Article **Chinese Science Bulletin**

Ecology

November 2013 Vol.58 No.31: 3738–3746
doi: 10.1007/s11434-013-5878-z

Molecular phylogeny of European and African *Barbus* and their West Asian relatives in the Cyprininae (Teleostei: Cypriniformes) and orogenesis of the Qinghai-Tibetan Plateau

WANG Jing[1†], WU XiaoYun[1,2†], CHEN ZiMing[3†], YUE ZhaoPing[3], MA Wei[1],
CHEN ShanYuan[1], XIAO Heng[3], MURPHY Robert W[2,4], ZHANG YaPing[1,2],
ZAN RuiGuang[3*] & LUO Jing[1*]

[1] *Laboratory of Conservation and Utilization of Bio-resources and Key Laboratory for Animal Genetic Diversity and Evolution of High Education in Yunnan Province, School of Life Sciences, Yunnan University, Kunming 650091, China;*

[2] *State Key Laboratory of Genetic Resources and Evolution and Yunnan Laboratory of Molecular Biology of Domestic Animals, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China;*

[3] *School of Life Sciences, Yunnan University, Kunming 650091, China;*

[4] *Centre for Biodiversity and Conservation Biology, Department of Natural History, Royal Ontario Museum, 100 Queen's Park, Toronto, ON, M5S 2C6, Canada*

Received January 8, 2013; accepted March 18, 2013; published online July 25, 2013

The phylogenetic relationships of European and African *Barbus* and their West Asian relatives in Cyprininae remain largely unresolved. Consequently, little is known about the drivers of their evolution, including the possible association of uplifting of the Qinghai-Tibetan Plateau (QTP) with the early divergence of the subfamily. We use complete sequence data of the mitochondrial DNA gene encoding the protein cytochrome *b* (*Cytb*) to hypothesize the phylogeny of 85 species belonging to 47 genera in the Cyprininae plus 6 species from the Leuciscinae. We employ 6 other species from Cypriniformes as outgroup taxa and estimate divergence times. Our results indicate that European *Barbus sensu stricto* lineage including *Aulopyge* shares a common ancestor with specialized and highly specialized schizothoracins and the genera *Cyprinion* and *Scaphiodonichtys*. The common ancestor appears to have originated in the Qinghai-Tibetan Plateau (QTP) region about 19.4–17.8 Ma. *Barbus sensu stricto* lineage appears to have originated about 16.6–15.5 Ma. Small to medium sized African *Barbus sensu lato* appear to have had an Oriental origin about 19.1–15.3 Ma and are closely related to Asian *Puntius*. West Asian *Carasobarbus* lineage including large African *Barbus sensu lato* might have originated about 9.94 Ma, also in Oriental Realm and has a close relationship to Asian *Neolissochilus* and *Tor*. The large-sized *Barbus sensu lato* appear to have diverged from *Carasobarbus* about 7.7 Ma. Finally, the Cyprininae appear to have radiated rapidly into nine lineages and many sublineages from about 27.8 to 17.8 Ma, close to the time of the second-stage tectonic movements of the QTP. Our analyses provide evidence that the uplifting of the QTP drove early diversification of the Cyprininae. Our extensive sampling of species involving all of the important areas results in clear evolutionary scenario for the Cyprininae.

***Barbus*, cyprininae, molecular clock, Qinghai-Tibetan Plateau**

Citation: Wang J, Wu X Y, Chen Z M, et al. Molecular phylogeny of European and African *Barbus* and their West Asian relatives in the Cyprininae (Teleostei: Cypriniformes) and orogenesis of the Qinghai-Tibetan Plateau. Chin Sci Bull, 2013, 58: 3738–3746, doi: 10.1007/s11434-013-5878-z

The Cyprininae *sensu* Cavender and Coburn [1] contains three tribes: Cyprinini, Labeonini, and Barbini (including the schizothoracines). It is one of the two largest lineages within Cyprinidae and was named "series Barbini" by some Chinese authors [2–4]. The subfamily has an extensive distribution in Eurasia and Africa and its members share similar morphological characteristics, such as the third neural

---

†These authors contributed equally to this work.
*Corresponding authors (email: zanruiguang@gmail.com; jl.jingluo@gmail.com)

spine being a single plate without a dissociative (unbounded) supraneural and the anal fin having five branched rays. Morphology indicates monophyly of this group of fishes [4].

Genus *Barbus*, one of numerous genera forming the Cyprininae, once included several hundred species spread widely across Asia, Europe, and Africa. Thought to be a polyphyletic assemblage of species, it was often referred to as "*Barbus sensu lato*" [5], hereafter termed "barbs". Nowadays, Asian species of barbs are in other genera (e.g. *Puntius*, *Acrossocheilus*, *Tor*) except for West Asian forms [6–8]. In Europe, all species of barbs and some species from North Africa and West Asia are in *Barbus sensu stricto* including subgenera *Barbus* and *Luciobarbus* [6,9–12]. The genera *Aulopyge* and *Capoeta* belong to the *Barbus sensu stricto* lineage and the *Barbus sensu stricto* respectively [9,13]. Africa barbs comprise 60.5% of the 477 African cyprinids [14] and these occur in two groups: large size and small–medium size [5,13,14]. The large fishes now belong to West Asian *Carasobarbus* lineage and West Asia now has appears to have one species only of barbs [13].

Taxonomists do not present consistent viewpoints on the relationships of species of Euro-Mediterranean *Barbus* and African barbs, as well as for Cyprininae from China, Southeast Asia, and South Asia [6,15]. Previous molecular phylogenetic studies of Cyprinidae and Cypriniformes evaluate too few species to obtain an overall suite of relationships for the group, though these studies achieve their major objectives [16–34].

