninae is a group of fishes with small to medium sized and inferior mouths within a clade of the Cyprinidae (Order Cypriniformes). The labeonine fishes are adapted to swift currents and mostly scrape algae off the benthic substrate. These fishes display the greatest diversity in lip morphology and other structures associated with the mouth among the Cyprinidae (Zhang et al., 2000). Diverse morphology of the mouth and associated structures distinguishes labeonines from other cyprinid fishes and also serves as the main basis of generic taxonomy and phylogenetic analyses. Consequently, several new genera have been successively erected according to special oromandibular morphology (e.g. *Cophecheilus* Zhu et al., 2011, *Hongshuia* Zhang et al., 2008, and *Qianlabeo* Zhang and Chen, 2004). Several morphology-based phylogenetic studies about the labeonines have been undertaken, but the phylogenetic results based on different data sets have been inconsistent. This incongruence may be the result of insufficient or different sampling, but these morphology-based phylogenetic studies were mainly based on the characters of the mouth and associated structures (Zhang, 1994; Zhang,

1998; Su, 2001; Zhang, 2005; Li et al., 2005).

As molecular techniques provided a new perspective in phylogenetic studies, the morphological phylogeny of the Labeoninae has been challenged. A few earlier molecular studies did not resolve the phylogeny of the labeonines due to insufficient samples (e.g. Li et al., 2005). Two recent studies present relatively detailed phylogenetic relationships (Yang and Mayden, 2010; Zheng et al., 2010) but with limited sampling localities. Yang and Mayden (2010) was mainly based on material from outside China, whereas Zheng et al. (2010) dealt primarily with the relationships of the Chinese representatives of the subfamily. A more comprehensive phylogeny of labeonines from both Asia and Africa has not been attempted. Of particular interest, whether these morphological characters have potential utility for phylogenetic inference remains unknown. Here, we explore the systematic significance of the morphologies, especially of the mouth and associated structures, on the basis of the comprehensive molecular phylogeny.

Labeoninae are distributed in Asia and Africa, making them well suited for examination of their historical

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biogeography. Tang et al. (2009) suggested that the origin centre of the Labeoninae was East Asia and two dispersal events of labeonines from Asia into Africa. Yang and Mayden (2010) also supported this dispersal opinion.

1 Material and Methods

1.1 Sample collection

To simplify the phylogenetic tree, one individual was retained to represent one species in the final analysis; therefore, 73 individuals representing 24 genera, and 73 species of Labeoninae were analyzed in this study. Four nuclear gene (exon 3 of recombination activating protein 1 (RAG1), Rhodopsin (RH), early growth response protein 2B gene (EGR2B) and interphotoreceptor retinoid binding protein gene (IRBP)) sequences from 36 species from Africa, southern Asia, southeastern Asia and eastern Asia were taken from Yang and Mayden (2010), and two nuclear gene (RAG1 and Rhodopsin) sequences from 37 species in eastern Asia (China) were derived from Zheng et al. (2010). In addition, two other nuclear genes (EGR2B and IRBP) of the latter 37 species were sequenced in this study and the five data sets (four separate and a combined ncDNA sequences) were combined for analysis. Seven species were selected as hierarchical outgroups with rooting on *Nipponocypris sieboldii* and *Opsariichthys uncirostris*, following Mayden et al. (2009), Yang and Mayden (2010) and Zheng et al. (2010). All sequences used here are listed in Appendix. The specimens sequenced in this study were the same as those used by Zheng et al. (2010). Species identification and collection localities are given in Appendix. The collection localities of our own samples can be found in Zheng et al. (2010). All specimens sequenced for use in this study were collected between 2004 and 2009 and are deposited at the Kunming Institute of Zoology, Chinese Academy of Sciences.

1.2 DNA extraction, PCR amplification and sequencing

Genomic DNA extraction and PCR amplifications follow the methods detailed in Zheng et al. (2010). Primers used in this study for polymerase chain reaction (PCR) amplification of the EGR2B locus were 278F^b AGT TTT CCA TCG ACT CSC AGT A, and 1117R^b AGG TGG ATT TTG GTG TGT CTY TT; primers for IRBP locus were 109F^b AAC TAC TGC TCR CCA GAA AAR C, and 1001R^b GGA AAT GCA TAG TTG TCT GCA A (Chen et al. 2008). Primers for direct sequencing of the PCR products were the same as those used for PCR amplification. PCR products were sent to

Biotake (Beijing, China) for purifying and sequencing. Sequences specifically obtained for this study have been deposited in GenBank under Accession Nos. JN16020–JN160274.

1.3 Sequence alignment and analyses

The initial sequence alignment was done with ClustalX v1.83 (Thompson et al., 1997) using the default parameters and adjusted by eye. To test for the possible saturation of substitution types, DAMBE was used to plot the number of transitions (Ti) and transversions (Tv) against the uncorrected pairwise distances for each marker (Xia and Lemey, 2009).

1.4 Phylogenetic reconstructions

Phylogenetic trees were estimated for each data set using Maximum Likelihood (ML), Bayesian Inference (BI) and maximum parsimony (MP). The most appropriate evolutionary model was selected by Modeltest v3.7 (Posada and Crandall, 1998) for ML and BI using Akaike information criterion (AIC, Akaike, 1973) before phylogenetic analyses. ML analysis was performed with PhyML3.0 (Guindon and Gascuel, 2003). Bayesian analysis was conducted using the program MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001). Four chains (three hot, one cold) were run for 10,000,000 generations, sampling trees every 100 generations. We confirmed that our Bayesian runs achieved sufficient convergence by ascertaining that the average standard deviation of split frequencies between chains was below 0.01 at the end of the run. The 1st 25,000 generations were discarded as burn-in from the calculation of posterior probabilities. MP analyses was carried out with PAUP* v4.0b10 (Swofford, 2002). The MP method was performed using heuristic searches with 1000 random-addition sequence replicates and TBR branch swapping.

1.5 Character evolution

Characters were tabulated and mapped using Mesquite 2.74 (Maddison and Maddison, 2010), and reconstructions were estimated based on parsimony considerations. We chose six particularly important characters (Reid, 1985; Rahman, 1989; Roberts, 1989; Talwar and Jhingran, 1992; Rainboth, 1996; Jayaram, 1999; Jayaram and Dhas, 2000; Zhang et al., 2000; Kottelat, 2001; Kullander and Fang, 2004; Zhang and Chen, 2004; Stiassny and Getahun, 2007), and these characters were mapped on our molecular phylogenetic tree. *Spinibarbus denticulatus* was used as the outgroup when the characters were polarized. All character states are listed in Table 1 and illustrated in Fig. 1.

