



PHYLOGENETIC POSITION AND OSTEOLOGY OF *PETHIA SETNAI* (CHHAPGAR & SANE, 1992), AN ENDEMIC BARB (TELEOSTEI: CYPRINIDAE) OF THE WESTERN GHATS, INDIA, WITH NOTES ON ITS DISTRIBUTION AND THREATS

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Abstract: *Pethia setnai* is an endemic and threatened freshwater fish of the Western Ghats of India. It has a restricted distribution in the west flowing rivers in the states of Maharashtra, Goa and Karnataka. We clarify the phylogenetic position of *Pethia setnai*, provide osteological details of topotypic material, and morphometric data of specimens from Maharashtra, Goa and Karnataka. We also provide details on micro-level distribution, habitat and threats to the species in its native range.

Keywords: Biometrics, *Puntius setnai*, threatened, topotype.

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INTRODUCTION

Two major hurdles in conservation of threatened and endemic species, especially those that occur in biodiversity hotspots is the Wallacean shortfall (arising from the incomplete information regarding the distribution of species) and Linnean shortfall (several species still not formally described) (Bini et al. 2006). The Western Ghats region, part of the Western Ghats-Sri Lanka biodiversity hotspot is no exception to this, as it has already been suggested that the freshwater fish fauna (especially endemic and threatened species) of this region is poorly known with regard to their taxonomy and distribution (Dahanukar et al. 2011; Raghavan et al. 2012). One reason for this gap in knowledge regarding geographical distribution is the lack of detailed descriptions and reliable genetic data, which can ultimately lead to misidentifications. As a result, genetic sequences from topotypic material and detailed morphometric data will not only help in understanding the systematics of the species, but also provide reliable identification criteria.

In a recent review of the South Asian barbs previously placed under the genus *Puntius*, Pethiyagoda et al. (2012) tentatively placed *Puntius setnai* Chhappgar & Sane, 1992 in the genus *Pethia* based on the data available in the original description of the species. The tentative placement was probably due to the limited information on the morphology, osteology and/or genetic data of the species. In the current study, we provide genetic information of *Pethia setnai* from topotypic material, osteological details to confirm the placement of the species under *Pethia*, and reliable genetic and morphometric data for accurate identification of the species. We also provide information on the distribution, habitat and threats to the species.

MATERIALS AND METHODS

Study site

Topotypes of *Pethia setnai* were collected from Salaulim River, a tributary of Zuari River in Sanguem (15.234°N & 74.182°E, 19m), Goa. Additional material was also collected from Terekhol River at Madkhol Village (15.935°N & 73.910°E, 43m), Maharashtra (Fig. 1). Collections were done responsibly following the guidelines set by IUCN (2008). Material collected in the present study is deposited in the museum of Bombay Natural History Society (BNHS), Mumbai; Wildlife Information Liaison Development (WILD), Coimbatore,

and the Zoological Survey of India, Western Regional Center (ZSI-WRC), Pune.

Morphometry

Measurements were taken point to point using dial calipers to the nearest 0.01mm and then rounded to 0.1mm. Subunits of the body are presented as percent of standard length (SL), and subunits of the head are presented as percent of head length (HL). All pored scales were counted when reporting the lateral line scales. Methods for taking counts and measurements follow Kullander (2008) and Pethiyagoda et al. (2012).

Materials examined

Pethia setnai: 9 exs., BNHS FWF 53, 63 to 70, 10.viii.2013, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar; 3 exs., WILD-13-PIS-043 to 045, 10.viii.2013, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar; 2 exs., ZSI-WRC P/3567, 10.viii.2013, collected from Sanguem, Goa, by U. Katwate, M. Paingankar and N. Dahanukar; 9 exs., BNHS FWF 54 to 62, 12.vi.2013, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate and N. Dahanukar; 3 exs., WILD-13-PIS-046 to 48, 12.vi.2013, collected from Terekhol River at Madkhol, Maharashtra, by Unmesh Katwate and Neelesh Dahanukar; 2 exs., ZSI-WRC P/3568, 12.vi.2013, collected from Terekhol River at Madkhol, Maharashtra, by U. Katwate and N. Dahanukar; 1 ex., ZSI-WRC P/3572, 5.ii.1971, collected from Kaneri River at Gund, District Karwar (Mysore), by B.S. Lamba and Party; 3 exs., ZSI-WRC P/3571, 17.ii.1971, collected from Ramanguli, District Karwar (Mysore), by B. S. Lamba and Party; 26 exs. BNHS FWF 17-42, collected from Sanguem, Goa by S.R. Sane.

Photographs examined

Pethia setnai: Holotype, ZSI Kolkata FF2766, 01.iii.1985, collected from Sanguem, Goa, by S.R. Sane; Paratypes, 6 exs., ZSI Kolkata FF2767, 01.iii.1985, collected from Sanguem, Goa, by S.R. Sane.

Pethia narayani: Syntypes, 2 exs., ZSI Kolkata F12180/1, collected from Cauvery River, Coorg, by C.R.N. Rao.