Herein, we hypothesize the evolutionary relationships of European and African *Barbus*, their West Asian relatives, and Cyprininae in China, Southeast Asia, and South Asia. We assess 85 species belonging to 47 genera of Cyprininae as well as six species of Leuciscinae and five outgroup taxa. We estimate divergence times and investigate the biogeography of *Barbus*. We explore the possibility that orogenesis of the Qinghai-Tibetan Plateau (QTP) drove the early radiation of the Cyprininae. To maximize taxonomic representation, we employ data from the mitochondrial DNA (mtDNA) gene encoding cytochrome *b* (*Cytb*) because this marker is widely used in phylogenetic studies of these fishes and it is a useful marker for studying higher-level relationships of teleosts [10,13,16,19,20,35–39].

1 Materials and methods

1.1 Sample collection and DNA extraction

Thirty one species were sampled *de novo* mainly from Yunnan, China (Table 1). Tissue samples were preserved in 95% ethanol and voucher specimens were deposited in the Zoological Museum of Yunnan University. Total DNA was extracted from muscle tissues using the standard phenol-chloroform extraction method. Sequences of *cytb* from 67 species were downloaded from GenBank (Accession numbers were listed as Table 1).

1.2 PCR amplification and sequencing

The complete sequences of *Cytb* were amplified with the primers L14724, L14737, and H15915 [33,40]. PCR amplifications were carried out in 50 μL reaction mixture containing 5 μL 10×PCR buffer (TaKaRa, Japan), 0.2 mmol L^{-1} dNTPs, 0.2 μmol L^{-1} each primer, with 1.5 U *Taq* DNA polymerase (TaKaRa) and approximately 50–100 ng genomic DNA. Reactions involved 33 cycles, each including denaturation at 94°C for 1 min, annealing at 52°C for 1min, and extension at 72°C for 1 min. PCR products were purified with Gel Extraction Mini Kit (Waston BioTechnologies). PCR products were sequenced in an ABI Prism 3730 (Applied Biosystems) automatic sequencer. Sequencing used both the PCR primers and internal primers (L15138, L15286, L15519, H15374, and H15560) [40,41].

1.3 Data analysis

All sequences were aligned using the MegAlign implemented in DNAStar 6.0 (DNASTAR, Madison, USA). The nucleotides were translated to amino acids to confirm the absence of pseudogenes and for alignment. Polymorphism assessments were made using MEGA 4 [42].

Phylogenetic reconstructions were performed using maximum parsimony (MP) as implemented in PAUP* 4.0b10 [Swofford], maximum likelihood (ML) by RAxML [43], and Bayesian inference (BI) using MrBayes 3.2 [44]. We selected the best-fitting models for ML and BI analyses using likelihood ratio tests as implemented in jModelTest 0.1.1 [45–49]. In the BI analysis, four independent Markov Chain Monte Carlo (MCMC) chains were simultaneously run for 5000000 generations while sampling one tree per 500 replicates, Burnin = 0, and Burninfrac = 0.10, 0.20, 0.30, 0.40, and 0.50, with two runs conducted independently. Sampled trees were used to construct a 50% majority rule consensus tree. The frequency of nodal resolution, termed a Bayesian posterior probability (BPP), was mapped on the BI tree. For MP and ML analyses, nodal support was assessed using nonparametric bootstrapping of 1000 pseudoreplicates [50].

1.4 Estimated divergence times

We used BEAST 1.5.3 to estimate divergence times under a relaxed clock with branch-specific rates following a lognormal distribution [51,52]. Four time-points were used to calibrate clock dates. A date of 55.8–40.4 Ma was used for estimating divergence of the Cyprinidae from the outgroup taxa based on Cavender [53], who believed the oldest cyprinid fossils were from the Middle Eocene. The second calibration point was 37.2–28.4 Ma for divergence of Cyprininae and Leuciscinae also based on Cavender [53], who

