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Molecular phylogeny of the fishes traditionally referred to Cyprinini *sensu stricto* (Teleostei: Cypriniformes)

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Carps (e.g. Koi) of the genus *Cyprinus* and Crucian carps (e.g. Goldfish) of the genus *Carassius* are among the most popular freshwater fishes around the world. However, their phylogenetic positions within the subfamily Cyprininae, relationships with their allies (e.g. *Procypris*, *Carassioides*), and the monophyly of the group formed by them and their allies, which is referred as the tribe Cyprinini *sensu stricto*, are far from clear. Historically, the Cyprinini was defined by different people according to whether a cyprinine fish possessed a spinous anal-fin ray (or anal spine), the spine was serrated or not, and occasionally, the number of branched dorsal-fin rays. Some definitions were established without providing any diagnostic characters. In this study, we investigated the monophyly of the tribe Cyprinini *sensu stricto*, based on four different historical definitions, and explored the phylogenetic relationships of these members in the subfamily Cyprininae. Using five mitochondrial genes as markers, both maximum-likelihood and Bayesian trees were constructed using the optimal partitioning strategy. Both analyses successfully resolved a monophyletic Cyprininae and recovered seven major clades from this subfamily. The diagnosis limiting the tribe Cyprinini *sensu stricto* to four genera, *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, received most support. We propose that only those cyprinines that possess a serrated anal spine and have no <10 branched dorsal-fin rays should be considered members of this tribe. Cyprinini is sister to the *Sinocyclocheilus* clade, a group traditionally considered a barbin, and together they form the 'Cyprinini-*Sinocyclocheilus*' clade. *Procypris* forms the basal clade of the Cyprinini, whereas species of *Carassius* and *Carassioides* locate at the top.

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Introduction

The Cyprininae is the largest subfamily of the Order Cypriniformes, which is the most diverse group of freshwater fishes on the planet (Nelson 2006). It currently contains roughly 110 genera and 1300 species. This subfamily generally includes barbins (e.g. *Barbus*, *Puntius*), labeonins (e.g. *Labeo*, *Garra*), cyprinins (e.g. common carp, goldfish), oreinins (snow trouts), etc. The classification of these fishes has been extremely chaotic, as various numbers of groups have been recognized within this subfamily (Rainboth 1981, 1991, 1996; Chen *et al.* 1984; Howes 1987, 1991; Cavender & Coburn 1992; Nelson 1994, 2006; Rüber *et al.* 2007; Wang *et al.* 2007; Fang *et al.* 2009) and many of these studies lack broad sampling of taxa and characters. For convenience and also as a working hypothesis, here we use the following recognized tribes to represent cyprinins (tribe Cyprinini), barbins (tribe Barbini), labeonins (tribe Labeonini) and oreinins (tribe Oreinini).

The monophyly of these tribes and their relationships to one another requires further investigation despite the few studies that have already been conducted (e.g. Rüber *et al.* 2007; Wang *et al.* 2007; Li *et al.* 2008; Fang *et al.* 2009; Yang & Mayden 2010).

The tribe Cyprinini as used herein is equivalent to the subfamily Cyprininae of Chen *et al.* (1984). It should be noted that, historically, the name Cyprinini has been used either to refer to a small group of fishes formed by *Cyprinus*, *Carassius* and their allies (Rendahl 1928; Lin 1931, 1933, 1935; Fang 1936a; Zhang 1959; Wu 1964; Chen & Huang 1977; Rainboth 1981) or to refer to a large group of fishes, which contains barbins, oreinins and cyprinins (Nichols & Pope 1927; Chu 1935; Smith 1945; Wang *et al.* 2007). These groupings as described above are called Cyprinini *sensu stricto* and Cyprinini *sensu lato*, respectively. Some researchers implicitly or explicitly put *Cyprinus* within the tribe Barbini (e.g. Gosline 1978; Howes 1987, 1991). They are wrong, because the family-group name Cyprinini Rafinesque, 1815 is earlier than the family-group name Barbini Bleeker, 1859. Our current study focuses on the Cyprinini *sensu stricto*.

Considerable debate exists on how the tribe Cyprinini *sensu stricto* should be defined. Depending on the different definitions used, various numbers of genera and species can be included in this tribe (Tables 1 and 2). Rendahl (1928) classified the tribe Cyprinini as species possessing a serrated spinous anal-fin ray or serrated anal spine (i.e. the last unbranched fin ray ossified and its latter part serrated) and an elongate dorsal fin with not <14 branched rays. These two characters were mainly used to differentiate cyprinins from the hypothesized closely related barbins. According to this definition, the Cyprinini should now include four genera: *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, although Rendahl (1928) only included *Cyprinus* and *Carassius* at that time. It should be noted that, although all *Cyprinus* species possess serrated anal spine, some of them have fewer than 14 branched dorsal-fin rays, e.g. *Cyprinus micristius* (10–12), *Cyprinus fuxianensis* (9–10) and *Cyprinus yilongensis* (13–15) (Table 1). *Cyprinus micristius* was described by Regan in 1906, well before Rendahl (1928) was published. This evidence rendered Rendahl's (1928) classification of the tribe Cyprinini *sensu stricto* inadequate, and his opinion was largely neglected thereafter.

Fang (1936a) proposed to use only one character to define the tribe Cyprinini, the possession of a serrated anal spine, and many later studies concurred with this definition (e.g. Zhang 1959; Wu 1964; Wang 1979; Zhou & Chu 1986). Fang (1936a) assigned six genera to the tribe Cyprinini, including *Cyprinus*, *Carassius*, *Mesocyprinus*, *Paraprocypris*, *Procypris* and *Puntioplites*. This was the first study that assigns *Puntioplites* within the Cyprinini. *Mesocyprinus* was originally created by Fang (1936a) to reflect the distinctive nature of *Cyprinus micristius* in having fewer branched dorsal-fin rays and the structure of its pharyngeal teeth. *Mesocyprinus* was treated by later studies either as a synonym of *Cyprinus* Linnaeus, 1758, but a valid subgenus (Chen & Huang 1977; Luo & Yue 2000), or an invalid taxon (Wang 1979; Zhou & Chu 1986; Wu & Wu 1992; Chen & Yang 2002). The genus *Paraprocypris* is now widely accepted as a synonym of *Procypris* Lin, 1933 (Chen & Huang 1977; Zhou & Chu 1986; Luo & Yue 2000). *Carassioides*, despite possessing a serrated anal spine, was treated as invalid genus by Fang (1936a) because he considered it a hybrid form between *Cyprinus carpio* and *Carassius auratus*, a conclusion also shared by some earlier and later studies of the genus (Nichols & Pope 1927; Lin 1933; Herre 1936; Chevey & Lemasson 1937). However, Chu (1935), Liu & Wu (1945) and Wang (1979) carefully compared *Carassioides* with known hybrids between *Cyprinus* and *Carassius* and argued that *Carassioides* is not of hybrid origin but a valid genus. Nguyen & Doan (1969) described *Laichowcypris* from Vietnam and placed it within the tribe Cyprinini, but Kottelat (2001) regarded it as a synonym of *Cyprinus*. Wang (1979) conducted a comprehensive study on the classification of the tribe Cyprinini and included the following five genera: *Cyprinus* Linnaeus, 1758; *Carassius* Nilsson, 1832; *Carassioides* Oshima, 1926; *Procypris* Lin, 1933; and *Puntioplites* Smith, 1929 (Tables 1 and 2). Bănărescu (1972) also suggested treating the genus *Puntioplites* as a member of Cyprinini. However, it seems that he changed his opinion in later studies (e.g. Bănărescu 1978).

In Wu's (1977) *The Cyprinid Fishes of China* (Vol. 2), Chen & Huang (1977) examined the tribe Cyprinini and expanded it to include all species of Cyprininae that possess an anal spine no matter whether it is serrated or not. In their study, Chen & Huang (1977) believed that species of *Puntius* possessing an un-serrated anal spine should be put into this tribe, including *Puntius bulu*, *Puntius waandersi*, *Puntius nini* and *Puntius lawak*. Interestingly, these four species are currently valid as *Puntioplites bulu*, *Puntioplites waandersi*, *Puntioplites waandersi* and *Kalimantania lawak*, respectively. The revision by Chen & Huang (1977) was also based on comparisons of species of *Puntius* with *Puntioplites proctozysron* and the above species were determined to be very similar in the shape of their pharyngeal bones, arrangement and shape of pharyngeal teeth, number of branched fin rays in dorsal and anal fins, and lips and associated structures. These authors also moved *Scaphognathops* from the Barbini into the Cyprinini. The anal spine in *Scaphognathops* is un-serrated. Therefore, using the definition of Cyprinini by Chen & Huang (1977), the tribe

Table 1 Genera and species included in Cyprinini *sensu* Chen & Huang, 1977. The distribution and number of branched dorsal rays were also shown. The first species for each genus is the type species

Genus	Species	Distribution*	Branched dorsal-fin ray*
Cyprinus	*carpio*	Eastern Europe	16–22
Linnaeus, 1758	*acutidorsalis*	Hainan Island, Guangxi (Qingjiang River), China	15–18
	barbatus	Erhai Lake and Yilong Lake, Yunnan, China	16–19
	centralus	Vietnam	17–20
	chilia	Lakes of Yunnan, China	16–19
	dai	Vietnam	18–20
	daliensis	Erhai Lake, Yunnan, China	16–19
	exophthalmus	Vietnam	17–20
	fuxianensis	Fuxianhu Lake and Xingyunhu Lake, Yunnan, China	9–10
	hyperdorsalis	Vietnam	17–21
	ilishaestomus	Qiluhu Lake, Yunnan, China	16–19
	intha	Inlè Lake, Myanmar	17
	longipectoralis	Lake Dianci, Yunnan, China	16–18
	longzhouensis	Zuojiang River, Longzhou to Shangjin, Guangxi, China	20–22
	megalophthalmus	Erhai Lake, Yunnan, China	15–18
	micristius	Lake Dianci, Yunnan, China	10–12
	multitaeniatus	Upper Xijiang River, China; Vietnam	17–20
	pellegrini	Xingyunhu Lake and Qiluhu Lake, Yunnan, China	15–18
	qionghaiensis	Qionghai Lake, Sichuan, China	17
	rubrofuscus	East Asia	18–22$^{1/2}$
	yilongensis	Yilong Lake, Yunnan, China	13–15
	yunnanensis	Qilu Lake, Yunnan, China	16–18
Carassius	*carassius*	Eurasia	16–18
Nilsson, 1832	*auratus*	East Asia	15–19
	cuvieri	China and Japan	15–18
	gibelio	Eurasia	16–19
Procypris	*mera*	Upper Xijiang River, China	15–17
Lin, 1933	*rabaudi*	Upper Changjiang River, China	19–21
Carassioides	*acuminatus*	Vietnam and China	15–20
Oshima, 1926	*argenteus*	Vietnam	17–20
	macropterus	Vietnam	17–20
Puntioplites	*proctozystron*	Maeklong basin, Chao Phraya basin, Mekong basin, Malay Peninsula	8–9
Smith, 1929	*falcifer*	Mekong basin	8
	bulu	Indonesia, Cambodia, peninsular Thailand	8–9
	waandersi	Indochina, Sumatra, Borneo, Malaya	8–9
Scaphognathops	*stejnegeri*	Mekong basin in Laos, Thailand, Cambodia, Vietnam	13–15
Smith, 1945	*bandanensis*	Mekong basin in Laos, Thailand, Cambodia	9
	theunensis	Nam Theun of the Mekong basin in Laos	15$^{1/2}$
Kalimantania	*lawak*	Western Borneo (Kapuas) and Java, Indonesia	8–8$^{1/2}$
Bănărescu, 1980			

This table is provided to guide our molecular analyses. The species information for *Cyprinus* and *Carassius* may not be accurate due to significant difficulty in their classification (Kottelat 2001). The validity of several Vietnamese species of *Cyprinus* and *Carassioides* needs further examination.

