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## The origin and divergence of Gobioninae fishes (Teleostei: Cyprinidae) based on complete mitochondrial genome sequences

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### Summary

The gudgeons (subfamily Gobioninae) are a group of cyprinid fishes primarily distributed in East Asia. However, studies on their origins and divergence are scarce. Here the whole mitochondrial genome sequences of 27 gudgeon species (including one newly determined), 22 other cyprinid species, and two non-cyprinids as outgroups are applied to infer the evolution of the gudgeons. Based on Bayesian and maximum likelihood phylogenetic analyses, the gudgeons were determined to be a monophyletic group which can be further subdivided into four monophyletic clades with strong supports. The divergence times of the gudgeons were estimated using a relaxed molecular clock method; the results indicate that these fishes originated in the early Paleocene (approx. 63.5 Mya) and that the basal *Hemibarbus* group diverged from the other gudgeon fishes (approx. 58.3 Mya). As an independent group the *Coreius* began to diverge from the remaining two groups (approx. 54.6 Mya); the most derived two groups diverged from each other (approx. 53.6 Mya). The divergences of the four gudgeon groups were within a relatively short time frame (approx. 58–53 Mya). Based on the reconstruction of evolutionary trends of gudgeon habitat, evidence is provided that supports the origin and differentiation of this fauna as being associated with some special paleo-environmental events occurring from the early Paleocene to the Pliocene. The study represents comprehensive molecular dating and character evolution analyses of the gudgeons, and providing a valuable framework for future research in the evolution of the Gobioninae fishes.

### Introduction

The fishes of the cyprinid subfamily Gobioninae (also called gudgeons) are widely distributed in Europe and Asia with 29 genera and approximately 200 species (Rainboth, 1991; Bănărescu, 1992; Nelson, 2006; Yang et al., 2006; Liu et al., 2010; Tang et al., 2011). Most members of the subfamily are concentrated in East Asia (e.g. China, Japanese archipelago, and Korean peninsula), except the genus *Gobio* that has a wide distribution throughout Europe and northern Asia (Turan et al., 2012) and the genus *Romanogobio* that is native to Europe (Rainboth, 1991; Bănărescu, 1992; Nelson,

2006). The Gobioninae fishes are generally small- to medium-sized cyprinids that display large morphological, ecological, and behavioral variations (Bănărescu, 1992; Yue, 1998). For example, most of the gudgeons are benthic but a few genera are medial dwellers (e.g. *Gobiocypris*), and *Hemibarbus* fishes are lower or medial dwellers. Consequently, they also exhibit modifications associated with the various lifestyles; the benthic fish groups are generally elongated, cylindrical, and have inferior mouths with longer barbels and smaller eyes, while the non-benthic groups are either elongate and compressed or oblong and compressed and have superior or terminal-to-inferior mouths with short barbels and moderate or large eyes (Yang, 2005). These characteristics are directly linked to their living environment. At present, it remains unknown whether their origin and evolution were associated with some special paleo-environmental events (e.g. the formation of the monsoon climate; Liu et al., 2010).

Previous phylogenetic analyses have focused largely on the relationships among species of this subfamily (Yang et al., 2006; Liu et al., 2010; Tang et al., 2011), but rarely involved the analysis of origin and divergence of this group of fishes. To date, only Liu et al. (2010) estimated the divergence times and speciation tempo of this group based on mitochondrial cytochrome b sequences merely with two fossil records as calibration points.

To address the questions regarding the origin and evolution of Gobioninae fishes, we utilized whole mitochondrial genome sequences from 27 Gobioninae species, 22 other cyprinids, and two non-cyprinids as outgroups. This is the first time that a large number of whole mitochondrial genome sequences were used as molecular markers to analyze the relationships and divergence times of Gobioninae fishes, since the complete mitochondrial genome has been proven to give more reliable estimations in numerous phylogenetic analyses of vertebrate relationships (Saccone et al., 1999), and may improve the accuracy of divergence time estimates (Mueller et al., 2004).

