



***Betadevario ramachandrani*, a new danionine genus and species from the Western Ghats of India (Teleostei: Cyprinidae: Danioninae)**

P. K. PRAMOD¹, FANG FANG², K. REMA DEVI³, TE-YU LIAO^{2,4}, T.J. INDRA⁵,
K.S. JAMEELA BEEVI⁶ & SVEN O. KULLANDER^{2,7}

¹Marine Products Export Development Authority, Sri Vinayaka Kripa, Opp. Ananda shetty Circle, Attavar, Mangalore, Karnataka 575 001, India. E-mail: pramodmohan@yahoo.com

²Department of Vertebrate Zoology, Swedish Museum of Natural History, POB 50007, SE-104 05 Stockholm, Sweden
E-mail: fang.kullander@nrm.se; teyu.liao@nrm.se; sven.kullander@nrm.se

³Marine Biological Station, Zoological Survey of India, 130, Santhome High Road, Chennai, India

⁴Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden

⁵Southern Regional Station, Zoological Survey of India, 130, Santhome High Road, Chennai, India

⁶Department of Zoology, Maharajas College, Ernakulam, India

⁷Corresponding author. E-mail: sven.kullander@nrm.se

Abstract

Betadevario, new genus, with the single species *B. ramachandrani*, new species, from Karnataka, southwestern India, is closely related to *Devario* but differs from it in having two pairs of long barbels (vs. two pairs of short or rudimentary barbels, or barbels absent), wider cleithral spot which extends to cover three scales horizontally (vs. covering only one scale in width), long and low laminar preorbital process (vs. absent or a slender pointed spine-like process) along the anterior margin of the orbit, a unique flank colour pattern with a wide dark band along the lower side, bordered dorsally by a wide light stripe (vs. vertical bars, or stripes narrow and usually in greater number).

Key words: Karnataka, endemism, *Devario*, *Danio*, cytochrome *b*, rhodopsin, phylogeny

Introduction

The tribe Danionini within the cyprinid subfamily Danioninae comprises about 70 species in eight genera, mainly distributed in South and Southeast Asia (Fang *et al.* 2009; Fang & Kullander 2009; Kullander & Fang 2009). Most taxa belong to one of the genera, *Danio* Hamilton, with 16 species (Kullander & Fang 2009), or *Devario* Bleeker, with 39 species (Fang & Kullander 2009). Species of *Devario* occur in lowlands and low hills in nearly all areas of South and Southeast Asia (Fang & Kullander 2001). With the exception of *Danio rerio* (Hamilton), which is widespread in India, Nepal, and Bangladesh (Engeszer *et al.* 2007; Spence *et al.* 2006), the species of *Danio* occur mainly in Myanmar and adjacent parts of neighboring countries, occupying smaller lentic or lotic water bodies in both lowlands and hilly regions (Fang & Kullander 2001). The genera *Danionella* Roberts, *Microdevario* Fang *et al.*, and *Microrasbora* Annandale, are also mainly distributed in Myanmar (Britz 2009; Kottelat & Witte 1999). *Chela* Hamilton is confined to India and Bangladesh, and *Esomus* Swainson and *Laubuca* Bleeker are found over much of Sri Lanka, India, Bangladesh, Myanmar, and Thailand (Pethiyagoda *et al.* 2008; Ahl 1923). Phylogenetic analyses show that *Devario*, *Microdevario*, *Microrasbora*, *Chela* and *Laubuca* form a monophyletic group, sister group of *Danio*, and with *Esomus* as sister group of *Danio* or positioned as a basal danionin lineage, whereas the position of *Danionella* varies between analyses (Fang *et al.* 2009).

Recently, the senior author discovered a highly distinctive danionin species in the Western Ghats of India. It combines characters of *Danio*, such as two pairs of very long barbels and a dark stripe on the anal fin, together with characters of *Devario*, such as a well-developed skin groove on the supraorbital shelves, a

prominent cleithral spot, and the presence of a preorbital process. This species represents a distinct lineage among danionine cyprinids and is described herein.

Material and methods

The description is based on alcohol-preserved specimens. Measurements were taken with digital calipers to 0.1 mm. Methods for obtaining measurements, counts, and coloration follow Fang (1997a, 1997b, 1998). Standard length (SL) is measured from the tip of the upper jaw to the base of the caudal fin. All measurements were made on the left side of the specimens whenever possible. The number of specimens exhibiting a given count is indicated in parentheses.

Radiographs were made with a Philips MG-105 low voltage X-ray unit and Kodak X-Omat V film. Dorsal, anal, caudal-fin rays and vertebral counts were made from radiographs. The first caudal vertebra is that having its haemal spine posterior to the first anal pterygiophore; the caudal vertebrae count includes the last half centrum. The abdominal vertebrae count includes the first four vertebrae of the Weberian apparatus. One specimen was cleared and stained using trypsin for maceration, Alcian blue for staining cartilage, Alizarin red for staining bone, and glycerol for clearing, following the procedure of Taylor & Van Dyke (1985) with slight modifications. Drawings were made using a drawing tube fitted to a WILD MZ8 stereo microscope.

The phylogenetic analysis using morphological characters is based on the matrix of Fang (2003) with modifications (Table 1). Small species suggested later (Rüber *et al.* 2007; Fang *et al.* 2009) not to be danionins (*Sundadanio*), or to be questionable in the position in Fang (2003) due to constraints on skeletal development of small body size (*Danionella*), were removed. The recently described danionin genus *Microdevario* (Fang *et al.* 2009) was added. The phylogenetic analysis was made with PAUP* 4 (Swofford 2002), using the heuristic parsimony algorithm and default settings. Tree stability was assessed using the bootstrap procedure in PAUP* 4, with 1000 replications; and a combination of TreeRot v. 3 (Sorensen & Franzosa 2007) and PAUP* 4 to calculate Bremer Decay indexes. WinClada (Nixon 2002) and NONA (Goloboff 1993) were used in concert to visualize character state transformations on the parsimony tree.

Fragments of the mitochondrial cytochrome *b* (cyt *b*) and the nuclear rhodopsin genes for species of the *Devario* clade (sensu Fang *et al.* 2009) are abundantly available from Genbank. Therefore these two genes were selected for the molecular analysis. Nuclear and mitochondrial DNA was extracted from one specimen of *Betadevario ramachandrani* (NRM 57780) using the GeneMole automated DNA extraction system (Mole Genetics) with recommended protocol.

FishcytB-F and TruccytB-R were used with DNA extract for PCR amplification of 1131 bp of the mitochondrial cytochrome *b* gene (cyt *b*), and Rod-F2B and Rod-4R were used for a 454 bp fragment of the rhodopsin. Primer sequences are given in Sevilla *et al.* (2007). The cyt *b* and rhodopsin genes PCR protocol is the same, 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 then purified using the ExoSAP (Fermentas).

The sequencing reactions were performed using BigDye 3.1 and the same primers as for respective PCR reactions with recommended protocol (Qiagen) and CytBI-7F and CytBI-5R were used for cyt *b* proofreading (Sevilla *et al.* 2007). The products of sequencing reactions were purified with the DyeEx 96 Kit (Qiagen), and then were screened at the final stage in an automatic sequencer (ABI 3700, Applied Biosystems, Inc., Foster City, CA). Both strands were sequenced, and the obtained fragments were proof-read and assembled using the Lasergene software package (DNASTAR, Madison, USA).