*Mainly according to Taki (1974); Taki & Katsuyama (1979); Mai (1978); Wang (1979); Roberts (1989); Balon (1995); Rainboth (1996); Luo & Yue (2000); Nguyen & Ngo (2001); Kohlmann *et al.* (2003); Kottelat & Freyhof (2007). The number of branched dorsal-fin rays for several species were counted directly from their pictures.

should contain at least seven genera, those listed above and *Scaphognathops* Smith, 1945 and *Kalimantania* Bănărescu, 1980 (Tables 1 and 2). In *Fauna Sinica*, Luo & Yue (2000) adopted the classification of Chen & Huang (1977), but did not include *Scaphognathops* and *Kalimantania*, as these genera do not occur in China. Thus, the difference between the definition of Cyprinini *sensu* Fang, 1936a and the definition of Cyprinini *sensu* Chen & Huang, 1977 lies in whether or not one should use the single character, the presence or absence of serrations on the anal spine, as a diagnostic trait for the tribe. It should be noted, however, that among the four currently recognized species of *Puntioplites*, *P. bulu* and *P. waandersi* possess un-serrated anal spines, whereas *Puntioplites proctozystron* (type species of genus) and *Puntioplites falcifer* possess serrated anal spines. Hence, if one strictly follows these definitions, *Puntioplites sensu* Fang, 1936a would be limited to only *P. proctozystron* and *P. falcifer*, whereas *Puntioplites sensu* Chen & Huang,

Table 2 List of definitions of the tribe Cyprinini *sensu stricto*

Definition	Diagnose character	Genus
Rendahl (1928)	1. Possession of serrated anal spine 2. With no <14 branched dorsal-fin rays	*Cyprinus* (partial), *Carassius*, *Carassioides*, *Procypris*
Fang (1936a)	1. Possession of serrated anal spine	*Cyprinus*, *Carassius*, *Carassioides*, *Procypris*, *Puntioplites* (*P. proctozystron*, *P. falcifer*)
Chen & Huang (1977)	1. Possession of anal spine (serrated or not)	*Cyprinus*, *Carassius*, *Carassioides*, *Procypris*, *Puntioplites*, *Scaphognathops*, *Kalimantania*
Rainboth (1981)	1. Possession of serrated anal spine* 2. With no <10 branched dorsal-fin rays*†	*Cyprinus*, *Carassius*, *Carassioides*, *Procypris*
Rainboth (1991)	(not provided)	*Cyprinus*, *Carassius*, *Carassioides*, *Procypris*, *Puntioplites*, *Catlocarpio*, *Thynnichthys*, *Barbus* (*Incertae sedis*)

*Diagnose characters for Cyprinini *sensu* Rainboth, 1981 were provided by the current study (see Discussion).
†*Cyprinus fuxianensis* occasionally have nine branched dorsal-fin rays (Table 1; see Discussion).

1977 would contain all four species listed above (Table 2). It is noteworthy that the definition of Cyprinini by Chen & Huang (1977) seems only shared by some Chinese ichthyologists.

Taki & Katsuyama (1979) suggested that *Puntioplites* should belong to the tribe Barbini instead of Cyprinini, based on some morphological characters (i.e. features of the snout region; pharyngeal bones and teeth; the number of dorsal-fin rays). In his unpublished dissertation, Rainboth (1981) expressed the same opinion. According to Taki & Katsuyama (1979) and Rainboth (1981), the tribe Cyprinini should be limited to four genera: *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*. As Rainboth (1981) specifically listed the above four genera under his tribe Cyprinini, we refer to this definition as Cyprinini *sensu* Rainboth, 1981. While both Taki & Katsuyama (1979) and Rainboth (1981) identified these four genera in this group, neither author provided any specific character(s) diagnosic for this tribe; they only discussed their close relationship. It should be noted that this definition of the Cyprinini is different from that of Rendahl (1928), because the latter author could not even diagnose all *Cyprinus* species. Rainboth (1991) changed his opinion and listed the following taxa under the tribe Cyprinini: *Carassioides*, *Carassius*, *Catlocarpio*, *Cyprinus* (*Mesocyprinus*), *Cyprinus* (*Cyprinus*), *Laichowcypris*, *Procypris*, *Thynnichthys* and *Barbus* (*Incertae sedis*). The seven valid genera included are: *Cyprinus* Linnaeus, 1758; *Carassius* Nilsson, 1832; *Carassioides* Oshima, 1926; *Procypris* Lin, 1933; *Puntioplites* Smith, 1929; *Catlocarpio* Boulenger, 1898; and *Thynnichthys* Bleeker, 1860 and maybe also *Barbus* Cuvier & Cloquet, 1816, but Rainboth provided no justification for this group or any diagnostic characters. Here, we refer to this particular definition as Cyprinini *sensu* Rainboth, 1991. This opinion of taxon composition is very different from his previous opinion in Rainboth (1981) and the opinions of all other previous researchers. As far as we know, no later studies have provided evidence to support the monophyly of this latter group and no other researchers have expressed similar opinions.

The monophyly of the tribe Cyprinini *sensu* Fang, 1936a has been supported by morphological studies of Chen *et al.* (1984) and Cavender & Coburn (1992). Chen & Huang (1977) and Wang (1979) investigated the subdivision and relationships among cyprinin genera based only on the morphology of their pharyngeal teeth and the number of barbels. Zhou (1989) conducted the first phylogenetic study on the tribe Cyprinini based on 25 morphological characters. In this study, he addressed the debate as to the definition of Cyprinini and conducted analyses without agreeing with either the definition of Fang (1936a) or the definition of Chen & Huang (1977), although his results tend to support those of Fang (Fig. 1). Zhou's (1989) study also supported the monophyly of the group formed by *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, but he seemed reluctant to limit the tribe Cyprinini to these four genera as Rainboth (1981) did. Zhou (1989) stated clearly in the paper that his study was based on the opinion that the tribe Cyprinini is monophyletic and derived from the tribe Barbini. The author even used barbins as outgroups to identify the relative character states as being either plesiomorphic or apomorphic. However, the limited taxonomic scope of Zhou's (1989) analysis draws into question the phylogenetic relationships proposed among the included cyprinin genera. As the study by Zhou (1989) was published in Chinese and was largely unknown to people of other countries, we have redrawn the phylogenetic relationships proposed (Fig. 1) and translated into English the Chinese descriptions of all the morphological characters used (see Appendix S1). Most molecular studies on cyprinids usually use a limited sampling of species, i.e. *Cyprinus carpio* and/or one or two *Carassius* species, to represent the tribe Cyprinini, which not only makes the test of the monophyly of this tribe impossible but also increases the difficulty of thoroughly exploring the relationships between the Cyprinini and other cyprinine tribes.

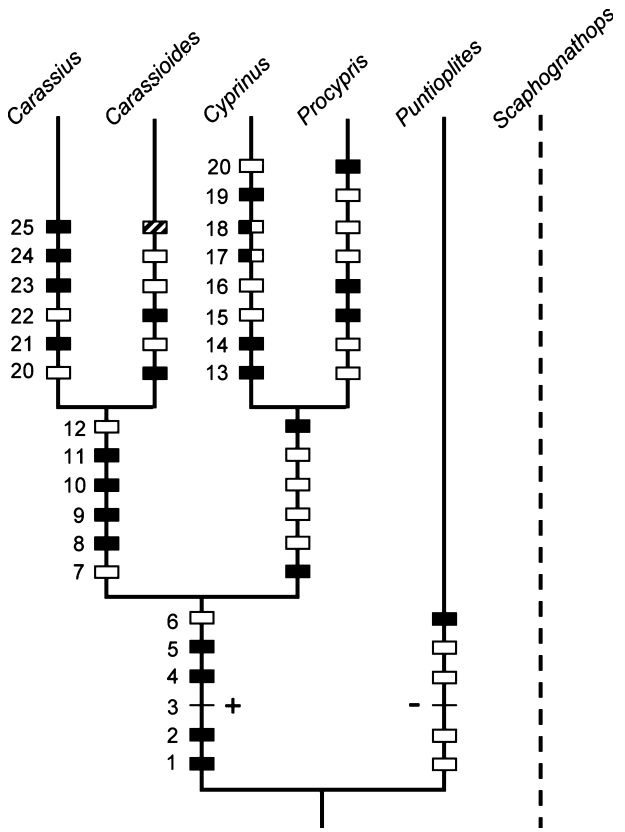


Fig. 1 Cladogram depicting the phylogenetic relationships among genera of the tribe Cyprinini. This figure was modified from the fig. 7 of Zhou (1989) without changing its topology. Open rectangles denote plesiomorphies; rectangles filled with black refer to apomorphies; rectangles filled with half black and half white mean multiple states of characters exist; rectangles with dark upward diagonals denote the character showing medial state; '+' and '–' mean with or without a character whose state cannot be determined. Number (1–25) beside each rectangle corresponds to a morphological character listed in the Appendix S1. The branch of the genus *Scaphognathops* was shown as dashes line because its overall osteological morphology is significantly different from other four genera (Zhou 1989).

The major objectives of this study were to use DNA sequences from five mitochondrial genes to: (i) test the monophyly of the tribe Cyprinini *sensu stricto* basing on the definitions of the tribe derived from different researchers, i.e. Fang (1936a), Chen & Huang (1977), Rainboth (1981), and Rainboth (1991) and (ii) investigate the phylogenetic relationships of members of the tribe Cyprinini *sensu stricto* within the subfamily Cyprininae.

Materials and methods

Taxa sampling

A total of 85 cypriniform fishes were used in this study. Among them, 66 species from 41 genera belong to the subfamily Cyprininae. All four tribes of this subfamily, i.e. Cyprinini, Barbini, Oreinini and Labeonini, were represented. For Cyprinini *sensu* Fang, 1936a; all five genera were sampled (Table 3). For Cyprinini *sensu* Chen & Huang, 1977, a total of six genera were included: *Cyprinus*, *Carassius*, *Carassioides*, *Procypris*, *Puntioplites* and *Scaphognathops* (Table 3). The only genus that we failed to sample is the monotypic *Kalimantania*, which has a restricted distribution in Western Borneo (Kapuas) and Java, Indonesia (Table 1). For Cyprinini *sensu* Rainboth, 1981, all four genera were included. For Cyprinini *sensu* Rainboth, 1991, we sampled six genera: *Cyprinus*, *Carassius*, *Carassioides*, *Procypris*, *Catlocarpio* and *Barbus* (including one species of *Barbus sensu stricto* and two species of *Barbus*). Many species of *Cyprinus* have very restricted distributions in one or only a few lakes. Some species are now very rare or even extinct for a series of reasons, e.g. habitat loss, introduced species or pollution (Xie & Chen 1999). In this study, although only two species of *Cyprinus* were sampled to represent the genus due to sampling difficulty, this may not be a problem, as our objectives were not to investigate species-level relationships within each genus and we have included the type species for the genus, *Cyprinus carpio*, in the analyses. *Cyprinus* was divided into two subgenera, *Mesocyprinus* and *Cyprinus*, by some researchers (e.g. Chen & Huang 1977). The two species used in this study, *Cyprinus multitaeniata* and *Cyprinus carpio*, belong to the first and the second subgenera, respectively. All four species of *Carassius* were sampled in this study. One species of *Procypris*, one species of *Carassioides*, three species of *Puntioplites*, and two species of *Scaphognathops* were used to represent other genera. To investigate the phylogenetic position of cyprinins within the subfamily Cyprininae and relationships with other cyprinines, we also sampled 38 barbins from 22 genera, six oreinins from five genera, and nine labeonins from eight genera. A total of 18 species from other cyprind subfamilies and other cypriniform families were chosen as outgroups following previous hypotheses regarding the phylogenetic relationships of the Cypriniformes (e.g. Cavender & Coburn 1992; Saitoh *et al.* 2006; Mayden *et al.* 2009). All samples used in this study were sampled by the authors or obtained from collaborators in the Cypriniformes Tree of Life (CToL) Project. Vouchered specimens were deposited at Saint Louis University and other institutes of our collaborators.