Osteology

Two specimens (BNHS FWF 55 and BNHS FWF 70) were cleared and stained using the procedure described by Potthoff (1984). Osteological nomenclature follow Conway (2011) and descriptions of osteology follow Pethiyagoda et al. (2012) so as to allow easy comparison

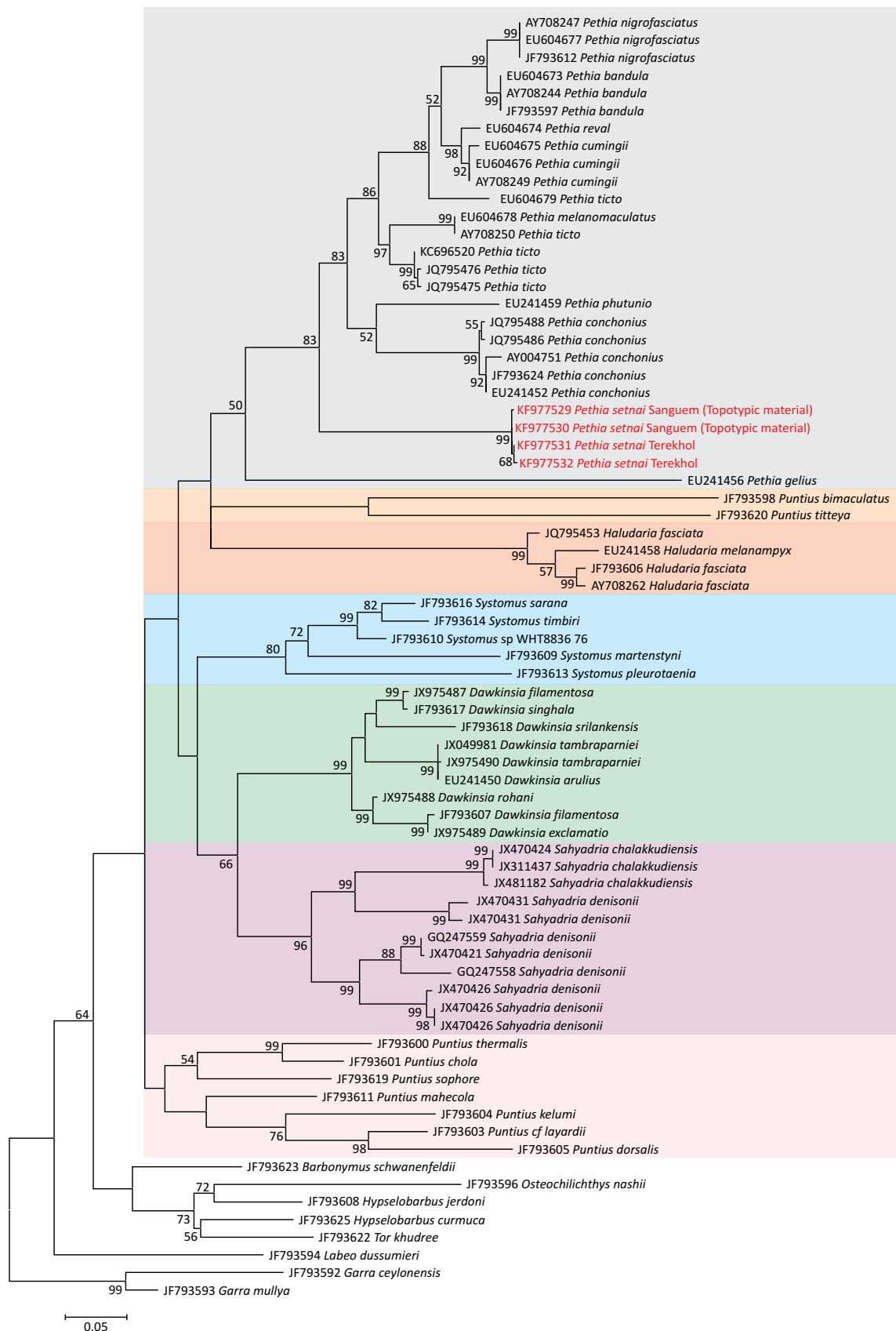


Figure 1. Phylogenetic position of *Pethia setnai*. Maximum likelihood tree based on mitochondrial cytb gene. Bootstrap support with more than 50% based on 1000 iterations for maximum likelihood. *Garra* species are used as outgroup.

with other related taxa. Osteological illustrations were made from images captured by a digital camera fitted on stereo-zoom light microscope (Leica S8 APO, USA).