Sequences obtained from GenBank and the specimens used for molecular analysis are listed in Table 2. Most sequences are from Fang *et al.* (2009). In the analysis *Kottelatia brittani* (Axelrod) was designated as outgroup for tree rooting. The 10 bp from the 3' end was deleted and excluded from the analysis due to the difference of length (Fang *et al.* 2009). Alignment, available from the authors, was made using the Lasergene software package and checked by eye. Bayesian analysis was performed using MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The dataset was divided into six partitions based on gene

(rhodopsin and *cyt b*) and codon position (first, second and third), and all parameters except topology and branch length were allowed to vary independently using the unlink command in MrBayes v3.1.2. Analyses were conducted with the GTR+I+G model as suggested by MrModeltest (Nylander 2004) sampling for four million generations (two concurrent analyses, nruns = 2; three heated chains, nchains = 4; chain temperature 0.1; sample frequency 1000; burnin = 25%; the average standard deviation of split frequencies of 0.002511).

TABLE 2. Genbank accession numbers for rhodopsin and cytochrome *b* sequences used in this paper.

Taxon	Genbank accession number		Voucher
	rhodopsin	cytochrome <i>b</i>	
<i>Betadevario ramachandrani</i>	GU327622	GU327623	NRM 57780
<i>Chela cachius</i>	EU241350	EU241416	NRM 50821
<i>Danio aesculapii</i>	EU241365	EU241430	NRM 52542
<i>Danio albolineatus</i>	EU241355	EU241419	NRM 41640
<i>Danio choprae</i>	EU241356	EU241421	NRM 41671
<i>Danio dangila</i>	EU241357	EU241422	NRM 42645
<i>Danio erythromicron</i>	EU241358	EU241423	NRM 51833
<i>Danio kerri</i>	EU241359	EU241424	NRM 41653
<i>Danio kyathit</i>	EU241360	EU241425	NRM 41644
<i>Danio margaritatus</i>	EU241349	EU241426	NRM 56722
<i>Danio rerio</i>	EU241362	EU241427	NRM 41655
<i>Danio cf. rerio 'Assam'</i>	EU241353	EU241420	NRM 41663
<i>Danio roseus</i>	EU241363	EU241428	NRM 47156
<i>Danio sp.Ozelot</i>	EU241364	EU241429	NRM 50198
<i>Devario apogon</i>	EU241366	EU241431	NRM 32304
<i>Devario auropurpureus</i>	EU241377	EU241442	NRM 50122
<i>Devario cf. malabaricus</i>	EU241367	EU241432	NRM 40304
<i>Devario chrysoaeniatus</i>	EU241368	EU241433	NRM 32302
<i>Devario devario</i>	EU241369	EU241434	NRM 41658
<i>Devario kakhienensis</i>	EU241370	EU241435	NRM 32303
<i>Devario maetaengensis</i>	EU241371	EU241436	NRM 47157
<i>Devario pathirana</i>	EU241372	EU241437	NRM 41679
<i>Devario regina</i>	EF151100	FJ531348	No vouchers known
<i>Devario shanensis</i>	EU241373	EU241438	NRM 49490
<i>Devario xyrops</i>	EU241374	EU241439	NRM 41674
<i>Esomus caudicellatus</i>	EU241375	EU241440	NRM 50426
<i>Esomus metallicus</i>	EU241376	EU241441	NRM 44786
<i>Kottelatia brittani</i>	EU241399	EU241464	NRM 52521
<i>Laubuca dadiburjori</i>	EU241351	EU241417	NRM 50246
<i>Malayochela maassi</i>	EU241379	EU241444	NRM 50167
<i>Microdevario gatesi</i>	EU241380	EU241445	NRM 50961
<i>Microdevario kubotai</i>	EU241381	EU241446	NRM 50120
<i>Microdevario nanus</i>	EU241382	EU241447	NRM 51968
<i>Microrasbora rubescens</i>	EU241383	EU241448	NRM 41652

Specimens have been deposited in the following collections: BMNH, Natural History Museum, London; CAS, California Academy of Sciences, San Francisco; NRM, Swedish Museum of Natural History, Stockholm, and ZSI/SRS, Zoological Survey of India, Southern Regional Station, Chennai.

Comparative material. *Devario devario* (Hamilton), NRM 40537, 46955. *Devario acrostomus* (Fang & Kottelat), NRM 41281. *Devario annandalei* (Chaudhuri), NRM 58128. *Devario malabaricus* (Jerdon), NRM 32020. *Devario neilgherriensis* (Day), BMNH 1889.1.2:1735–1744. *Devario sondhii* (Hora & Mukerji), NRM 58134. *Microdevario kubotai* (Kottelat & Witte), NRM 51603. See Fang (2003) for additional material used in the morphological phylogenetic analysis.

***Betadevario*, new genus**

Diagnosis. Similar to *Devario*, *Microrasbora*, *Laubuca*, and *Chela* in the presence of a supraorbital groove and to *Devario* also in the presence of an infraorbital process; similar to *Devario*, *Microdevario*, *Chela*, and *Laubuca* in the presence of the two-ligament connection between the kinethmoid and premaxillary ascending process (Fang *et al.* 2009: fig. 6), and the presence of a U-shaped and vertically orientated kinethmoid (Fang *et al.* 2009). *Betadevario* is distinguished from species of the genera *Devario*, *Microdevario*, *Chela*, and *Microrasbora* by having long rostral and maxillary barbels (vs. barbels absent, short, or rudimentary), a wide cleithral spot which extends to cover three scales in width horizontally (vs. absent, or small and rounded, covering one scale in width), a broad and low preorbital process along the anterior margin of the orbit (vs. preorbital process narrow and pointed, or absent, except in *Devario xyrops*, in which it is broad), and a dark stripe across the anal-fin rays resembling the A stripe in *Danio* (vs. A stripe absent). The new genus is distinguished also from *Chela* by having the pelvic fin posterior to the pectoral fin (vs. beneath the pectoral fin), from *Chela* and *Laubuca* by the absence of a ventral keel, from *Microdevario* by the straight (vs. indented) parasphenoid (Fang *et al.* 2009), and from *Microrasbora* by the presence of a two-ligament (vs. a single-ligament) connection between the kinethmoid and premaxillary ascending process. Flank colour pattern of *Betadevario* is unique among danionins, comprised of a broad light stripe along the middle of the side, and a wide dark band paralleling it ventrally.

Type species. *Betadevario ramachandrani*.

Etymology. *Beta-*, the second letter in Greek alphabet, also representing the numeral 2, and used in the meaning of second, and also the name of Beta Mahatvaraj who made great efforts to make the material available for this study; and *-devario*. With reference to its similarity to the genus *Devario*, in the meaning of second *Devario*. The gender is masculine.

***Betadevario ramachandrani*, new species**

(Fig. 1)

Holotype. ZSI/SRS F.8147. Adult male, 45.1 mm SL. India: Karnataka: Dakshina Kannada: Shimoga District: Agumbe, small stream tributary to Sita River, 2 km upstream from Onake Abbi Fall (13°30'79"N 75°4'49"E), 558 mASL; P.K. Pramod, Jan 2008.