Table 1 The Taxa used in this study

Taxon[a)]	Common name	Locality	Accession No.
Subfamily Cyprininae			
Tribe Barbins			
Puntius ticto ♣	Ticto barb or Firefin barb	Ruili, Yunnan, China	KC696520
Puntius ticto	Ticto barb or Firefin barb	Southeast Asia	NC008658
Puntius tetrazona	rosy barb or puthi	Southeast Asia	NC010110
Puntius conchonius	rosy barb or puthi	Southeast Asia, South Asia	AY004751
Puntius semifasciolatus ♣	Chinese barb or Gold barb	Luoping, Yunnan, China	KC696521
Puntius semifasciolatus	Chinese barb or Gold barb	Guangxi, China	AY856116
Capoeta capoeta	Seven khramulya or Transcaucasian barb	West Asia, Armenia (the genus also in Europe)	AF145951
Poropuntius huangchuchieni *♣	–	Menglun, Yunnan, China	KC696522
Poropuntius opisthoptera *♣	Parmička zadoploutvá	Ximeng, Yunnan, China	KC696523
Neolissochilus wynaadensis *♣	Parmička keralská	Baoshan, Yunnan, China	KC696524
Neolissochilus hexagonolepis *♣	Boka or Copper mahseer	Longchuan River, Yunnan, China	KC696525
Neolissochilus benasi *♣	Parmička Benasova	Yuanjiang, Yunnan, China	KC696526
Neolissochilus benasi *♣	Parmička Benasova	Yuanjiang, Yunnan, China	KC696527
Hypsibarbus vernayi *♣	Parma Vernayova	Mengla, Yunnan, China	KC696528
Hypsibarbus pierrei *♣	Parma thajská	Mengla, Yunnan, China	KC696529
Barbodes carnaticus	Carnatic carp or Carnatica carp	Southeast Asia	HM010725
Barbonymus gonionotus	Java barb or Tawes	Southeast Asia	NC008655
Barbus barbus	Barbel	Europe, France	AB238965
Barbus cyclolepis	Parmička kruhošupinná	Greece, Turkey	AY026412
Barbus meridionalis	Mediterranean barbel	French, Spain	AF045977
Barbus callensis	Algerian barb	Algeria	AF045974
Barbus capito	Bulatmai barbel or Turkestan barbel	Caucasia	AF045975
Barbus bocagei	Barbo do Norte	Spain	AF112125
Barbus albanicus	Parma albánská	Greece	AF090779
Barbus intermedius	Baringo barb	Ethiopia	AF112406
Barbus trimaculatus	Threespot barb	South of Sahara in Africa	AB239600
Barbus grypus	Shabbout	Tigris R., Turkey	AF145945
Barbus andrewi	Cape Whitefish or Whitefish	South Africa	AF180843
Barbus reinii	Parma Reinova	Morocco	AF145946
Barbus bigornei	Parmička Bigornova	Guinea	AY004752
Barbus anoplus	Chubbyhead barb	South Africa	AF112405
Barbus fasciolatus	African banded barb or Orange barb	The Central African Republic	HM536811
Tor sinensis ♣	Trey khaor	Menglun, Yunnan, China	KC696530
Tor douronensis ♣	Semah mahseer or River carp	Jinghong, Yunnan, China	KC696531
Spinibarbus denticulatus yunnanensis ♣	Ostropysk junanský	Fuxian Lake, Yunnan, China	KC696532
Spinibarbus denticulatus yunnanensis ♣	Ostropysk junanský	Fuxian Lake, Yunnan, China	KC696533
Acrossocheilus yunnanensis ♣	Ostropysk junanský	Luoping, Yunnan, China	KC696534
Acrossocheilus yunnanensis ♣	Ostropysk junanský	Luoping, Yunnan, China	KC696535
Acrossocheilus iridescens yuanjiangensis ♣	Ostropysk hajnanský	Jiangcheng, Yunnan, China	KC696536
Acrossocheilus iridescens yuanjiangensis ♣	Ostropysk hajnanský	Jiangcheng, Yunnan, China	KC696537
Sinocyclocheilus grahami	Golden-line barbel	Dianchi, Yunnan, China	GQ148557
Sinocyclocheilus altishoulderus	–	Guangxi, China	FJ984568
Sinocyclocheilus jii	Gara Jiova	Guangxi, China	AY854728
Sikukia flavicaudata ♣	–	Simao, Yunnan, China	KC696538
Sikukia flavicaudata ♣	–	Menglun, Yunnan, China	KC696539
Sikukia stejnegeri ♣	Parmička Stejnegerova	Menghan, Yunnan, China	KC696540
Mystacoleucus marginatus ♣	Trey tim proeak	Mengla, Yunnan, China	KC696541
Mystacoleucus lepturus ♣	Palang junanský	Mengla, Yunnan, China	KC696542
Mystacoleucus lepturus ♣	Palang junanský	Mengla, Yunnan, China	KC696543
Cosmochilus cardinalis ♣	Squared-jaw barbel	Mekong River, Yunnan, China	KC696544
Hampala macrolepidota ♣	Hampala barb or Grooved-isthmus barbel	Menglun, Yunnan, China	KC696545
Percocypris pingi pingi ♣	Parma Pingova	Jingsha River, Yunnan, China	KC696546
Varicorhinus beso	Chramule východoafrická	Ethiopia	AF180862
Onychostoma gerlachi ♣	Chramule Gerlachova	Mengla, Yunnan, China	KC696547

(*To be continued on the next page*)

(*Continued*)

Taxon	Common name	Locality	Accession No.
Onychostoma gerlachi ♣	Chramule Gerlachova	Mengla, Yunnan, China	KC696548
Cyprinion macrostomus	Parmoušice velkoústá	Tigris R., Turkey (the genus main in Indus basin)	AF180826
Kosswigobarbus kosswigi	Parmička Kosswigova	Tigris R., Turkey	AF180853
Carasobarbus canis	Binit gdolat kaskas	Tiberias Laike, Israel	AF145947
Carasobarbus luteus	Himri	Euphrates R, Turkey	AF145944
Aulopyge hyegelii	–	Europe, Dalmatia	AF112133
Scaphiodonichthys acanthopterus ♣	Parmánka trnoploutvá	Mengla, Yunnan, China	KC696549
Scaphognathops stejnegeri	Keltapyrstökiekkobarbi	Southeast Asia	HM536807
Scaphognathops bandanensis	Ankat prak	Southeast Asia	HM536828
Puntioplites waandersi	Kepiat	Southeast Asia	HM536829
Puntioplites proctozysron	Serrated anal-spine carp or Smith's barb	Southeast Asia	HM536813
Probarbus jullieni	Isok barb	Southeast Asia	HM536810
Catlocarpio siamensis	Giant barb	Southeast Asia	HM536812
Tribe Labeonins			
Linichthys laticeps *	Parmička široká	Zhenning, Guizhou, China	AY854739
Sinilabeo laticeps ♣	Labeo šanské	Menglun, Yunnan, China	KC696550
Gara pingi pingi ♣	–	Menglun, Yunnan, China	KC696551
Discogobio tetrabarbatus ♣	–	Yunnan, China	KC696552
Discogobio tetrabarbatus ♣	Hrouzek čtyřvousý	Yunnan, China	KC696553
Labeo senegalensis	African carp	Africa	AB238968
Labeo batesii	Labeo Batesovo	Africa	AB238967
Cirrhinus microlepis	Small scale mud carp	Southeast Asia	HM536825
Tribe Schizothoracins			
Schizothorax lissolabiatus ♣	Marinka říční	Yunnan, China	KC696554
Schizothorax chongi	Marinka sečuánská	Min River, Sichuan, China	AY095325
Schizothorax argentatus	Balkhash marinka	Lli River, Kazakhstan	AY954269
Schizothorax prenanti	Marinka Prenantova	Min River, Sichuan, China	GQ466605
Gymnocypris przewalskii	Osman Przewalského	Qinghai Lake, China	AB239595
Gymnocypris namensis	Osman namenský	Nam Lake, Xizang, China	DQ309353
Ptychobarbus chungtienensis	Heavy lips fish or Chungtien schizothoracin	Zhongdian, Yunnan, China	AY403506
Chuanchia labiosa	Huanghe naked carp	Huang River, China	FJ601012
Platypharodon extremus	Wide-tooth Schizothoracin	Maqu, Gansu, China	FJ601029
Schizopygopsis pylzori	–	Linxia, Gansu, China	FJ601005
Schizopygopsis malacanthus malacanthus	–	Ya-lung River, Jingsha River, China	DQ533794
Oxygymnocypris stewartii	Naked schizothoracin	Lasa, Xizang, China	DQ491114
Gymnodiptychus integrigymnatus	Osman junanský	Mekong River, Yunnan, China	FJ601041
Tribe Cyprinins			
Procypris rabaudi	Rock carp	Mudong, Chongqing, China	NL011192
Procypris merus ♣	Chinese-ink carp	Xijiang, Guangxi, China	KC696555
Cyprinus carpio	Aischgrund carp or Common carp	Taiwan, China	X61010
Cyprinus multitaeniata ♣	Kapr mnohopruhý	Guangxi, China	KC696556
Cyprinus multitaeniata ♣	Kapr mnohopruhý	Guangxi, China	KC696557
Carassius carassius	Cyprin or Golden carp	Xinjiang, China	AY714387
Carassius auratus langsdorfi	Gin-buna	Japan	AB006953
Carassoides cantonensis ♣	Karasovec kantonský or Cá Nhung	Qinzhou, Guangxi, China	KC696558
Subfamily Leuciscinae			
Zacco platypus	Freshwater minnow	Japan	AY958194
Ctenopharyngodon idellus	Grass carp or White amur	Yangtze River, China	NC010288
Distoechodon tumirostris	Jeleček čínský	Yangtze River, China	NC011208
Megalobrama terminalis	Black Amur bream	Yangtze River, China	AF475156
Rutilus rutilus ♣	Roach	Save River, Europe	KC696559
Alburnus alburnus	Bleak	Europe	NC008659
Outgroups			
Gyrinocheilus aymonieri	Siamese algae eater or Sucker loach	Southeast Asia	NC008672
Leptobotia mantschurica	Manchurian spiny loach	Russia, Amur	AB242170
Vaillantella maassi	Nauhanuoliainen	Southeast Asia	NC008680
Carpiodes carpio	River carpsucker	Mississippi River	NC005257
Myxocyprinus asiaticus	Chinese sucker	Yangtze River, China	NC006401