Genetic analysis

Gills were taken from four fresh specimens (BNHS FWF 53, BNHS FWF 54, WILD-13-PIS-043 and WILD-13-PIS-046) and preserved in absolute ethanol. The tissue was digested at 60°C for two hours using the STE buffer (0.1M NaCl, 0.05M Tris-HCl, 0.01M EDTA, 1%SDS) with 15µl Proteinase K (20mg/ml) per 500ml of STE buffer. DNA was extracted using conventional phenol-chloroform method and re-suspended in TE (10mM Tris, 1mM EDTA, pH 8) buffer. Polymerase chain reaction was performed to amplify cytochrome b (cytb) gene using primer pair L14724 (5'-GACTTGAAAAACCACCGTTG-3') and H15915 (5'-CTCCGATCTCCGGATTACAAGAC-3') (Chen et al. 2007). PCR reaction was performed in a 25µl reaction volume containing 5µl of template DNA (~200ng), 5µl of 10X reaction buffer (100 mM Tris pH 9.0, 500 mM KCl, 15 mM MgCl₂, 0.1% Gelatin), 3µl of 25mM MgCl₂, 1µl of 10mM dNTPs, 1µl of each primer, 0.5µl Taq polymerase (2.5 units) and 8.5µl nuclease free water. The thermal profile was 10 minutes at 95°C, and 35 cycles of 1 minute at 94°C, 1 minute at 52°C and 2 min at 72°C, followed by extension of 10 min at 72°C. Amplified DNA fragments were purified using the Wizard Gel and PCR clean up system (Promega, USA). The purified PCR products were sequenced using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) and ABI prism 3730 sequencer (Applied Biosystems, USA). Sequences were analyzed by BLAST tool (Altschul et al. 1990). All sequences used in the paper have been deposited in GenBank.

We used the cytb gene dataset of South Asian cyprinid fishes by Pethiyagoda et al. (2012), Raghavan et al. (2013a) and additional sequences of *Pethia*, *Dawkinsia* and *Haludaria* available from NCBI GenBank (<http://www.ncbi.nlm.nih.gov/>). Accession numbers for the sequences used for the analysis are provided in Fig. 1. Gene sequences were aligned using MUSCLE (Edgar 2004). Phylogeny was performed using the freeware MEGA 5 (Tamura et al. 2011). Best fit model for nucleotide substitution was selected from 24 models available in MEGA 5 based on minimum Akaike Information Criterion (AIC) value (Posada & Crandall 2001). Tamura & Nei (1993) nucleotide substitution model including invariant sites and a Gamma parameter was obtained as a best fit model (TN93+G+I, AIC = 12894.38, lnL = -6328.70). This model was then used for constructing a phylogenetic tree using the maximum likelihood method. Reliability

of the phylogenetic tree was estimated using bootstrap values run for 1000 iterations.

RESULTS AND DISCUSSION

Before it was formally described, *Pethia setnai* collected from Ponda, Goa, was first reported as *Pethia nigrofasciata* (Günther, 1868) by Yazdani (1977). Chhapgar & Sane (1980) criticized this report, after collecting this species from Sanguem, Goa, as well as examining the specimens studied by Yazdani (1977), and suggested that the species was *Pethia narayani* (Hora, 1937). However, subsequently, Chhapgar & Sane (1992) described the species as *Puntius setnai*. Subsequently, Yazdani & Ghate (1994) provided further information on this species based on the specimens collected from Hosangadi in Karnataka.

Phylogenetic position

Pethia setnai forms a monophyletic group (Fig. 1) with the genus *Pethia* sensu stricto as defined by Pethiyagoda et al. (2012). The genetic divergence in the cytb gene between the topotypic material collected from Sanguem, Goa and additional material collected from Terekhol River at Madkhol, Maharashtra, was 0.325%, while the divergence between the two Terekhol specimens as well as two Sanguem specimens was 0.2%. Because of the very low genetic divergence, the two populations have to be considered as genetically similar. We could not collect fresh specimens of the species for genetic analysis from Karnataka State, and as a result, future studies are essential to establish the extent of genetic variation in different known populations of the species. Nevertheless, as we are providing the genetic information of the topotypic material, future studies can have a more reliable comparative material.

Taxonomy

***Pethia setnai* (Chhapgar & Sane, 1992)**

Puntius nigrofasciata (non Günther, 1868): Yazdani (1977)

Puntius narayani (non Hora, 1937): Chhapgar & Sane (1980)

Puntius setnai Chhapgar & Sane, 1992: Chhapgar & Sane (1992), Yazdani & Ghate (1994), Knight et al. (2012)

Pethia setnai (Chhapgar & Sane, 1992): Pethiyagoda et al. (2012)

FF 2766 [HOLOTYPE]
Puntius setnai Chhapgar
 + Sane
 Loc: Sanguem, Goa.
 Coll: Mr. S.R. Sane
 Date: 1-3-1985

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Type material information

Holotype and six paratypes of *Pethia setnai* collected from Sanguem, Goa, by S.R. Sane on 1.iii.1985 are available in good condition in the museum collection of ZSI Kolkata under the accession numbers FF2766 (Image 1) and FF2767 (Image 2). Chhapgar & Sane (1992) described the species based on 25 specimens (the holotype and 24 paratypes) with the note that "... holotype and some of the paratypes will be deposited with the Zoological Survey of India, Calcutta". The whereabouts of remaining 18 paratypes is not known. Interestingly, 26 specimens of *P. setnai* are in the museum collection of BHNS under the accession numbers BNHS FWF 17-42 (see Materials examined). However, whether some of the specimens from BNHS constitute the missing paratypes could not be deciphered.

Image 1. Holotype of *Pethia setnai* ZSI Kolkata FF 2766.