Paratypes. NRM 57779, 3, 43.2–48.7 mm SL; NRM 44980, 1, 48.3 mm SL, cleared and stained; NRM 57780, 1, 45.0 mm TL, tissue; NRM 57781, 1, 60 mm TL, tissue; India: Karnataka: Dakshina Kannada: Sita River close to Barkana Falls, bordering Shimoga and Someshwara regions, between Hebri and Somaishwara. (Approximately 13°30'N, 75°00'N). No date, A. Manjunath. Kept in aquarium, preserved 2008. — NRM 60838. 9, 35.1–36.6 mm SL. Same data as holotype. — ZSI/SRS F.8147. 10, 49.0–61.0 mm SL; Same locality as holotype; P.K. Pramod, 16 Dec 2007.

Diagnosis. As for the genus. For field identification purposes *B. ramachandrani* is readily distinguished from all species of *Devario* (see Fig. 1) by its colour pattern including a wide light horizontal band along the middle of the side bordering a wide dark lateral band along the abdominal side, and the long rostral and

maxillary barbels.

Description. Measurements and counts were taken from the holotype and nine paratypes, ZSI/SRS F.8147, and NRM 60838 (Table 3). Counts were taken also from NRM 57779 and NRM 44980. General body features and pigmentation are illustrated in Figs. 1–2.



FIGURE 1. *Betadevario ramachandrani*. Holotype, ZSI/SRS F.8147, male, 45.1 mm SL, from Sita River, Karnataka, India.



FIGURE 2. *Betadevario ramachandrani*. Paratype, NRM 60838, female, 45.5 mm SL, from Sita River, Karnataka, India.

Body laterally compressed, elongate. Predorsal contour slightly curved, with minor occipital indentation, slightly ascending, sloping posteriorly from dorsal-fin insertion. Prepelvic contour slightly curved. Chest conspicuously more compressed below pectoral-fin base, not keeled. Snout short, rounded in dorsal aspect, obtuse in lateral aspect, about as long as eye diameter. Infraorbital process very low, long, with slightly curved margin. Danionine notch caudally margined by well developed anteromedial projecting laminar dentary process, processes of opposite sides meeting medially when mouth closed. Skin cover absent from distal part of infraorbital process, dentary process, and narrowly anterior margin of supraorbital. Mouth terminal, obliquely directed upwards. Small skin-covered bony knob at dentary symphysis fitting into depression in upper jaw. Maxilla reaching to below anterior margin of orbit. Jaws equal anteriorly; lower jaw ending anteriorly at horizontal through about middle or upper $\frac{1}{4}$ of eye. Small tubercles with broad base and sharp pointed tip arranged in 2–3 rows along adlabial lateral margin of dentary. Minute tubercles present in groups along anterior pectoral fin rays in six of 10 specimens. Rostral barbel extending beyond to posterior margin of vertical limb of preopercle. Maxillary barbel longer than rostral barbels, reaching to slightly beyond vertical from posterior margin of opercle. Sexual dimorphism expressed only in presence of tubercles on pectoral-fin rays, and slightly more developed tubercles on dentary in males.

Lateral line complete, along 32 (3), 33(8), 34 (2) scales, and 1–2 scales on caudal-fin base; comprising six tubed scales running caudoventrad followed by tubed scales paralleling ventral outline and ending on lower half of caudal peduncle. Median predorsal scales 16 (10), 17 (3). Lateral scale rows passing between dorsal and pelvic fins $\frac{1}{2}6+1+1$. Circumpeduncular scale rows 7 above, 3 below lateral lines (total 12) in five

specimens; 9 above, 3 below (total 14) in eight specimens. A row of scales along anal-fin base. Slightly less than ¼ of caudal-fin length scaled basally.

TABLE 3. Morphometry of *Betadevario ramachandrani*, ZSI/SRS F.8147 (holotype) and NRM 60838 (9 paratypes). Measurements are in per cent of SL, except for SL and TL (in mm). SD = standard deviation.

	N	Min	Max	Mean	SD
SL (mm)	10	35.1	53.8	45.1	5.47
Total length	8	46.7	71.0	59.1	8.04
Body depth	10	29.0	30.8	30.0	0.60
Head length	10	24.6	26.5	25.3	0.53
Snout length	10	7.1	7.9	7.4	0.23
Head depth	10	17.6	19.7	18.8	0.66
Head width	10	12.9	14.5	13.7	0.52
Upper jaw length	10	11.0	11.7	11.3	0.28
Lower jaw length	10	12.8	14.3	13.5	0.54
Orbital diameter	10	7.5	8.8	8.1	0.44
Interorbital width	10	10.8	11.7	11.3	0.29
Caudal peduncle length	10	19.1	22.1	21.2	1.03
Caudal peduncle depth	10	12.3	13.4	12.9	0.41
Dorsal-fin base length	10	13.4	15.1	14.1	0.58
Anal-fin base length	10	17.5	18.8	18.1	0.47
Predorsal length	10	58.9	62.4	60.6	1.12
Preanal length	10	60.8	64.7	63.5	1.25
Prepelvic length	10	44.1	49.5	46.7	1.43
Pectoral-fin length	10	22.7	25.3	23.8	0.98
Pelvic-fin length	10	15.0	17.0	16.2	0.58
Rostral barbel length	10	13.0	15.2	14.0	0.70
Maxillary barbel length	10	18.4	22.0	20.1	1.27

Dorsal-fin rays ii.7½ (4), ii.8½(10). Anal-fin rays iii.11½ (7), iii.12½(7). Pectoral-fin rays i.12 (2), i.13 (1). Pelvic-fin rays i.6 (2), i.7 (10). Dorsal fin inserted at highest point of dorsum, little posterior to middle of body. Anal fin inserted below anterior rays of dorsal fin. Pectoral-fin insertion at about vertical through posterior margin of osseous opercle; pectoral fin reaching slightly past origin of pelvic fin. Pectoral-fin axial lobe well developed. Pelvic fin inserted slightly anterior to midbody, not reaching anal-fin origin. Pelvic axillary scale present. Caudal fin forked; procurrent rays 6 in both lobes, except in one specimen with 7 ventrally; principal rays 10+9 (14).

Vertebrae 16+19=35 (4), 16+20=36 (2), 17+18=35 (5), 17+19=36 (3). Predorsal vertebrae 14 (14). Vertebral centra contained within caudal peduncle 8(6), 9 (8). Pharyngeal teeth 5,3,2/2,3,5 (one specimen dissected, Fig. 3).

Coloration in preservative. No sexual dichromatism. Dark brown stripe marking dorsal midline from occiput to dorsal-fin base. Dorsal side and dorsal margin of caudal peduncle brown, except slightly lighter zone on each side of mid-dorsal brown stripe. Contrasting yellowish-white horizontal band, about 1½ scales deep from orbit to dorsal half of caudal-fin base. Dark brown cleithral spot covering three scales posterior to cleithrum, immediately ventral to light horizontal band. Side ventral to light horizontal stripe grayish brown to a depth of about three scale rows. Abdomen, area close to anal fin base, and ventral margin of caudal

peduncle, pale yellowish. Top of head brownish. Cheek yellowish, peppered with brown melanophores. Gill cover and jaws grayish to hyaline. Dorsal fin basally light grey; dark grey to blackish stripe across middle of fin, margined distally by wide whitish zone and grey margin. Anal fin pale grayish or hyaline basally; blackish stripe from middle of anterior rays to tips of posterior rays; anterior margin white or hyaline. Pelvic and pectoral fins hyaline, pectoral fin lightly pigmented along first fin-ray. Caudal fin basally grayish, continuing dark body pigmentation, gradually fading along middle rays; pale grayish along dorsal and ventral margins of fin.