(1) Upper lip: 0, present, entire; 1, present, fringed; 2,

Table 1 Matrix of the morphological characters used with Mesquite

Species	Characters	1	2	3	4	5	6	Species	Characters	1	2	3	4	5	6
<i>Labeo bata</i>		1	0	0	0	0	0	<i>Henicorhynchus lobatus</i>		1	0	0	0	1	0
<i>Bangana behri</i>		0	0	0	0	0	0	<i>Henicorhynchus siamensis</i>		1	0	0	0	1	0
<i>Labeo boga</i>		1	0	0	0	0	0	<i>Crossocheilus reticulatus</i>		0	1	0	0	1	0
<i>Cirrhinus microlepis</i>		0	0	0	0	0	0	<i>Epalzeorhynchus kalopterus</i>		2	1	0	1	0	0
<i>Cirrhinus mrigala</i>		0	0	0	0	0	0	<i>Epalzeorhynchus bicolor</i>		2	1	0	1	0	0
<i>Labeo rohita</i>		1	0	0	0	0	0	<i>Epalzeorhynchus frenatum</i>		2	1	0	1	0	0
<i>Labeo stolizkae</i>		1	0	0	0	0	0	<i>Bangana lemassoni</i>		0	0	0	0	1	0
<i>Labeo calbasu</i>		1	1	0	0	0	0	<i>Garra micropulvinus</i>		2	1	1	1	0	1
<i>Labeo chrysophekadion</i>		1	0	0	0	0	0	<i>Garra imberba</i>		2	1	1	1	0	0
<i>Labeo pierrei</i>		1	0	0	0	0	0	<i>Placocheilus cryptonemus</i>		2	1	1	1	0	1
<i>Labeo batesii</i>		1	0	0	0	0	0	<i>Mekongina erythrospila</i>		0	1	0	1	1	0
<i>Labeo weeksii</i>		1	0	0	0	0	0	<i>Bangana tonkinensis</i>		0	0	0	0	1	0
<i>Labeo senegalensis</i>		1	0	0	0	0	0	<i>Bangana lippus</i>		0	0	0	0	1	0
<i>Garra fasciacauda</i>		2	1	1	1	0	0	<i>Parasinilabeo assimilis</i>		2	0	0	0	0	0
<i>Garra flavatra</i>		2	1	1	1	0	0	<i>Semilabeo obscurus</i>		2	0	0	0	0	0
<i>Garra spilota</i>		0	1	1	1	0	0	<i>Rectoris posehensis</i>		2	1	0	0	0	0
<i>Garra annandalei</i>		0	1	1	1	0	0	<i>Stenorynchoacrum xijiangensis</i>		1	0	0	0	1	0
<i>Garra gotyla gotyla</i>		0	1	1	1	0	0	<i>Pseudocrossocheilus liuchengensis</i>		2	1	0	1	1	0
<i>Garra fuliginosa</i>		2	1	1	1	0	0	<i>Pseudocrossocheilus tridentis</i>		2	1	0	1	1	0
<i>Garra orientalis</i>		2	1	1	1	0	0	<i>Pseudocrossocheilus papillolabrus</i>		2	1	0	1	1	0
<i>Garra mirofrontis</i>		2	1	1	1	0	0	<i>Pseudocrossocheilus longibullus</i>		2	1	0	1	1	0
<i>Garra ceylonensis</i>		0	1	1	1	0	0	<i>Pseudocrossocheilus bamaensis</i>		2	1	0	1	1	0
<i>Garra congoensis</i>		2	1	1	1	0	0	<i>Pseudocrossocheilus nigrovittatus</i>		2	1	0	1	1	0
<i>Garra makiensis</i>		2	1	1	1	0	0	<i>Pseudogyrinocheilus prochilus</i>		2	0	0	0	0	0
<i>Garra tengchongensis</i>		2	1	1	1	0	0	<i>Qianlabeo striatus</i>		0	1	0	0	0	0
<i>Crossocheilus burmanicus</i>		0	1	0	0	1	0	<i>Ptychidio jordani</i>		2	1	0	0	1	1
<i>Akrokolioplax bicornis</i>		2	1	0	1	1	0	<i>Sinocrossocheilus labiatus</i>		2	1	0	1	0	1
<i>Labiobarbus lineatus</i>		1	0	0	0	0	0	<i>Hongshuia megalophthalmus</i>		2	1	0	1	0	1
<i>Labiobarbus siamensis</i>		1	0	0	0	0	0	<i>Hongshuia microstomatus</i>		2	1	0	1	0	1
<i>Osteochilus melanopleurus</i>		1	0	0	0	0	0	<i>Hongshuia paoli</i>		2	1	0	1	0	1
<i>Osteochilus microcephalus</i>		1	0	0	0	0	0	<i>Discogobio yunnanensis</i>		2	1	2	1	0	1
<i>Osteochilus waandersii</i>		1	0	0	0	0	0	<i>Discocheilus wui</i>		2	0	2	1	0	1
<i>Osteochilus lini</i>		1	0	0	0	0	0	<i>Discocheilus wuluheensis</i>		2	0	2	1	0	1
<i>Osteochilus salsburyi</i>		1	0	0	0	0	0	<i>Discogobio tetrabarbatus</i>		2	1	2	1	0	1
<i>Cirrhinus molitorella</i>		1	0	0	0	0	0	<i>Discogobio brachyphysallidos</i>		2	1	2	1	0	1
<i>Lobocheilos melanotaenia</i>		0	0	0	0	1	0	<i>Discogobio macrophysallidos</i>		2	1	2	1	0	1
<i>Henicorhynchus lineatus</i>		1	0	0	0	1	0								

absent. Figs. 1A and C show the developed upper lip, and Figure 1D illustrates the present but vestigial upper lip.

(2) Margin of rostral fold: 0, entire or only with median incision (Fig. 1A); 1, fringed (Figs. 1B, E-G).

(3) Lower lip: 0, no disc (Fig. 1A); 1, with a disc, without protrusion (Fig. 1B, F); 2, with a disc, protruded to form a horseshoe-shaped, pocket-like or ring-like fold

around central fleshy pad (Fig. 2G).

(4) Postlabial groove: 0, present; 1, absent.

(5) Mental groove: 0, absent (Figs. 1A, B, F, and G); 1, present (Fig. 1E).

(6) Pharyngeal teeth rows: 0, three; 1, two.

