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Western boundary of the subfamily Danioninae in Asia (Teleostei, Cyprinidae): derived from the systematic position of *Barilius mesopotamicus* based on molecular and morphological data

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Abstract

Analysis of mitochondrial cytochrome *b* sequences from 198 species confirms the position of *Barilius mesopotamicus* as a species of the Asian genus *Barilius* within the subtribe Chedrina in the subfamily Danioninae. This relationship is supported by a parsimony analysis based on 43 morphological characters, and the morphological examination confirms that *B. mesopotamicus* possesses the synapomorphies of the Danioninae and Chedrina, *viz*. absence of the Y-shaped ligament and the greatly reduced postcleithrum, respectively. *Barilius mesopotamicus* greatly expands the western boundary of the Asian Danioninae into the Tigris-Euphrates basin.

Key words: cytochrome *b*; Tigris-Euphrates; disjunct distribution; freshwater fish; biogeography

Introduction

The Danioninae are one of the most species-rich subfamilies of the Cyprinidae, comprising some 200 species. The distribution of the Danioninae is disjunct, with two well separated geographic groups, one in tropical Africa and the other in southern and eastern Asia. The Asian distribution extends from the Makran River basin in south-eastern Iran, where the subfamily is represented by *Cabdio morar* (Hamilton) (Coad 2010; as *Aspidoparia morar*), eastward to China and Japan (Howes 1991: fig. 1.7). *Barilius mesopotamicus* Berg has a more western distribution in the Tigris-Euphrates basin, but its position among the Danioninae has been contested (Bănărescu & Coad 1991; Howes 1991).

Barilius mesopotamicus is a small species, generally not surpassing 50.7 mm SL, diagnosed by the presence of an opsariichthin colour pattern (i.e., vertical bars similar to those of *Opsariichthys*) and barbels (Coad 2010). Howes (1980: 181), in the analysis of the systematics of his bariliine group, stated that *B. mesopotamicus* is not a *Barilius* and should be included in the "*Leucaspius* generic complex". *Leucaspius* is a monotypic genus of the cyprinid subfamily Leuciscinae, widely distributed in Europe (Briolay *et al*. 1998; Kottelat & Freyhof 2007). Despite Howes (1980) claiming the proposed affinity to be based on synapomorphies, characters supporting a close interrelationship between *Leucaspius* and *B. mesopotamicus* were not provided. Bianco & Bănărescu (1982) agreed with Howes (1980) that *B. mesopotamicus* may be generically distinct from South Asian *Barilius* based on the number of pharyngeal tooth rows (two, vs. three in *Barilius*). However, they doubted an affinity between *B. mesopotamicus* and species of *Leucaspius*. Howes (1991), in the overview of systematics and biogeography of cyprinids, retained *B. mesopotamicus* in *Barilius* and considered this species to be a member of the Danioninae. In his map (Howes 1991: fig. 1.7), however, the distribution of the Danioninae was not illustrated accordingly. Bănărescu & Coad (1991) suggested that *B. mesopotamicus* could be a member of the Danioninae, but of uncertain relationships and generic placement. According to Coad (2010), *B. mesopotamicus* can be distinguished from *Leucaspius* by the possession of barbels (absent in *Leucaspius*), lateral line running along the ventral outline (vs. short

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and running along the middle of the side), broad suborbital bones, presence of flank bars, and absence of genital papillae unique to female *Leucaspius*.

Inclusion of this species in the Danioninae would expand the distribution of the Asian lineage westward from India to nearly the Mediterranean Sea. The systematic position of this species is important to the understanding of the biogeography of the Danioninae. However, the systematic position of *B. mesopotamicus* is contentious and the classification in *Barilius* was doubted (Howes 1980; Bianco & Bănărescu 1982; Bănărescu & Coad 1991; Bănărescu 1992). The aim of this paper is to present the results of phylogenetic analyses based on cytochrome *b* (cyt *b*) sequences and morphological characters to resolve the issue of the phylogenetic position of *B. mesopotamicus*, and therewith to establish the western boundary of the Asian Danioninae.

Material and methods

Vernacular family group names followed Fang *et al*. (2009), i.e. with the ending –ine for subfamily name and –in for tribe names. Thus danionines refer to the Danioninae, and chedrins, danionins and rasborins refer to the Chedrina, Danionina and Rasborini, respectively. Subdivisions of the Danioninae followed Liao *et al*. (in press).