Live coloration (Fig. 4). Bluish green dorsally, light horizontal stripe golden yellow, dark lateral band deep blue. Abdomen silvery. Dorsal fin yellow along middle, bluish distally. Anal fin yellow basally, white distal to dark band. Pelvic fins lemon yellow. Caudal fin, except for dark stripes lemon yellow.

Geographical distribution. Known only from the type locality area in the upper Sita River drainage on the southwestern coast of India (Fig. 5). The type locality, at 558 m a.s.l. is a small, high-altitude stream with a cascade and riffle-pool habitat (Fig. 6). The width of the stream was 2–2.7 m, the depth at most about 30 cm. As recorded at 1245h on 16 December 2007, the water temperature was 18.9°C, pH 6.4. The area has a notably high precipitation, Agumbe receiving an average rainfall of 7640 mm annually.



FIGURE 3. *Betadevario ramachandrani*. Left side pharyngeal bone in anterolateral aspect. NRM 57781, 60 mm TL.



FIGURE 4. *Betadevario ramachandrani*. Freshly collected specimen from the type locality showing live coloration.

Phylogenetic position. Addition of *Betadevario ramachandrani* to the morphological matrix of danionines used by Fang (2003), as well as to the molecular dataset used by Fang *et al.* (2009) places the species with *Devario* (Figs. 7–8). In the molecular tree (Fig. 8), *Betadevario* is the sister group of other *Devario*, including the type species *D. devario* (Hamilton), whereas in the morphological tree (Fig. 7) *D. devario* is more basal.

Etymology. Named for Professor Alappat Ramachandran, School of Industrial Fisheries, Cochin University of Science and Technology, Kochi, in recognition of his contributions fisheries and sea food production management and studies on indigenous ornamental fishes.

Discussion

Betadevario ramachandrani is a noteworthy species for several reasons. Superficially, it resembles a *Danio* species because of the long barbels, much longer than in any species of *Devario*. The dark stripe across the anal fin is shared with most species of *Danio*, but not present in species of *Devario*. The colour pattern is similar to that of *Danio dangila* (Hamilton), *D. feegradei* Hora, and many species of *Devario* in the presence of a dark blotch anteriorly on the side—the humeral blotch. *Betadevario ramachandrani* is also the first danionin to be reported as endemic from the Western Ghats, where it is apparently restricted to one or a few hill streams.

Danionin systematics was recently revised by Fang (2003) and Fang *et al.* (2009), recognizing in the latter paper (Fang *et al.* 2009: fig. 4) two well separated clades, one containing *Danio* and the other *Devario*, *Microrasbora*, *Microdevario*, *Chela*, and *Laubuca*.

Danio is recognized by the shape of the dentary, with an anterior lateral expansion that supports a group of spiny tubercles which are best developed in males; a median projection on the pleural rib of the fourth vertebra, two dark stripes on the caudal fin and a dark stripe (the ‘A stripe’) across the anal fin (Fang 2003). Except for a stripe across the anal fin, those character states are absent in *B. ramachandrani*.

The monophyly *Devario* is supported by the following morphological synapomorphies: infraorbital 2 not or only slightly reduced in size; kinethmoid vertically oriented and almost entirely posterior to premaxilla; premaxillary ascending process short and wide and with the kinethmoid apophysis on its posterior end (Fang 2003). These character states are also present in *B. ramachandrani*

Two groups of *Devario* species can be recognized according to the similarities in colour pattern: striped *Devario* and barred *Devario*. While all *Devario* species share a horizontal dark P stripe along the middle of the side, the stripe ends at the end of caudal peduncle in all barred *Devario* species, and extends on to the end of caudal-fin rays in all striped *Devario* species. In some of the striped *Devario* species, a small pointed preorbital spine can be found at the lower posterior margin of the first infraorbital bone (Fang 2003, fig. 4A). In *D. xyrops* Fang & Kullander (2009), however, it was reported as broad and low. This preorbital spine is unknown from other cyprinid genera.

Although unique, the colour pattern of *B. ramachandrani* is homologous with that of other striped *Devario*, retaining the light horizontal stripe above the P stripe, but not developing any light horizontal stripes above or below that. The broad dark band below the yellow stripe thus corresponds to the P and P-1 stripes in other striped *Devario*. The colour pattern of *B. ramachandrani* is similar to that of *Devario sondhii* which also has a simple colour pattern composed mainly of a light stripe along the upper middle part of the side, and a wide dark band along the abdominal side and lower caudal peduncle. In *D. sondhii*, however, males are reddish on the lower part of the caudal peduncle and cranial onto the posterior part of the abdomen. Work in progress suggests that *D. sondhii* has a basal position among *Devario*, and the similarity in colour pattern may be ancestral for the *Devario* clade. The dark stripe across the anal fin (A stripe) characterizes all species of

Danio, with exception of *D. erythromicron* (Annandale) (Conway *et al.* 2008), but a less distinct wide stripe is present in the same position in some species of *Devario* in which the anterior corner of the anal fin is whitish (*D. acrostomus*, *D. annandalei*, *D. xyrops*).