### Materials and methods

#### Taxon sampling for mitochondrial genomes

We determined the complete mitochondrial genome for a Gobioninae fish, the *Abbottina obtusirostris*. The others were

retrieved from GenBank. Our current taxon sampling represented diversity in each of the major cyprinid lineages with at least two species per subfamily, thereby subdividing long branches according to the strategies of Hillis (1998) and Miya and Nishida (2000) for all except the monogeneric groups. In total, 27 species from 15 genera of the subfamily Gobioninae plus 22 species from other cyprinid subfamilies were included for phylogenetic analysis. Two species, *Carpio* *carpio* and *Myxocyprinus asiaticus*, were used as outgroups (Table 1) based on the current knowledge of the

Table 1  
Fish species used in the present analysis

Subfamily	Species	Accession No.
Gobioninae	<i>Biwia zezera</i>	AB250108
	<i>Coreius guichenoti</i>	JF906108
	<i>Coreius heterodon</i>	JF906110
	<i>Coreoleuciscus splendidus</i>	DQ347951
	<i>Gnathopogon elongatus</i>	AB218687
	<i>Gobio gobio</i>	AB239596
	<i>Gobiocypris rarus</i>	JN116719
	<i>Hemibarbus barbus</i>	AB070241
	<i>Hemibarbus labeo</i>	DQ347953
	<i>Hemibarbus longirostris</i>	DQ347952
	<i>Hemibarbus maculatus</i>	JF906109
	<i>Hemibarbus mylodon</i>	DQ345787
	<i>Microphysogobio alticorpus</i>	KC762939
	<i>Microphysogobio koreensis</i>	FJ515920
	<i>Pseudogobio esocinus</i>	AP009310
	<i>Pseudopungtungia nigra</i>	EU332752
	<i>Pseudopungtungia tenuicorpus</i>	FJ515917
	<i>Pseudorasbora parva</i>	JF802126
	<i>Pseudorasbora pumila</i>	AB239599
	<i>Pungtungia herzi</i>	AB239598
	<i>Sarcocheilichthys nigripinnis</i>	JX401522
	<i>Sarcocheilichthys parvus</i>	JX456224
	<i>Sarcocheilichthys variegatus microoculus</i>	AB054124
	<i>Gobiobotia macrocephala</i>	FJ515918
	<i>Gobiobotia naktongensis</i>	KC353467
	<i>Gobiobotia brevisbarba</i>	FJ515919
	<i>Abbottina obtusirostris</i>	This study
Tincinae	<i>Tinca tinca</i>	AB218686
	<i>Acheilognathus typus</i>	AB239602
Acheilognathinae	<i>Acheilognathus yamatsutae</i>	EF483936
	<i>Rhodeus uyekii</i>	EF483937
Leueiscinae	<i>Rhodeus ocellatus kurumeus</i>	AB070205
	<i>Ctenopharyngodon idella</i>	EU391390
	<i>Cyprinella lutrensis</i>	AB070206
	<i>Gila conspersa</i>	AP009315
	<i>Mylocheilus caurinus</i>	AP010779
Cultrinae	<i>Mylopharyngodon piceus</i>	EU979305
	<i>Aphyocypris chinensis</i>	AB218688
	<i>Ischikauia steenackeri</i>	AB239601
	<i>Opsariichthys bidens</i>	DQ367044
	<i>Zacco (Nipponocypris) sieboldii</i>	AB218898
Cyprininae	<i>Barbus barbus</i>	AB238965
	<i>Barbus trimaculatus</i>	AB239600
	<i>Carassius auratus</i>	EF483931
	<i>Cyprinus carpio</i>	AP009047
	<i>Labeo bata</i>	AP011198
Danioninae	<i>Labeo senegalensis</i>	AB238968
	<i>Danio rerio</i>	AC024175
Outgroup	<i>Esomus metallicus</i>	AB239594
	<i>Carpio carpio</i>	AY366087
Catostomidae	<i>Myxocyprinus asiaticus</i>	AY986503

phylogenetic relationships among cypriniform fishes (Mayden et al., 2009).

#### DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from fin tissue using the DNeasy Blood and Tissue Kit (Qiagen, Shanghai, China) following the manufacturer's protocol. We amplified the mitochondrial genome of *A. obtusirostris* in its entirety by polymerase chain reaction (PCR) (Miya and Nishida, 1999). The PCR reactions were conducted in a 50  $\mu$ l reaction volume containing 4  $\mu$ l dNTPs (2.5 mM), 5  $\mu$ l of 10 $\times$  buffer ( $Mg^{2+}$  free), 3  $\mu$ l  $MgCl_2$  (25 mM), 1  $\mu$ l of each primer (10  $\mu$ M), 0.2  $\mu$ l (2.5 U) of Taq DNA Polymerase (rTaq, TaKaRa, Dalian, China), 2  $\mu$ l template DNA, and 33.8  $\mu$ l  $H_2O$ . The reaction conditions were 2 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 50–60°C (depending on primer specificity), 1–4 min at 72°C, and a final extension at 72°C for 6 min. The PCR products were sequenced with the same primers offered by commercial sequencing companies. The complete mitochondrial genome sequence of *A. obtusirostris* obtained in the present study has been deposited in the GenBank under the accession number KF955012.

#### Sequence alignment

Contig assembly was performed using the program CONTIGEXPRESS (Invitrogen). Multiple alignments were performed with MAFFT version 6 with the default parameters (Katoh and Toh, 2008). The aligned sequences were imported into Gblocks\_0.91b (Castresana, 2000) to exclude divergent or ambiguously aligned regions from an alignment prior to phylogenetic analysis.

#### Phylogenetic analysis

Prior to analyses, the JMODELTEST 2.0.2 (Posada and Buckley, 2004) was used to ascertain the best-fit model of nucleotide substitution for the sequences with Bayesian information criterion. Two phylogenetic analysis methods, Bayesian analysis (BI) and maximum likelihood (ML), were used to reconstruct the phylogenetic trees. Bayesian phylogenetic analyses were conducted with MRBAYES 3.1.2 (Ronquist and Huelsenbeck, 2003) based on the most appropriate model estimated by JMODELTEST. Starting from a random tree, four Markov chains were run for 10 000 000 generations to estimate the posterior probability (PP) distribution (sampling one tree/1000 replicates for each run). After discarding the first 1000 trees as burn-in with non-stationary log likelihood values, 50% majority-rule consensus trees were estimated for the remaining trees. ML analysis was performed using the program PHYML 3.0 (Guindon et al., 2010) with the HKY model. Bootstrap values were calculated using 100 replicates to assess node support.

#### Estimation of divergence time

The tree from BI was used as the reference topology to perform the molecular dating analyses. We used a relaxed molecular clock method for dating analysis, which was

developed by Thorne and Kishino (2002), and selected the default F84 +  $\Gamma$  model. The MCMCTREE program allows for minimum (lower) and maximum (upper) time constraints, and multiple calibration points were used to provide overall realistic divergence time estimates (Benton and Donoghue, 2006); nine age calibration points (Table 2) were therefore used in the present study. A series of applications in the software package MULTIDISTRIBUTE were used for these analyses. BASEML in PAML 3.14 was used to estimate the model parameters for each partition separately under the F84 +  $\Gamma$  model of sequence evolution (the most parameter-rich model implemented in MULTIDISTRIBUTE). Based on the outputs from BASEML, branch lengths and the variance–covariance matrix were estimated using ESTBRANCHES in MULTIDISTRIBUTE for each partition. Finally, MULTIDIVTIME in MULTIDISTRIBUTE was used to perform Bayesian MCMC analyses to approximate the posterior distribution of substitution rates, divergence times, and 95% credible intervals. MCMC approximation with a burn-in period of 100 000 cycles was obtained and every 100 cycles were taken to create a total of 100 000 samples. To check for the convergence of MCMC, analyses were run from at least two different starting points.

#### Tracing character evolution

The evolutionary trends of several characteristics including habitat, body shape, mouth position, eye size, rows of pharyngeal teeth, and barbel lengths of Gobioninae fishes (Yang, 2005) were reconstructed based on the time tree, which was performed using MACCLADE 4.08a (Maddison and Maddison, 2005).

## Results

#### Mitogenome organization

As observed in most published vertebrate mitogenomes, the mitogenome of *A. obtusirostris* contains 13 protein-coding genes, two rRNA subunits, 22 tRNAs, and the putative control region. Their gene arrangements are identical to the typical gene order in vertebrates.