DNA extraction, PCR and sequencing

Genomic DNA was isolated from ethanol-preserved tissue samples (muscle or fin clips) using DNeasy Blood & Tissue Kit (QIAGEN Sciences, Inc. Germantown, Maryland. U.S.A.). Five mitochondrial genes were used in this study: cytochrome oxidase subunit I (COI), cytochrome *b* (Cyt *b*),

Table 3 Taxa examined in this study with GenBank accession numbers for each gene

Taxa	COI	Cyt *b*	ND4	ND5	16S
Order Cypriniformes					
Family Catostomidae					
Catostomus commersonii	AB127394	AB127394	AB127394	AB127394	AB127394
Family Gyrinocheilidae					
Gyrinocheilus aymonieri	AB242164	AB242164	AB242164	AB242164	AB242164
Family Cobitidae					
Cobitis striata	AB054125	AB054125	AB054125	AB054125	AB054125
Family Balitoridae					
Homaloptera leonardi	AB242165	AB242165	AB242165	AB242165	AB242165
Family Cyprinidae					
Subfamily Acheilognathinae					
Acheilognathus typus	AB239602	AB239602	AB239602	AB239602	AB239602
Rhodeus ocellatus	AB070205	AB070205	AB070205	AB070205	AB070205
Subfamily Gobioninae					
Gobio gobio	AB239596	AB239596	AB239596	AB239596	AB239596
Hemibarbus labeo	AB070241	AB070241	AB070241	AB070241	AB070241
Subfamily Leuciscinae					
Alburnus alburnus	AB239593	AB239593	AB239593	AB239593	AB239593
Ctenopharyngodon idella	EU391390	EU391390	EU391390	EU391390	EU391390
Notropis stramineus	DQ536429	DQ536429	DQ536429	DQ536429	DQ536429
Subfamily Phoxininae					
Phoxinus perenurus	AP009061	AP009061	AP009061	AP009061	AP009061
Subfamily Xenocyprinae					
Xenocypris macrolepis	AP009059	AP009059	AP009059	AP009059	AP009059
Subfamily Cultrinae					
Ischikauia steenackeri	AB239601	AB239601	AB239601	AB239601	AB239601
Subfamily Danioninae					
Danio rerio	AC024175	AC024175	AC024175	AC024175	AC024175
Esomus metallicus	AB239594	AB239594	AB239594	AB239594	AB239594
Incertae sedis					
Nipponocypris sieboldii	AB218898	AB218898	AB218898	AB218898	AB218898
Opsariichthys uncirostris	AB218897	AB218897	AB218897	AB218897	AB218897
Subfamily Cyprininae					
Tribe Cyprinini *sensu* Rainboth, 1981					
Carassioides acuminatus	HM536905	HM536806	HM536704	HM536869	HM536763
Carassius auratus	AB006953	AB006953	AB006953	AB006953	AB006953
Carassius auratus 2	AB006953	AB006953	AB006953	AB006953	AB006953
Carassius carassius	AY714387	AY714387	AY714387	AY714387	AY714387
Carassius cuvieri	AB045144	AB045144	AB045144	AB045144	AB045144
Carassius gibelio	AB379922	AB379922	HM536748	AB378300	AB379922
Cyprinus carpio	X61010	X61010	X61010	X61010	X61010
Cyprinus multitaeniatus	HM536896	HM536798	HM536718	HM536866	DQ845845
Procypris rabaudi	EU082030	EU082030	EU082030	EU082030	EU082030
Tribe Barbini					
Acrossocheilus monticola	HM536893	HM536795	HM536715	HM536839	HM536756
Balantiocheilos melanopterus	HM536894	HM536796	HM536716	HM536875	HM536757
Barbodes carnaticus	HM010713	HM010725	HM010737	HM010737	HM010701
Barbonymus gonionotus	AB238966	AB238966	AB238966	AB238966	AB238966
Barbus barbus	AB238965	AB238965	AB238965	AB238965	AB238965
Barbus fasciolatus	HM536910	HM536811	HM536730	HM536877	HM536768
Barbus trimaculatus	AB239600	AB239600	AB239600	AB239600	AB239600
Capoeta capoeta	HM536852	HM536882	HM536707	HM536850	HM536751
Catlocarpio siamensis	HM536911	HM536812	HM536731	HM536878	HM536769
Cyclocheilichthys armatus	HM536926	HM536827	HM536745	HM536861	HM536784
Cyclocheilichthys janthochir	HM536907	HM536808	HM536727	HM536870	HM536765
Eirmotus octozona	HM536918	HM536819	HM536737	HM536855	HM536776
Hampala macrolepidota	HM536886	HM536790	HM536709	HM536832	DQ845863

Table 3 (Continued).

Taxa	COI	Cyt *b*	ND4	ND5	16S
Hypsibarbus malcolmi	HM536915	HM536816	HM536735	HM536872	HM536773
Hypsibarbus vernayi	HM536892	HM536794	HM536714	HM536838	DQ845870
Mystacoleucus marginatus	HM536913	HM536814	HM536733	HM536880	HM536771
Neolissochilus stracheyi	HM536922	HM536823	HM536741	HM536857	HM536780
Onychostoma simum	HM536899	HM536801	HM536721	HM536843	DQ845861
Oreichthys cosuatis	HM536921	HM536822	HM536740	HM536856	HM536779
Poropuntius opisthoptera	HM536891	HM536793	HM536713	HM536837	HM536755
Probarbus jullieni	HM536909	HM536810	HM536729	HM536848	HM536767
Puntioplites falcifer	HM536904	HM536805	HM536726	HM536868	HM536762
Puntioplites proctozysron	HM536912	HM536813	HM536732	HM536879	HM536770
Puntioplites waandersi	HM536928	HM536829	HM536747	HM536863	HM536786
Puntius brevis	HM536914	HM536815	HM536734	HM536871	HM536772
Puntius nigrofasciatus	HM536920	HM536821	HM536739	HM536849	HM536778
Puntius oligolepis	HM536919	HM536820	HM536738	HM536881	HM536777
Puntius sp.	HM536916	HM536817	HM536736	HM536873	HM536774
Puntius tetrazona	EU287909	EU287909	EU287909	EU287909	EU287909
Puntius ticto	AB238969	AB238969	AB238969	AB238969	AB238969
Puntius titteya	HM536908	HM536809	HM536728	HM536876	HM536766
Scaphognathops bandanensis	HM536927	HM536828	HM536746	HM536862	HM536785
Scaphognathops stejnegeri	HM536906	HM536807	HM536705	HM536847	HM536764
Sikukia stejnegeri	HM536898	HM536800	HM536720	HM536842	DQ845872
Sinocyclocheilus altishoulderus	FJ984568	FJ984568	FJ984568	FJ984568	FJ984568
Sinocyclocheilus grahami	GQ148557	GQ148557	GQ148557	GQ148557	GQ148557
Sinocyclocheilus macrophthalmus	HM536889	HM536792	HM536711	HM536835	HM536754
Sinocyclocheilus microphthalmus	HM536888	AY854690	HM536703	HM536834	HM536753
Sinocyclocheilus xunlensis	HM536887	HM536791	HM536710	HM536833	HM536752
Spinibarbus hollandi	HM536890	AY195629	HM536712	HM536836	DQ845865
Spinibarbus sinensis	HM536895	HM536797	HM536717	HM536840	DQ845864
Tor sinensis	HM536900	HM536802	HM536722	HM536844	DQ845876
Tor tambroides	HM536923	HM536824	HM536742	HM536858	HM536781
Tribe Oreinini					
Chuanchia labiosa	HM536897	HM536799	HM536719	HM536841	HM536758
Gymnocypris przewalskii	AB239595	AB239595	AB239595	AB239595	AB239595
Oxygymnocypris stewartii	HM536853	DQ491114	HM536749	HM536864	DQ845918
Platypharodon extremus	HM536854	AY463498	HM536750	HM536851	DQ845855
Schizothorax oconnori	HM536902	AY463519	HM536724	HM536846	HM536760
Schizothorax waltoni	HM536903	HM536804	HM536725	HM536867	HM536761
Tribe Labeonini					
Cirrhinus microlepis	HM536924	HM536825	HM536743	HM536859	HM536782
Crossocheilus reticulatus	HM536925	HM536826	HM536744	HM536860	HM536783
Epalzeorhynchos bicolor	HM536917	HM536818	HM536706	HM536874	HM536775
Garra orientalis	HM536884	HM536788	HM536702	HM536831	DQ845884
Henicorhynchus lineatus	HM536901	HM536803	HM536723	HM536845	HM536759
Labeo batesii	AB238967	AB238967	AB238967	AB238967	AB238967
Labeo senegalensis	AB238968	AB238968	AB238968	AB238968	AB238968
Labiobarbus lineatus	HM536885	HM536789	HM536708	HM536865	DQ845914
Osteochilus salsburyi	HM536883	HM536787	HM536701	HM536830	DQ845892
Expanded dataset (additional taxa)					
Linichthys laticeps		AY854683	AY854740		
Procypris rabaudi 2		DQ366226			
Procypris rabaudi 3		DQ366238			
Procypris rabaudi 4		DQ366243			
Procypris rabaudi 5		DQ366250			
Sinocyclocheilus anatirostris		AY854708	AY854765		
Sinocyclocheilus angustiporus		AY854702	AY854759		
Sinocyclocheilus anophthalmus		AY854697	AY854754		
Sinocyclocheilus bicornutus		AY854730	AY854787		

Table 3 (Continued).

Taxa	COI	Cyt *b*	ND4	ND5	16S
Sinocyclocheilus cyphotergous		AY854711	AY854768		
Sinocyclocheilus furcodorsalis		AY854709	AY854766		
Sinocyclocheilus guishanensis		AY854725	AY854782		
Sinocyclocheilus halfibindus		AY854723	AY854780		
Sinocyclocheilus huaningensis		AY854718	AY854775		
Sinocyclocheilus hyalinus		AY854721	AY854778		
Sinocyclocheilus jii		AY854727	AY854784		
Sinocyclocheilus jiuxuensis		AY854736	AY854793		
Sinocyclocheilus lateristriatus		AY854703	AY854760		
Sinocyclocheilus lingyunensis		AY854691	AY854748		
Sinocyclocheilus longibarbatus		AY854714	AY854771		
Sinocyclocheilus lunanensis		AY854710	AY854767		
Sinocyclocheilus macrocephalus		AY854685	AY854742		
Sinocyclocheilus macrolepis		AY854729	AY854786		
Sinocyclocheilus maculates		EU366193	EU366183		
Sinocyclocheilus maitianheensis		AY854715	AY854772		
Sinocyclocheilus malacopterus		AY854722	AY854779		
Sinocyclocheilus multipunctatus		AY854712	AY854769		
Sinocyclocheilus oxycephalus		AY854686	AY854743		
Sinocyclocheilus purpureus		EU366189	EU366178		
Sinocyclocheilus qiubeinsis		EU366188	EU366182		
Sinocyclocheilus qujingensis		AY854720	AY854777		
Sinocyclocheilus rhinocerous		AY854683	AY854740		
Sinocyclocheilus tianeensis		AY854717	AY854774		
Sinocyclocheilus tingi		AY854701	AY854758		
Sinocyclocheilus yangzongensis		AY854719	AY854776		
Sinocyclocheilus yimenensis		EU366191	EU366180		

16S ribosomal RNA (16S), NADH dehydrogenase subunits 4 (ND4) and subunits 5 (ND5). These genes have been commonly used in phylogenetic investigations of Cypriniformes fishes to resolve relationships across broad phylogenetic levels. The combination of these genes was expected to provide good resolution across different levels of the hierarchy. Nuclear genes were not used in this study simply due to the fact that some polyploids exist in cyprinins (e.g. *Cyprinus* and *Carassius*), barbins (e.g. *Barbus* and *Tor*) and oreinins (e.g. *Schizothorax* and *Gymnocypris*) (Yu *et al.* 1987; Klinkhardt *et al.* 1995). As misidentification of homologous characters in the nuclear genome can be a significant source of homoplasy in polyploidy species, these genes were excluded until such time that more specific primers are available to establish homology. All primers used to amplify the five mitochondrial genes were listed in Table 4. Information on the amplification of COI and Cyt *b* was provided in Mayden *et al.* (2007). Protocols used to amplify genes ND4 and ND5 were from Miya *et al.* (2006). For the 16S rRNA gene, amplifications were carried out in 25 μL reactions [5 μL (10×) reaction buffer, 2.5 μL dNTP (2.5 mM each), 2.5 μL $MgCl_2$ (25 mM), 1 μL (10 μM) each primer, 1 μL template DNA (10 ng/μL) and 0.2 μL ExTaq *Taq* DNA polymerase (Takara Bio Inc. Japan)]. The following thermal cycling profiles were adopted: 94 °C predenaturing (3 min), 94 °C denaturing (45 s), 50 °C annealing (45 s), 72 °C extension (60 s), for 35 cycles and 72 °C final extension (7 min). Amplified products were either directly purified using Agencourt Ampure (Agencourt Bioscience Corporation. Beverly, Maryland. U.S.A.) or loaded onto agarose gels and electrophoresed, followed by excision of the target DNA from the gel and purification using QIAquick Gel Extraction Kit (QIAGEN Sciences, Inc. Germantown, Maryland. U.S.A.). Primers used for PCR amplifications were also used for sequencing. All sequences generated from this study were deposited in GenBank and accession numbers for these and other sequences downloaded from GenBank are provided in Table 3.