1.6 Biogeography

Statistical dispersal-vicariance analysis (S-DIVA 1.5, Yu et al., 2010) was employed to infer historical distri-

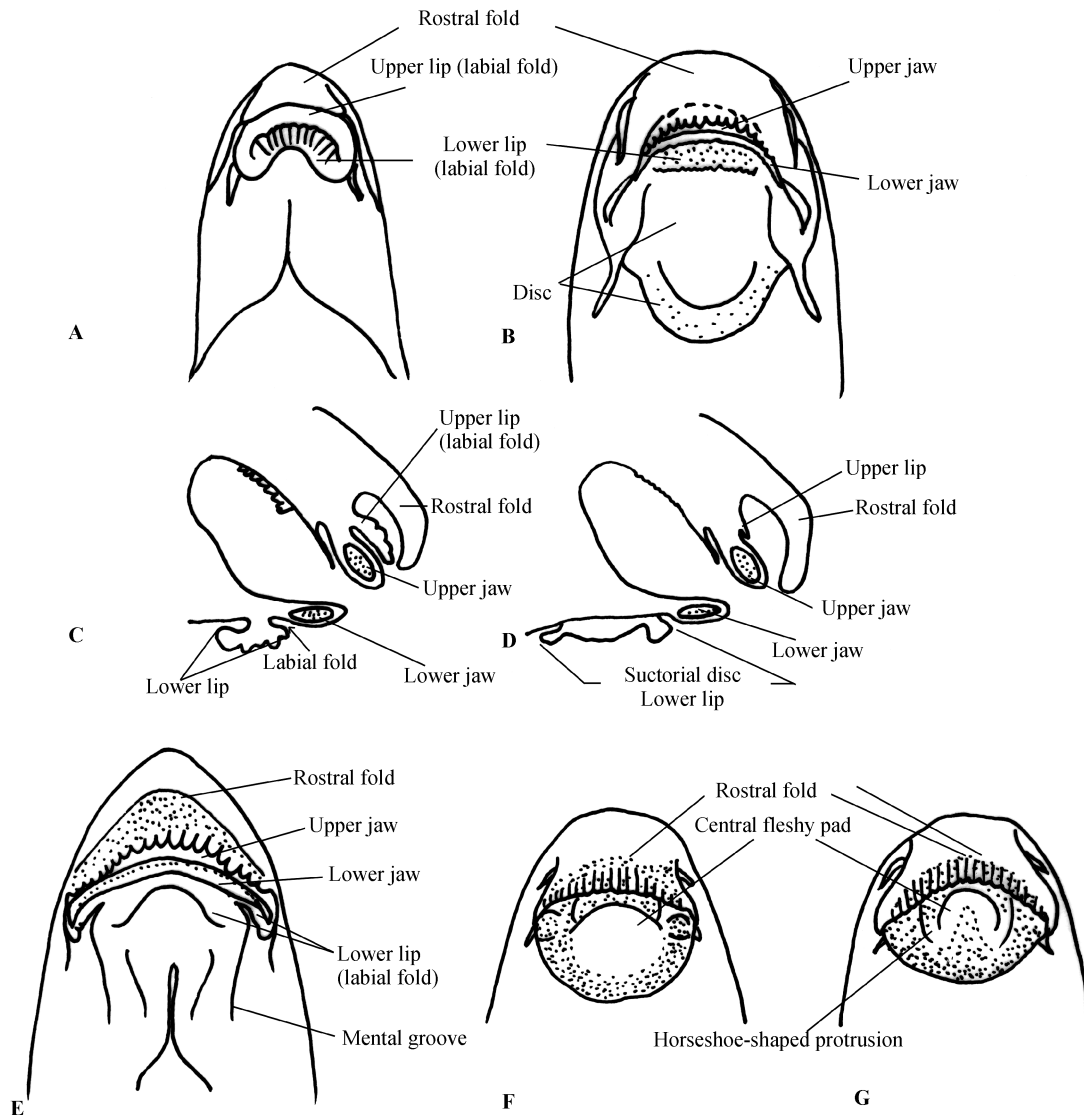


Fig. 1 Characters of oromandibular morphology of Labeoninae discussed in the text

Ventral view of head: **A.** *Labeo nasus*, **B.** *Garra dembeensis*, and **E.** *Mekongina erythrospila* (after Stiassny and Getahun, 2007). **F.** *Placocheilus robustus*, and **G.** *Discogobio tetrabarbus* (after Zhang et al., 2002). In cross-sectional schematic view: **C.** *Labeo*, and **D.** *Garra* (after Stiassny and Getahun, 2007).

butions of lineages based on the ML tree. For this analysis, four geographic regions were defined on the basis of current distribution. A represents Africa, B represents East Asia (China), C represents South Asia, and D represents Southeast Asia.

2 Results

2.1 Sequence analyses

A total of 292 nucleotide sequences were used in this phylogenetic analysis, of which 74 sequences were obtained in this study. Of the 1443 bp aligned for RAG1, 581 sites were variable and 421 were parsimony informative. Of the 488 aligned bp for Rhodopsin, 177 sites were variable and 143 were parsimony informative. Of

the 751 bp aligned for EGR2B, 198 sites were variable and 122 were parsimony informative, and of the 810 aligned bp for IRBP, 410 sites were variable and 309 were parsimony informative. No signal of saturation was observed among sequences. The final aligned data matrix used for analyses consisted of 3492 bp for 73 ingroup taxa. Mean base composition of the combined dataset is as follows: A, 0.2368; C, 0.2785; G, 0.2465, and T, 0.2382. No significant compositional biases existed in either ingroup or outgroup taxa. Models selected by Modeltest for each of the four loci and the combined dataset are presented in Table 2.

2.2 Phylogenetic analyses

The ML tree of the combined dataset is presented

Table 2 The best substitution models selected for each gene for the Bayesian analysis using Modeltest

Loci	Model	Nst	Invariable sites (I)	Gamma-shape parameter (α)
RAG1	GTR+I+G	6	0.4585	1.0525
Rhodopsin	HKY+I+G	2	0.5522	0.9960
EGR2B	TVM+I+G	6	0.5150	0.7323
IRBP	TrNef+I+G	6	0.3623	1.0330
Combined	GTR+I+G	6	0.4729	0.8900

here together with the nodal support values generated by ML, BPP and MP bootstrap analysis, respectively (Fig. 2). The subfamily Labeoninae was resolved as a monophyletic group with high bootstrap values (100%, 100%, and 100%, respectively). All nuclear gene datasets except for Rhodopsin supported the monophyly of the Labeoninae (trees not shown). A nested set of four major clades (A–D in Fig. 2) was resolved within the Labeoninae.

Clade A included all the species of *Labeo* plus *Bangana behri*, *Cirrhinus microlepis* and *C. mrigala*, and this result was well supported by three gene sets (but not by Rhodopsin). Clade B was comprised of most of the species currently assigned to *Garra* (12 species), *Crossocheilus burmanicus* and *Akrokolioplax bicornis*. The species of *Cirrhinus molitorella*, *Epalzeorhynchus*, *Henicorhynchus*, *Labiobarbus*, *Lobocheilos*, *Osteochilus*, and *Crossocheilus reticulatus* were clustered in Clade C. The remaining species included in our analysis formed Clade D, which can be further divided into three monophyletic lineages (D1, D2 and D3). Clade D1 was the sister lineage to the clade consisting of Clade D2 plus D3. *Bangana lemasoni* is the sole member of D1; *Bangana* (2 species), *Mekongina* (1 species), *Placochilus* (1 species) and two species of *Garra* from China formed group D2; and the other species, all from karst regions of southern China, clustered into D3. This latter group (D3), which includes most of the species endemic to China, is characterized by diversified oromandibular morphology. Table 3 shows how each gene supported the eight monophyletic lineages recovered from the maximum likelihood tree of the combined dataset.