DNA extraction and PCR amplification. Voucher specimens for new sequences are kept at the Swedish Museum of Natural History (NRM), catalogued as NRM 60683 (*B. mesopotamicus*), NRM 58599 (*Opsarius* sp.), and NRM 59315 [*Raiamas guttatus* (Day)]. Further information on voucher specimens is available from the webpage of the collection database (http://artedi.nrm.se/nrmfish/). The mitochondrial cyt *b* gene was selected for the molecular analysis. This gene is shown to be suitable for the research of cyprinid interrelationships (Rüber *et al*. 2007; Sevilla *et al*. 2007; Fang *et al*. 2009) and subfamily assignment of cyprinids (Rüber *et al*. 2007; Fang *et al*. 2009). In addition to the new sequences generated for this study, a further 195 sequences of cyt *b* were downloaded from GenBank, amounting to a total of 198 sequences for the phylogenetic analysis. GenBank accession numbers of sequences are given after the species names in Figure 1.

Mitochondrial DNA was extracted from specimens using the GeneMole automated DNA extraction system (Mole genetics) with recommended protocol. The primer set, FishCyt*b*-F and TrucCyt*b*-R (Sevilla *et al*. 2007), was used to amplify the fragment of cyt *b* gene. The PCR protocol was as follows: PCR cycling: 94˚C 4′; 4* (94˚C 30″; 55˚C 30″; 72˚C 1′), 4* (94˚C 30″; 53˚C 30″; 72˚C 1′), 35* (94˚C 30″; 51˚C 30″; 72˚C 1′); 72˚C 8′. PCR products were checked on minigel and purified by using the ExoSAP (Fermentas).

The sequencing reactions were performed using BigDye 3.1 (Qiagen, Inc.) with recommended protocol. The products of sequencing reactions were purified with the DyeEx 96 Kit (Qiagen, Inc.) and sequenced in an automatic sequencer (ABI 3700, Applied Biosystems, Inc.). Each sequence was composed of four fragments obtained with the same primers as for PCR amplification and two internal primers, Cyt*b*I-3R and Cyt*b*I-7F (Sevilla *et al*. 2007). Assemblage of the fragments was conducted using the Lasergene software package (DNASTAR).

Sequences downloaded from GenBank and those generated by this study were aligned by using the Lasergene software package (DNASTAR) and modified by eye. The 10 bps from the 3' end of the cyt *b* were deleted to avoid alignment uncertainty (Fang *et al*. 2009), and eventually the length of each sequence was 1131 bps. Alignments are available from the authors.

Morphological character coding. Liao *et al*. (in press) concluded that the Danioninae (sensu stricto) are characterized by absence of the Y-shaped ligament connecting the kinethmoid to the mesethmoid, while the Chedrina are diagnosed by (1) the postcleithrum greatly reduced or absent (character 26 [state 1]) and (2) the postcleithrum normal to abdominal ribs (character 27 [state 1]). We therefore used the character matrix of Liao *et al*. (in press) to verify whether *B. mesopotamicus* is a chedrin or not.

Cleared and stained (C&S) specimens were prepared following the protocol described by Taylor & Van Dyke (1985) with slight modifications. Vertebral counts were made as described by Liao *et al*. (2010). The terminology of characters followed Liao *et al*. (in press). For the morphological analyses, character states were recorded from *B. mesopotamicus* (NRM 60864) and *L. delineatus* (Heckel) (NRM 60809) according to the protocol in Liao *et al*. (in press; refer to Appendix 1 for characters and states) and these two species were appended to the character matrix of Liao *et al*. (in press: table S2) as the 35th and 36th taxon, respectively.

Phylogenetic analysis. The superorder Ostariophysi is divided into two groups, Anotophysi and Otophysi. The former comprises the Gonorynchiformes and the latter includes Characiformes, Cypriniformes, Siluriformes and Gymnotiformes (Nelson 2006). In the present study, *Chanos chanos*, a representative of the Gonorynchiformes, was designated as outgroup for tree rooting in the molecular analysis. Cytochrome *b* sequences were applied for Bayesian analysis by using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with partitions based on codon position (first, second and third), allowing all parameters except topology and branch length to vary independently using the unlink command in MrBayes. The GTR+I+G model as suggested by MrModeltest (Nylander 2004) was adopted for the analyses with sampling for 23 million generations. Convergence was checked using the software Tracer v1.5 (Rambaut & Drummond 2003) and the SUMP command of MrBayes (two concurrent analyses, nruns = 2; three heated chains, nchains = 4; chain temperature 0.1; sample frequency 1000; burn in = 22 million generations).

In the morphological analyses *Opsariichthys bidens* was designated as outgroup for tree rooting following Liao *et al*. (in press). The character matrix was analyzed with PAUP* 4 (ver. 4.0b10; Swofford 1998) for phylogenetic parsimony reconstruction using the heuristic algorithm with zero-length branches permitted. All characters were unordered and of equal weight. Bootstrap support was calculated with 100 reiterations. Three analyses were conducted respectively with the same setting. Two analyses included *L. delineatus*, one with all characters and the other excluding characters 26 and 27; the third analysis includes all characters, but *L. delineatus* is excluded from the analysis. Refer to the discussion for reasons for conducting three analyses.