Sequence alignment and phylogenetic analyses

Multiple alignment of the protein-coding gene sequences was performed using CLUSTALX (Thompson *et al.* 1997), SEAVIEW alignment editor (Galtier *et al.* 1996), and verified by eye. Alignment of the 16S rRNA gene followed the methods of Li *et al.* (2008). Partitioned maximum likelihood (ML) and partitioned Bayesian analyses (BA) were conducted using RAxML 7.0.4 (randomized axelerated

Table 4 Lists of primers used in this study and their sequences

Marker	Primer	Sequence (5′–3′)	Source
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* (1994)
Cyt *b*	LA-cyp	ATGGCAAGCCTACGAAAAAC	Tang *et al.* (in press)
	LA-danio	GACTYGAARAACCACYGTTG	Mayden *et al.* (2007)
	HA-cyp	TCGGATTACAAGACCGATGCTT	Tang *et al.* (in press)
	HA-danio	CTCCGATCTTCGGATTACAAG	Mayden *et al.* (2007)
16S	16Sar_L	CGCCTGTTTACCAAAAACATCGCCT	Palumbi (1996)
	16Sbr_H	CCGGTCTGAACTCAGATCACGT	Palumbi (1996)
ND4–5	L10444	AAGACCTCTGATTTCGGCTCA	Mayden *et al.* (2007)
	L10474-Arg-C	GGTTWGAKTCCGYGGTTCCCTTATGA	Miya *et al.* (2006)
	L10681-ND4-C	GCKTTTTCTGCKTGTGARGC	Miya *et al.* (2006)
	L11427-ND4-C	CCWAAGGCSCATGTWGARGC	Miya *et al.* (2006)
	L12170-His-C	GTAAGTATAGTTTAAKTWAAATRTTAGATTGTG	Miya *et al.* (2006)
	L12319-Leu-C	TTGGTCTTAGGAACCAAAAACTCTTGGTGC	Miya *et al.* (2006)
	L12328-Leu-C	AACTCTTGGTGCAAMTCCAAG	Miya *et al.* (2006)
	L13058-ND5-C	TCKGCTATGGAGGGYCCKAC	Miya *et al.* (2006)
	L13559-ND5-C	TCKTATCTKAACGCCTGRGC	Miya *et al.* (2006)
	H11618-ND4-C	TGGCTKACKGAKGAGTAKGC	Miya *et al.* (2006)
	H12296-Leu-C	CAAGAGTTTTTGGTTCCTAAG	Miya *et al.* (2006)
	H12632-ND5-C	TTCTAGGATKGATCAGGTGACGWAKAGKGC	Miya *et al.* (2006)
	H13393-ND5-C	CCTATTTTKCGGATGTCTTGYTC	Miya *et al.* (2006)
	H13721-ND5-C	ATGCTTCCTCAGGCRAGKCG	Miya *et al.* (2006)
	H14473-ND6-C	GCGGCWTTGGCKGCKGAGCC	Miya *et al.* (2006)
	H14710-Glu-C	CTTGTAGTTGAATWACAACGGTGGTTYTTC	Miya *et al.* (2006)

maximum likelihood) (Stamatakis 2006) and MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), respectively.

Partitioned ML search was first performed using RAxML (parallelized version) on the high-performance cluster computing facility (36 nodes) located at Saint Louis University. Nucleotide data were partitioned into 14 partitions according to the codon positions of each of the four protein-coding genes and the stems and loops of 16s rRNA gene. The GTR+Γ+I model (with four discrete rate categories) was chosen for each partition. A total of 100 distinct runs were performed based on 100 random starting trees using the default algorithm of the program. The tree with ML scores was chosen as the final tree. ML bootstrap analysis (MLBS) was conducted using RAxML (sequential version) (Felsenstein 1985; Stamatakis *et al.* 2008). The same partitioning strategy was used as in the initial ML search. The number of nonparametric bootstrap replications was set at 1000, and other parameters were set as default. The resulting trees were imported into paup*4.0.b10 (Swofford 2002) to obtain the 50% majority rule consensus tree.

Partitioned BA that implements multiple models in a single analysis were conducted using MrBayes on our cluster computing facility. ModelTest 3.8 (ModelTest Server 1.0: http://darwin.uvigo.es/software/modeltest_server.html) (Posada & Crandall 1998; Posada 2006) and Bayesian Information Criterion (BIC) were used to select the best evolution model for each gene, the combined dataset and the subsets of them. For BIC, sampling sizes were set as the total number of characters of each dataset. Models selected are provided in Table 5. To increase the accuracy of modelling data and decrease the chance of overpartitioning at the same time, 13 independent BA under different partitioning schemes were conducted to choose the best-fit partitioning strategy using the methods of Brandley *et al.* (2005). Partitions were chosen *a priori* based on gene types (protein-coding or rRNA), genes, codon positions (COI, Cyt *b*, ND4 and ND5), stems and loops (16S). In five of the 13 BA conducted, we combined ND4 and ND5 sequences because they have similar functional and evolutionary constraints and are expected to evolve similarly. The combined dataset for the five genes was partitioned into 1 (one model for the whole dataset) to 14 partitions (one model each for the codon positions of each protein-coding gene and for the stem and loop regions of 16S rRNA gene). Names and descriptions of each partitioning strategy used to analyze the combined dataset are summarized in Table 6. The Bayes factors (B_{10}), which can be calculated as the ratio of the harmonic means of the likelihoods of the two analyses being tested, were used to select the optimal partitioning strategy for analysis of the combined data. The harmonic means of the likelihoods for each analysis were retrieved by executing the 'sump' command. The criterion of 2ln Bayes factor ≥10 was used as very strong evidence of one hypothesis against the alternative hypothesis. Finally,

Table 5 Models chosen by BIC (Bayesian Information Criterion) for each dataset using ModelTest and the models implemented in MrBayes

Loci	Length (bp)	Model selected by ModelTest	Model implemented in MrBayes
COI	678	HKY+I+G	HKY+I+G
1st position	226	GTR+I+G	GTR+I+G
2nd position	226	HKY+G	HKY+G
3rd position	226	TrN+I+G	GTR+I+G
1st + 2nd position	452	SYM+I+G	SYM+I+G
Cyt *b*	1141	TVM+I+G	GTR+I+G
1st position	381	GTR+I+G	GTR+I+G
2nd position	380	HKY+I+G	HKY+I+G
3rd position	380	TrN+I+G	GTR+I+G
1st + 2nd position	761	GTR+I+G	GTR+I+G
16S	559	GTR+I+G	GTR+I+G
Stem	302	SYM+I+G	SYM+I+G
Loop	257	TIM+I+G	GTR+I+G
ND4	1381	GTR+I+G	GTR+I+G
1st position	461	GTR+I+G	GTR+I+G
2nd position	460	TVM+I+G	GTR+I+G
3rd position	460	GTR+I+G	GTR+I+G
1st + 2nd position	921	GTR+I+G	GTR+I+G
ND5	1842	K81uf+I+G	GTR+I+G
1st position	614	GTR+I+G	GTR+I+G
2nd position	614	TVM+I+G	GTR+I+G
3rd position	614	GTR+I+G	GTR+I+G
1st + 2nd position	1228	TrN+I+G	GTR+I+G
ND4 + ND5	3223	HKY+I+G	HKY+I+G
1st position	1075	GTR+I+G	GTR+I+G
2nd position	1074	GTR+I+G	GTR+I+G
3rd position	1074	HKY+I+G	HKY+I+G
1st + 2nd position	2149	HKY+I+G	HKY+I+G
COI + Cyt *b* + ND4 + ND5	5042	TVM+I+G	GTR+I+G
1st position	1682	GTR+I+G	GTR+I+G
2nd position	1680	TVM+I+G	GTR+I+G
3rd position	1680	GTR+I+G	GTR+I+G
1st + 2nd position	3362	GTR+I+G	GTR+I+G
All gene combined	5601	TVM+I+G	GTR+I+G

the partitioning strategy that explains the variability in the data better than other strategies and with fewer partitions was considered as the best-fit strategy.

For each of the 13 partitioned BA, random tree was used as the starting tree. Models implemented in these analyses are listed in Table 5. The parameters 'shape', 'pinvar', 'statefreq' and 'revmat' were unlinked for all data partitions. The parameter 'prsetratepr' was set as 'variable' to make rate multiplier variable across all partitions. Eight (one cold chain and seven heated chains) simultaneous Markov chains were run for 5 000 000 generations, with trees being sampled every 100 generations for a total of 50 001 trees in the initial sample. The log-likelihood scores were plotted against generation time to determine when the Markov chains reached stationary. Based on the plotting results (not shown), the first 5000 trees were discarded as burn-in and the remaining 45 001 trees were imported into PAUP to compute the 50% majority rule consensus trees. The frequency with which clades were recovered is interpreted as the posterior probability of that clade. Two independent analyses were conducted for each dataset.

Five constraint trees were constructed based on the hypotheses proposed by Fang (1936a), Chen & Huang (1977) and Rainboth (1981, 1991) (with/without *Barbus*). Partitioned ML searches were carried out using RAxML (parallelized version). Data were partitioned into 14 sections as before and the GTR+Γ+I model was adopted for each partition. A total of 100 distinct runs were performed based on each of the five constraint trees. The tree with ML scores was chosen as the best tree. Shimodaira and Hasegawa tests (SH tests; Shimodaira & Hasegawa 1999) were then conducted using RAxML (sequential version) to investigate whether the best ML trees resulting from constraint searches are significantly worse than the best ML tree obtained from the original non-constraint search.

To further investigate the phylogenetic position of the genus *Sinocyclocheilus* and its relationships with *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, we downloaded Cyt *b* and ND4 sequences for another 31 *Sinocyclocheilus* species from the GenBank. Most of these sequences were from Xiao *et al.* (2005). For comparative purpose, the sequences for the outgroup species *Barbodes laticeps* (now valid as: *Linichthys laticeps*) in Xiao *et al.* (2005) were also downloaded. We also downloaded the Cyt *b* gene sequences for four additional individuals of *Procypris rabaudi* from the GenBank. The downloaded data and the dataset that we used in previous analyses (original dataset) were combined to form the expanded dataset (Table 3). A constraint tree was built by pruning the five *Sinocyclocheilus* species and *Procypris rabaudi* from the most likelihood tree resulting from the original dataset. Partitioned ML searches were conducted using RAxML based on the constraint tree with parameters set as the same as in our previous constraint ML analyses. The tree with the ML score was chosen as the best tree. MLBS and BA were conducted to evaluate node support for the best ML trees.