a) ♣ Sequences new determined. * Chinese *Barbodes* species revised by Chen and Yang (2003) and Zhang and Fang (2005) [58,59].

concluded "by early Oligocene times, representatives of the two major Cyprinid phyletic groups (Leuciscinae and Cyprininae) were in evidence". The third calibration point was 8.1±0.4 Ma for divergence of subgenera *Barbus* and *Luciobarbus* [10]. The fourth and final calibration point was 5.0 ±0.3 Ma for the divergence of Iberian and African species of subgenus *Luciobarbus* [10].

2 Results

2.1 Phylogeny

Cyprininae was monophyletic and this was strongly supported in our analyses (BPP = 100%, ML = 97%). The group contained nine lineages (Figure 1; MP not shown), of which lineages I, II, III, IV, VII, and IX corresponded to clades 3, 2, 1, 6, 7, and 4, reported by Yang et al. [34], respectively, who did not assay West Asian *Carasobarbus* and large African and West Asian barbs.

Lineage I (BPP=100%) contained sublineages I-1 and I-2 (Figure 1). Sublineage I-1 contained Southeast and South Asian *Barbodes carnaticus*. Sublineage I-2 contained four small lineages. From Yunnan, two species of *Tor* formed lineage I-2a, two species of *Neolissochilus* comprised lineage I-2b, and two individuals of *N. benasi* formed I-2c. Large African barbs (including *Varicorhinus beso*), West Asian *Carasobarbus* plus *Kosswigobarbus kosswigi* and *Barbus grypus* formed lineage I-2d, which was *Carasobarbus* lineage of Durand et al. [13].

Major lineage IV (BPP = 100%) contained four sublineages. *Hampala macrolepidota* (IV-1) from Yunnan of Southwest China was the sister group of the other sublineages. Sublineage IV-2 was comprised of *Puntius tetrazona* from Southeast Asia. The two species of *Puntius* from Yunnan and Southeast Asia formed sublineage IV-3, and the small–medium sized African barbs comprised IV-4. Lineage VI (BPP = 95%) consisted of *Sinocyclocheilus* from Southwest China.

Lineage IX (BPP = 97%) was the largest group. Relationships among its four sublineages were not well-resolved. *Spinibarbus yunnanensis* (IX-1) formed the sister group to the other sublineages followed by IX-2 (*Schizothorax* from highland Asia and *Percocypris pingi pingi* from Yunnan; BPP = 100%), and then IX-3 (BPP = 100%) (*Acrossocheilus* and *Onychostoma* from Yunnan) and IX-4 (BPP = 94%). The latter group contained *Barbus sensu stricto*, *Aulopyge*, *Capoeta*, *Cyprinion*, from Europe, West Asia, and North Africa, *Scaphiodonichthys acanthopterus* from Yunnan, and specialized and highly specialized, high-elevation Asian schizothoracins [36,37,54]. This sublineage further divided into four groups (IX-4a, IX-4b, IX-4c, and IX-4d). Lineage IX-4d (BPP = 100%, ML = 100%) consisted of *Barbus sensu stricto*, *Aulopyge hyegeilii*, and *Capoeta capoeta*; it corresponded to the *Barbus sensu stricto* lineage of Tsigenopoulos and Berrebi [9].