Results

Molecular phylogeny. The Bayesian analysis based on the cyt *b* gene shows that subfamilies recognized by Fang *et al*. (2009), including Acheilognathinae, Cultrinae, Cyprininae, Danioninae, Gobioninae, Leuciscinae, Opsariichthyinae (= EX-Danioninae), and Tincinae, are recovered. The Danioninae are at the base of the Cyprinidae. The remaining cyprinids are divided into two groups. The first group comprises the Cyprininae. The second group consists of *Acrossocheilus yunnanensis*, *Sinocyclocheilus grahami* and a multifurcation clade comprising the Acheilognathinae, Cultrinae, Gobioninae, Leuciscinae, Opsariichthyinae, Tincinae, and the genus *Tanichthys*. The most basal danionines are *Paedocypris* and *Sundadanio*. The remaining danionines are divided into three groups of unresolved interrelationships, corresponding to chedrins, danionins and rasborins. *Barilius mesopotamicus* is a chedrin nested within the Asian genus *Barilius* of the Danioninae. One sequence of *R. guttatus* downloaded from GenBank (accession number: AF051875), coming out in the Xenocypridinae, is probably a misidentification. *Leucaspius delineatus* is recovered within the Leuciscinae, remote from *B. mesopotamicus* (Fig. 1).

Morphological phylogeny. The character states of *B. mesopotamicus* and *L. delineatus* are summarized in Table 1. In the analysis including *L. delineatus* and all characters, rasborins are a sister group of danionins, which form a polytomy with chedrins and two clades comprising *Aphyocypris* and *Metzia*+*Parachela*, respectively (Fig. 2a). *Barilius mesopotamicus* and *L. delineatus* form two monotypic clades successively at the base of the Chedrina, characterized by two lateral processes on each side of the kinethmoid (character 7 [state 1]) and two autapomorphies, *viz*. the postcleithrum greatly reduced and the postcleithrum normal to abdominal ribs.

TABLE 1. Morphological character matrix appended to the matrix in Liao *et al.* (in press). Character states are explained in Appendix 1.

	Characters																					
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Barilius mesopotamicus	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1
Leucaspius delineatus	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1

continued.

	Characters																				
Taxon	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Barilius mesopotamicus	1	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Leucaspius delineatus	1	0	0	1	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0