Results

A total of 5601-bp nucleotides were sequenced for the five mitochondrial genes: COI (678 bp), Cyt *b* (1141 bp), ND4 (1381 bp), ND5 (1842 bp) and 16S (559 bp/nt). For 16S rRNA, 302 bp belong to stems, and 257 nt belong to loops. Nucleotide sequences of the first four protein-coding genes code for 226, 380, 460 and 614 amino acids, respectively. A total of 425 nucleotide sequences were used for the original dataset, with all five genes sequenced or downloaded from GenBank for all ingroup and outgroup

Table 6 Description of partitioning strategies used in the partitioned Bayesian analyses

Partitions	Free model parameters	Partition identity	Harmonic mean of marginal likelihood
×1	10	Entire dataset	−134342.02
×2	20	COI + Cyt *b* + ND4 + ND5; 16S	−134229.91
×3	30	COI + Cyt *b* + ND4 + ND5; 16S stem; 16S loop	−134147.02
×4	32	COI; Cyt *b*; ND4 + ND5; 16S	−134107.11
×5	46	COI; Cyt *b*; ND4; ND5; 16S	−134037.78
×5	42	COI; Cyt *b*; ND4 + ND5; 16s stem; 16S loop	−134022.66
×6	56	COI; Cyt *b*; ND4; ND5; 16S stem; 16S loop	−133946.01
×7	62	COI 1st + 2nd; Cyt *b* 1st + 2nd; [ND4 + ND5] 1st + 2nd; 16S	−132550.25
×8	72	COI 1st + 2nd; Cyt *b* 1st + 2nd; [ND4 + ND5] 1st + 2nd; 16S stem; 16S loop	−132443.26
×9	90	COI 1st + 2nd; Cyt *b* 1st + 2nd; ND4 1st + 2nd; ND5 1st + 2nd; 16s	−132314.64
×10	100	COI 1st + 2nd; Cyt *b* 1st + 2nd; ND4 1st + 2nd; ND5 1st + 2nd; 16S stem; 16S loop	−132222.25
×11	96	COI 1st; COI 2nd; COI 3rd; Cyt *b* 1st; Cyt *b* 2nd; Cyt *b* 3rd; [ND4 + ND5] 1st; [ND4 + ND5] 2nd; [ND4 + ND5] 3rd; 16S stem; 16S loop	−132043.08
×14	130	COI 1st; COI 2nd; COI 3rd; Cyt *b* 1st; Cyt *b* 2nd; Cyt *b* 3rd; ND4 1st; ND4 2nd; ND4 3rd; ND5 1st; ND5 2nd; ND5 3rd; 16S stem; 16S loop	−131827.97

taxa (Table 3). For the combined dataset, 3186 base locations in aligned sequences (characters) were variable, and 2767 characters were parsimony informative. Mean base composition of the combined dataset was A, 0.30333; C, 0.27239; G, 0.15296; and T, 0.27133. No significant compositional biases existed in either ingroup and outgroup taxa. Base composition among cyprinines and also among all taxa analyzed was homogenous at all three codon positions (results not shown). Models selected by ModelTest under BIC criterion for each of the five genes, the combined dataset, and subsets of the data are provided in Table 5. Some models were revised, e.g. from TrN to GTR, so that they could be implemented in MrBayes, as this program cannot address the difference in the rate matrix of different models.

Maximum-likelihood analysis yielded one best likelihood tree (−ln *L* = 130684.195637) (Fig. 2). The subfamily Cyprininae was resolved as monophyletic, and seven major clades (Clades 1–7) were successfully recovered. Clade 1, which is the basal-most group, contains only two species: *Catlocarpio siamensis* and *Probarbus jullieni*. Clade 2 is comprised exclusively of labeonin species. Clade 3 includes three species from the two genera *Tor* and *Neolissochilus*. All species of the tribe Orieinini clustered within Clade 4 together with six other barbins: *Onychostoma simum*, *Acrossocheilus monticola*, *Spinibarbus sinensis*, *Spinibarbus hollandi*, *Barbus barbus* and *Capoeta capoeta*. Some taxa of the tribe Cyprinini and one genus, *Sinocyclocheilus*, of the tribe Barbini formed Clade 5. Clade 6 was comprised exclusively of 12 barbins, including species of the genera *Puntius*, *Barbus* and their allies. Clade 7 contains five species of the genera *Puntioplites* and *Scaphognathops* and nine barbin species.

The harmonic means of the likelihoods for BA based on each partitioning strategy are shown in Table 6. The 2ln Bayes factors (B_{10}) results from comparisons of alternative partitioning strategies are shown in Table 7. All Bayes factor estimates were much larger than the required criterion for strong evidence against a hypothesis. Partitioning the combined dataset into 14 partitions and estimating the evolution model for each partition independently can provide a decisively better explanation of the data than all other analyses according to the Bayes factor (Table 7). Thus, this phylogeny is our preferred hypothesis of cyprinines, and subsequent discussion will be limited to this tree (Fig. 3). The BA under the 14-partition scheme also recovered a monophyletic Cyprininae and the seven major clades resolved by the ML tree (Fig. 3). The topologies of these two trees are nearly identical (Figs 2 and 3). The only difference lies in the Clade 3 regarding whether the grouping of *Neolissochilus stracheyi* and *Tor tambroides* was strongly supported.

The monophyly of the subfamily Cyprininae was robustly supported by both partitioned MLBS analysis and partitioned BA (14-partition scheme). All seven clades recovered were supported with high bootstrap values (BP) (≥80%) in the MLBS analysis (except the Clade 5, BP = 64%) and high Bayesian posterior probability values (BPP) (≥95%) for the BA. The Cyprinini, regardless of its composition by Fang (1936a), Chen & Huang (1977) or Rainboth (1991), was not monophyletic. SH tests revealed that the ML trees resulting from constraint searches were significantly worse that the tree obtained from non-constraint search (Table 8). Cyprinini *sensu* Rainboth, 1981 was non-monophyletic in the phylogenetic trees resulting

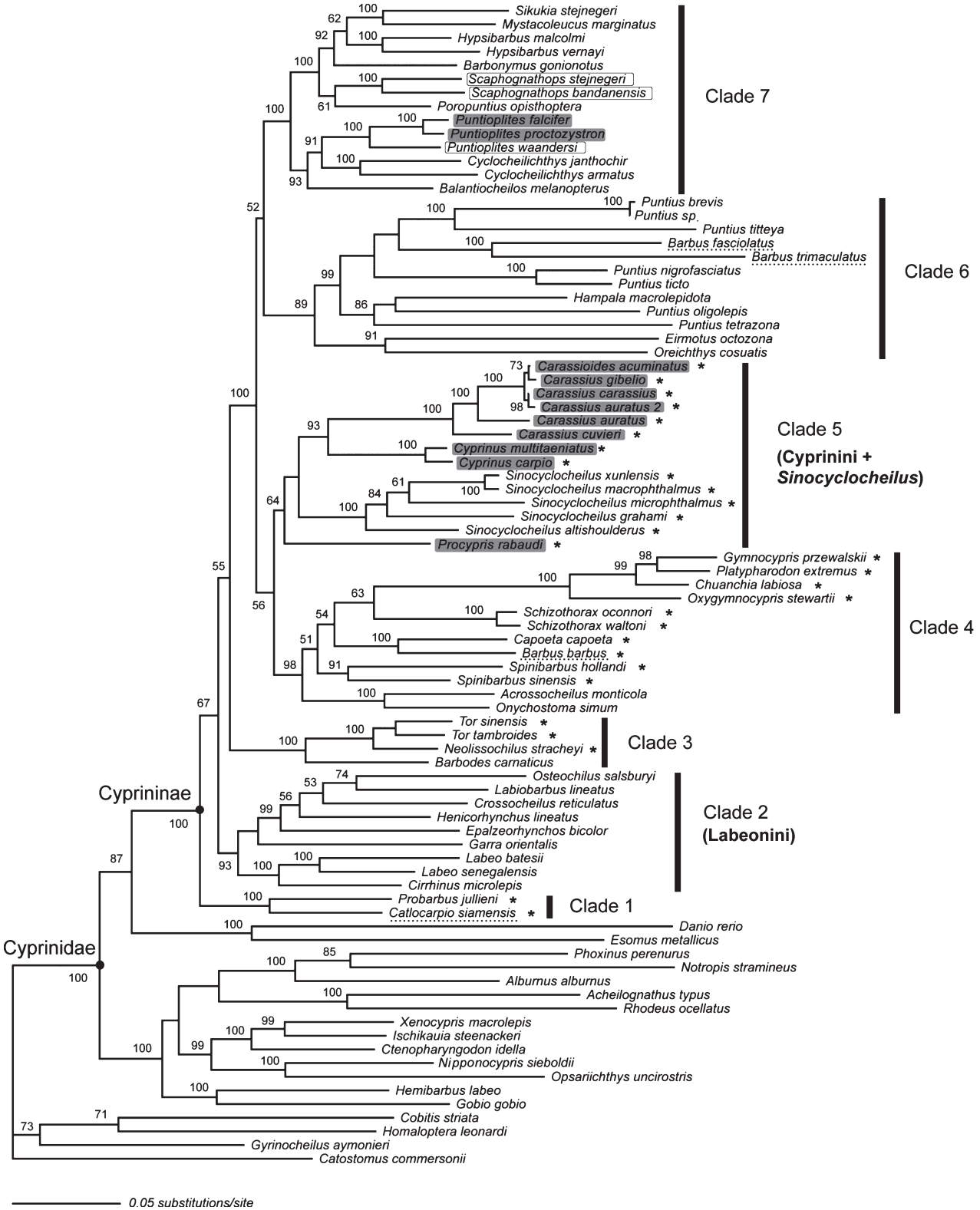


Table 7 The 2ln Bayes factors (B_{10}) results of comparisons of alternative partitioning strategies

	M_1												
M_0	×1	×2	×3	×4	×5	×5	×6	×7	×8	×9	×10	×11	×14
×1		**224.22**	**390.00**	**469.82**	**608.48**	**638.72**	**792.02**	**3583.54**	**3797.52**	**4054.76**	**4239.54**	**4597.88**	**5028.10**
×2	−224.22		**165.78**	**245.60**	**384.26**	**414.50**	**567.80**	**3359.32**	**3573.30**	**3830.54**	**4015.32**	**4373.66**	**4803.88**
×3	−390.00	−165.78		**79.82**	**218.48**	**248.72**	**402.02**	**3193.54**	**3407.52**	**3664.76**	**3849.54**	**4207.88**	**4638.10**
×4	−469.82	−245.60	−79.82		**138.66**	**168.90**	**322.20**	**3113.72**	**3227.70**	**3584.64**	**3769.72**	**4128.06**	**4558.28**
×5	−608.48	−384.26	−218.48	−138.66		**30.24**	**183.54**	**2975.06**	**3189.04**	**3446.28**	**3631.06**	**3989.40**	**4419.62**
×5	−638.72	−414.50	−248.72	−168.90	−30.24		**153.30**	**2944.82**	**3158.80**	**3416.04**	**3600.82**	**3959.16**	**4389.38**
×6	−792.02	−567.80	−402.02	−322.20	−183.54	−153.30		**2791.52**	**3005.50**	**3262.74**	**3447.52**	**3805.86**	**4236.08**
×7	−3583.54	−3359.32	−3193.54	−3113.72	−2975.06	−2944.82	−2791.52		**213.98**	**471.22**	**656.00**	**1014.34**	**1444.56**
×8	−3797.52	−3573.30	−3407.52	−3227.70	−3189.04	−3158.80	−3005.50	−213.98		**257.24**	**442.02**	**800.36**	**1230.58**
×9	−4054.76	−3830.54	−3664.76	−3584.64	−3446.28	−3416.04	−3262.74	−471.22	−257.24		**184.78**	**543.12**	**973.34**
×10	−4239.54	−4015.32	−3849.54	−3769.72	−3631.06	−3600.82	−3447.52	−656.00	−442.02	−184.78		**358.34**	**788.56**
×11	−4597.88	−4373.66	−4207.88	−4128.06	−3989.40	−3959.16	−3805.86	−1014.34	−800.36	−543.12	−358.34		**430.22**
×14	−5028.10	−4803.88	−4638.10	−4558.28	−4419.62	−4389.38	−4236.08	−1444.56	−1230.58	−973.34	−788.56	−430.22	

Values above the diagonal show the Bayes factor support for model M_1 over model M_0, whereas values below the diagonal show Bayes factor (2ln B_{10}) support for M_0 over M_1. Bold indicates strong evidence (2ln $B_{10} \geq 10$) for one model over the alternative models.

from the original dataset, whereas it is monophyletic in the phylogenetic trees resulting from the expanded dataset. SH tests revealed that the ML trees resulting from constraint searches were not significantly worse that the tree obtained from non-constraint search (Table 8). Members of either Cyprinini *sensu* Fang, 1936a or Cyprinini *sensu* Chen & Huang, 1977 are found in two clades, Clade 5 and Clade 7 (Figs 2 and 3). Members of Cyprinini *sensu* Rainboth, 1981 are confined to Clade 5. Members of Cyprinini *sensu* Rainboth, 1991 scatter in Clades 1, 4, 5 and 6. In Clade 5, *Procypris rabaudi* forms the basal sister group to remaining members. Five species of *Sinocyclocheilus* form a monophyletic group sister to a clade composed of *Cyprinus*, *Carassius* and *Carassioides*. *Carassius* is resolved as paraphylectic with respect to *Carassioides* as it is within the former genus. This clade is strongly supported by both MLBS (BP = 100%) and BA (BPP = 100%). The *Carassius* clade is sister to a clade of two species of *Cyprinus* representing both of the subgenera, a relationship that is also strongly supported (BP = 93%; BPP = 100%). SH tests also revealed that the new diagnosis proposed in this study for the tribe Cyprinini (see Discussion) reflects a topology that is not significantly worse than that of the best ML tree (Table 8). ML analysis based on the expanded dataset yielded one best likelihood tree (−ln *L* = 139108.542125) (Fig. 4). All *Sinocyclocheilus* species formed a monophyletic group (BP = 55%; BPP = 98%), and it is sister to a clade comprised *Cyprinus*, *Carassius*, *Carassioides* and *Procypris* (BP < 50%; BPP = 62%). This sister group relationship was supported by both MLBS analysis (BP < 50%) and BA (BPP = 98%).