#### Phylogenetic analysis

The aligned data matrix used for analyses consisted of 14 889 base pairs for 51 taxa. Bayesian and ML phylogenetic

analyses yielded congruent and well-supported tree topologies, which recovered the monophyly of the subfamily Gobioninae, strongly supported by PP = 100 (Fig. 1). The phylogenetic position of Gobioninae closer to Acheilognathinae and Leueiscinae than Cyprininae was recovered with a significant PP = 100. Within Gobioninae, four monophyletic clades were identified with strong support values. *Hemibarbus* consists of the most basal fishes as an independent group and forms Clade A; *Gobio*, *Gobiobotia*, *Pseudogobio*, *Abbotina*, *Biwia*, and *Microphysogobio* were placed in Clade C; Clade D consists of *Pseudopungtungia*, *Coreoleuciscus*, *Gnathopogon*, *Gobiocypris*, *Sarcocheilichthys*, *Pungtungia*, and *Pseudorasbora*. The genus *Coreius* as a distinct group (Clade B) is a sister to Clades C and D. The sister group relationship between Clade C and Clade D was confidently recovered (PP = 100).

#### Estimation of divergence time

Based on the tree topology yielded by BI, the divergent times of the subfamily Gobioninae were estimated using a relaxed molecular clock method implemented in PAML/MULTIDIVTIME. The analysis indicated that Gobioninae originated in the early Paleocene (approx. 63.5 Mya) and that the basal *Hemibarbus* group diverged from the other gobionid fishes (approx. 58.3 Mya). In the early Eocene (approx. 54.6 Mya), the *Coreius* as an independent group began to diverge from the remaining two Gobioninae groups. The divergence time between Clade C (mentioned in the results of phylogenetic analysis) and Clade D was around 53.6 Mya (Fig. 2).

#### Tracing character evolution

Character evolution analysis of Gobioninae fishes suggested that as the most basal clade, all members of the *Hemibarbus* group are lower or medial dwellers, with an elongate body, laterally compressed in shape, subinferior mouth position, moderate eye size, short barbels, and three rows of pharyngeal teeth (vs two or fewer in other gobionines); these features represent the ancestral characters of Gobioninae fishes. The *Coreius* group of fishes, at the second primitive position, are bottom dwellers, with the body elongated and cylindrical in cross-section, inferior mouth position, short eye size, long barbels, and single rows of pharyngeal teeth, which makes it a distinct group. In Clade D, all elongate and laterally

Table 2  
Time constraints used for divergence time estimation

Node/constraint	Lower (most recent)	Upper (most ancient)	Note (Reference)
African <i>labeonines</i>	17.0		Oldest African <i>Labeo</i> -like fossil (Van Couvering, 1977)
<i>Mylopharyngodon wui</i>	13.8		Oldest known <i>Mylopharyngodon</i> (Chen and Arratia, 2010)
<i>Aphyocypris chinensis</i>	34.0		<i>Aphyocypris</i> -like fossil (Zhou, 1990)
Basal to <i>Hemibarbus</i>	5.3		<i>Hemibarbus barbatus</i> and <i>H. labeo</i> fossil from Late Miocene (Zhou, 1990)
Basal to <i>Gnathopogon</i>	5.3		<i>Gnathopogon macrocephala</i> fossil from Late Miocene (Zhou, 1990)
<i>Pseudorasbora</i>	3.0		Oldest <i>Pseudorasbora</i> -like (Young and Tchang, 1936)
Cypriniformes		94.9	From the result of Near et al., 2012
Basal to Gobioninae	33.9		Oldest Gobioninae fossil (Zhou, 1990)
<i>Gobio</i> sp.	13.8		Oldest <i>Gobio</i> -like fossil (Rutte, 1962)