3 Discussion

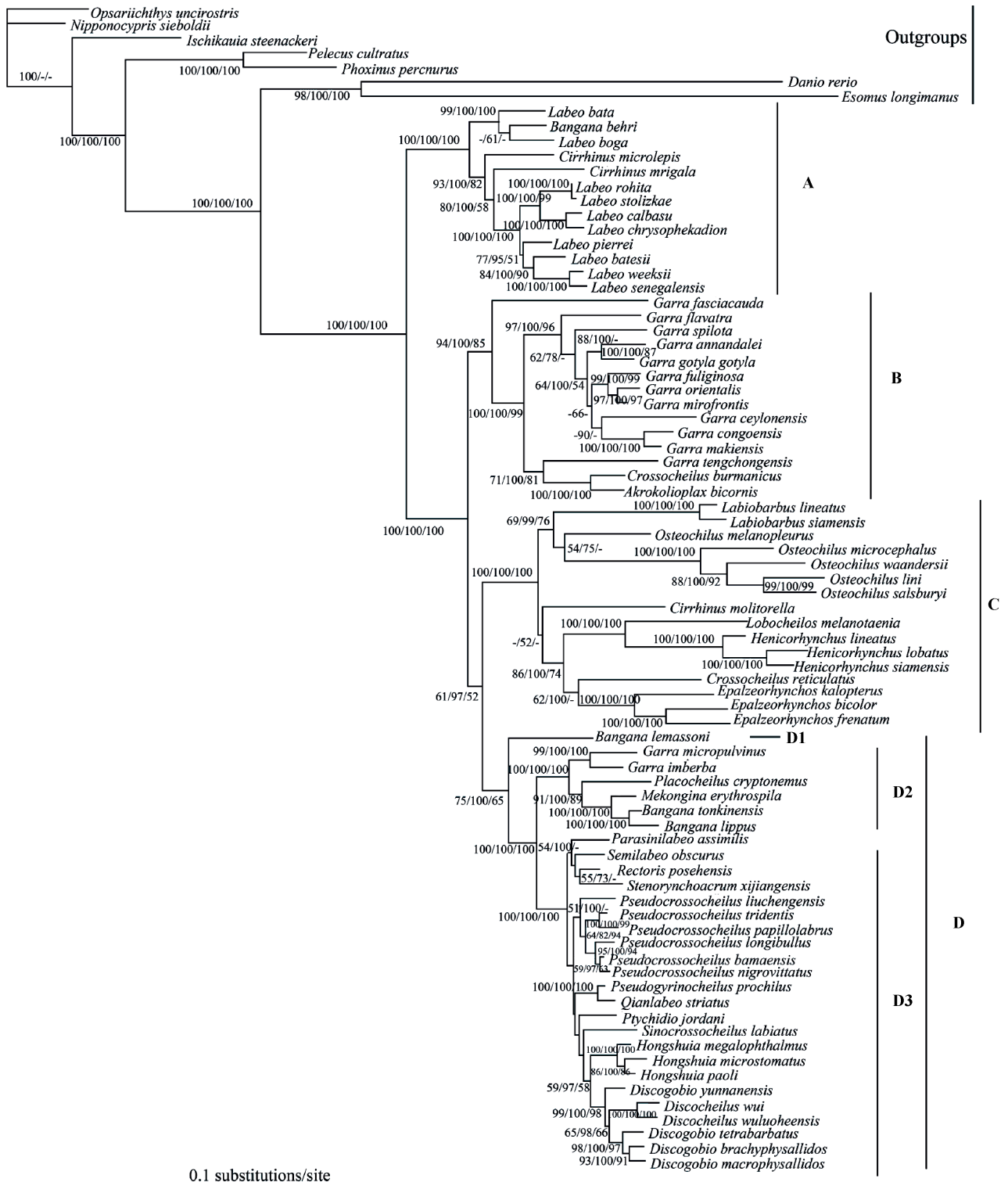
3.1 Phylogeny and subdivision within Labeoninae

The monophyly of the Labeoninae has been well supported, but subdivision within the Labeoninae has been controversial. Rainboth (1991, 1996) considered that two subgroups, Labeones and Garrae, could be recognized within the Labeonini (= Labeoninae) based on morphology, and Zhang et al. (2000) subdivided the

subfamily into three groups based on oromandibular structures. The subdivision presented by Stiassny and Getahun (2007) was basically consistent with that of Rainboth (1991, 1996). Recently, two major clades and three monophyletic subgroups within the second clade were resolved within this subfamily based on nuclear gene variation (Yang and Mayden, 2010), and six clades were recovered by Zheng et al. (2010) based on nuclear and mitochondrial genes. When more samples from Asia and Africa were included (this study), four major clades and three smaller clades within the fourth clade were recognized within this group of fishes. Our results are incongruent with those of previous authors based on morphology, but integrated the conclusions of Yang and Mayden (2010) and Zheng et al. (2010). Comparing our results with two recent results, our findings represent the most comprehensive and detailed hypothesis of the phylogenetic relationships within the Labeoninae.

The morphological analyses cited above suggested that our Clade A was close to Clade C, and Clade B was close to Clade D. In the current sampling scheme, our results show that *Labeo* is the first lineage to diverge from all other clades within the Labeoninae. In Clade B, *Garra* itself as currently defined is clearly polyphyletic, with a minimum of four separate lineages included. This genus has been characterized, at least in part, by the putatively apomorphic presence of a disc on the lower jaw (e. g. Wu, 1977; Zhang et al., 2000; Stiassny and Getahun, 2007). The nesting of *Crossocheilus burmanensis* and *Akrokolioplax bicornis* (both of which lack a lobe on the lower lip) within the clade B species currently assigned to *Garra* emphasizes the need for a much larger sampling of the species and a thorough re-examination of all of these taxa.