2.2 Estimated divergence times

The common ancestor of Cyprininae originated about 32.3 Ma (Figure 1). Small-medium sized African barbs originated about 19.1 Ma. The common ancestor of large sized African barbs (including *Varicorhinus beso*) and West Asian *Carasobarbus* originated about 9.9 Ma. Divergence of African large barbs from West Asian *Carasobarbus* occurred about 7.7 Ma. Sublineage IX-4 was estimated to have originated about 19.4–17.8 Ma. European *Barbus sensu stricto* lineage may have originated about 16.6–15.5 Ma. Radiation of Cyprininae into its nine clades and subclades took place from about 27.8 to 17.8 Ma.

3 Discussion

We do not discuss the species and topologies of lineages II, III, V and VIII (Figure 1) because they are not the focus of this work. The species are important for confirming relationships of our targeted taxa.

3.1 Relationship of European and African *Barbus* to Asian Cyprininae

European *Barbus sensu stricto* lineage (including *Aulopyge* and *Capoeta*) and Asian *Cyprinion*, *Scaphiodonichthys* and specialized and highly specialized schizothoracins have a close relationship because they together from the sublineage IX-4 (Figure 1). Howes [15] stated "the barbin lineage (i.e., *Barbus sensu stricto*) may be more closely related to the schizothoracins than to any of the other so far identified". Banarescu and Coad [6] also stated *Barbus* "appears to be related to the East Asian *Sinocyclocheilus* and High Asian Schizothoracini" [15]. Our results support these hypotheses to a certain extant (Figure 1). Many previous molecular trees displayed close relationships between European *Barbus* and schizothoracins. Differences among relationships owe mainly to sampling strategy, and in particular the absence of critical samples [13,20–24,26,27,30–34]. Our study, the first to provide detailed relationships for these fishes (Figure 1).

Our tree depicts divides African barbs into large and small–medium sized fishes and this is congruent with previous morphological and molecular studies [5,13,14]. Large sized African barbs (including *Varicorhinus beso*) belong to West Asian *Carasobarbus* lineage (including *Kosswigobarbus*) [13]. Our results closely associate this lineage with *Neolissochilus* and *Tor* from Southwest China, Southeast Asia, and South Asia (Figure 1). Previous molecular studies did not reveal this relationship owing to the absence of critical species, such as *Carasobarbus* and large sized barbs, and/or *Neolissochilus* and *Tor* [9,13,26,27,34]. Small–medium size African barbs have a close relationship to Oriental *Puntius*, perhaps exclusive of *P. semifasciolatus*; this resolution is consistent with most previous analysis