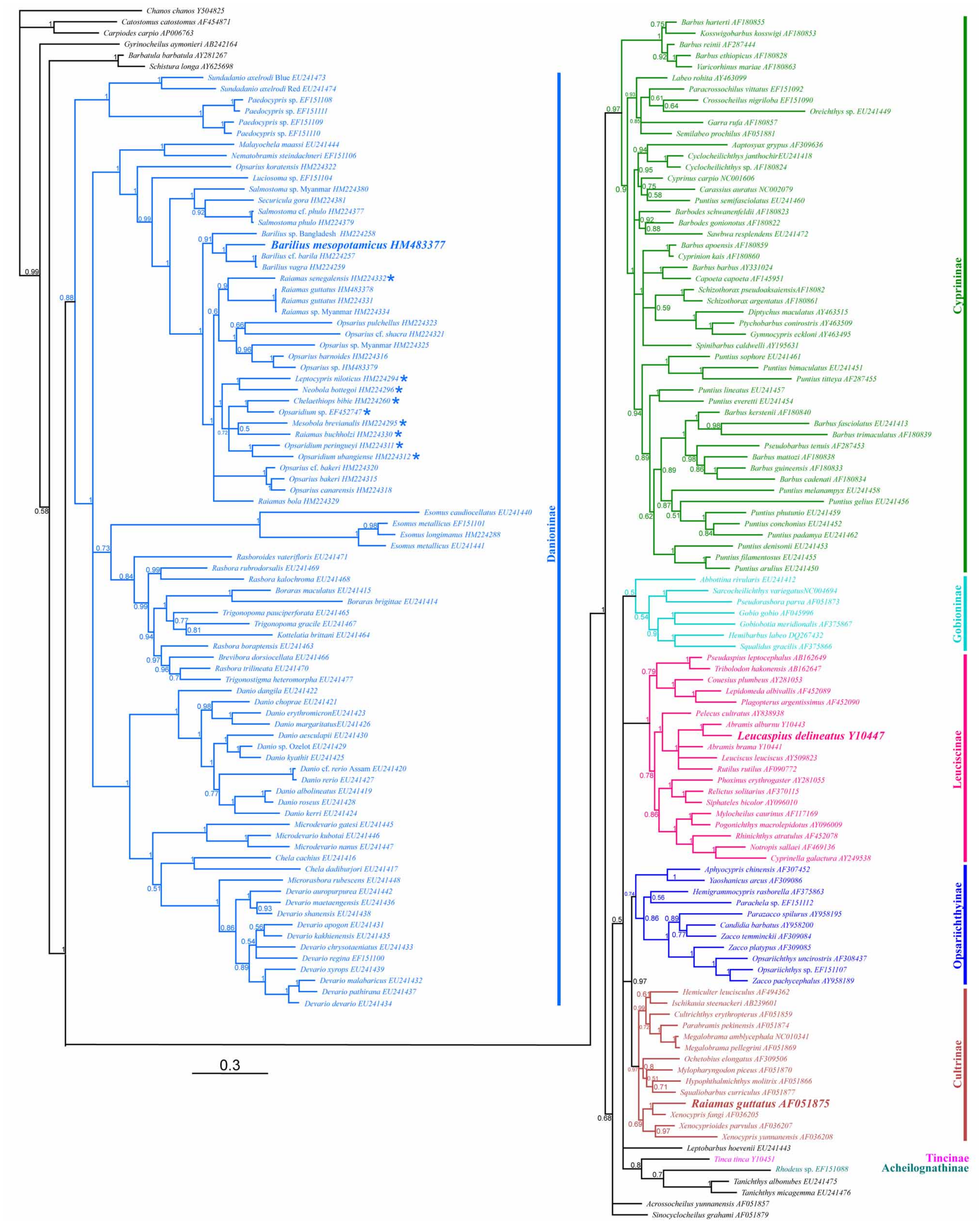

FIGURE 1. Systematic position of *Barilius mesopotamicus* among cyprinids. The 50 % majority rule consensus tree based on nucleotide sequences of the cytochrome *b* gene. Subfamily attribution follows Fang *et al*. (2009). The subfamily name, Opsariichthyinae Rendahl, 1928, is used to replace their EX-Danioninae. Asterisks denote African chedrins. Numbers around nodes denote posterior probability.

In the analysis including *L. delineatus,* but excluding characters 26 and 27, *L. delineatus* is clustered with species in which the Y-shaped ligament is present. *Barilius mesopotamicus* is at the base of the danionines (Fig. 2b).

In the analysis including all characters, but excluding *L. delineatus,* the topology of the consensus tree (Fig. 2c) is similar to that of Liao *et al*. (in press), in which *B. mesopotamicus* is closely related to *R. guttatus* and *Opsarius bakeri,* but the relationships among these three species is not resolved. Monophyly of *B. mesopotamicus, R. guttatus* and *O. bakeri* is supported by three homoplasies (character 14 [state 0], 34 [0], 37 [0]).

The Y-shaped ligament is present in *L. delineatus*, but absent in *B. mesopotamicus*. The postcleithrum is greatly reduced and normal to the abdominal ribs in both species.