Discussion

Phylogenetic positions of members of the tribe Cyprinini sensu stricto in the subfamily Cyprininae

The monophyly of the subfamily Cyprininae is robustly supported in our analyses, a finding that is consistent with previous morphological (Chen *et al.* 1984; Cavender & Coburn 1992) and molecular studies (Wang *et al.* 2007; Li *et al.* 2008; Mayden *et al.* 2009). Among its four tribes, however, only the Labeonini and Oreinini are monophyletic. The tribe Barbini is not a natural grouping; its traditionally identified members are in all the major clades recovered except Clade 2 that includes all labeonin species. The tribe Cyprinini as defined by Fang (1936a), Chen & Huang (1977) or Rainboth (1991) never formed a mono-

Fig. 2 The best likelihood tree (−ln *L* = 130684.195637) resulting from partitioned maximum-likelihood analysis. Numbers above (or below) branches indicate the bootstrap values from the maximum-likelihood bootstrap analysis (1000 replicates). Only those values larger than 50% were shown. The boxes (open plus shaded) denote species included in the tribe Cyprinini *sensu* Chen & Huang, 1977; whereas the shaded boxes show species contained in the Cyprinini *sensu* Fang, 1936a. The shaded boxes in the Clade 5 denote species included in tribe Cyprinini *sensu* Rainboth, 1981. Cyprinini *sensu* Rainboth, 1991 includes the shaded species, the underlined species and *Puntioplites waandersi* in the Clade 7. The '*' marks denote cyprinine fishes that are polyploids (including tetraploids and hexaploids; mainly according to Yu *et al.* 1987 and Klinkhardt *et al.* 1995).

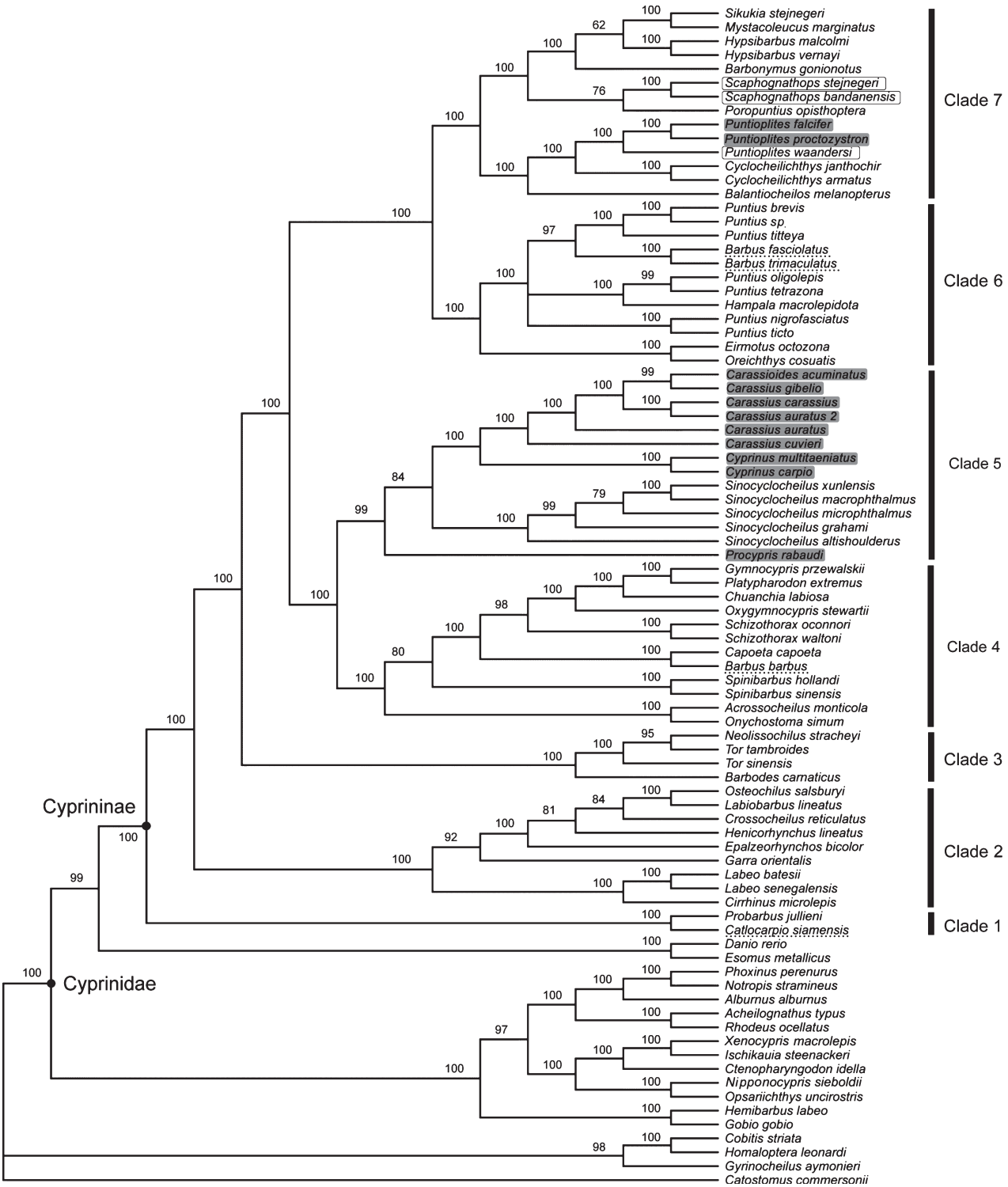


Fig. 3 The 50% Bayesian consensus tree generated from partitioned Bayesian analysis basing on the best partitioning strategy (14 partition). Numbers above (or below) branches indicated the posterior probability values (shown as percentage). Only those values larger than 50% were shown. The boxes (open plus shaded) denote species included in the tribe Cyprinini *sensu* Chen & Huang, 1977; whereas the shaded boxes show species included in the Cyprinini *sensu* Fang, 1936a. The shaded boxes in the Clade 5 denote species included in the Cyprinini *sensu* Rainboth, 1981. Cyprinini *sensu* Rainboth, 1991 includes the shaded species, the underlined species and *Puntioplites waandersi* in the Clade 7.

Table 8 Statistical comparison of various hypotheses using Shimodaira & Hasegawa (1999) test as implemented in RAxML

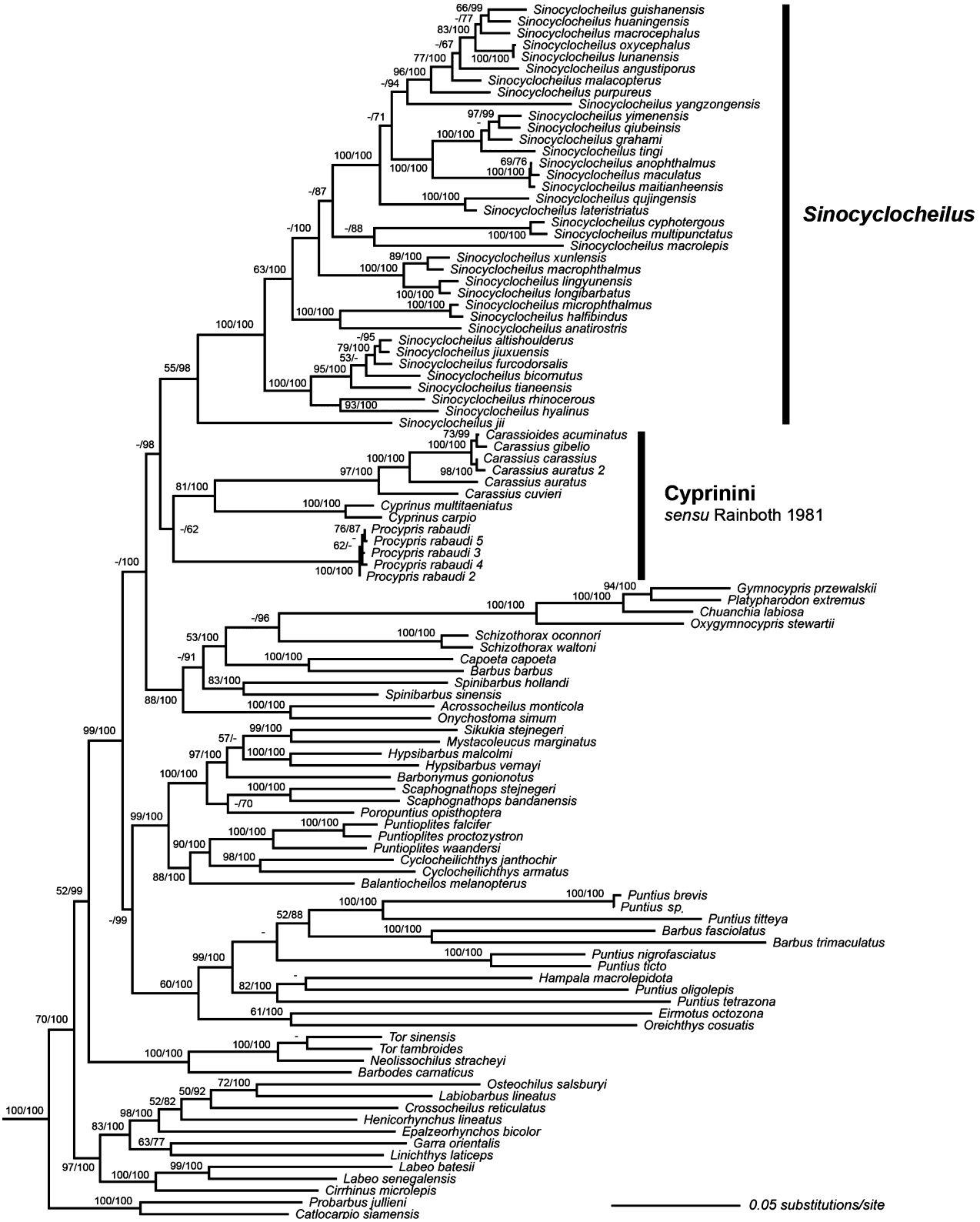
Hypotheses	−ln *L*	−ln *L* Diff.	SD	Significantly worse
This study (best ML tree)	130684.195637			
Fang (1936a)*	131024.477950	340.282312	35.745536	Yes
Chen & Huang (1977)*	130967.170358	282.974721	34.559113	Yes
Rainboth (1981)*	130686.812794	2.617157	5.324941	No
Rainboth (1991)* (with *Barbus*)	131137.177186	452.981549	44.430036	Yes
Rainboth (1991)* (without *Barbus*)	130918.375135	234.179498	28.289070	Yes

*Best maximum-likelihood tree recovered from constraint search using RAxML.

phyletic group. Species of Cyprinini *sensu* Rainboth, 1981 formed a monophyletic group in those phylogenetic trees resulting from the expanded dataset, but failed to do so in the phylogenetic trees resulting from the original dataset. Four genera, *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, were treated as members of the tribe Cyprinini in all previous definitions. In our phylogenetic analyses, these genera and species were found in Clade 5. *Cyprinus*, *Carassius* and *Carassioides* formed a monophyletic group in all analyses, whereas the position of *Procypris* varied. Whether *Procypris* formed a monophyletic group with the other three genera is related to the phylogenetic position of the genus *Sinocyclocheilus*, which varied in its placement in different analyses. Our later discussions focus on these genera.