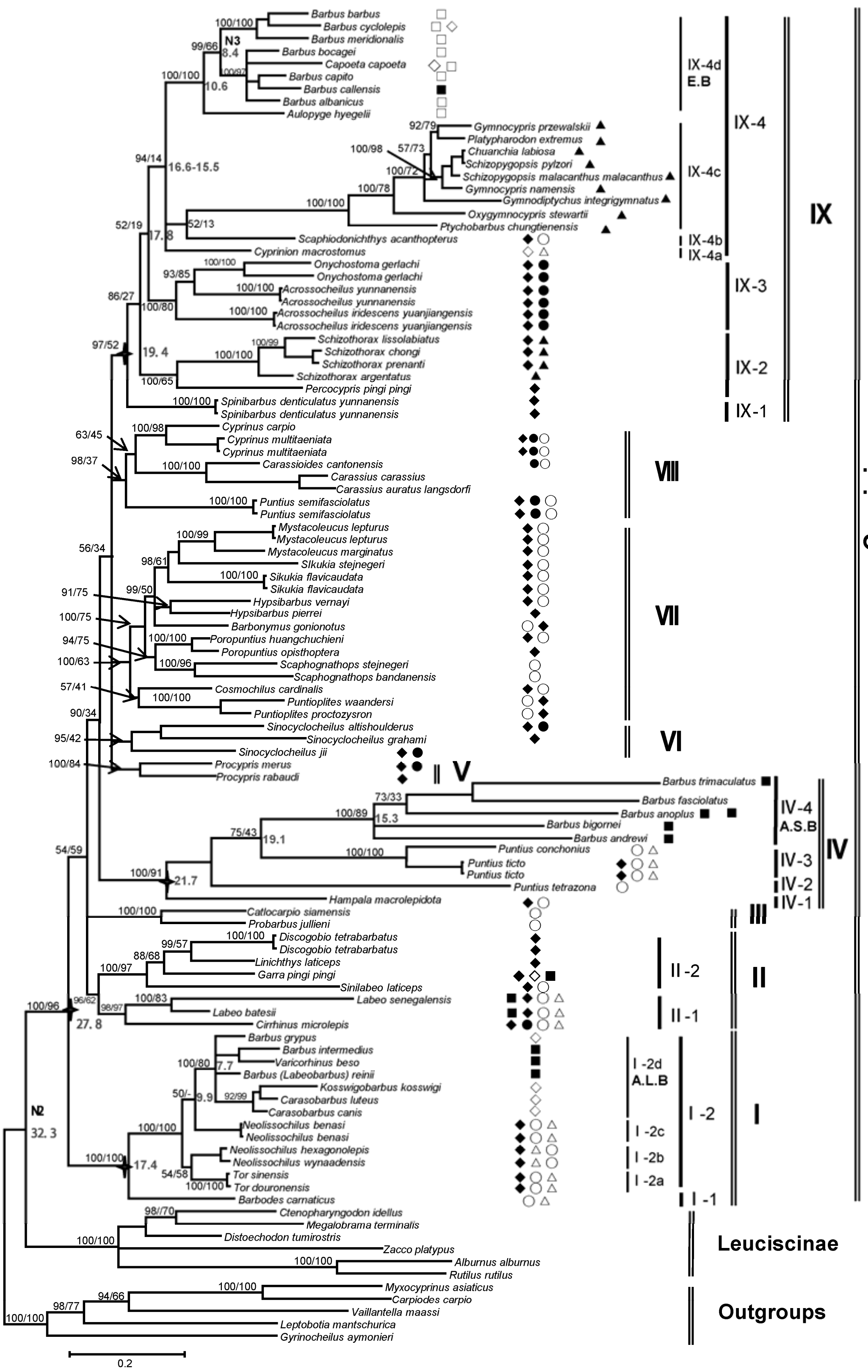

Figure 1 Bayesian inference matrilineal genealogies of *Cytb* for 85 species belong to 46 genera of the Cyprininae, six Leuciscinae, and five outgroup species. Nodal support values are Bayesian posterior probabilities and bootstrap values for maximum likelihood. Black bold numbers indicate time estimations in millions of years. N2 and N3 denote nodes used for calibrating molecular data estimates. Distribution areas of species and their genera: ○: Southeast Asia ●: Southern China △: South Asia ▲: Qinghai-Tibetan Plateau ◇: West Asia ◆: Southwest China □: Europe ■: Africa. A.L.B. represents large size African barbs (*Barbus sensu lato*); A.S.B represents small–medium size African *Barbus*; E.B represents European *Barbus*.

[23,24,30–34] but conflicts with the proposal of Howes [15], who claimed African *Barbus* was not related to *Puntius*.

3.2 Origin of European *Barbus sensu stricto* and African *Barbus sensu lato*

European *Barbus sensu stricto* lineage including *Aulopyge* appear to have shared a common ancestor with *Cyprinion*, *Scaphiodonichthys*, and the specialized and highly specialized schizothoracins. Taken together, our phylogeny and the geographical distribution of the genera in lineage IX and sublineage IX-4 (Figure 1, Table 1) suggests that the common ancestor had an origin on the Qinghai-Tibetan Plateau (QTP) about 19.4–17.8 Ma (Early Miocene; Figure 1). European *Barbus sensu stricto* lineage might have originated in northern areas adjacent to the QTP and dispersed to Europe about 16.6–15.5 Ma (Figure 1). This finding is concordant with oldest European fossil records of *Barbus* dated at 15–11 Ma [10]. Dispersal might have started from Xinjiang, China, and then, as advocated by Banarescu and Coad [6], first reached western Siberia (Kazakhstan). As proposed by Tsigenopoulos and Berrebi [9], the common ancestor may have then crossed the Paratethys Sea—the Middle Miocene nearly brackish interior sea that once covered Southeast Europe and West Asia, extending as far east as the present Caspian Sea—or its coastal water system on its way to Europe.

The topologies of lineages I and IV (Figure 1), and the geographical distributions of the genera, indicate that West Asian *Carasobarbus* lineage including large African barbs might have originated in the Oriental Region about 9.94 Ma. Separation of large sized African barbs from West Asian *Carasobarbus* might have taken place about 7.7 Ma. The recent ancestor of the African small-medium barbs may also have an Oriental origin (Figure 1). The origin of this ancestor and its colonization of Africa might have occurred about 19.1–15.3 Ma. The oldest African fossil *Barbus* and *Barbus*-like species date to the Middle and Late Miocene [55]. This correspondence corresponds to our estimated divergence time. No previous molecular study has analyzed the biogeographical relationships between African *Barbus* and Chinese, Southeast Asian, and South Asian cyprinine fishes, except for Durand et al. [13], who proposed that the West Asian *Carasoburbus* lineage might have originated from adjacent countries, such as India.

3.3 Radiation of Cyprininae and tectonic movements of the QTP

The Cyprininae appears to have radiated into nine lineages and some sublineages starting from about 27.8 to 17.78 Ma (Figure 1). This time frame corresponds with the second stage tectonic movement and uplifting of the QTP (29–18 Ma), which occurred more frequently at its southeastern margin [56]. Further, extant schizothoracins appear to be adapted to high-elevation environments because most species live on the QTP. Almost all other members of the subfamily inhabit the surrounding areas, especially Southwest China and Indochina; in the latter two areas, all the tribes and most genera of the subfamily have their own distribution [6–8,57]. A new fossil genus of barbins dates to 29–27 Ma in Qaidam Basin, QTP [58] and it appears to be among the oldest fossil genera in Cyprininae [4,53,58].

Our analyses suggest that the recent ancestor of the Cyprininae likely had a wide distribution on the QTP and adjacent southeastern areas before 27.8 Ma. Diversification starting from about 27.8 to 17.8 Ma is associated with the second stage of intense tectonic movement of the QTP. This orogenesis is associated with the origins of the major lineages and some sublineages. Orogenesis of the QTP appears to be the driver of current patterns, as also appears to be true for frogs in the tribe Paini [59]. Divergence of European *Barbus* from other sublineages within IX-4 about 16.6–15.5 Ma may also have been driven by uplifting of the QTP.

This work was supported by the National Basic Research Program of China (2007CB411600), the National Natural Science Foundation of China (30870291, 30930071, 31272335), State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, the Chinese Academy of Sciences, NSERC Discovery Grant 3148, and a Visiting Professorship for Senior International Scientists from the Chinese Academy of Sciences for RWM.

1 Cavender T M, Coburn M M. Phylogenetic relationships of North American Cyprinidae. In: Mayden R L, ed. Systematics, Historical Ecology and North American Freshwater Fishes. Palo Alto, CA: Stanford University Press, 1992. 293–327

2 Chen X L, Yue P Q, Lin R D. Major groups within the family Cyprinidae and their phylogenetic relationships (in Chinese). Acta Zoo Sin, 1984, 9: 424–440

3 Chen X Y. Studies on the phylogenetic relationships of Chinese leuciscine fishes (pieces: Cypriniformes) (in Chinese). Acta Zoo Sin, 1987, 12: 427–438

4 Chen Y Y. General Introduction to Cyprinidae. In Fauna Sinica, Osteichthyes, Cypriniformes (II) (in Chinese). Beijing: Science Press, 1998. 1–18

5 Agnese J F, Berrebi P, Leveque C, et al. Two lineages, diploid and tetraploid, demonstrated in African species *Barbus* (Osteichthyes, Cyprinidae). Aquat Living Resour, 1990, 3: 305–311