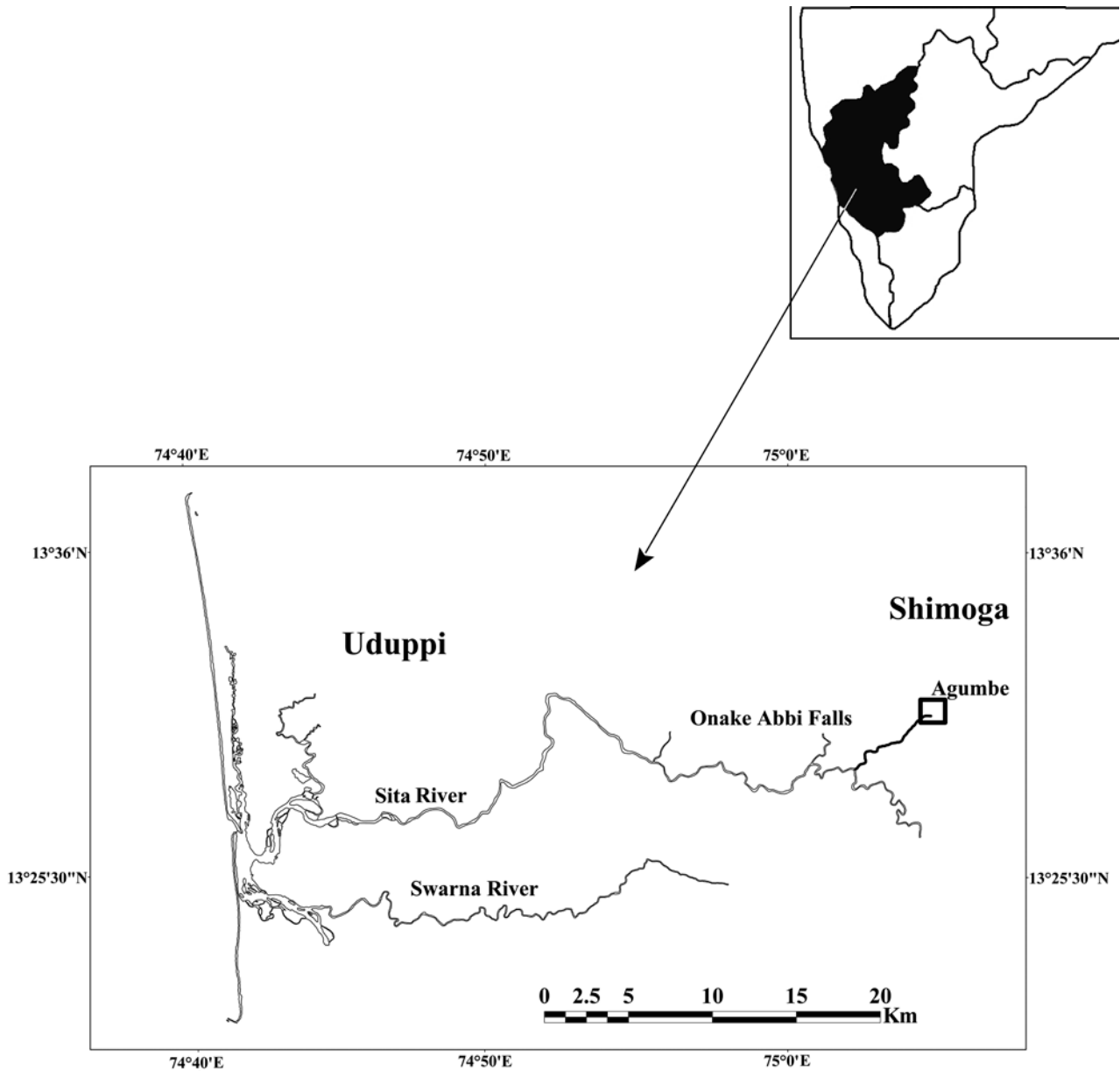


FIGURE 5. *Betadevario ramachandrani*. Location of type locality.

Morphological and nucleotide data agree on a basal position for *B. ramachandrani* relative to species of *Devario*, but the respective trees are quite dissimilar (Figs. 7–8).

In Fang's (2003) morphological assessment of danionin cyprinids, the small species *Sundadanio axelrodi* (Brittan), *Danionella translucida* Roberts, *Microrasbora rubescens* Annandale, and *Danio erythromicron* grouped as a monophyletic sister group of *Esomus* and *Danio*. Apparently the analysis suffered from homoplasies reflecting morphological rather than phylogenetic constraints. Later studies have demonstrated that *D. erythromicron* is a species of *Danio* (Conway *et al.* 2008; Fang *et al.* 2009), and that *Microrasbora rubescens* is closely related to *Devario* (Conway *et al.* 2008; Fang *et al.* 2009), although the precise position

of *M. rubescens* could not be ascertained. The phylogenetic relationships of *Sundadanio* (Kottelat & Witte) and *Danionella* vary among studies. It seems that *Danionella* is a danionin, but outside the clade containing *Devario* and *Danio* (Conway *et al.* 2008; Fang *et al.* 2009; Britz *et al.* 2009). *Sundadanio* is not supported as a danionin (Conway *et al.* 2008; Fang *et al.* 2009).



FIGURE 6. *Betadevario ramachandrani*. Type locality. Photo P. K. Pramod.

To assess the relationships of *Betadevario ramachandrani* we used the matrix of Fang (2003), but based on the above-mentioned conflicting data we modified it slightly. We removed *Danionella* and *Sundadanio*, both because they seem to be irrelevant for danionin interrelationships, and because many characters could not be coded for *Danionella* because the corresponding morphological components are absent. Reexamination of *Microrasbora rubescens* showed that although it does not have the typical deep supraorbital groove characteristic of *Devario* and *Chela*, it does have a depression in the same position, and the earlier coding as absence of the supraorbital groove was in error. We code the shallow and the deep groove as a single character state. This groove is an exclusive character state for *Devario*, *Chela*, *Laubuca*, and *Microrasbora*. A similar groove has been observed in *Chelaethiops* (Howes 1984: fig. 19), however, and it should be analyzed in a context of both African and Asian danionine cyprinids.

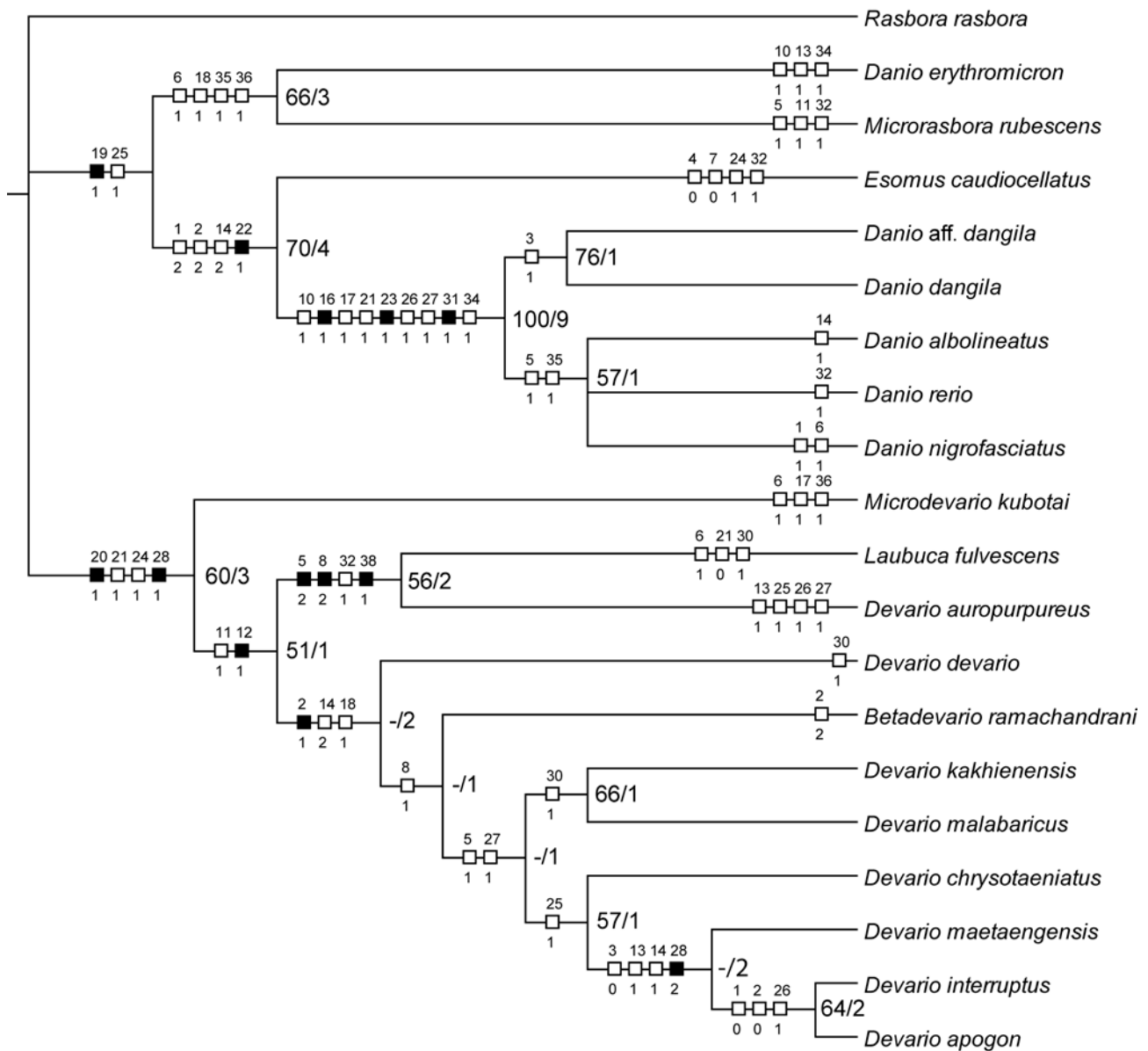


FIGURE 7. Cladogram of relationships among danionin cyprinids, based on morphological characters. Strict consensus tree from two most parsimonious trees, tree length 96 steps, consistency index 0.4688, homoplasy index 0.5313, retention index 0.7488, rescaled consistency index 0.3510. Numbers at nodes are bootstrap values in per cent from 1000 replications to the left of the slash, and the Bremer Decay Index to the right. Bootstrap values less than 50% are not shown. Squares on branches show character states for each branch, the character number above the square, the state below. Only unambiguous transformations are shown; open squares are homoplasies, solid squares are synapomorphies.