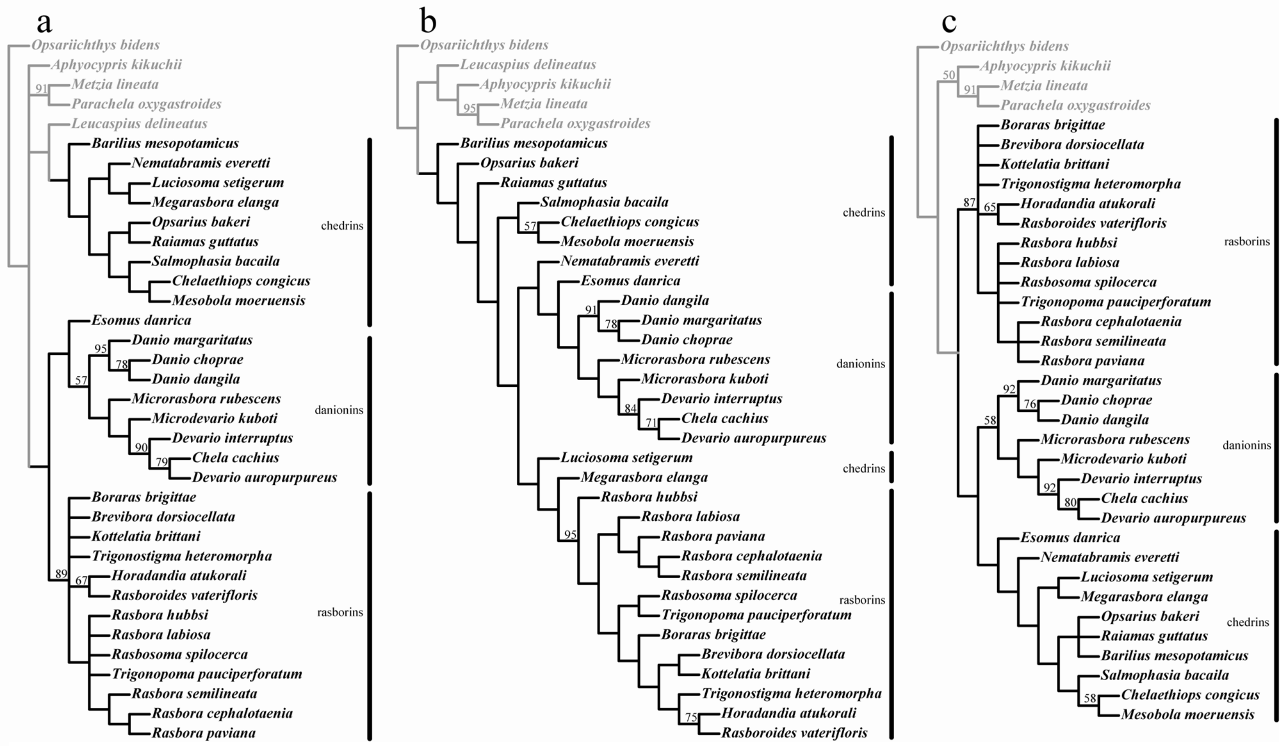

FIGURE 2. Strict consensus trees of maximum parsimony analysis. (a) *Leucaspius delineatus* and all characters are included (303 most parsimonious trees with 116 steps; CI: 0.4138; RI: 0.7882). (b) *Leucaspius delineatus* is included but not characters 26 and 27 (60 most parsimonious trees with 108 steps; CI: 0.4259; RI: 0.7967). (c) All characters are included but *L. delineatus* is excluded (50 most parsimonious trees with 110 steps; CI: 4324; RI: 0.7968). Species without the Y-shaped ligament are in black and numbers above nodes denote bootstrap support.

Discussion

Systematic position of *Barilius mesopotamicus.* In the molecular analysis, *B. mesopotamicus* is clustered with Asian Chedrina (Fig. 1), remote from the postulated relative *Leucaspius* (Howes, 1980), which is placed in the Leuciscinae (Fig. 1a; Briolay *et al*. 1998; Kottelat & Freyhof 2007). Interrelationships among the Chedrina (Fig. 1) vary slightly from the results of Tang *et al*. (2010), probably reflecting the different datasets. The present tree (Fig. 1) is reconstructed from the cytochrome *b* gene alone (vs. four genes in Tang *et al*. 2010) and only sequences longer than 1131 bps are included in the present study (vs. all sequences of different length in Tang *et al*. 2010). Morphological phylogenetic analyses also confirm *B. mesopotamicus* to belong with the Chedrina. In the phylogenetic analysis including *L. delineatus* and all morphological characters, both *B. mesopotamicus* and *L. delineatus* are placed in the Chedrina (Fig. 2a). This contradiction between molecular and morphological analyses is probably a consequence of homoplastic characters being erroneously recovered as synapomorphies. *Leucaspius delineatus*, a member of the Leuciscinae, is clustered with the Chedrina because of the greatly reduced postcleithrum that is normal to abdominal ribs (characters 26 and 27). The greatly reduced postcleithrum, however, is known to be derived independently in the Danioninae, Leuciscinae and some benthic cyprinids (Cavender & Coburn 1992; Liao *et* *al*. in press). *Leucaspius delineatus* is the only leuciscine in the phylogenetic analysis based on morphological data. Lack of representative taxa of the Leuciscinae may result in failure to provide informative characters to distinguish leuciscines, *L. delineatus* herein, from danionines. For instance, the separation of infraorbital and supraorbital canals is a synapomorphic character for the Leuciscinae and Phoxininae (Cavender & Coburn 1992). This disconnection is only present in *L. delineatus* and hence not informative in the present study. When characters 26 and 27 are excluded from the analysis, the Danioninae remain monophyletic, but the Chedrina are not recovered because of the absence of synapomorphies (Fig. 2b). Since inclusion of *L. delineatus* may interfere with the phylogenetic analysis based on morphological data, this species is excluded and the phylogenetic tree constructed accordingly shows that *B. mesopotamicus* is a chedrin closely related to *R. guttatus* and *O. bakeri* (Fig. 2c), which is congruent with the molecular tree (Fig. 1). In conclusion, the phylogenetic analyses confirm *B. mesopotamicus* as a chedrin species rather than a relative of *Leucaspius* as postulated by Howes (1980).

Bianco & Bănărescu (1982) considered *B. mesopotamicus* to be generically distinct from South Asian *Barilius* based on the biserial pharyngeal teeth (vs. triserial in *Barilius*). However, variation of the number of tooth-rows within *Devario* and *Rasbora* has been reported (Liao *et al*. 2010). This character alone may not substantially support a split of this species from *Barilius*. In the present molecular study, *B. mesopotamicus* is nested with species of *Barilius* (Fig. 1). However, morphological characters that confine this species to *Barilius* remain contentious. Howes (1980) identified two groups within *Barilius* (sensu lato), corresponding to the genera *Barilius* and *Opsarius* (Rainboth 1991). Howes (1980) diagnosed *Barilius* by (1) long jaws, (2) hyomandibular condyles well separated, (3) scales with many radii, (4) body shallow, (5) barbels in two pairs and (6) tubercles small and poorly developed. *Barilius mesopotamicus* possesses most of the characters of *Barilius* except for the number of radii (Table 2). The scale extracted from the belly that we examined has ten radii. However, Howes (1980) did not provide a quantitative definition so that we are not able to assign a character state. Later Howes (1983: fig. 3) provided a different set of diagnostic characters to depict the interrelationships of his bariliines and chelines. According to the latter diagnosis, *B. mesopotamicus* shares only one character with *Barilius*, i.e., 1st vertebra with expanded lateral processes, but possesses all characters diagnosing *Opsarius*, including (1) pectoral and pelvic axial scales lobate or fleshy, (2) palatine extended laterally, supporting rostral barbel when present and (3) parallel rows of tubercles on dentary (Table 2). The generic placement of *B. mesopotamicus* varies with the diagnostic character set that is used. This conflict implies that the definition of *Barilius* is contentious and the classification of species currently included in *Barilius* and *Opsarius*, including *B. mesopotamicus*, is in need of a deeper systematic revision.