Cirrhinus, *Henicorhynchus*, *Labiobarbus*, *Lobocheilos* and *Osteochilus*, which belong in the first group as defined by Zhang et al. (2000), were included in our Clade C. *Cirrhinus* is obviously not monophyletic in our results, and the monophyly of all other genera except for *Henicorhynchus* could not be confirmed because of limited sampling. The monophyly of *Henicorhynchus*



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Fig. 2 Maximum likelihood tree inferred from combined dataset (3492 bp of RAG1, RH, EGR2B and IRBP) for all species. The nodal numbers are ML, BPP, and MP bootstrap values, respectively. Only values above 50% are given.

seems likely because three of its four species were used in this analysis. In addition, *Crossocheilus reticulatus* and *Epalzeorhynchus*, assigned to the "*Crossocheilus* group" by Bănărescu (1986), form a well-supported monophyletic unit nested as one of the terminal taxa

within Clade C. It is noteworthy that the other species of *Crossocheilus* included in our analysis, *C. burmanicus*, was separately located in Clade B. Thus, *Crossocheilus* as presently constituted is not monophyletic, and we suggest that the taxonomy of *Crossocheilus* needs to be

Table 3 Support for major clades of the ML tree (Fig. 1) as shown by independent analyses of each gene. In cases where a clade was supported in the ML tree of each gene, ML, BPP, and MP bootstrap values > 50% are shown sequentially

	RAG1	RH	EGR2B	IRBP
Labeoninae	Yes 100/100/100	No	Yes 81/100/96	Yes -/62/89
Clade A	Yes 100/100/100	No	Yes 60/91/68	Yes 94/78/77
Clade B	Yes 98/100/97	No	Yes -/96/-	No
Clade C	Yes 90/100/90	Yes -/98/-	Yes -/63/-	Yes -/71
Clade D	No	Yes -/85/-	No	Yes 89/-/-
Subclade D1	Yes	Yes	Yes	Yes
Subclade D2	Yes -/69/-	Yes 63/85/73	Yes -/53/-	Yes 87/83/73
Subclade D3	Yes 88/100/88	Yes 69/100/69	No	No

The “-” denote those clades with support values lower than 50%.

further examined.

Clade D consists of most of the species from China and contains the majority of genera in the Labeoninae. The species included in Clade D3 are all from the karst regions of southern China, therefore, Clade D3 is designated the "karst group" herein. The phylogenetic position of clade D3 was supported by both morphological results (e.g. Zhang et al., 2000) and molecular data (Zheng et al., 2010 and this study). In addition, the branch lengths between taxa within the karst group are relatively short in our tree, which suggests that these species have evolved rapidly within a relatively short time period. It is also noteworthy that the three species of *Bangana* analyzed here were subdivided into two lineages in this study, and this result was consistent with that of Zheng et al. (2010). Further work on the taxonomy and phylogeny of *Bangana* is essential.

3.2 Character evolution of oromandibular morphology

The Labeoninae display an amazing diversity of oromandibular morphology, and as knowledge of the molecular phylogeny rapidly grows it is necessary to discuss the phylogenetic significance of this structural complex. The characters listed in this paper have been hypothesized to be of phylogenetic significance in labeonines by various authors (e. g. Zhang, 1994, 1998, 2005; Su, 2001; Zhang and Chen, 2004; Stiassny and Getahun, 2007), but our molecular phylogenetic results do not fully support their conclusions. Our results show that all these characters of oromandibular morphology of the Labeoninae were homoplastically distributed on the molecular phylogenetic tree (Fig. 3).

The presence of the upper lip (Character 1) was considered as the plesiomorphic character state by above mentioned morphological studies and mostly present in the relatively generalized taxa (e.g. *Cirrhinus*, *Hemicorhynchus*, *Labeo*, *Labiobarbus*, *Lobocheilos* and *Os-*

teochilus). However, the results of character evolution show that the evolution of the upper lip of labeonine fishes is very complex. Another relevant character is the margin of the rostral fold (Character 2). The rostral fold was considered to evolve from "entire" to "fringed" by morphological studies. Our results suggest that the entire rostral fold appears to be plesiomorphic, but the evolution of the rostral fold has also gone through a complex process. The fringed rostral fold has been acquired at least six times and the entire rostral fold has been reacquired (reversion to the plesiomorphic state) three times within the Labeoninae. Similarly, the fishes with a disc on the lower lip (Character 3) were considered as relatively derived species by morphological studies. The results of character evolution also show that the lower lip with a disc seems to be apomorphic, but this character state homoplastically occurs within Clade B and D. The presence of the disc on the lower lip seems to have been acquired three times; the absence of the disc has been reacquired twice within the Labeoninae. It can be seen that although both *Garra* and *Discogobio* have a disc on the lower lip, the discs of these two genera have independent origins. The postlabial groove and the mental groove have been also used as important taxonomic characters in labeonine systematics. When these two characters (Characters 4 and 5) are mapped on the molecular phylogenetic tree, the presence of the postlabial groove and the absence of the mental groove are considered as plesiomorphic states. However, the character evolution suggests that the absence of the postlabial groove is homoplastically distributed within Clade B, C and D. The results indicate that the absence of the postlabial groove has been acquired five times and the presence of the postlabial groove has been reacquired twice within the Labeoninae. The mental groove also exhibits extensive homoplasy throughout the tree as the whole, being present within