Danio erythromicron was demonstrated as most closely related to *D. margaritatus* (Roberts) by Conway *et al.* (2008) and Fang *et al.* (2009), and both are clearly species of *Danio*, although *D. margaritatus* was first placed in a monotypic genus, *Celestichthys* Roberts. Two synapomorphies of other *Danio* are absent in *D. erythromicron* but present in *D. margaritatus*, viz., a dark stripe across the anal fin (the A stripe), and two horizontal stripes on the caudal fin (Fang 2003; Conway *et al.* 2008). The latter two character states are absent in *D. erythromicron*. When we re-run Fang's matrix without *Sundadanio* and *Danionella*, and with the supraorbital groove character corrected for *Microrasbora rubescens*, we still obtain *D. erythromicron* and *M. rubescens* as sister taxa, and separate from the *Esomus*+*Danio* and *Devario*+*Microdevario*+*Betadevario*+*Laubuca* clades. This is due to characters 6 (reduced number of abdominal vertebrae, a common character state among small danionins (Fang 2003)), 18 (normal infraorbital 5, shared with *Devario*, but not with the outgroup or

Danio), 19 (narrow and long ascending process of premaxilla, shared with *Danio*), 35 (hypural 6 absent), and 36 (uroneural 2 absent), the latter two character states shared with *Danionella* and *Sundadanio*, and probably size-constrained (Fang *et al.* 2009). Except for the wide infraorbital 5, where one would rather expect a reduced bone as is more characteristic of small cyprinids (Conway *et al.* 2008), and the long ascending process of the premaxilla, the characters uniting *D. erythromicron* and *M. rubescens* are correlated with small size and conflict with very strong autapomorphic traits within the genus *Danio* and within the *Devario* clade. We analyzed the matrix both with and without characters 6, 35, and 36. Since they provided a change of resolution and bootstrap support only within the *Danio* clade, and did not exclude *Microrasbora* from the *Danio* clade, we nevertheless kept those characters in the final analysis.

We also re-examined *Devario devario*, the type species of *Devario*. It is unusual in the genus for having a very long dorsal fin with 16 branched rays and, as observed by Fang (2003), a typical danionin notch is absent. The danionin notch is present in all other species of *Devario*, also in *D. auropurpureus* contra Fang (2003). We find that in *D. devario* the dentary is relatively slender, lacking the long mediad projections that define the posterior margin of the danionin notch, but it is gently concave anteriorly, and could be viewed as an extremely shallow danionin notch. We thus coded the danionin notch as present in this species. Conway *et al.* (2008: fig. 10D), interpret the shape of the lower jaw in *D. devario* as possessing a short danionin notch. *Devario devario* also lacks a preorbital process and consequently has a smoother head than most other species of *Devario*, and it is possible that the absence of a typical danionin notch, which is marked in other *Devario* by a very strong mediad process terminating the notch caudally and the absence of the preorbital process may indicate a functional correlation. A danionin notch has also been recorded from two rasborin species, viz. *Boraras brigittae* and *Rasbora hubbsi* (Liao *et al.* 2010).

The result is two most parsimonious trees that only differ in arrangements within *Danio* (consensus tree, Fig. 7). Both trees have *Microrasbora rubescens* in the *Danio*+*Esomus* clade, sharing as synapomorphy the long and narrow premaxillary ascending process (Fig. 7: character 19). This clade, however, has no bootstrap or Bremer support, which is strong only for *Danio* without *D. erythromicron*. *Devario devario* has a more basal position than *Betadevario*, which turns out to be the sister group of the remaining *Devario*. The consensus tree (Fig. 7) shows a *Devario*+*Laubuca*+*Betadevario* clade with poor or absent bootstrap and Bremer support for most of the subclades, and consequently with limited information on relationships among *Devario*, *Laubuca*, and *Betadevario*.

The topology of the Bayesian tree (Fig. 8) resulting from analysis of the molecular data is mostly similar to that of the combined tree of Fang *et al.* (2009: fig. 4), in which danionins are divided into *Danio* and *Devario* clades. *Esomus* is the sister group of danionins. The *Devario* clade is further divided into three clades, comprising species of *Microdevario*, *Chela*+*Laubuca* and *Betadevario*+*Microrasbora*+*Devario*, respectively. *Betadevario ramachandrani* is the sister taxon of the *Microrasbora*+*Devario* clade. *Microrasbora rubescens*, nested with species of *Devario* in figure 4 of Fang *et al.* (2009), is the sister taxon of *Devario* in the present analysis.

In the parsimony analysis of molecular data, five most parsimonious trees, 3908 steps, were obtained. The strict consensus tree of the parsimony analysis (not shown) is similar to that of the Bayesian analysis (Fig. 8), but with less resolution. The systematic position of *Betadevario ramachandrani* and the interrelationships among *Betadevario*, *Microrasbora* and *Devario* are the same in both analytic methods, with strong reiteration support.

The molecular analysis places *Betadevario* as the sister group of *Devario*+*Microrasbora* (Fig. 8), which is in agreement with the presence of a supraorbital groove. Although we use a different outgroup here, viz. *Kottelatia brittani* (Axelrod), representing the Rasborini, and *Malayochela maassi* (Weber & de Beaufort), placed in the Chedrini by Fang *et al.* (2009), the tree obtained is in agreement with that obtained for the same ingroup in Fang *et al.* (2009). *Devario devario* ends up far removed from the base of the tree, suggesting that its atypical absence of a preorbital process, very shallow danionin notch, like the long dorsal fin, are reversals or autapomorphies, possibly associated with its very deep body shape, also unique within the genus.