The three species of *Puntioplites*, whether anal spines are serrated or not, form a robustly supported clade in the Clade 7, sister to the two species of *Cyclocheilichthys*, with *P. waandersi* sister to *P. falcifer* plus *P. proctozystron*. Therefore, the genus *Puntioplites* should be treated as a member of the tribe Barbini. This agrees with the opinions of Taki & Katsuyama (1979) and Rainboth (1981). Within the same clade, the species *Scaphognathops stejnegeri* and *Scaphognathops bandanensis* form a strongly supported clade, sister to *Poropuntius*. Therefore, *Scaphognathops* is also a barbin. Actually, this opinion is widely accepted by those ichthyologists that have examined the external morphology of fishes of this genus. In Zhou's (1989) English abstract (not in Chinese abstract or any other part of the paper), he also stated, 'According to comparisons of skeletons, the author is inclined to classify the genus *Scaphognathops* under Barbinae rather than Cypininae'. Previous karyotypical studies also supported the opinion of treating the genera *Puntioplites* and *Scaphognathops* as barbins. Species of these two genera usually have a chromosome number of 2n = 50 (Magtoon & Arai 1993; Donsakul *et al.* 2007), whereas *Cyprinus*, *Carassius*, *Carassioides* and *Procypris* usually have a chromosome number of 2n = *c.* 100 (some may have more chromosomes, 2n = *c.* 150 or *c.* 200) (Yu *et al.* 1987; Klinkhardt *et al.* 1995). Few studies have been conducted on the taxonomic affiliation of the monotypic genus *Kalimantania* due to its rarity. In the original description of this genus, Bănărescu (1980) stated that '…These data do not suggest any closer relationship between *Kalimantania* and the Barbini; on the contrary, the genus seems, in respect of scale shape, closer to *Xenocheilichthys*'. *Xenocheilichthys* is currently a junior synonym of *Sikukia*. Based on the results of the current study, we predict that *Kalimantania* is not a cyprinin. No karyotypical study has been conducted on this genus. Wang (1979) suggested creating a new tribe to accommodate those cyprinin species that possess a smooth anal spine (no serrations). He also considered the distributions of these species and their distinct mouth structures. Luo & Yue (2000) argued that one cannot determine the tribe to which a species really belongs simply based on its distribution, and mouth structures are problematic (e.g. homoplasious) because they are closely associated with habitats and feeding behaviour. In this study, *Puntioplites proctozystron*, *Scaphognathops stejnegeri* and *Scaphognathops bandanensis* are all species that have smooth anal spines. These three species did not form a monophyletic group but were members of Clade 7, with the *P. proctozystron* and the species of *Scaphognathops* being located in different subclades in both ML and BA (Figs 2 and 3). This pattern of relationships among these species is not likely to change even when more species are included in future analysis, including *Kalimantania*. Therefore, we cannot agree with Wang (1979) with the establishment of a new tribe to accommodate species with a smooth anal spine. It is likely that both the anal spine and the occurrence of serrations have evolved independently multiple times in cyprinin fishes. Using the phylogenetic tree derived from our expanded dataset (Fig. 4), anal spines appear to have evolved independently at least three times, whereas serrations on the anal spine have evolved independently at least twice.

Rainboth (1991) suggested putting the genera *Catlocarpio*, *Thynnichthys* and maybe also *Barbus* in the Cyprinini. The genus *Catlocarpio* is certainly not a cyprinin, because it is located in the Clade 1. The only species of this genus,



C. siamensis, has a chromosome number of 2n = 98 (Suzuki & Taki 1988), which is similar to that of *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*. However, it might be a product of an independent polyploidization event. The genus *Barbus* cannot be treated as a cyprinin either. This genus is a very typical barbin. In our phylogenetic trees, a species of *Barbus sensu stricto* (*B. barbus*) is found in Clade 4, whereas two species of '*Barbus*' (*B. fasciolatus* and *B. trimaculatus*) are found in the Clade 7. For the genus *Thynnichthys*, our unpublished data reveal that it should be a member of the tribe Labeonini.

Evidences for the monophyly of the 'Cyprinini–Sinocyclocheilus' clade

Clade 5 comprised four traditionally recognized cyprinin genera (*Cyprinus*, *Carassius* and *Carassioides*, *Procypris*) and the monophyletic and traditionally recognized barbin genus *Sinocyclocheilus*, and is supported as a monophyletic group in all of our analyses. We name this group as the 'Cyprinini–*Sinocyclocheilus*' clade. MLBS based either on the original dataset or on the expanded dataset supported this clade despite weakly (BP = 64% and BP < 50%, respectively). BA based on these datasets supported this clade with posterior possibility values (BPP) of 99% and 98%, respectively. The 'Cyprinini–*Sinocyclocheilus*' clade was also successfully recovered and strongly supported (BP = 100% for MLBS; BPP = 100%) by a recent phylogenetic study based on whole mitochondrial genome sequences albeit with a limited taxa sampling (15 taxa) (Wu *et al.* 2010). Increased genomic sampling may increase support values for the 'Cyprinini–*Sinocyclocheilus*' clade.

Species of *Sinocyclocheilus* are an amazingly diverse group and many have various morphological adaptations for cave dwelling. They are endemic to the Yunnan–Guizhou Plateau and adjacent Guangxi Autonomous Region of China and are usually found in karst cave waters and surface rivers or lakes (Xiao *et al.* 2005). Historically, *Sinocyclocheilus* was almost always considered a member of the tribe Barbini. Species of this genus have been placed in the genera *Barbus* (Regan 1904) and *Schizothorax* (Pellegrin 1931). Fang (1936b) established the genus *Sinocyclocheilus*. Wu (1963) once put species of *Sinocyclocheilus* under the genus *Percocypris* but moved it back later (Wu 1977). Fang (1936b) believed that *Sinocyclocheilus* was closely related to the barbin genus *Cyclocheilichthys*. Wu (1977) put *Sinocyclocheilus* under the tribe Barbini and found that this genus resembles European *Barbus* based on the small-sized scales, similar lip structure, and barbel count as *Barbodes*. Shan & Yue (1994) found that *Sinocyclocheilus* share more synapomorphies with *Barbodes* than with any other barbin genera. Wang *et al.* (1999) also found that *Sinocyclocheilus macrolepis*, which was thought by them as the most primitive species of this genus, is similar to *Barbodes vernayi* (now valid as *Hypsibarbus vernayi*) on 22 of the 28 morphological characters examined. In *Fauna Sinica*, Shan *et al.* (2000) put *Sinocyclocheilus* under the tribe Barbini. The unpublished dissertation of Zhao (2006) represents the latest and most comprehensive study on this genus. Both morphological and molecular characters were used. However, his study was not focused on the phylogenetic position of the genus. It is the same case for the molecular study of Xiao *et al.* (2005). As far as we know, no morphological study has ever been conducted to explore the phylogenetic position of *Sinocyclocheilus* in the family Cyprinidae. Although many researchers believed that the genus should belong to the tribe Barbini, no one has ever presented synapomorphies shared by *Sinocyclocheilus* and many other barbin genera. In the classic study by Chen *et al.* (1984), this genus was not examined. The molecular studies of Wang *et al.* (2007) and Li *et al.* (2008) included several representatives of *Sinocyclocheilus* in their phylogenetic trees of the family Cyprinidae and the subfamily Cyprininae. However, both studies had potential flaws. Wang *et al.* (2007) directly amplified nuclear RAG1 gene from the genome and sent out for sequencing without considering the fact that many cyprinines including *Sinocyclocheilus* are polyploids; Li *et al.* (2008) only used the 16S rRNA gene as marker, which is hard to align.

No morphological studies have ever suggesting a close relationship between *Sinocyclocheilus* and any of the four cyprinin genera, *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*. It has been challenging to find one or several synapomorphies to unite these cyprinin genera and *Sinocyclocheilus* as a monophyletic group. They do share two morphological characters: (i) compressed basioccipital and (ii) a parasphenoid without a dorsally projecting lamina behind the orbital section. However, these characters are not unique to these genera but are shared among all non-labeonin cyprinin fishes (i.e. barbins, oreinins, cyprinins) (Chen *et al.* 1984; Cavender & Coburn 1992). Zhou (1989) actually included two *Sinocyclocheilus* subspecies (species), *Sinocyclocheilus grahami yangzongensis* (now valid

Fig. 4 The best likelihood tree (–ln *L* = 139108.542125) resulting from partitioned and constrained maximum-likelihood search based on the expanded dataset. Outgroup taxa and branches were not shown. Numbers above branches show the bootstrap values from the maximum-likelihood bootstrap analysis (1000 replicates) and the posterior probability values of the partitioned Bayesian analyses sequentially. Those values smaller than 50% were either not shown or shown as '–'.

as *S. yangzongensis*) and *Sinocyclocheilus grahami tingi* (now valid as *S. tingi*) as outgroups for analysis of the Cyprinini, but he did not mention any apomorphic character(s) shared between cyprinins and *Sinocyclocheilus*. While we have not examined the morphology of all of these species, the analysis by Zhou (1989) provides evidence that the morphology of *Sinocyclocheilus* includes only characters similar to other barbin outgroups. Zhou (1989) did, however, identify four morphological characters (characters 1, 2, 4 and 5) that are synapomorphic for a lineage inclusive of *Procypris*, *Cyprinus*, *Carassius* and *Carassioides* (see Fig. 1, Appendix S1). We have not examined these characters in *Sinocyclocheilus* specimens, but appropriate comparisons are possible from previous studies in the literature. For character 1 (i.e. enlarged pore presents at the dorsal side of the lateral process of frontal), Zhou (1989) stated clearly that *Sinocyclocheilus* only possesses the plesiomorphy condition, a small pore at the dorsal side of the lateral process of frontal. The condition of character 2 (i.e. fifth suborbital narrower than second suborbital), for *Sinocyclocheilus* is illustrated by Wang *et al.* (1999; fig. 3) showing that *Sinocyclocheilus* lacks the apomorphic condition. Furthermore, we also know from Wang *et al.* (1999) that some *Sinocyclocheilus* species (e.g. *Sinocyclocheilus anatirostris*) are completely blind and have lost all of the circumorbital series. For the character 4 (i.e. branched dorsal-fin rays 10–22), it is also not true for *Sinocyclocheilus*. According to Shan *et al.* (2000), *Sinocyclocheilus* species only have 7–9 branched dorsal-fin rays, a lot fewer than that of those four genera. The condition of character 5 (i.e. second preethmoid is a well-developed bone) in *Sinocyclocheilus* is unknown at this time; however, it should be emphasized that Zhou (1989) did not mention that species of this genus possess the derived condition.

Wang *et al.* (1999) examined morphological characters of 22 species of *Sinocyclocheilus* and a hypothesized primitive barbin outgroup *Barbodes vernayi* (now valid as *Hypsibarbus vernayi*). They summarized three synapomorphies for fishes of *Sinocyclocheilus*: (i) in addition to the lateral-line system shared by all cyprinid fishes, they have short sensory canals beside the lateral-line canal, which are very well developed especially on the dorsal side of the head and cheek areas; (ii) cave-dwelling habitat shared by the whole genus; and (iii) frontal bone narrow and extended, length is at least three times its width above the highest point of the supraorbital area. Zhao (2006) agreed with Wang *et al.* (1999) and did not go any further than them in finding synapomorphies for the genus *Sinocyclocheilus*. We have included the outgroup species used by Wang *et al.* (1999), *Hypsibarbus vernayi*, in our molecular analyses, and it is within Clade 7 and is not closely related to the genus *Sinocyclocheilus*. As the three synapomorphies of *Sinocyclocheilus* were determined through comparison with this species, we may find one or more of these characters in the cyprinin taxa that we have found to be most closely related to *Sinocyclocheilus* through our molecular analyses. However, based on the studies of Zhou (1989) and Luo & Yue (2000), these characters are not shared by all (or most) members of the genera *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*.

In the 'Cyprinini–*Sinocyclocheilus*' clade, *Sinocyclocheilus* and the other four genera share one important character: they are mainly tetraploids (2n = *c.* 100; some cyprinins may have more chromosomes, 2n = *c.* 150 or *c.* 200) (Yu *et al.* 1987; Klinkhardt *et al.* 1995). However, in our phylogenetic trees, tetraploids can also be found in Clade 1 (*Catlocarpio* and *Probarbus*, 2n = 98), Clade 3 (*Tor* and *Neolissochilus*, 2n = 100) and Clade 4 (all species except *Acrossocheilus monticola* and *Onychostoma simum*, 2n =*c.* 100) (Yu *et al.* 1987; Klinkhardt *et al.* 1995). Only the species in the Clades 2, 6 and 7 are not polyploids (2n = 48, 50) (Yu *et al.* 1987; Klinkhardt *et al.* 1995). Therefore, whether or not chromosome serves as a synapomorphy for the 'Cyprinini–*Sinocyclocheilus*' clade is unknown.