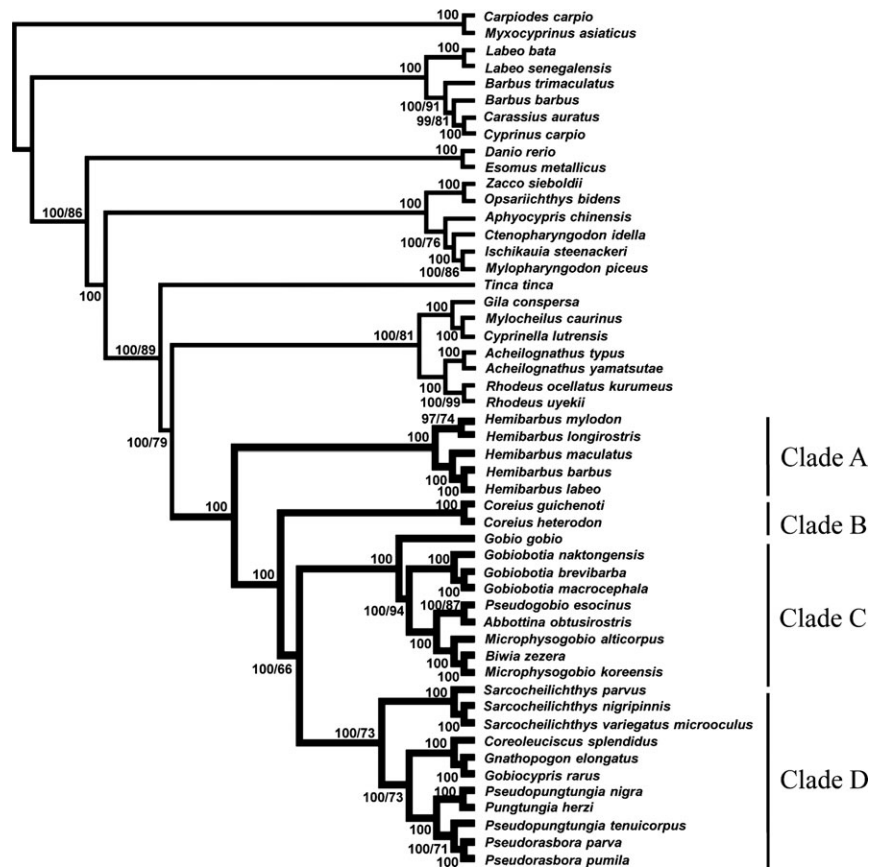


Fig. 1. Bayesian (BI) phylogram based on complete mitochondrial genomes sequences. Topology identical to Maximum likelihood (ML) analysis. Numbers at nodes = Bayesian posterior probabilities (left) and bootstrap support values (right) (only different numbers with BI label). Bolder branch = gobionid fishes

compressed species are medial dwellers with the exception of *Pseudopungtungia nigra*, *P. tenuicorpus*, and *Coreoleuciscus splendidus*; in Clade C, all elongate and cylindrical species are almost all bottom dwellers, except *Gobio gobio*. Species in Clades C and D have different characteristics in the mouth position, eye size, barbel length, and rows of pharyngeal teeth (Fig. 3). Overall, the body shape of Gobioninae evolved roughly from elongate and laterally compressed fishes (*Hemibarbus* group; lower or medial dwellers) to an elongate and cylindrical shape in cross-section (*Coreius* species; bottom dwellers). The body shape then evolved from elongate and cylindrical in cross-section in two directions: one retained the shape of elongate and cylindrical in cross-section (Clade C; bottom dwellers); the other evolved back to elongate and laterally compressed (Clade D; medial dwellers).

## Discussion

### Phylogenetic analysis

With the whole mitogenomic data, the phylogenetic analyses yielded well-resolved trees for the subfamily Gobioninae, supporting the notion that large datasets demonstrate strong phylogenetic resolving power (Rogers, 2001). Our results support the view that Gobioninae is a monophyletic subfamily within Cyprinidae, as described previously (Bănărescu and Nalbant, 1973; Bănărescu, 1992; Yang et al., 2006; Liu

et al., 2010; Tang et al., 2011). Regarding its sister group, our results demonstrate that Gobioninae is closely related to the clade (Acheilognathinae plus partial Leuciscinae species) that is similar to the Bayesian and ML topologies described by Tang et al. (2011), who suggested that Acheilognathinae is the sister group of Gobioninae.