6 Banarescu P, Coad B W. Cyprinids of Eurasia. In: Winfield I J, Nelson J S, eds. Cyprinid Fishtematics: Systematic, Biology and Exploitation. London: Chapman and Hall, 1991. 127–15

7 Rainboth W J. Cyprinids of South East Asia. In: Winfield I J, Nelson J S, eds. Cyprinid Fishes: Systematics, Biology and Exploitation. London: Chapman and Hall, 1991. 156–210

8 Yue P Q. Fauna Sinica, Osteichthyes, Cypriniformes (III) (in Chinese). Beijing: Science Press, 2000

9 Tsigenopoulos C S, Berreb P. Molecular phylogenetics of north Mediterranean freshwater barbs (genus *Barbus*: Cyprinidae) inferred from cytochrome *b* sequences: Biogeographic and systematic implications. Mol Phylogen Evol, 2000, 14: 165–179

10 Zardoya R, Doadrio I. Molecular evidence on the evolutionary and biogeographical pattern of European Cyprinids. J Mol Evol, 1999, 49: 227–237

11 Machordom A, Doadrio I. Evidence of a Cenozoic Betic–Kabilian connection based on freshwater fish phylogeography (*Luciobarbus*, Cyprinidae). Mol Phylogen Evol, 2001, 18: 252–263

12 Callejas C C, Ochando M D. Phylogenetic relationships among

Spanish *Barbus* species (Pisces: Cyprinidae) shown by RAPD markers. Heredity, 2002, 89: 36–43

13 Durand J D, Tsigenopoulos C S, Unlue E, et al. Phylogeny and biogeography of the family Cyprinidae in the Middle East inferred from Cytochrome *b* DNA evolutionary significance of this region. Mol Phylogen Evol, 2002, 22: 91–100

14 Skelton P H, Tweddle D, Jackson P B N. Cyprinids of African. In: Winfield I J, Nelson J S, eds. Cyprinid Fishes: Systematics, Biology and Exploitation. London: Chapman and Hall, 1991. 211–219

15 Howes G J. Systematics and biogeography: An overview. In: Winfield I J, Nelson T S, eds. Cyprinid fishes. Systematics, Biology and Exploitation. London: Chapman and Hall, 1991. 1–33

16 Briolay J, Galtier N, Brito R M, et al. Molecular phylogeny of Cyprinidae inferred from cytochrome *b* DNA sequence. Mol Phylogen Evol, 1998, 9: 100–108

17 Gilles A, Lecointre G, Faure E, et al. Mitochondrial phylogeny of the European Cyprinides: Implications for their systematics, reticulate evolution, and colonization time. Mol Phylogen Evol, 1998, 10: 132–143

18 Gille A, Lecointre G, Miquelis A, et al. Partial combination applied to phylogeny of European Cyprinids using the mitochondrial control region. Mol Phylogen Evol, 2001, 19: 22–33

19 Zardoya R, Economidis P S, Doadrio I. Phylogenetic relationships of the Greek Cyprinidae: Molecular evidence for at least two origins of the Greek Cyprinid fauna. Mol Phylogen Evol, 1999, 13: 122–131

20 Cunha C, Mesquita N, Dowling T E, et al. Phylogenetic relationships of Eurasian and American cyprinids using cytochrome *b* sequences. J Fish Biol, 2002, 61: 929–944

21 Liu H Z, Chen Y Y. Phylogeny of the East Asian cyprinids inferred from sequences of the mitochondrial DNA control region. Can J Zool, 2003, 81: 1938–1946

22 He S P, Liu H Z, Chen Y Y, et al. Molecular phylogenetic relationships of Eastern Asian Cyprinidae (Pisces: Cypriniformes) inferred from cytochrome *b* sequences. Sci China Life Sci, 2004, 47: 130–138

23 Saitoh K, Sado T, Mayden R L, et al. Mitogenomic evolution and interrelationships of the Cypriniformes (Actinopterygii: Ostariophysi): The first evidence toward resolution of higher-level relationships of the world's largest freshwater fish clade based on 59 whole mitogenome sequences. J Mol Evol, 2006, 63: 826–841

24 Saitoh K, Sado T, Doosey M H, et al. Evidence from mitochondrial genomics supports the lower Mesozoic of South Asia as the time and place of basal divergence of Cypriniformes fishes (Actinopterygii: Ostariophysi). Zool J Linnean Soc, 2011, 161: 633–662

25 Kong X H, Wang X Z, Gan X N, et al. The c-myc coding DNA sequences of Cyprinids (Teleostei: Cypriniformes): Implication for phylogeny. Chin Sc Bull, 2007, 52: 1491–1500

26 Wang X Z, Li J B, He S P. Molecular evidence for the monophyly of East Asian groups of Cyprinidae (Teleostei: Cypriniformes) derived from the nuclear recombination activating gene 2 sequences. Mol Phylogen Evol, 2007, 42: 157–170

27 Li J B, Wang X Z, Kong X H, et al. Variation pattern of the mitochondrial 16srRNA gene with secondary structure constraints and their application to phylogeny of cyprinine fishes (Teleostei: Cypriniformes). Mol Phylogen Evol, 2008, 47: 472–478

28 Mayden R L, Chen W J, Bart H L, et al. Reconstructing the phylogenetic relationships of the earth's most diverse clade of freshwater fishes-order Cypriniformes (Actinopterygii: Ostariophysi): A case study using multiple nuclear loci and the mitochondrial genome. Mol Phylogen Evol, 2009, 51: 500–514

29 He S P, Mayden R L, Wang X Z, et al. Molecular phylogenetics of the family Cyprinidae (Actinopterygii: Cypriniformes) as evidenced by sequence variation in the first intron of S7 ribosomal protein-coding gene: Further evidence from a nuclear gene of the systematic chaos in the family. Mol Phylogen Evol, 2008, 46: 818–829