In summary, although our molecular analyses and another molecular study by Wu *et al*. (2010) support *Sinocyclocheilus* as closely related to *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, and form the 'Cyprinini–*Sinocyclocheilus*' clade, we have not found any morphological synapomorphies for this clade at this time. Thus, we call for a thorough morphological systematic study to be conducted on this clade.

Definition of the tribe Cyprinini sensu stricto and phylogenetic relationships among its genera

Within the 'Cyprinini–*Sinocyclocheilus*' clade, molecular analyses are not consistent as to whether the four cyprinin genera, *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*, form a monophyletic group. In the best ML tree based on the original dataset, these genera did not form a monophyletic group; *Sinocyclocheilus* was found between *Procypris* and the other three genera. However, this relationship was weakly supported by MLBS (BP < 50%) and BA (BPP = 84%). In Wu *et al*.'s (2010) phylogenetic tree based on the whole mitochondrial genome sequences, species of *Sinocyclocheilus* first clustered with *Procypris rabaudi* (BP = 23% for MLBS; BPP = 77%), and this clade formed a clade with *Cyprinus carpio* (BP = 24%; BPP = 94%) that was sister to the clade formed by two *Carassius* species. It is clear that this relationship is also not robustly supported by bootstrap analyses. There exists some evidence supporting the monophyly of the group formed by these four genera. The SH test showed that if

we forced this group to be monophyletic, the resulting phylogeny is not significantly worse than the optimal ML tree (Table 8). Analyses on the expanded dataset also support this group as monophyletic but the support values are low (BP < 50%; BPP = 62%). However, as discussed above, morphological characters presented by Zhou (1989) provide a very important line of evidence to support that *Procypris*, *Cyprinus*, *Carassius* and *Carassioides* form a monophyletic group (Fig. 1; Appendix S1). The genus *Procypris*, whose position is not consistent in different analyses, has been identified as a member of Cyprinini *sensu stricto* by all the researchers ever since it was established by Lin (1933). According to Luo & Yue (2000), the only two species of this genus are quite similar to *Cyprinus* in external morphology. Zhou (1989) identifies this genus as most closely related to *Cyprinus* (Fig. 1). As described above, currently there are no synapomorphies to unite *Sinocyclocheilus* with any of the other four cyprinin genera or the groups formed by them. It is curious that the analyses based on our original dataset and those of Wu *et al*. (2010) failed to recover these four genera as a monophyletic group. We argue that the most likely reason is taxon sampling. Wu *et al*. (2010) only sampled six taxa for the 'Cyprinini–*Sinocyclocheilus*' clade, including one species each of *Procypris* and *Cyprinus* and two species each of *Carassius*, and *Sinocyclocheilus*. In our expanded dataset 1, which includes more intensive sampling of species of *Sinocyclocheilus*, this group is supported as monophyletic. To further increase taxon sampling in the 'Cyprinini–*Sinocyclocheilus*' clade, the most important and efficient effort would be to increase sampling of species of *Cyprinus*. Given that one already has intensive taxon sampling for the 'Cyprinini–*Sinocyclocheilus*' clade and other major clades of the subfamily Cyprininae, then increasing genome sampling is predicted to assist in resolving the relationships in this group. Nuclear gene markers have proven to be extremely useful in resolving phylogenetic relationships in the Cypriniformes (e.g. Chen & Mayden 2009; Mayden *et al.* 2009; Yang & Mayden 2010) and should be included in future studies of this group but only after effective methods are developed to isolate and sequences homologous sequences as these species are polyploids (mainly tetraploids; Yu *et al.* 1987; Xiao *et al.* 2002). For this study, we are recognizing the monophyly of the group formed by *Procypris*, *Cyprinus*, *Carassius* and *Carassioides*.

Therefore, we agree with Rainboth's (1981) definition on the tribe Cyprinini *sensu stricto* and restrict the tribe to *Cyprinus*, *Carassius*, *Carassioides* and *Procypris*. A combination of two characters (equally important) can be used to successfully diagnose members of this revised tribe: (i) possession of serrated anal spine and (ii) dorsal fin with no <10 branched fin rays (Table 2). We are uncertain of *Cyprinus fuxianensis* occasionally possessing nine branched dorsal-fin rays (Table 1), but this species can only be found in the Fuxianhu Lake and Xingyunhu Lake of Yunnan Province, China. If necessary, it can be successfully identified as a member of this clade with future molecular analyses of the species and/or with the assistance of character 2 (i.e. fifth suborbital narrower than second suborbital) of Zhou (1989) (Fig. 1; Appendix S1). Our delineation and diagnosis of the tribe Cyprinini *sensu stricto* appears similar to that of Rendahl (1928), which was given more than 80 years ago. The only difference lies in the number of branched dorsal-fin rays (ours 10 vs. his 14) that should be used in diagnosis. One reason for the difference in our diagnoses is that Rendahl intentionally ignored the fact that *C. micristius* has <14 branched dorsal-fin rays. The other reason lies in the fact that several species of *Cyprinus* have fewer branched dorsal-fin rays, e.g. *C. fuxianensis* and *C. yilongensis*, were not described until the late 1970s, well after Rendahl's (1928) study. While it appears that after many years of study and classification of these genera we have converged on the results of an earlier study of the group, this is not the case. Rendahl (1928) did not provide a scientific explanation for his classification, whereas our diagnosis of this clade is based on phylogenetic systematic studies of molecular and morphological data, the latter from Zhou (1989). As for the phylogenetic relationships within the Cyprinini *sensu stricto*, the genus *Procypris* forms the basal clade of the Cyprinini, whereas the '*Carassius–Carassioides*' clade and *Cyprinus* have sister relationships more apical in the tree. These relationships are different from the results of Zhou (1989) (Fig. 1), where *Cyprinus* and *Procypris* formed sister groups. In the '*Carassius–Carassioides*' clade, the two individuals of *Carassius auratus*, whose sequences were downloaded from the GenBank, were not clustered with each other (Figs 2–4; Table 3).

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Appendix

Listed below are the characters (translated from Chinese) used by Zhou (1989) to construct the cyprinin cladogram shown in Fig. 1. For related figures, please refer to Zhou (1989). **P**: plesiomorphy; **A**: apomorphy; **M**: medial state; **U**: unknown, character state cannot be determined. Plesiomorphies were recognized through comparing with barbins (including two *Sinocyclocheilus* species). The cyprinin genera that show a specific state of character were listed in the brackets following each character state. It should be noted that, the characters of *Scaphognathops* were not listed because they were very different from those of other Cyprinin genera (Zhou, 1989).

1. **P**: small pore presents at the dorsal side of the lateral process of frontal (*Puntioplites*);
   A: enlarged pore presents at the dorsal side of the lateral process of frontal (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*).
2. **P**: 5th suborbital broader than 2nd suborbital (*Puntioplites*);
   A: 5th suborbital narrower than 2nd suborbital (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*).
3. **U**: presence of a frontoparietal fontanelle (*Puntioplites*);
   U: absence of frontoparietal fontanelle (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*).
4. **P**: branched dorsal-fin rays 8-9 (*Puntioplites*);
   A: branched dorsal-fin rays 10-22 (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*).
5. **P**: 2nd preethmoid is connective tissue, located between preethmoid and maxilla (*Puntioplites*);
   A: 2nd preethmoid is a well-developed bone (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*).
6. **P**: middle part of the pleural rib of 4th vertebra does not form a "<" shape (*Procypris*, *Cyprinus*, *Carassioides*, *Carassius*);
   A: middle part of the pleural rib of 4th vertebra bend backward and is shaped like a "<" (*Puntioplites*).
7. **P**: absences of a small pore in proctic for cranial nerve IX (*Puntioplites*, *Carassioides*, *Carassius*);
   A: presence of a small pore in proctic for cranial nerve IX (*Procypris*, *Cyprinus*).
8. **P**: an incision (big or small) present between posterior process of pterotic and exoccipital (*Puntioplites*, *Procypris*, *Cyprinus*);
   A: absence of incision between posterior process of pterotic and exoccipital (*Carassioides*, *Carassius*).
9. **P**: absence of the crests at the ventral side of basioccipital and the latter part of parasphenoid (*Carassioides*, *Carassius*);
   A: two paralleled crests present at the ventral side of basioccipital and the latter part of parasphenoid (*Puntioplites*, *Procypris*, *Cyprinus*).
10. **P**: absence of bufferfly shaped articula near the middle of the ventral part of parasphenoid (*Puntioplites*, *Procypris*, *Cyprinus*);
    A: presence of bufferfly shaped articula near the middle of the ventral part of parasphenoid (*Carassioides*, *Carassius*).
11. **P**: ventral crest of pelvic girdle short and incomplete (*Puntioplites*, *Procypris*, *Cyprinus*);
    A: ventral crest of pelvic girdle long and extends to the tip of the outer limb (*Carassioides Carassius*).
12. **P**: dorsal trough of pelvic girdle complete (*Puntioplites*, *Carassioides*, *Carassius*);
    A: forward part of the dorsal tough of pelvic girdle not clear, dorsal tough leans down towards forward (*Procypris*, *Cyprinus*).
13. **P**: pharyngeal teeth somewhat slender and pointed (*Puntioplites*, *Procypris*);
    A: pharyngeal teeth, shaped like a molar (*Cyprinus*), or shaped like a shovel (*Carassioides*, *Carassius*).
14. **P**: 4 pharyngeal teeth of main row (*Puntioplites*, *Procypris*, *Carassioides*, *Carassius*);
    A: 3 pharyngeal teeth of main row (*Cyprinus*).
15. **P**: fewer scales on lateral line (*Puntioplites*, *Procypris*, *Carassioides*, *Carassius*);
    A: more scales on lateral line (*Procypris*).
16. **P**: fewer vertebrae (*Puntioplites*, *Cyprinus*, *Carassioides*, *Carassius*);
    A: more vertebrae (*Procypris*).
17. **P**: occipital process with a single process (*Procypris*);
    A: occipital process is a trough (*Carassioides*, *Carassius*);
    M: basal part of occipital process is a single process, but the latter part is a trough (*Puntioplites*);
    P,**A** (multiple states): both situations exist (*Cyprinus*).
18. **P**: transverse process of the 1st vertebra long, later side extends the outer edge of tripus (*Puntioplites*, *Procypris*);
    A: transverse process of the 1st vertebra short, later side far from the outer edge of tripus (*Carassioides*, *Carassius*);
    P,**A** (multiple states): both situations exist (*Cyprinus*).
19. **P**: anterior end of cleithrum with its inside and outside almost aligned, the outer angle rounded (*Puntioplites*, *Procypris*);
    A: anterior end of cleithrum depressed in the middle, the outer angle a little sharp (*Cyprinus*, *Carassioides*, *Carassius*).

20. **P**: front lateral leaf of dorsal limb of cleithrum is narrow and equal in width to the back lateral leaf (*Puntioplites*, *Cyprinus*, *Carassius*);
    A: front lateral leaf of dorsal limb of cleithrum is well developed and wider than the back lateral leaf (*Procypris*, *Carassioides*).
21. **P**: size of the front connected surface is 1/2 or equal to that of the back connected surface of cleithrum and coracoid (*Puntioplites*, *Procypris*, *Cyprinus*, *Carassioides*);
    A: size of the front connected surface is 1/3 of that of the back connected surface of cleithrum and coracoid (*Carassius*).
22. **P**: dorsal part of parasphenoid does not project upward and contact with pterosphenoid to form a barrier (*Puntioplites*, *Procypris*, *Cyprinus*, *Carassius*);
    A: dorsal part of parasphenoid projects upwards and contact with pterosphenoid to form a barrier (*Carassioides*).
23. **P**: 2 pairs of barbels (*Procypris*, *Carassioides*);
    A: 0 pairs of barbels (*Puntioplites*, *Carassius*);
    P,**M**,**A** (multiple states): 0-2 pairs of barbels (*Cyprinus*).
24. **P**: two ends and the middle of the third suborbital similar in width, the widest part narrower than the width of the first suborbital (*Puntioplites*, *Procypris*, *Cyprinus*, *Carassioides*);
    A: middle of the third suborbital projects significantly backwards and downwards, renders its width larger than that of the first suborbital (*Carassius*).
25. **P**: pharyngeal teeth in 3 rows (*Puntioplites*, *Procypris*, *Cyprinus*);
    A: pharyngeal teeth in 1 row (*Carassius*);
    M: pharyngeal teeth in 2 rows (*Carassioides*).