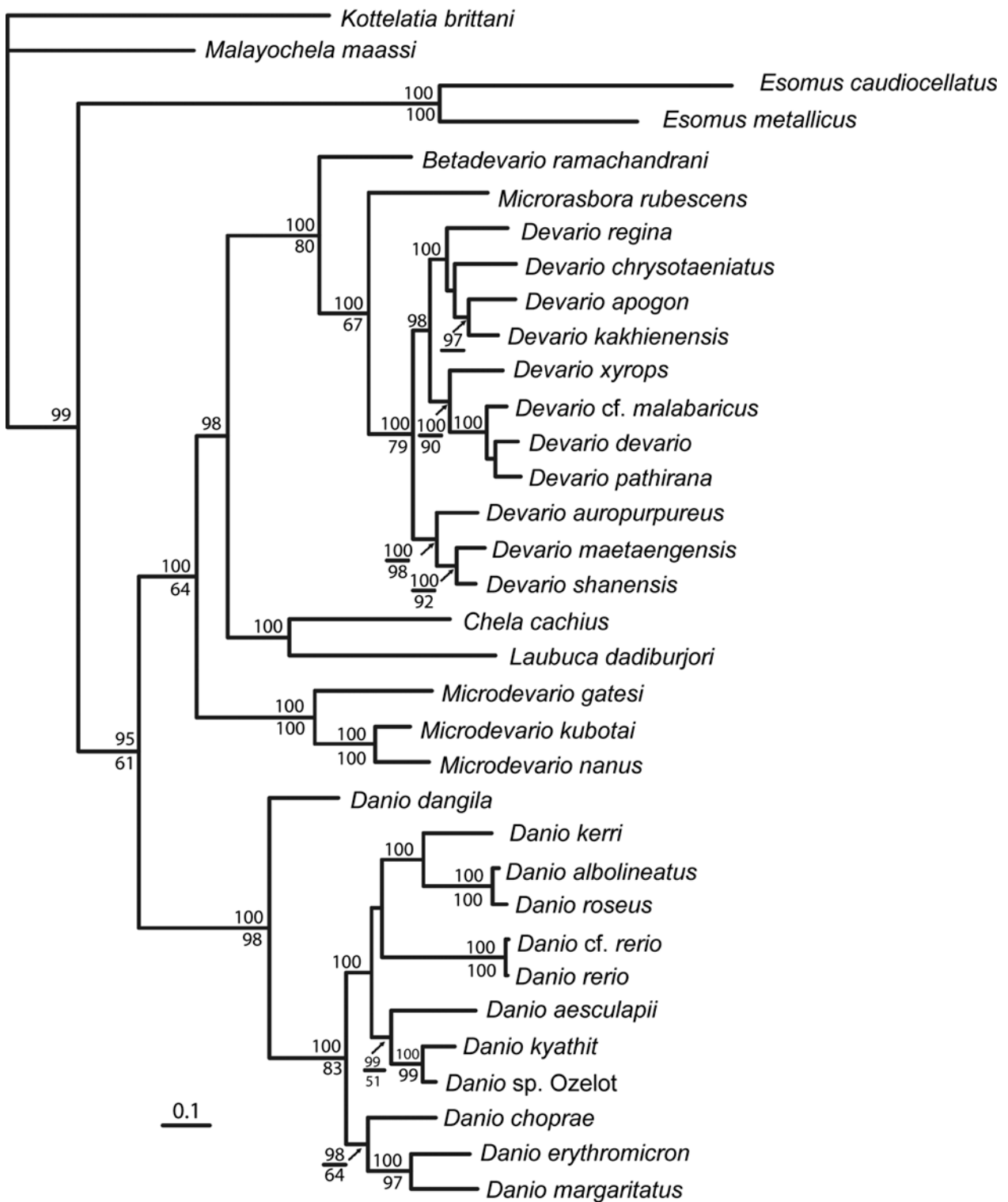


FIGURE 8. Phylogram of relationships among danionin cyprinids. 50 % majority-rule consensus tree obtained from the Bayesian analysis based on nucleotide sequences of mitochondrial cytochrome *b* and nuclear rhodopsin genes. Values above and below nodes indicate posterior probability and bootstrap support, respectively. Values lower than 95 (posterior probability) and 50 (bootstrap support) are not shown. Branch lengths are proportional to estimated change indicated on the lower left of the figure.

The morphological tree (Fig. 7) is apparently problematic. *Danio* is diagnosed by three strong synapomorphies, the A stripe (character 16), the lateral expansion anteriorly on the dentary (character 23), and an expanded nasal (character 31). The median process on the os suspensorium (character 34), suggested as a

Danio autapomorphy by Fang (2003) and Conway *et al.* (2008), appears here as a homoplasy shared with *Danio erythromicron*. *Devario*+*Laubuca*+*Chela*+*Microrasbora* are characterized by a unique skin groove on the supraorbital (character 28), which here is a homoplasy for *Microrasbora rubescens* and *Laubuca*+*Devario*+*Betadevario*. The *Devario* clade, including *Microdevario* is rather diagnosed here by the kinethmoid apophysis on the premaxilla (character 20), and the U-shaped kinethmoid (character 28). The latter character is correlated with the presence of two kinethmoid-ethmoid ligaments, whereas in other cyprinids there is a single ligament (Fang *et al.* 2009). The remaining characters apparently are not informative for resolving taxa within the *Devario* clade, and further analysis must be based on an expanded morphological data set. Whereas the morphological tree does provide support for the recognition of *Danio* as a genus distinct from *Devario* and the original analysis was focused on demonstrating this (Fang 2003), it contains apparent conflict and too low support values to provide a reliable hypothesis of relationships among *Devario* and similar genera. We thus refer to the molecular result, with consistent strong support for all clades, in combination with morphological autapomorphies within the *Devario* clade in our decision to give generic status to *Betadevario ramachandrani*. *Betadevario* then remains diagnosed only by the unique colour pattern and presence of long barbels. The latter character is shared with many species of *Danio* and with *Esomus*, but not with *Devario*, *Microdevario*, *Microrasbora*, *Laubuca* or *Chela*.

Betadevario ramachandrani is a basal species in the clade containing *Devario* in both morphological and molecular analyses, and possibly a relict species being obviously confined to small streams in the Western Ghats, draining to the Laccadive Sea. Dahanukar *et al.* (2004) listed 288 fish species from the Western Ghats, of which 118 (41%) are endemic. There are practically no useful taxonomic revisions available for the fishes of the region, but given the overall endemism reported for other organismal groups in the area (Gunawardene *et al.* 2007), the estimates may be realistic.

Two other species of *Devario* have been reported from the western slope of the Western Ghats. One of them is identified as *D. malabaricus* or *D. aequipinnatus* (M'Clelland) in the literature (Jayaram 1991), occurring in all major south Indian rivers, also in western slope rivers (Chandrashekhariah *et al.* 2000) and in Sri Lanka (Pethiyagoda 1991). The group of striped devarios to which *D. malabaricus* belongs has not yet been subjected to taxonomic revision, and similar species occur also in northern India and Myanmar. Whereas *D. malabaricus* was described from the Malabar coast ("common in all the streams of Malabar out of reach of the tides", Jerdon 1849) and presumably then from rivers draining to the Laccadive Sea, *D. aequipinnatus* was described from Assam (M'Clelland 1839), and it seems unlikely that the latter species would also be present on the southwestern coast of India. The only other south Indian species of *Devario* is *D. neilgherriensis* from the Nilgiri Hills at the extreme south of the Western Ghats (Day 1867). *Devario malabaricus* and *D. neilgherriensis* both differ from *Betadevario ramachandrani* in short rostral barbel and short or absent maxillary barbel, longer dorsal fin (9½–12½ branched rays), and different colour pattern. In *D. neilgherriensis*, there is a narrow dark P stripe bordered by narrow light stripes; in *D. malabaricus* several light and dark horizontal stripes.

Danio is represented in southern India only by *Danio rerio* (Hamilton) which is widespread over much of northern India and Bangladesh, and reaches south to the Cauvery River, which it ascends to the region of Mysore (CAS 81661, NRM 26408), but it has not been recorded to occur in western slope drainages.

Acknowledgements

We are grateful to Ananthaiah Manjunath for field work together with the first author, G. Sankar for photography, Beta Mahatvaraj and Ananthaiah Manjunath for assisting with access to specimens, and two anonymous referees who made constructive comments on the manuscript.