With regard to the phylogenetic relationships among Gobioninae fishes, our results are largely in agreement with those of Tang et al. (2011), except the position of the *Coreius* clade. The genus *Hemibarbus* is the most basal of Gobioninae fishes as an independent group, sister group to the remaining gobionines, and is thought to be the primitive group of Gobioninae. These features were widely supported by both morphological characteristics and molecular data (Bănărescu and Nalbant, 1965; Yang et al., 2006; Liu et al., 2010; Tang et al., 2011). The genus *Coreius*, which is at the second primitive position, was a sister group to the remaining groups, except for the *Hemibarbus* group with a strong PP = 100. The position of this genus was unresolved in studies conducted by Yang et al. (2006) and Tang et al. (2011). Bănărescu (1992) proposed a distinctive group for *Coreius* alone based on its features such as small eyes, long barbels, smooth lips, and molariform teeth (Bănărescu and Nalbant, 1965; Bănărescu, 1992). Hence, further work is needed to confirm the phylogenetic position of this genus. In Clade C, *Gobiobotia* was monophyletic due to its various specializations, located at the basal

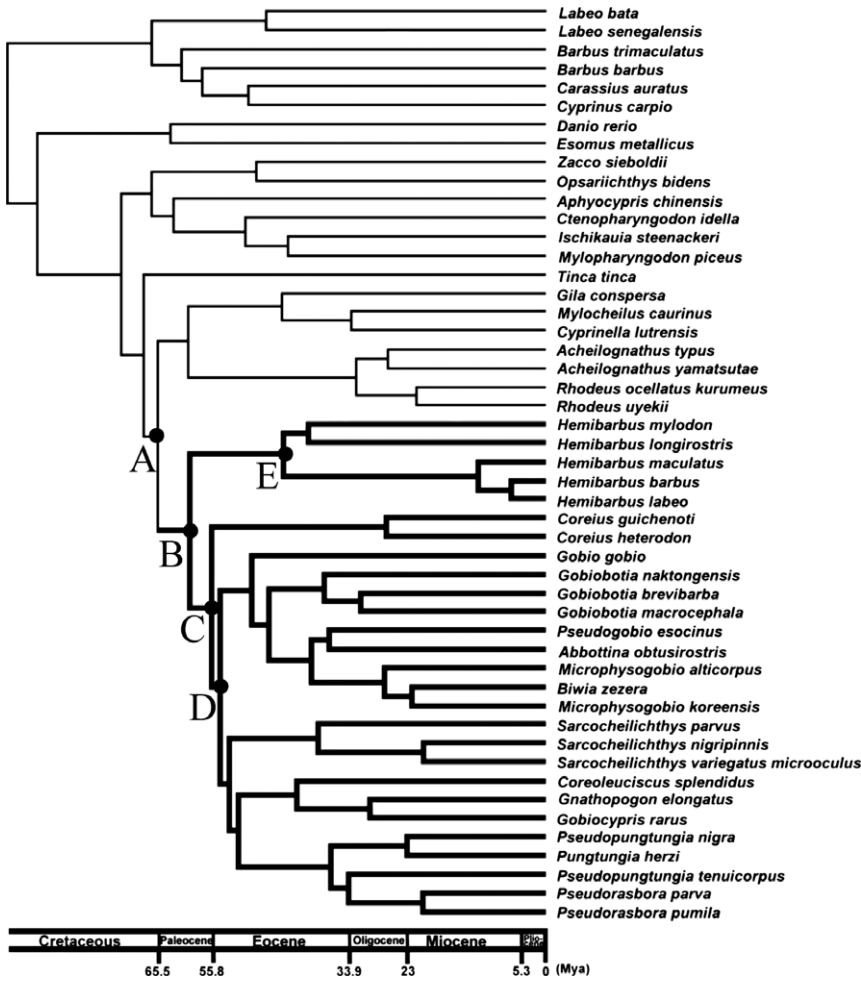


Fig. 2. Timetree derived from Bayesian relaxed-molecular clock method (without outgroups). Nine nodes total used for time constraints (details in Table 2). Bolder branch = gobionid fishes. Black dots = origin and key divergence times of gobionines. A. = Gobioninae originated in the Early Paleocene (about 63.5 Mya); B. = time when basal *Hemibarbus* group diverged from other gobionid fishes (about 58.3 Mya); C. = *Coreius* group began to diverge from remaining groups (about 26.5 Mya); D. = divergence time of the two groups in Clade D (about 53.6 Mya); E. = species separation occurred after 43.1 Mya