TABLE 2. Characters differentiating *Barilius* and *Opsarius*. Two character sets were proposed by Howes (1980, 1983). The characters possessed by *B. mesopotamicus* are in bold.

Howes (1980)		Howes (1983)	
Barilius	*Opsarius*	*Barilius*	*Opsarius*
jaws long	jaws short	pectoral and pelvic axial scales elongated	**pectoral and pelvic axial scales lobate or fleshy**
hyomandibular condyles well separated	hyomandibular condyles barely separated	**1st vertebra with expanded lateral processes**	
scales with many radii **(?)**	scales with few radii	omega-shaped ethmoid notch, vomerine arms extending straight forward	
body shallow	body deep		**palatine extended laterally, supports rostral barbel when present**
barbels in two pairs*	a single pair of barbels or no barbels		**parallel rows of tubercles on dentary**
tubercles small and poorly developed	tubercles large and well developed		

*****: one pair in the specimens we examined, but some with two pairs are reported by Coad (2010). ?: unknown, due to lack of a quantitative definition; see Discussion.

Western boundary of the Asian Danioninae. *Barilius mesopotamicus* is widely distributed in the Tigris-Euphrates basin of Turkey, Syria, Iraq and Iran (Coad 2010), and eastward to the Mand River in Iran (Abdoli 2000; refer to Coad & Krupp (1983) for localities with coordinates in these countries). It is absent from eastern Iran and western Pakistan, a desert region from which no other species of *Barilius* has been reported. This disjunct distribution of *Barilius* is probably explained as a consequence of desertification.

Barilius mesopotamicus is an Asian chedrin (Fig. 1). Inclusion of this species in the Danioninae greatly expands the western boundary of the Asian Danioninae to the Tigris-Euphrates basin. Another danionine cyprinid, *Cabdio morar* has been reported all the way from Thailand to the Straits of Hormuz in Iran (Coad 2010; Kiabi & Abdoli 2000). The distribution of the African and Asian danionines is thus only gapped by the Arabian Peninsula (Fig. 3), and this discontinuity might be caused by relatively recent desertification (Otero 2001). Liao *et al*. (in press) excluded some East Asian cyprinid genera from the Danioninae, including *Aphyocypris*, *Opsariichthys* and *Zacco*. Based on their conclusion and the present study, the Asian Danioninae occur from the Tigris-Euphrates basin to southern China and the southward area, including the Indian subcontinent, Indochina and Sundaland (Fig. 3).