FF 2767 [PARATYPE]
Puntius setnai /6 Nos.
 Chhapgar + Sane
 Loc: Sanguem, Goa.
 Coll: Mr. S.R. Sane
 Date: 1-3-1985.



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Image 2. Paratypes of *Pethia setnai* ZSI Kolkata FF 2767

Table 1. Morphometric characters and meristics of *Pethia setnai* collected from Sanguem, Madkhol and Karwar. Raw data for all the measurements are provided in Appendix A, B and C.

Character	Sanguem (n = 12) ^a		Madkhol (n = 12) ^b		Karwar (n = 4) ^c	
	Mean (sd)	Range	Mean (sd)	Range	Mean (sd)	Range
Total length (mm)	42.4 (6.0)	34.0–50.4	29.7 (4.7)	25.1–42.2	Caudal fin damaged	Caudal fin damaged
Standard length (SL, mm)	32.6 (4.8)	26.3–39.7	23.0 (3.6)	19.2–32.4	34.0 (3.2)	30.7–37.3
% SL						
Head length (HL)	29.9 (1.3)	27.7–32.4	29.3 (0.9)	27.7–31.0	30.0 (2.3)	28.2–33.1
Head depth	24.6 (0.9)	22.7–25.6	24.6 (1.1)	23.0–26.8	22.8 (1.0)	21.9–23.8
Head width	15.8 (0.5)	14.8–16.6	16.5 (0.4)	15.6–16.9	16.3 (0.2)	16.0–16.5
Body depth	40.5 (3.0)	35.8–44.4	35.3 (1.4)	33.4–38.6	40.9 (1.5)	39.1–42.3
Body width at dorsal fin origin	16.4 (1.1)	14.6–17.7	16.0 (1.5)	12.6–18.2	14.5 (0.6)	13.7–14.9
Body width at anal fin origin	11.9 (0.8)	9.9–12.5	12.5 (0.9)	11.2–14.9	9.1 (0.7)	8.3–9.9
Pre dorsal distance	52.5 (2.0)	47.1–54.8	52.0 (1.8)	49.9–56.6	51.8 (4.0)	46.1–55.4
Dorsal to hypural distance	47.4 (1.6)	43.2–50.1	47.7 (2.1)	43.1–49.9	52.2 (2.8)	48.8–55.0
Prepelvic distance	50.2 (2.7)	43.3–53.9	52.7 (2.3)	49.4–57.4	48.7 (1.4)	47.2–50.6
Preanal distance	72.3 (2.6)	66.7–76.3	71.5 (1.7)	69.2–74.2	71.0 (1.4)	69.8–72.6
Prepectoral distance	28.4 (1.4)	25.6–30.4	31.5 (1.3)	29.2–33.8	31.3 (2.3)	28.2–33.9
Dorsal fin length	26.8 (1.7)	24.1–30.1	26.1 (1.2)	23.8–27.3	27.4 (2.5)	24.8–29.8
Dorsal fin spine length	18.9 (1.0)	16.8–20.6	17.6 (1.0)	15.7–18.9	18.3 (2.2)	16.0–20.3
Length of dorsal fin base	18.6 (1.4)	16.3–20.3	16.9 (2.5)	12.0–19.6	19.1 (1.4)	17.4–20.3
Pectoral fin length	20.7 (3.6)	10.0–23.5	18.4 (1.4)	16.5–21.4	21.9 (3.5)	19.2–27.0
Anal fin depth	18.8 (1.8)	15.2–21.6	18.6 (1.0)	17.1–20.3	19.6 (1.2)	18.6–21.3
Caudal peduncle length	17.6 (1.4)	15.6–19.9	19.3 (0.8)	18.3–21.0	16.0 (1.2)	15.0–17.6
Caudal peduncle depth	15.0 (0.4)	14.4–15.5	14.6 (1.0)	11.9–15.4	16.1 (0.5)	15.5–16.7
% HL						
Head depth	82.4 (5.6)	69.9–92.2	83.9 (4.0)	77.8–90.8	76.5 (8.8)	66.3–84.2
Head width	52.8 (3.4)	46.9–58.3	56.2 (2.6)	51.5–60.5	54.7 (4.0)	50.0–58.4
Snout length	29.8 (2.2)	25.2–33.7	27.4 (1.8)	24.6–29.9	32.7 (2.4)	30.0–35.4
Eye diameter	30.2 (2.8)	26.7–37.1	34.1 (3.0)	27.1–37.6	30.8 (2.7)	28.7–34.6
Inter orbital width	34.4 (4.4)	21.6–39.6	30.2 (2.9)	26.2–35.8	37.0 (3.3)	33.9–41.6

^a BNHS FWF 63 to 70; WILD-13-PIS-044, 045; ZSI-WRC P/3567; ^b BNHS FWF 55 to 62; WILD-13-PIS-047, 48; ZSI-WRC P/3568; ^c ZSI-WRC P/3572 and P/3571

Morphometric and meristic data

Morphometric characters and meristics of the specimens collected for the present study from the type locality Sanguem, Goa; a newly discovered population from Terekhol River near Madkhol Village, Maharashtra and from Karwar, Karnataka are provided in Table 1 and Table 2, respectively. Photographs specimens collected for the present study are provided in Images 3 and 4.