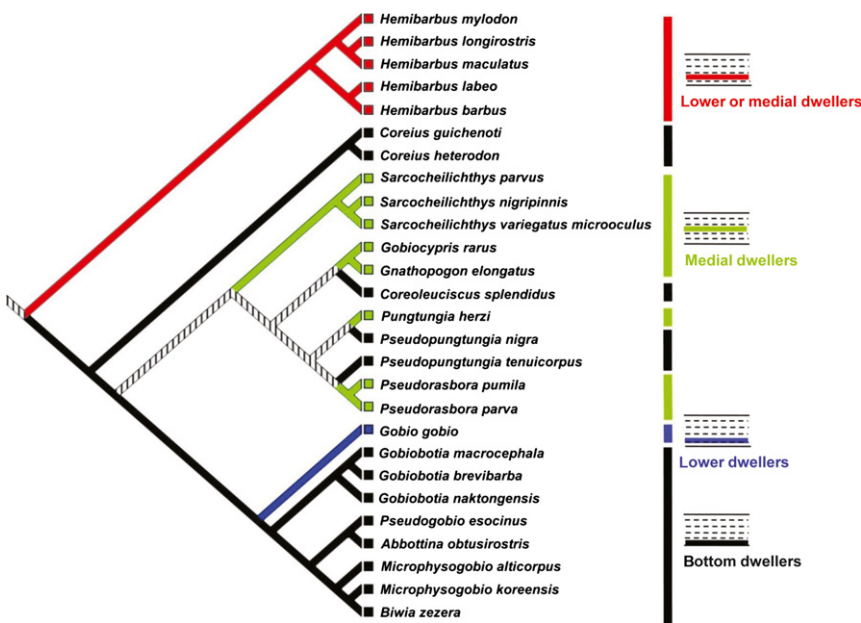


Fig. 3. Evolutionary trend of Gobioninae habitat based on timetree using MACCLADE 4.08a (Maddison and Maddison, 2005). Red = lower or medial dwellers; blue = lower dwellers; black = bottom dwellers; and yellow = medial dwellers. This suggests that the basal group of gobionines was lower or medial dwellers, then most became bottom dwellers; lower dwellers and medial dwellers recurred during the Eocene and through the Oligocene (Fig. 2)

position and isolated from the other members of this clade, with PP = 100 in this study, but it was uncertain in the studies by Yang et al. (2006) and Tang et al. (2011). *G. brevibarba* was sister to *G. macrocephala*. *Abbottina* was sister to *Pseudogobio* and was deemed to belong to Clade C within Gobioninae. In addition, both *Abbottina* and *Pseudogobio* have free swim bladders. *Abbottina* was recovered as a *Pseudogobio* group within Gobioninae (PP = 100), which is consistent with the results of Tang et al. (2011). *Microphysogobio* was non-monophyletic perhaps due to the deficiency of specimens and markers. The results of the study by Tang et al. (2011) were ambiguous on the phylogeny of this genus based on ML, MP, and BI analyses. Therefore, additional work is required to resolve this uncertainty.