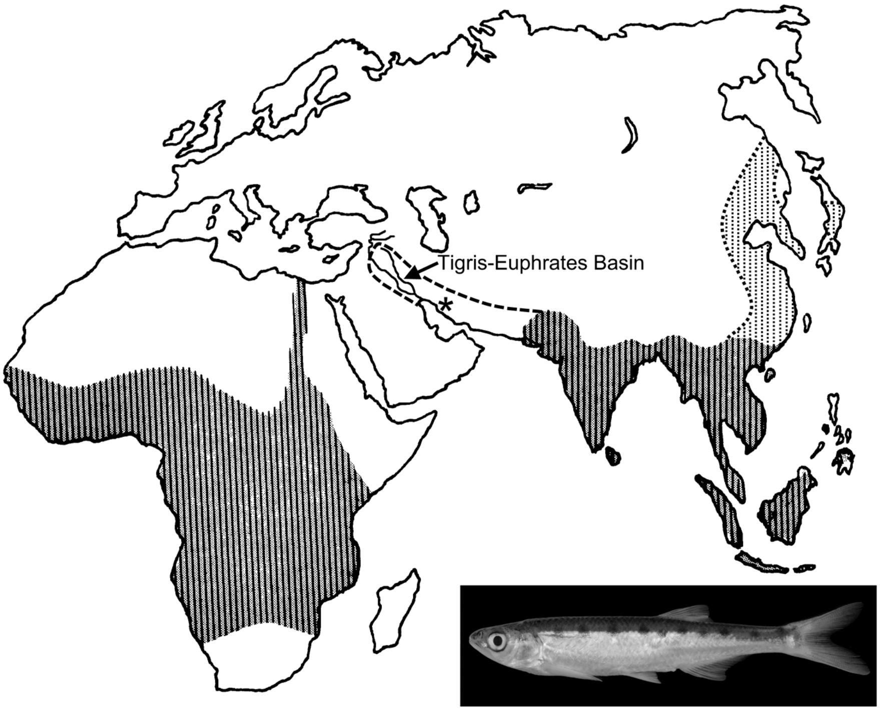

FIGURE 3. Distribution of the Danioninae (modified from Howes 1991: fig. 1.7). The shaded and dotted-line area shows the distribution proposed by Howes (1991). Expansion of the dotted-line area is due to the inclusion of *Opsariichthys*, *Zacco* and *Aphyocypris* in the Danioninae. Exclusion of these three genera from this subfamily has restricted the distribution in the shaded area. The dashed-line area indicates the westward expansion by inclusion of *Barilius mesopotamicus* and *Cabdio morar.* The asterisk indicates approximately the mouth of the Mand River, as the eastern distributional boundary of *B. mesopotamicus*. The fish on the lower right is *B. mesopotamicus* (62.3 mm SL; NRM 60863).

Fish families with disjunct distributions in Asia and Africa include the Anabantidae, Clariidae, Schilbeidae, Bagridae, Mastacembelidae, and Channidae (Berra 2007). The only other cyprinid subfamily distributed in both Africa and Asia is the Cyprininae, but cyprinines are also found throughout Eurasia, and on the Arabian Peninsula (Howes 1991). Durand *et al*. (2002), studying the cyprinid subfamilies Cyprininae and Leuciscinae, suggested the Middle East as an interchange area of freshwater ichthyofaunas, implying an intercontinental dispersal. The African Clariidae, a group of catfishes with a distribution pattern similar to that of the Danioninae, are proposed to have an Asian origin according to a molecular phylogeny as well as parasitological and paleontological data (Agnese & Teugels 2005 and literature therein). The westward dispersal pattern is also deduced in the genus *Garra* (Colli *et al*. 2009) and the Labeonini as a whole (Tang *et al*. 2009). The same scenario for the Danioninae may be revealed when a more targeted analysis of African danionines is conducted.

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APPENDIX 1. Characters and character-states used in the morphological phylogenetic analysis.

Ligament

1. Y-shaped ligament (Liao *et al*. in press: fig. 3).—States: [0], present; [1], absent.
2. Inner origin of Baudelot's ligament.—States: [0], lateral process of first vertebra; [1], exoccipital or basioccipital.
3. External origin of Baudelot's ligament.—States: [0], Baudelot's ligament attaching to upper cleithrum and then extending to supracleithrum bypassing the posterior side of upper cleithrum (Liao *et al.* in press: fig. 6A); [1], Baudelot's ligament attaching to upper cleithrum, but not to supracleithrum (Liao *et al.* in press: fig. 6B).

Neurocranium

4. Anterior outline of ethmo-vomerine region.—States: [0], rather straight or slightly concave; [1], U-shaped; [2], vomer significantly ahead of mesethmoid.
5. Kinethmoid position.—States: [0], horizontally oriented, mostly ventral to premaxilla ascending process; [1], vertically oriented, mostly posterior to premaxilla ascending process.
6. Number of ligaments for the connection between the kinethmoid and tip of the premaxilla ascending process.—States: [0], one (Liao *et al*. in press: fig. 3); [1], two (Liao *et al.* in press: fig. 8A & B).
7. Number of lateral processes on each side of the kinethmoid.—States: [0], 0 or 1; [1], 2 (Liao *et al*. 2010: fig. 10).
8. Lateral ethmoid.—States: [0], rather narrow, the ventral outline extended from parasphenoid smoothly, the overall shape pointed (Liao *et al*. in press: fig. 4B); [1], rather thick, a ventral expansion present immediately next to parasphenoid (Liao *et al.* in press: fig. 4A), the ventral outermost tip rather blunt as a rudimentary process in some species.
9. Frontoparietal fontanelle.—States: [0], absent; [1], present.

Weberian apparatus

10. The pleural rib of 4th vertebra.—States: [0], extremely short, the length below tripus in the lateral view approximately the same as that above, usually shorter than inner arm; [1], reaching or surpassing the ventral level of pharyngeal process, the length below tripus remarkably longer than that above.
11. Median projection on the pleural rib of 4th vertebra (Fang, 2003: fig. 8).—States: [0], absent; [1], present.
12. Tripus.—States: [0], anterior outline oblique, dolabriform in dorsal view (Liao *et al*. 2010: fig. 5A); [1], the outermost

anterior tip without or with a rudimentary process, anterior outline rather straight (Liao *et al*. 2010: fig. 5B).