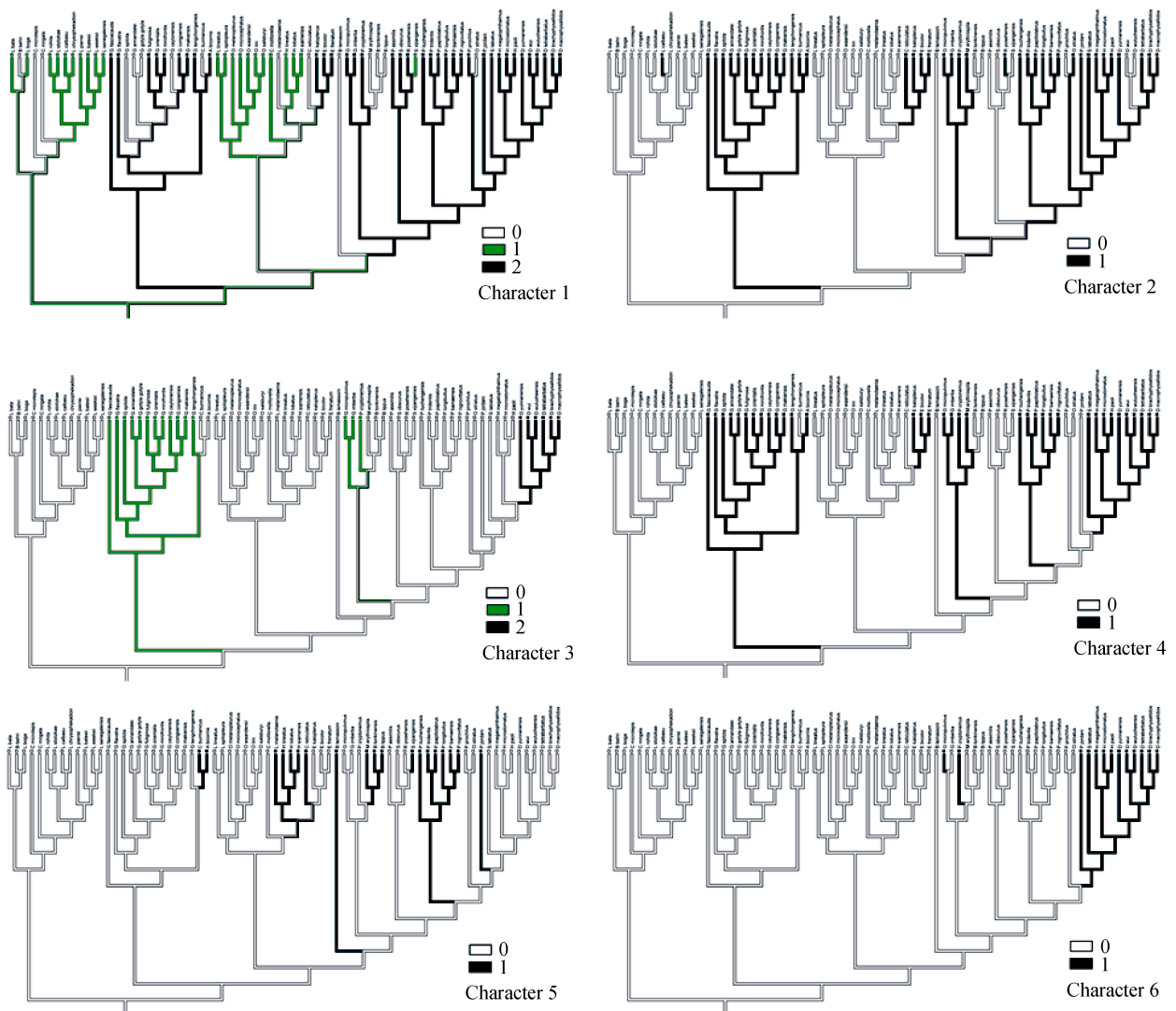


Fig. 3 Characters mapped on the phylogenetic hypothesis by Mesquite

Clades B, C and D (Fig. 3). However, the occurrence of this character is distributed differently from that of the postlabial groove in the subsets of each clade. The results suggest that the presence of the mental groove has been independently acquired eight times. Our results also show that the pharyngeal tooth pattern (Character 6) of 3 rows is plesiomorphic relative to 2 rows. Character evolution shows that the pharyngeal tooth pattern of 2 rows has been acquired three times within the Labeoninae. Although the rows of the pharyngeal teeth do not exhibit a phylogenetic signal congruent with our tree, they are still useful in making taxonomic distinctions.

Based on the analyses above, we can infer that the morphological characters, especially oromandibular characters, have not evolved unidirectionally, but have evolved several times in parallel. It also can be seen that although these morphological characters do not have the systematic significance originally proposed by morpho-

logical studies, they can exhibit taxonomic significance. Therefore, these morphological characters are still useful for taxonomy. The diversified characters are indicative of adaptive radiations within labeonine fishes, which frequently live in swift currents. These fishes appear to have adapted to local ecological environments through the development of an array of superficially similar morphological character states. Our study reinforces the power of phylogenies to unravel complex patterns of homoplasy resulting from independent morphological responses to similar ecological conditions.

3.3 Biogeographical implications of Labeoninae

Cyprinidae or Cypriniformes have been widely accepted to have an Oriental origin, either from South Asia (Briggs, 1979; Menon, 1986), East Asia (Bănărescu and Coad, 1991) or both South and East Asia (Sawada, 1982; Chu, 1986). Recently, Saitoh et al. (2011) suggested that Cypriniformes most likely originated

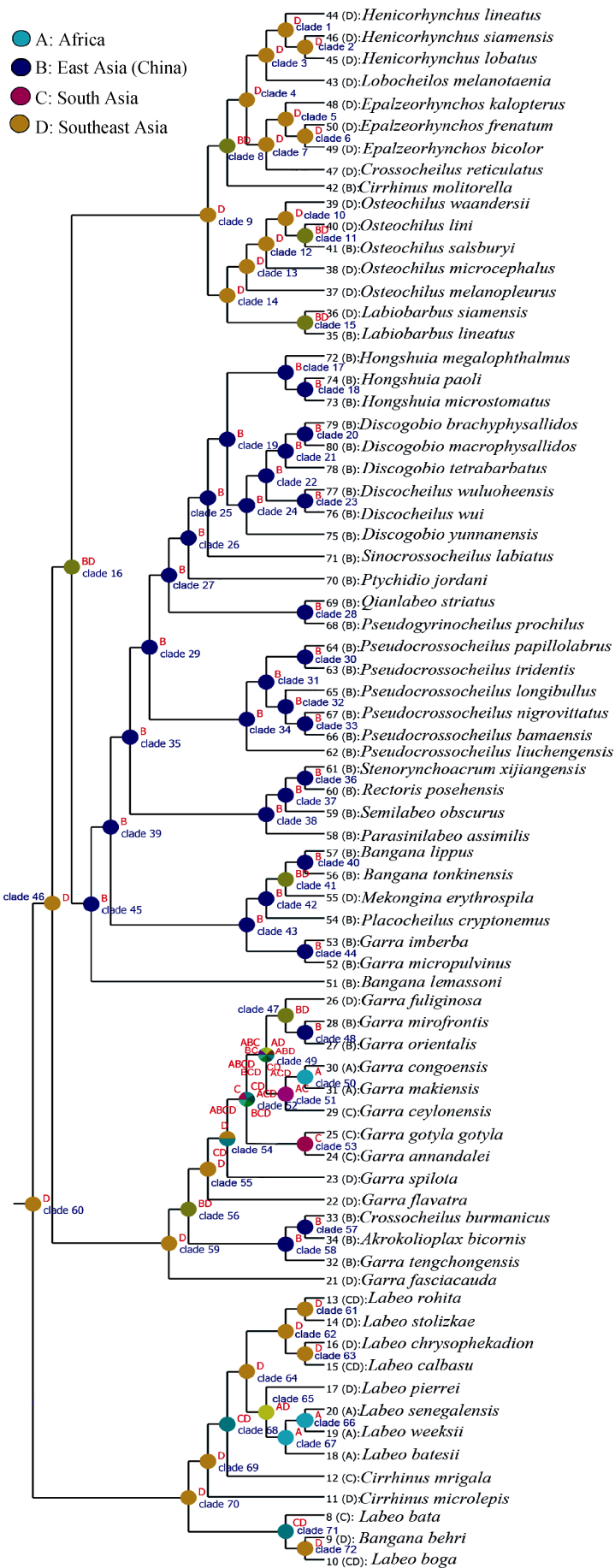


Fig. 4 Ancestral distribution ranges inferred by S-DIVA analysis within Labeoninae

Single color means the node has a specific ancestral range, and multicolor mean the node has multiple possible ancestral ranges.

from South Asia. Gaubert et al. (2009) proposed that the Oriental tropical region was the centre of origin of Cyprinidae, and identified three Oriental-to-Palaearctic, two Palaearctic-to-Nearctic, and one Oriental-to-Afro-tropical major migration events.