#### Divergence time

Before the present study, there were a few studies that provided time estimates for the major splits of Gobioninae (Liu et al., 2010). Our age estimation (approx. 63.5 Mya for the split of Gobioninae from other cyprinids) fell approximately at the lower limit of the confidence range as reported in the study by Saitoh et al. (2011), which estimated the split of gobionines/(tinca + leuciscines) to be 69.4–99.4 Mya based on the mitogenomes of seven gobionines. The divergence time is much earlier than the oldest Gobioninae fossil record dated back to Oligocene (33.9 Mya) because the fossil record usually implied the latest origin time of the species. However, due to limited sampling, Saitoh et al. (2011) did not mention the intergeneric or interspecific divergence times of Gobioninae. Time estimates by Liu et al. (2010) were based solely on mtDNA cytochrome b gene sequences from 59 Gobioninae fishes, calibrated using the fossil records of *Palaeogobio zhongyuanensis* appearing about early–middle Eocene (50 Mya) and of the genus *Pseudorasbora* (3 Mya). According to their molecular clock estimates, *Hemibarbus* as the basal group of Gobioninae diverged from the other groups at approx. 29.3 Mya (early Oligocene), then approximately at 27.1–25.8 Mya, the remaining groups successively departed from the others. However, our age estimates are remarkably older than those indicated in the study by Liu et al. (2010). The basal gobionine divergence estimated here was at 58.3 Mya, and the divergence of the other major clades occurred before the Oligocene (>34 Mya) (Fig. 2). The present study is the first to use nearly whole mitochondrial genome markers to estimate the divergence time of Gobioninae. The discrepancy between molecular estimates and fossil records may either be a result of the incompleteness of the fossil record, uncertainty in molecular dating involving remote calibration points, or both (Saitoh et al., 2011). On the other hand, the reliable calibration point is another key factor in divergence time estimation. In our study, seven calibration points, which were almost evenly distributed within the in- and out-groups, were selected (Table 2). Among them, the newly discovered fossil record of *Mylopharyngodon* (middle Miocene) implied that cyprinids might have a more ancient ancestor (Chen and Arratia, 2010).

#### Tracing character evolution

As mentioned in the results, Gobioninae fishes originated at approx. 63.5 Mya (early Paleocene). According to the classification of the world's freshwater ichthyofauna by Zhang et al. (2001), we propose a hypothesis that the origin of Gobioninae occurred after the transgressive event in the late Cretaceous in order to adapt to the unique East Asian climate and environment (Zhang, 2000), and that the divergence time of the four primary clades was during the early Paleocene to early Eocene. Nevertheless, there is evidence that faunal and environmental changes occurred across the Paleocene–Eocene boundary at approx. 55.8 Mya in Tibet. The Cretaceous–Paleocene boundary (approx. 65.5 Mya) was a time of particularly swift change due to one of the three largest mass extinctions (Schulte et al., 2010). Currently the two major hypotheses explaining the mass extinctions are either a large meteorite impact or a widespread volcanism. Whatever the cause, the quantities of water, dust, and climate-forcing gases would have drastically altered the climate system (Smit, 1990), which possibly yielded numerous novel habitats facilitating diversification of the fish located in the freshwater system. It is during this time that some great changes occurred in the habitats as well as in the habitat-related morphological characteristics in gobionid fishes. For example, the basal clade of this group is lower or medial dwellers (Fig. 3), and most of them changed to bottom dwellers during the Paleocene–Eocene, which may be attributable to the deterioration of the environment. Dust clouds may have suppressed sunlight, reducing photosynthesis on a global scale (Okada, 2000), which might have led to the decline of plants and algae debris as one of the food sources for lower or medial dwellers. However, for bottom dwellers, the primary source of food is biological organic debris, which would have been only slightly influenced by extinction events (Sheehan and Fastovsky, 1992). Furthermore, there is a more stable environment at the bottom to prevent fishes from being affected by large changes in the environment (light rays, temperature, carbon monoxide concentrations, etc.) (Smit, 1990). All of these factors might have promoted the gobionid fishes to evolve as bottom dwellers; the associated morphological characteristics also alternated to accommodate the benthic environment, such as most of them having an elongate and cylindrical body shape, inferior mouth position, and moderate eyes, rather than the elongate and compressed body shape, subinferior mouth position, and short barbels similar to the lower or medial dwellers. Subsequently, Gobioninae fishes began to diversify into each of the four clades during the Eocene and the early Miocene (Fig. 2), a period roughly corresponding to the beginning of the uplift of the Tibetan Plateau (Li and Fang, 1999; Tapponnier et al., 2001). These geological events produced a diversified monsoon climate in East Asia and brought about a series of eco-environmental effects (Zhang et al., 2000), which facilitated the diversification of gobionines including the recurrence of medial dwellers (Fig. 3). Further work is still warranted to clarify the cause of diversification of this group of fishes with additional comprehensive sampling and more genetic markers.

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