Osteology

Osteology of the specimen from the type locality is provided in Image 5. Gill rakers simple, acuminate (not

Table 2. Range for meristics of *Pethia setnai* (n = 28).

Meristics	Range
Lateral line scales	19-21
Transverse scale rows	(3½-4½)-1-(3-3½)
Predorsal scales	7-8
Prepelvic scales	10-11
Preanal scales	14-16
Dorsal fin rays	iii 8
Pectoral fin rays	i 12/13
Pelvic fin rays	i 7
Anal fin rays	iii 5

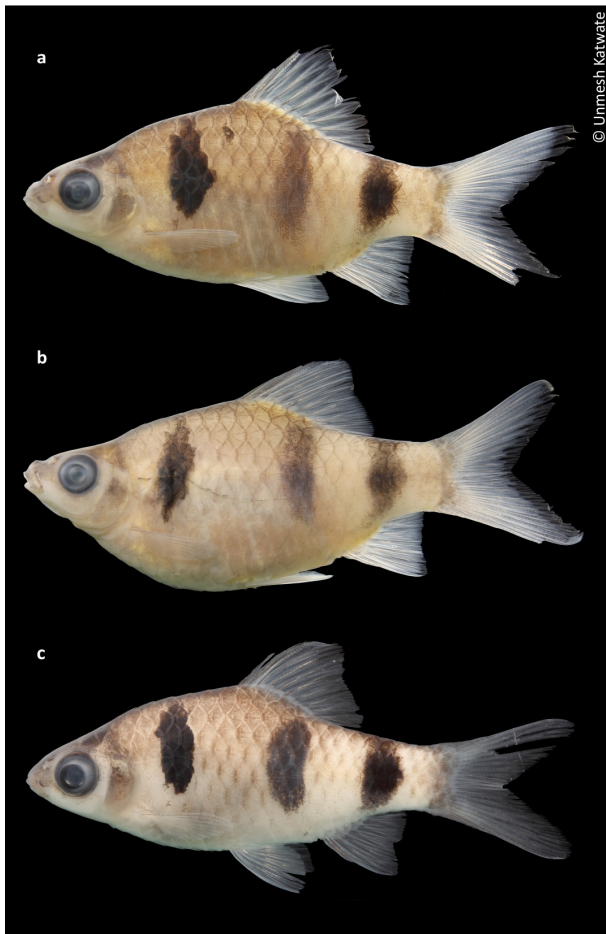


Image 3. *Pethia setnai* collected in the present study. a - Male from Sanguem, Goa, b - Female from Sanguem, Goa; c - unsexed individual from Madkhol, Maharashtra.

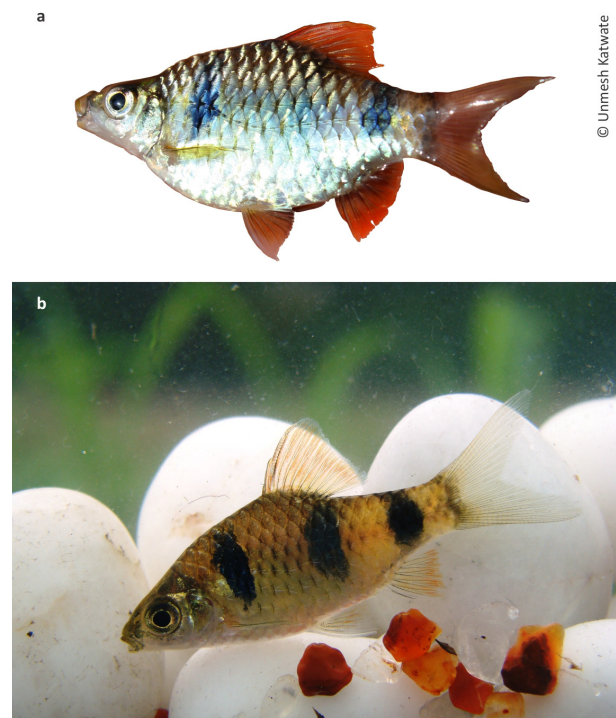


Image 4. *Pethia setnai* showing color in life. (a) fresh specimen from Sanguem, Goa; and (b) live specimen from Terekhol River near Madkhol, Maharashtra.

branched or laminate); post-epiphysal fontanelle absent (Image 5b); infraorbital 3 deep, partially overlapping the cheek and preoperculum (Image 5c); last unbranched dorsal-fin ray stiff, serrated (Image 5d); free uroneural absent (Image 5e); 4 supraneurals; 13 precaudal and 13 caudal vertebrae. Osteological details of *P. setnai* matches with the diagnosis of the genus *Pethia* provided

Table 3. Distribution of *Pethia setnai*. Latitude and longitude data is approximated using Google Earth from the locality details available in the references.