Our results of the S-DIVA analysis suggest that the Labeoninae originated from Southeast Asia and the Labeoninae followed three dispersal routes, including Southeast Asia-to-Africa, Southeast Asia-to-East Asia (China), and Southeast Asia-to-South Asia (Fig. 4). The labeonine fishes dispersed into Africa twice within the Labeoninae, one within the species of *Labeo*, and the other within the species of *Garra*. This result is consistent with the view of two dispersal events of labeonins from Asia into Africa in Tang et al. (2009). Labeoninae dispersed into East Asia (China) and South Asia several times from Southeast Asia, and a few East Asian species dispersed back into Southeast Asia. The connection of drainages between East Asia (China) and Southeast Asia may have contributed to this reverse dispersal. Our biogeographic results of Labeoninae coincide with Gaubert et al. (2009) about the Oriental tropical origin of Cyprinidae and our results also verify the Oriental-to-Afro-tropical migration events.

In particular, Clade D, which is located in the terminal clade in the phylogenetic tree, includes most endemic species in China. Our results indicate that the labeonine fishes dispersed into China from Southeast Asia. The diversified ecological environment in southern China, especially in the hilly and karst regions of southwestern China, facilitates speciation. Consequently, these species rapidly evolved in southern China, resulting in the high diversity and endemism found today.

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Appendix Taxa included in this study and accession numbers in GenBank

Subfamily	Taxon	Voucher specimen	Sampling location	Accession No.	RAG1	Rhodopsin	EGR2B	IRBP
Outgroups	<i>Cultrinae</i>							
		<i>Ischikaita steenackeri</i>			EU292687*	FJ197046*	EU409744*	FJ197096*
		<i>Pelecus cultratus</i>			EU711144*	FJ197045*	FJ531301*	FJ197095*
	<i>Danioninae</i>	<i>Danio rerio</i>			U71093*	L11014*	NM130997*	X85957*
		<i>Esomus longimanus</i>			FJ531248*	FJ531349*	FJ531292*	FJ531369*
		<i>Nipponocypris sieboldii</i>			EU292713*	FJ197069*	FJ531312*	FJ197120*
		<i>Opsariichthys uncirostris</i>			FJ197126*	FJ197068*	FJ531300*	FJ197119*
	<i>Leuciscinae</i>	<i>Phoxinus phoenurus</i>			EU409627*	EU409655*	EU409751*	EU409687*
	Ingroups							
<i>Laboninae</i>								
		<i>Akrokoloplax bicornis</i>	KIZ05207	Lushui, Yunnan Prov., China	GU086514*	GU086476*	JN160236	JN160272
		<i>Bangana behri</i>		Southeast Asia	GQ913450*	GQ913502*	GQ913554*	GQ913609*
		<i>Bangana lemassoni</i>	KIZCXY20070420013	Ivchun, Yunnan Prov., China	GU086499*	GU086461*	JN160222	JN160258
		<i>Bangana lippus</i>	KIZCXY2008077	Xishuangbanna, Yunnan Prov., China	GU086525*	GU086487*	JN160213	JN160249
		<i>Bangana tonkinensis</i>	KIZ200401163	Mojiang, Yunnan Prov., China	GU086524*	GU086486*	JN160212	JN160248
		<i>Cirrhinus microlepis</i>		Southeast Asia	GQ913451*	GQ913503*	GQ913555*	GQ913610*
		<i>Cirrhinus moliorella</i>	KIZCXY20071011	Hekou, Yunnan Prov., China	GU086500*	GU086462*	JN160223	JN160259
		<i>Cirrhinus mirigala</i>		South Asia	GQ913452*	GQ913504*	GQ913556*	GQ913611*
		<i>Crossocheilus burmanicus</i>	KIZCXY20060030	Longchuan, Yunnan Prov., China	GU086497*	GU086459*	JN160220	JN160256
		<i>Crossocheilus reticulatus</i>		Southeast Asia	GQ913454*	GQ913506*	GQ913558*	GQ913613*
		<i>Discocheilus wuluoheensis</i>	KIZMR20080429	Luoping, Yunnan Prov., China	GU086531*	GU086493*	JN160211	JN160247
		<i>Discocheilus wui</i>	KIZDLN20080045	Leye, Guangxi, Prov., China	GU086523*	GU086485*	JN160209	JN160245
		<i>Discogobio brachyphysallidos</i>	KIZMR20080368	Luoping, Yunnan Prov., China	GU086509*	GU086471*	JN160232	JN160268
		<i>Discogobio macrophysallidos</i>	KIZMR20080335	Luoping, Yunnan Prov., China	GU086511*	GU086473*	JN160233	JN160269
		<i>Discogobio tetrabarbatus</i>	KIZMR20080058	Libo, Guizhou Prov., China	GU086518*	GU086480*	JN160207	JN160243
		<i>Discogobio yunnanensis</i>	KIZ20060003377	Jinning, Yunnan Prov., China	GU086507*	GU086469*	JN160230	JN160266
		<i>Epalzeorhynchus bicolor</i>		Southeast Asia	GQ913456*	GQ913508*	GQ913560*	GQ913615*
		<i>Epalzeorhynchus frenatum</i>		Southeast Asia	GQ913457*	GQ913509*	GQ913561*	GQ913616*
	<i>Epalzeorhynchus kalopterus</i>		Southeast Asia	GQ913458*	GQ913510*	GQ913562*	GQ913617*	
	<i>Garra amandalei</i>		South Asia	GQ913460*	GQ913512*	GQ913564*	GQ913619*	
	<i>Garra ceylonensis</i>		South Asia	GQ913459*	GQ913511*	GQ913563*	GQ913618*	