References

- Ahl, E. (1923) Ichthyologische Mitteilungen, IV. Eine Revision der Cypriniden-Gattung *Esomus*. *Mitteilungen aus dem Zoologischen Museum in Berlin*, 11, 38–43.
- Britz, R. (2009) *Danionella priapus*, a new species of miniature cyprinid fish from West Bengal, India (Teleostei: Cypriniformes: Cyprinidae). *Zootaxa*, 2227, 53–60.
- Britz, R., Conway, C. & Rüber, L. (2009) Spectacular morphological novelty in a miniature cyprinid fish, *Danionella dracula* n. sp. *Proceedings of the Royal Society, B (Biological Sciences)*, 276, 2179–2186. Published online 11 March 2009, doi:10.1098/rspb.2009.0141.
- Chandrashekhariah, H.N., Rahman, M.F. & Lakshmi Raghavan, S. (2000) Status of fish fauna in Karnataka. Pp. 98–135 In Ponniah, A.G. & Gopalakrishnan, A. (eds.), *Endemic fish diversity of Western Ghats*. National Bureau of Fish Genetic Resources, Lucknow, 347 pp.
- Conway, K.W., Chen, W.-J. & Mayden, R. L. (2008). The “Celestial Pearl danio” is a miniature *Danio* (s.s) (Ostariophysi: Cyprinidae): evidence from morphology and molecules. *Zootaxa*, 1686, 1–28.
- Dahanukar, N., Raut, R. & Bhat, A. (2004) Distribution, endemism and threat status of freshwater fishes in the Western Ghats of India. *Journal of Biogeography*, 31, 123–136.
- Day, F. (1867) On the fishes of the Neilgherry Hills and rivers around their bases. *Proceedings of the Zoological Society of London*, 1867, 281–302.
- Engeszer, R.E., Patterson, L.B., Rao, A.A. & D.M. Parichy. 2007. Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish*, 4, 21–40.
- Fang F. (1997a) Redescription of *Danio kakhienensis*, a poorly known cyprinid fish from the Irrawaddy basin. *Ichthyological Exploration of Freshwaters*, 7, 289–298.
- Fang F. (1997b) *Danio maetaengensis*, a new species of cyprinid fish from northern Thailand. *Ichthyological Exploration of Freshwaters*, 8, 41–48.
- Fang, F. (1998) *Danio kyathit*, a new species of cyprinid fish from Myitkyina, northern Myanmar. *Ichthyological Exploration of Freshwaters*, 8, 273–280.
- Fang F. (2003) Phylogenetic analysis of the Asian cyprinid genus *Danio* (Teleostei, Cyprinidae). *Copeia*, 2003, 714–728.
- Fang, F., Norén, M., Liao, T.Y., Källersjö, M. & Kullander, S.O. (2009) Molecular phylogenetic interrelationships of the south Asian cyprinid genera *Danio*, *Devario* and *Microrasbora* (Teleostei, Cyprinidae, Danioninae). *Zoologica Scripta*, 38, 237–256.
- Fang F. & Kottelat, M. (1999) *Danio* species from northern Laos, with descriptions of three new species (Teleostei, Cyprinidae). *Ichthyological Exploration of Freshwaters*, 10, 281–295.
- Fang, F. & Kullander, S.O. (2009) *Devario xyrops*, a new species of danionine fish from south-western Myanmar (Teleostei: Cyprinidae). *Zootaxa*, 2164, 33–40.
- Fang Kullander, F. (2001) *Phylogeny and species diversity of the South and Southeast Asian cyprinid genus Danio Hamilton* (Teleostei, Cyprinidae). PhD dissertation, Stockholm University, Stockholm, 26 pp.
- Goloboff, P.A. (1993) *NONAME*, v. 1.6. Computer software distributed by the author.
- Gunawardene, N.R., Dulip Daniels, A.E., Gunatilleke, I.A.U.N., Gunatilleke, C.V.S., Karunakaran, P.V., Geetha Nayak, K., Prasad, S., Puyravaud, P., Ramesh, B.R., Subramanian, K.A. & Vasanthi, G. (2007) A brief overview of the Western Ghats – Sri Lanka biodiversity hotspot. *Current Science*, 93, 1567–1572.
- Howes, G.J. (1984) A review of the anatomy, taxonomy, phylogeny and biogeography of the African neoboline cyprinid fishes. *Bulletin of the British Museum (Natural History), Zoology series*, 47, 155–185.
- Huelsenbeck, J. P. & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17: 754–755.
- Jayaram, K.C. (1991) Systematic status of *Danio malabaricus* (Pisces: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 2, 109–112.
- Jerdon, T.C. (1849) On the fresh-water fishes of Southern India. *Madras Journal of Literature and Science*, 15, 302–346.
- Kottelat, M. & Witte, K.-E. (1999) Two new species of *Microrasbora* from Thailand and Myanmar, with two new generic names for small southeast Asian cyprinid fishes (Teleostei: Cyprinidae). *Journal of South Asian Natural History*, 4, 49–56.
- Kullander, S.O. & Fang, F. (2009) *Danio tinwini*, a new species of spotted danio from northern Myanmar (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 20, 223–228.
- Liao, T.Y., Kullander, S.O. & Fang, F. (2010) Phylogenetic analysis of the genus *Rasbora* (Teleostei: Cyprinidae). *Zoologica Scripta*, 39, 155–176.
- M'Clelland, J. (1839) Indian Cyprinidae. *Asiatic Researches*, 19, 217–471.
- Nixon, K. (2002) *WinClada 1.0*. Computer software distributed by the author.
- Nylander, J. A. A. (2004) *MrModeltest v2*. Computer software distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Pethiyagoda, R. (1991) *Freshwater fishes of Sri Lanka*. Wildlife Heritage Trust of Sri Lanka, Colombo, xiii+362 pp.

- Pethiyagoda, R., Kottelat, M., Silva, A., Maduwage, K. & Meegaskumbura, M. (2008) A review of the genus *Laubuca* in Sri Lanka, with description of three new species (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 19, 7–26.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Rüber, L., Kottelat, M., Tan, H.H., Ng, P.K.L. & Britz, R. (2007) Evolution of miniaturization and the phylogenetic position of *Paedocypris*, comprising the world's smallest vertebrate. *BMC Evolutionary Biology*, 7, 38.
- Sevilla, R. G., Diez, A., Norén, M., Mouchel, O., Jérôme, M., Verrez-Bagnis, V., van Pelt, H., Favre-Krey, L., Krey, G., The FishTrace Consortium & Bautista, J. M. (2007) Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome *b* and nuclear rhodopsin genes. *Molecular Ecology Notes*, 7, 730–734.
- Sorenson, M.D. & E.A. Franzosa (2007) *TreeRot, version 3*. Boston University, Boston, MA. Computer software distributed by the author.
- Spence, R., Fatema, M.K., Reichard, M., Huq, K.A., Wahab, M.A., Ahmed, Z.F. & C. Smith (2006) The distribution and habitat preferences of the zebrafish in Bangladesh. *Journal of Fish Biology*, 69, 1435–1448.
- Swofford, D.L. (2002) *PAUP*, phylogenetic analysis using parsimony (and other methods) 4.0 beta 10 for 32-bit Microsoft Windows*. Sunderland, Massachusetts: Sinauer Associates.
- Taylor, W.R. & Van Dyke, G.C. (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium*, 9, 107–119.