State	Location	River system	Latitude (°N)	Longitude (°E)	Altitude (m)	Reference
Maharashtra	Madkhol	Terekhol	15.94	73.91	43	Current study
Goa	Ponda	Zuari	15.40	74.00	18	Yazdani (1977)
Goa	Sanguem	Zuari	15.23	74.18	19	Chhappgar & Sane (1992); Current study
Karnataka	Karwar	Kalinadi	15.08	74.51	20	Current study
Karnataka	Karwar	Kalinadi	15.79	74.58	20	Current study
Karnataka	Souparnika	Souparnika	13.87	74.81	76	Knight et al. (2012)
Karnataka	Hosangadi	Varahi	13.68	74.97	58	Yazdani & Ghate (1994)
Karnataka	Agumbe	Sitanadi	13.50	75.09	669	Knight et al. (2012)
Karnataka	Gundia	Kumaradhara and Nethravati river systems	12.73	75.66	340	Gururaja et al. (2007)
Karnataka	Kukke Subramanya	Kumaradhara and Nethravati river systems	12.66	75.61	126	Knight et al. (2012)

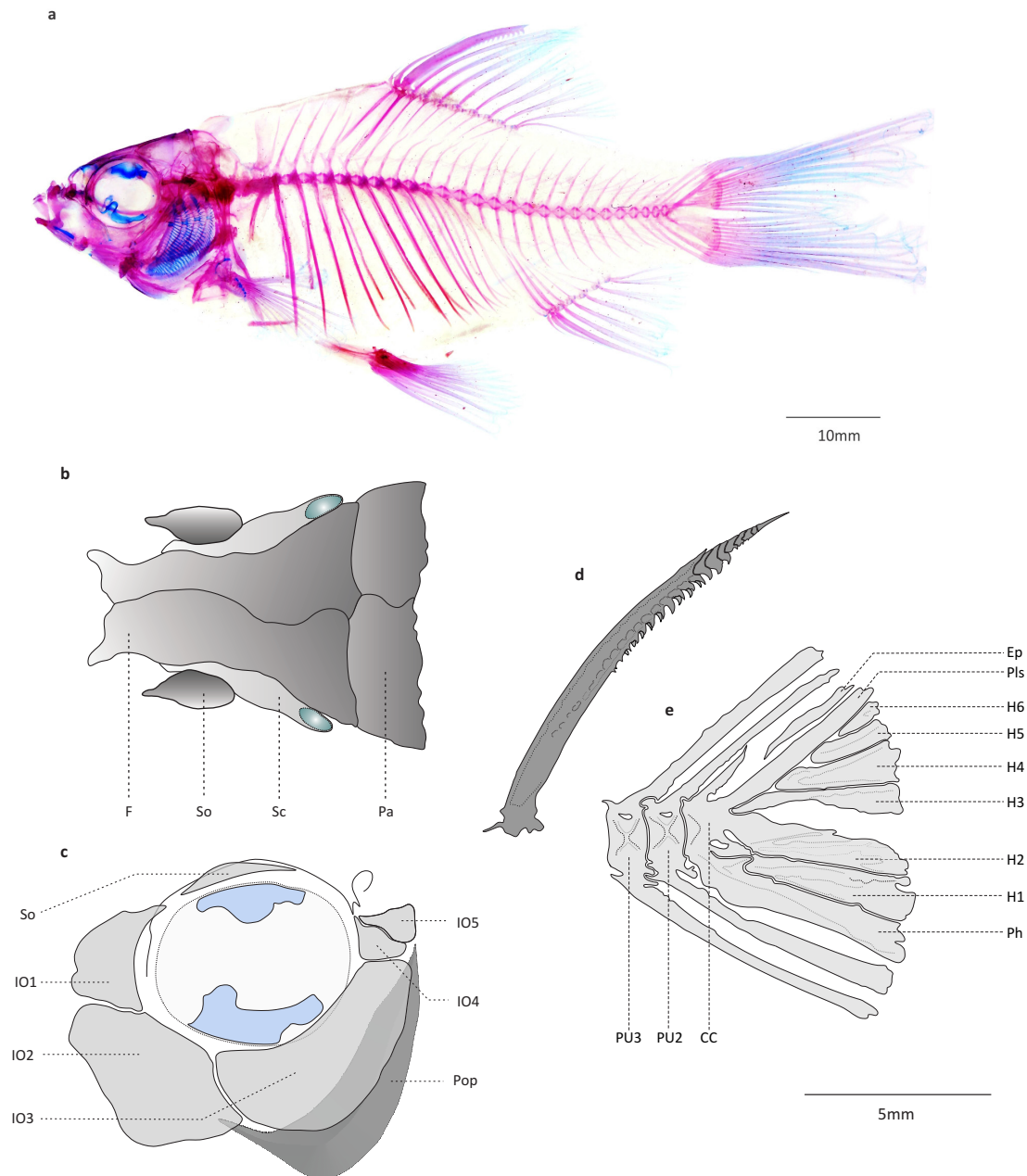


Image 5. *Pethia setnai* topotypic material from Sanguem, Goa (BNHS FWF 70, 32.1mm SL: (a) cleared and stained specimen; (b) dorsal view of orbital region of cranium (F, frontal; Pa, parietal; Sc, supraorbital sensory canal); (c) circumorbital series (So, supraorbital; IO1-5, infraorbitals 1-5; Pop, preopercle); (d) last unbranched dorsal-fin ray and (e) caudal skeleton (CC, compound centrum; Ep, epural; H1-6, hypurals 1-6; Ph, parhypural; Pls, pleurostyle; PU2-3, preural centra 2-3). Photo credit: Unmesh Katwate.

by Pethiyagoda et al. (2012).