To be continued

Subfamily	Taxon	Voucher specimen	Sampling location	Accession No.	Rhodopsin	EGR2B	IRBP
				RAG1			
	<i>Garra congoensis</i>		Africa	GQ911679*	GQ911687*	GQ911669*	GQ911666*
	<i>Garra fasciacauda</i>		Southeast Asia	GQ911683*	GQ911691*	GQ911673*	GQ911661*
	<i>Garra flavatra</i>		Southeast Asia	GQ913461*	GQ913513*	GQ913565*	GQ913620*
	<i>Garra fuliginosa</i>		Southeast Asia	GQ913462*	GQ913514*	GQ913566*	GQ913621*
	<i>Garra gotyla gotyla</i>		South Asia	GQ913463*	GQ913515*	GQ913567*	GQ913622*
	<i>Garra imberba</i>	KIZWWY20080051	Wenshan, Yunnan Prov., China	GU086530*	GU086492*	JN160217	JN160253
	<i>Garra makiensis</i>		Africa	GQ911677*	GQ911685*	GQ911675*	GQ911663*
	<i>Garra micropulvinus</i>	KIZWWY20080244	Wenshan, Yunnan Prov., China	GU086529*	GU086491*	JN160216	JN160252
	<i>Garra mirofrontis</i>	KIZ20050325008	Yunxian, Yunnan Prov., China	GU086527*	GU086489*	JN160215	JN160251
	<i>Garra orientalis</i>	KIZ200406230	Longlin, Guangxi Prov., China	GU086526*	GU086488*	JN160214	JN160250
	<i>Garra spilota</i>		Southeast Asia	EU409621*	EU409649*	EU409745*	EU409681*
	<i>Garra tengchongensis</i>	KIZCXY20060137	Tengchong, Yunnan Prov., China	GU086528*	GU086490*		
	<i>Henicorhynchus lineatus</i>		Southeast Asia	GQ913467*	GQ913520*	GQ913572*	GQ913627*
	<i>Henicorhynchus lobatus</i>		Southeast Asia	GQ913468*	GQ913521*	GQ913573*	GQ913628*
	<i>Henicorhynchus siamensis</i>		Southeast Asia	GQ913469*	GQ913522*	GQ913574*	GQ913629*
	<i>Hongshuia megalophthalmus</i>		Tian'e, Guangxi Prov., China	GU086517*	GU086479*	JN160204	JN160240
	<i>Hongshuia microstomatus</i>	KIZMR20080234	Libo, Guizhou Prov., China	GU086510*	GU086472*		
	<i>Hongshuia paoli</i>	KIZMR200800576	Lingyun, Guangxi, Prov., China	GU086522*	GU086484*	JN160238	JN160274
	<i>Labeo bata</i>	KIZDLN20080003	South Asia	GQ913470*	GQ913523*	GQ913575*	GQ913630*
	<i>Labeo batesii</i>		Africa	EU711150*	FJ197052*	GQ913576*	FJ197103*
	<i>Labeo boga</i>		South Asia and Southeast Asia	GQ913471*	GQ913524*	GQ913577*	GQ913631*
	<i>Labeo calbasu</i>		South Asia and Southeast Asia	GQ913472*	GQ913525*	GQ913578*	GQ913632*
	<i>Labeo chrysocephkadion</i>		Southeast Asia	EU409622*	EU409650*	EU409746*	EU409682*
	<i>Labeo pierrei</i>		Southeast Asia	GQ913475*	GQ913526*	GQ913581*	GQ913635*
	<i>Labeo rohita</i>		South Asia and Southeast Asia	GQ913476*	GQ913527*	GQ913582*	GQ913636*
	<i>Labeo senegalensis</i>		Africa	EU711151*	FJ197053*	GQ913583*	FJ197104*
	<i>Labeo stoliczkae</i>	KIZCXY20060059	Ruilu, Yunnan Prov., China	GU086498*	GU086460*	JN160221	JN160257
	<i>Labeo weeksii</i>		Africa	GQ911680*	GQ911688*	GQ911670*	GQ911665*
	<i>Labiobarbus lineatus</i>		East Asia	GQ913477*	GQ913528*	GQ913584*	GQ913637*

To be continued

Subfamily	Taxon	Voucher specimen	Sampling location	Accession No.	Rhodopsin	EGR2B	IRBP
				RAG1			
	<i>Labiobarbus siamensis</i>		Southeast Asia	GQ913479*	GQ913530*	GQ913586*	GQ913639*
	<i>Lobocheilus melanotaenia</i>		Southeast Asia	GQ913480*	GQ913531*	GQ913587*	GQ913640*
	<i>Mekongina erythrospila</i>		Southeast Asia	GQ911684*	GQ911692*	GQ911674*	GQ911668*
	<i>Osteochilus lini</i>		Southeast Asia	GQ913481*	GQ913532*	GQ913588*	GQ913641*
	<i>Osteochilus melanopleurus</i>		Southeast Asia	GQ911681*	GQ911689*	GQ911671*	GQ911664*
	<i>Osteochilus microcephalus</i>		Southeast Asia	GQ913482*	GQ913533*	GQ913589*	GQ913642*
	<i>Osteochilus salsburyi</i>	KIZCXY20071012	Hekou, Yunnan Prov., China	GU086501*	GU086463*	JN160224	JN160260
	<i>Osteochilus waandersii</i>		Southeast Asia	GQ911682*	GQ911690*	GQ911672*	GQ911662*
	<i>Parasinilabeo assimilis</i>	KIZ20050625013	Libo, Guizhou Prov., China	GU086515*	GU086477*	JN160237	JN160273
	<i>Placocheilus cryptonemus</i>	KIZ05169	Lushui, Yunnan Prov., China	GU086494*	GU086456*	JN160218	JN160254
	<i>Pseudocrossocheilus bamaensis</i>	KIZMR20080141	Du'an, Guangxi Prov., China	GU086516*	GU086478*	JN160203	JN160239
	<i>Pseudocrossocheilus liuchengensis</i>	KIZMR20080173	Du'an, Guangxi Prov., China	GU086502*	GU086464*	JN160225	JN160261
	<i>Pseudocrossocheilus longibullus</i>	KIZ20050625001	Libo, Guizhou Prov., China	GU086495*	GU086457*	JN160210	JN160246
	<i>Pseudocrossocheilus nigrovittatus</i>	KIZMR20080051	Libo, Guizhou Prov., China	GU086503*	GU086465*	JN160226	JN160262
	<i>Pseudocrossocheilus papillolabrus</i>	KIZMR20080514	Zhenfeng, Guizhou Prov., China	GU086513*	GU086475*	JN160235	JN160271
	<i>Pseudocrossocheilus tridentis</i>	KIZMR20080365	Luoping, Yunnan Prov., China	GU086508*	GU086470*	JN160231	JN160267
	<i>Pseudogyriinocheilus prochilus</i>	KIZ200405021	Yiliang, Yunnan Prov., China	GU086496*	GU086458*	JN160219	JN160255
	<i>Pyichidio jordani</i>	KIZMR20080087	Libo, Guizhou Prov., China	GU086504*	GU086466*	JN160227	JN160263
	<i>Qianlabeo striatus</i>	KIZMR20080505	Zunyi, Guizhou Prov., China	GU086512*	GU086474*	JN160234	JN160270
	<i>Rectoris posehensis</i>	KIZMR20080065	Libo, Guizhou Prov., China	GU086519*	GU086481*	JN160205	JN160241
	<i>Semilabeo obscurus</i>	KIZMR20080085	Libo, Guizhou Prov., China	GU086505*	GU086467*	JN160228	JN160264
	<i>Sinocrossocheilus labiatus</i>	KIZZLP20090021	Tongzi, Guizhou Prov., China	GU086520*	GU086482*	JN160206	JN160242
	<i>Stenorynchoacrum xijiangensis</i>	KIZDLN20080001	Gutlin, Guangxi, Prov., China	GU086521*	GU086483*	JN160208	JN160244

* Sequences downloaded from the NCBI.

Continued