13. Lateral process of first vertebra.—States: [0], long and expanded, visible from dorsal view or fused with the process of second vertebra; [1], absent or very short in length, not visible in dorsal view; [2], posterior half beneath second lateral process.
14. Shape of lateral process of second vertebra in dorsal view.—States: [0], straight and normal to vertebrae, some with distal end posteriorly pointed, (Liao *et al*. 2010: fig. 5B); [1], arced, distal end posteriorly pointed (Liao *et al*. 2010: fig. 5A).
15. Vertebra 2 and 3.—States: [0], separate; [1], merged.

Jaw bones

16. Dentary projection (Fang, 2003: fig. 2D, E & F).—States: [0], absent; [1], present.
17. Anterior margin of lower part of the maxilla.—States: [0], anterior margin of lower part of the maxilla straight (Liao *et al*. 2010: fig. 2D & G); [1], with an anteriorly pointed ridge (Fang 2003: fig. 2C); [2], with a downward projected-extension (Liao *et al*. 2010: fig. 2E) or blunt without process (Liao *et al*. 2010: fig. 2C).
18. Anterior part of the palatine.—States: [0], greatly reduced in width from lateral view (Fang 2003: fig. 2G) or rather slender; [1], rather thick in lateral view, anterior part not reduced in width, with a lateral plate-like expansion; [2], rather thick in lateral view, anterior part not reduced in width (Fang 2003: fig. 2H).

Gill arch

19. Rasborin process of epibranchial 4.—States: [0], absent (Liao *et al*. 2010: fig. 7A); [1], present (Liao *et al*. 2010: fig. 7B).
20. Interhyal.—States: [0], well ossified; [1], not ossified or absent.

Suspensorium bones

21. Opercular canal.—States: [0], absent (Liao *et al*. 2010: fig. 4A & C); [1], present (Liao *et al*. 2010: fig. 4B).
22. Metapterygoid-quadrate fenestra (Greenwood *et al*. 1966: fig. 9.—States: [0], present; [1], absent.
23. Dorsal posterior process of the metapterygoid (Greenwood *et al*. 1966: fig. 9).—States: [0], present; [1], absent.
24. Proximity of the quadrate and metapterygoid.—States: [0], rather close (Fang 2003: fig. 6B); [1], far separated by the remnant of the palatoquadrate cartilage (Fang 2003: fig. 6A).

Pectoral girdle

25. Danionin foramen (Liao *et al*. 2010: fig. 6A).—States: [0], absent; [1], present.
26. Postcleithrum.—States: [0], not greatly reduced (Liao *et al*. in press: fig. 5B & C); [1], greatly reduced or absent (Liao *et al*. in press: fig. 5A).
27. Orientation of the postcleithrum.—States: [0], parallel to abdominal ribs (Liao *et al*. in press: fig. 5B); [1], normal to abdominal ribs (Liao *et al*. in press: fig. 5A).
28. A mediad projecting process near the distal end of the upper part of the cleithrum.—States: [0], absent; [1], present.

Orbital series

29. Infraorbital 2.—States: [0], slightly or not reduced; [1], greatly reduced or absent.
30. Infraorbital 3.—States: [0], slightly or not reduced; [1], greatly reduced.
31. Infraorbital 4.—States: [0], slightly or not reduced; [1], greatly reduced.
32. Relative position of the supraorbital and infraorbital 5.—States: [0], well separated; [1], in contact.

Colour pattern

33. A stripe (Fang 1998: fig. 1).—States: [0], absent; [1], present.
34. Vertical bars on the side of body.—States: [0], present; [1], absent.
35. Supra-anal pigment and sub-peduncular streak (Brittan, 1972: fig. 51).—States: [0], absent; [1], present.

Other external characters

36. Skin groove on supraorbital shelf (Fang, 2003: fig. 3).—States: [0], absent; [1], present.
37. Relative position of dorsal fin and pelvic-fin insertions.—States: [0], dorsal-fin insertion approximately dorsal to pelvic-fin insertion; [1], dorsal-fin insertion between pelvic- and anal-fin insertions or approximately dorsal to anal-fin insertion.
38. Ventral keel.—States: [0], absent; [1], present.
39. Margin of dorsal fin.—States: [0], rather straight; [1], concave.
40. Rostral barbels.—States: [0], absent; [1], present.
41. Branched anal-fin rays.—States: [0], 7 or more; [1], 5-6.
42. Lateral-line pattern.—States: [0], with one gentle depression on anterior half (Liao *et al*. 2010: fig. 8A); [1], descending abruptly for first few scales (Fang 1997: fig. 1); [2], two slight depressions near the beginning of the lateral line, with three or four scales exhibiting a horizontal arrangement in between (Liao *et al*. 2010: fig. 8B).
43. Predorsal scales.—States: [0], not in a definite mid-dorsal line; [1], in a definite mid-dorsal line.