Color variation

In life *Pethia setnai* has silvery-grey or dull golden color with three black transverse bands; anterior one situated between the dorsal profile and below the lateral line encompassing 3rd and 4th lateral line scales; middle band between the posterior half of the dorsal fin base

and one scale below lateral line encompassing 9th to 11th lateral line scale; posterior band between the middle of end of dorsal fin base and caudal fin and the posterior half of the anal fin base (Image 4). Posterior most and anterior most bands are darker than the middle band (Image 4a). Dorsal, ventral and anal fins are bright red in certain specimens in their natural habitat (Image 4a) but the fin colors fade rapidly in captivity (Image 4b). Some

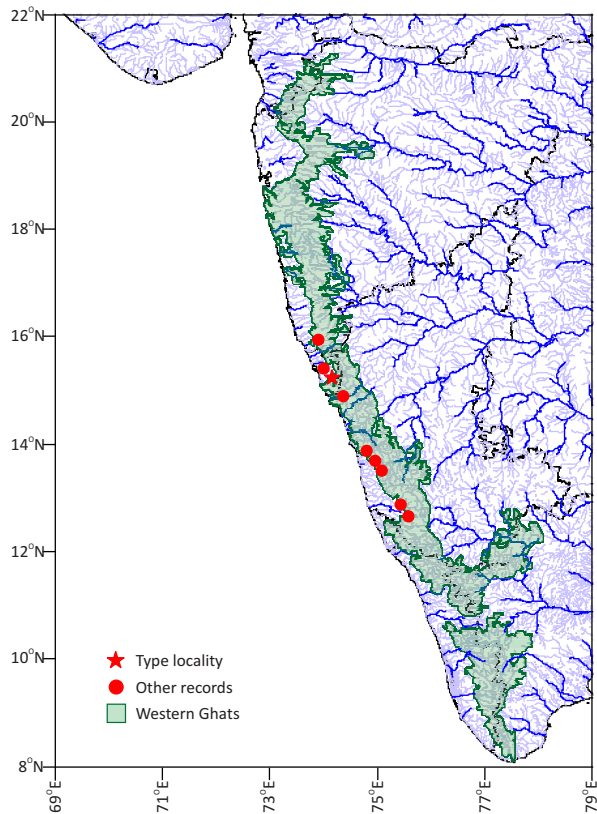


Figure 2. Distribution of *Pethia setnai*.

specimens have indigo blue tinge on the dorsolateral area and therefore the species is also known as indigo barb among hobbyists. While, specimens with yellow fins are also known from this species we have not come across such specimens in our study.

Distribution

Based on the current collections as well as locality information deciphered from literature (Table 3), *Pethia setnai* is known to be distributed in the west flowing rivers of the Western Ghats in the states of Karnataka, Goa and Maharashtra between 12.66°N to 15.94°N latitude (Fig. 2). The species is currently known from seven fragmented populations, most of them reported from lower altitudes (Table 3) except the one from Agumbe, Karnataka located at 669m (Knight et al. 2012).

To our knowledge, there are no published and reliable records of *Pethia setnai* from the Cauvery River system, or any other east flowing rivers. Anecdotal reference to the presence of this species in the Cauvery, especially among the aquarium fish hobbyists, is as a result of the misidentification of *Pethia setnai* as *Pethia narayani* by Chhapgar & Sane (1980). While criticizing the report of *Pethia nigrofasciata* from Goa by Yazdani (1977),



Image 6. Syntypes of *Pethia narayani* ZSI Kolkata F 12180/1.

Chhapgar & Sane (1980) suggested that the species was *Pethia narayani* (Image 6), a species originally described from Cauvery River system in Coorg by Hora (1937). Mr. S.R. Sane who was active in the aquarium fish trade marketed the species occurring in Goa as Narayan's Barb until Chhapgar & Sane (1992) realized that they had overlooked the presence of osseous and serrated last unbranched dorsal fin ray in the specimens from Goa (as opposed to non-osseous and non-serrated last unbranched dorsal fin ray in *Pethia narayani*), and described *Pethia setnai* (B.F. Chhapgar, pers. comm. July 2013). Apart from having strongly osseous and serrated (vs. smooth articulated) last unbranched dorsal fin ray, *P. setnai* differs from *P. narayani* based on 8 (vs. 9) branched dorsal fin rays, 5 (vs. 6) branched anal fin rays and 19–20 (vs. 22) lateral line scales. Because of the misleading common name 'Narayan's Barb' for *P. setnai*, it is likely that hobbyists have treated *P. narayani* from Cauvery River as *P. setnai*.

We failed to come across *P. setnai* during our repeated surveys in Mysore, Coorg and several other areas from Cauvery river system. As a result, unless a reliable report of *P. setnai* is available from Cauvery River system, backed up with genetic data, the species is considered as restricted to west flowing rivers of the Western Ghats based on Yadav (2003) and information presented in this paper.