30 Mayden R L, Chen W J, Bart H L, et al. Reconstructing the phylogenetic relationships of the earth's most diverse clade of freshwater fishes—order Cypriniformes (Actinopterygii: Ostariophysi): A case study using multiple nuclear loci and the mitochondrial genome. Mol Phylogen Evol, 2009, 51: 500–514

31 Chen W J, Mayden R L. Molecular systematics of the Cyprinoidea (Teleostei: Cypriniformes), the world's largest clade of freshwater fishes: Further evidence from six nuclear genes. Mol Phylogen Evol, 2009, 52: 544–549

32 Zhang X Y, Yue B S, Jiang W X, et al. The complete mitochondrial genome of rock carp *Procypris rabaudi* (Cypriniformes: Cyprinidae) and phylogenetic implications. Mol Bio Rep, 2009, 36: 981–991

33 Wu X Y, Wang L, Chen S Y, et al. The complete mitochondrial genomes of two species from Sinocyclocheilus (Cypriniformes: Cyprinidae) and a phylogenetic analysis within Cyprininae. Mol Bio Rep, 2010, 37: 2163–2171

34 Yang L, Mayden R L, Sado T, et al. Molecular phylogeny of the fishes traditionally referred to Cyprinini sensu stricto (Teleostei: Cypriniformes). Zool Scr, 2010, 39: 527–550

35 Dowling T E, Tibbets C A, Minckley W L, et al. Evolutionary Relationships of the Plagopterins (Teleostei: Cyprinidae) from Cytochrome *b* sequences. Copeia, 2002, 3: 665–678

36 He D K, Chen Y F, Chen Y Y, et al. Molecular phylogeny of the specialized Schizothoracine fishes (Teleostei: Cyprinidae), with their implications for the uplift of the Qinghai-Tibetan Plateau. Chin Sci Bull, 2004, 49: 39–48

37 He D K, Chen Y F. Molecular phylogeny and biogeography of the highly specialized grade Shizothoracine fishes (Teleostei: Cyprinidae) inferred from cytochrome *b* sequences. Chin Sci Bull, 2007, 52: 777–788

38 Schonhuth S, Doadrio I, Dominguez-Dominguez O, et al. Molecular evolution of southern North American Cyprinidae (Actinopterygii), with the description of the new genus *Tampichthys* from central Mexico. Mol Phylogen Evol, 2008, 47: 729–756

39 Miya N, Nishida M. Use of mitogenomic information in teleostean molecular phylogenetics: A tree-based exploration under the maximum-parsimony optimality criterion. Mol Phylogen Evol, 2000, 17: 437–455

40 Xiao H, Chen S Y, Liu Z M, et al. Molecular phylogeny of *Sinocyclochcilus* (Cypriniformes: Cyprinidae) inferred from mitochondria DNA sequences. Mol Phylogen Evol, 2005, 36: 67–77

41 Xiao W H, Zhang Y P, Liu H. Molecular systematics of Xenocyprinae (Teleostei: Cyprinidae): Taxonomy, biogeography, and coevolution of a special group restriccted in East Asia. Mol Phylogen Evol, 2001, 18: 163–173

42 Tamura K, Dudley J, Nei M, et al. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol, 2007, 24: 1596–1599

43 Stamatakis A. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics, 2006, 22: 2688–2690

44 Huelsenbeck J P, Ronquist F. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics, 2001, 17: 754–755

45 Goldman N. Simple diagnostic statistical tests of models of DNA substitution. J Mol Evol, 1993, 37: 650–661

46 Goldman N. Statistical tests of models of DNA substitution. J Mol Evol, 1993, 36: 182–198

47 Huelsenbeck J P, Crandall K A. Phylogeny estimation and hypothesis testing using maximum likelihood. Ann Rev Ecol Syst, 1997, 28: 437–466

48 Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol, 2003, 52: 696–704

49 Posada D. jModeltest: Phylogenetic model averaging. Mol Phylogen Evol, 2008, 25: 1253–1256

50 Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 1985, 39: 783–791

51 Drummond A J, Nicholls G K, Rodrigo A G, et al. Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. Genetics, 2002, 161: 1307–1320

52 Drummond A J, Rambaut A. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol, 2007, 7: 214–221

53 Cavender T M. The fossil record of Cyprinidae. In: Winfield I J, Nelson J S, eds. Cyprinid Fishes. Systematics, Biology and Exploitation. London: Chapman and Hall, 1991. 127–155

54 Cao W X, Chen Y Y, Wu Y F, et al. Origin and evolution of schizothorscine fishes in relation to the upheaval of the Qinghai-Xizang Plateau. In: The Comprehensive Scientific Expedition to the QinghaiXizang Plateau, CAS, ed. Studies on the Period, Amplitude and Type of the Uplift of the Qinghai-Xizang Plateau (in Chinese). Beijing: Science Press, 1981. 118–130

55 Stewart K M. The freshwater fish of Neogene Africa (Miocene-Pleistocene): Systematics and biogeography. Fish Fish, 2001, 2: 177–230

56 Zhong D L, Ding L, Zhang J J, et al. Coupling of the lithospheric convergence of west China and dispersion of East China in Cenozoic link with paleoenviromental changes (in Chinese). Quaternary Sci, 2001, 21: 303–312

57 Zheng L P, Yang J X, Cheng X Y. Phylogeny of the Labeoninae (Teleostei, Cypriniformes) based on nuclear DNA sequences and implications on character evolution and biogeography. Curr Zool, 2012, 58: 837–850

58 Chen G J, Liu J. First fossil *Barbin* (Cyprinidae, Teleostei) from Oligocene of Qaidam Basin in northern Tibetan Plateau. Vert Palasiatica, 2007, 45: 330–341

59 Che J, Zhou W W, Hua J S, et al. Spiny frogs (Paini) illuminate the history of the Himalayan region and Southeast Asia. Proc Natl Acad Sci USA, 2010, 107: 13765–13770