Habitat

Chhapgar & Sane (1992) described *Pethia setnai* from clear streams of Sanguem and Ponda in Goa. However, they did not provide any information on the habitat in which the species was found. As part of the present study, fresh collection of *P. setnai* was made from the Salaulim River, a tributary of Zuari River near Sanguem, Goa (Image 7). The habitat consisted of a slow flowing, clear water stream with maximum substratum composed of sand and mud. Riparian cover was rich in



Image 7. Habitat in Zuari River near Sanguem, the type locality of *Pethia setnai*. [Image taken on 10.viii.2013]



Image 8. Habitat of *Pethia setnai* in Terekhol River at Madkhol Village, Sindhudurga District, Maharashtra [Image taken on 12.vi.2013]

vegetation, and mostly dominated by *Pundanus* plant species. *Pethia setnai* was mostly found in small shoals with 7–8 individuals in each shoal. During each collection attempt, maximum catch was dominated by females with one to two males in each shoal. Juveniles were observed to be confined at shallow depth (0.1–0.5 m), whereas adults were confined to greater depths (1–2 m). We also discovered a new population of *P. setnai* in the main river channel of Terekhol River at Madkhol Village (Image 8). In contrast with type locality, at Terekhol, the individuals of *P. setnai* were found in deep pools and fast flowing rapids (Image 8). Co-occurring species included *Dawkinsia* cf. *filamentosa*, *Haludaria pradhani*, *Devario aequipinnatus*, *Rasbora* sp. and *Salmophasia* sp.

Threats

Based on IUCN Categories and Criteria (IUCN 2001), Dahanukar (2011) assessed *Pethia setnai* as a 'Vulnerable' species owing to its restricted distribution and on-going threats to habitat because of tourism, urbanization and agricultural pollution. The Goa populations are subjected to heavy mining operations in the headwaters, apart from the pressure from increasing tourism (Image 9). Habitats of the species in Terekhol River are severely threatened by organic wastes and sewage. Further, the species is also known to occur in aquarium trade under the common name Indigo Barb (Chhappgar & Sane 1992). Although no statistics is available on the extent of wild caught *Pethia setnai* in the trade, it has already been suggested that the unregulated aquarium trade is a plausible threat to the endemic and threatened species of freshwater fishes in India (Raghavan et al. 2013b). Further studies on the extent of collection and trade in *P. setnai* is therefore essential.



Image 9. Heavy mining operation near type locality of *Pethia setnai* at Sanguem, Goa. A possible threat to *Pethia setnai*. [Image taken on 10.viii.2013]

CONCLUSIONS

A comprehensive assessment of the status of freshwater fishes of Western Ghats for the IUCN Red List of Threatened Species revealed that little to no information exists regarding distribution, population status and life history of several threatened and endemic species (Dahanukar et al. 2011). This has led to renewed research on various aspects including taxonomic issues and those explaining detailed distribution of several threatened freshwater fishes of the Western Ghats (Ali et al. 2013a,b; Emmanuel et al. 2013; Katwate et al. 2012; Knight et al. 2013). We hope that the detailed biometric and molecular data of topotypic material of *Pethia setnai* will aid in the reliable identification of this threatened taxon, and the information on its distribution and threats will be helpful in directing future conservation efforts.

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Appendix A. Morphometry of specimens collected from Terekhol River at Madkhol.

Characters	BNHS FWF 55	BNHS FWF 56	BNHS FWF 57	BNHS FWF 58	BNHS FWF 59	BNHS FWF 60	BNHS FWF 61	BNHS FWF 62	WILD- 13- PIS- 047	WILD- 13- PIS- 048	ZSI- WRC P/3568	ZSI- WRC P/3568
Total Length (mm)	42.20	32.17	30.30	32.74	26.15	27.53	25.18	25.10	30.47	29.11	29.13	26.56
Standard Length (mm)	32.44	25.34	23.79	25.20	20.37	20.94	19.17	19.79	23.81	22.26	22.63	20.15
Head length (mm)	8.97	7.48	6.91	7.11	6.01	6.09	5.95	5.94	7.12	6.42	6.85	5.83
Head depth (mm)	7.46	6.07	5.73	6.16	5.46	5.27	4.88	4.62	5.94	5.82	5.52	4.76
Head width (mm)	5.43	4.10	3.80	4.22	3.41	3.54	3.21	3.25	3.87	3.68	3.53	3.36
Snout length (mm)	2.55	1.93	1.73	2.01	1.64	1.82	1.54	2.00	1.98	1.78	1.96	1.74
Eye diameter (mm)	3.22	2.65	2.50	2.67	2.04	2.25	1.88	1.61	2.38	2.18	2.14	2.09
Inter orbital width (mm)	2.45	2.21	1.81	2.40	1.90	2.18	1.77	1.58	2.02	2.03	2.07	1.83
Body depth (mm)	12.51	9.12	8.23	9.20	7.04	7.18	6.92	6.73	7.96	8.07	7.94	6.98
Body width at dorsal fin origin (mm)	5.89	4.14	3.90	4.32	3.35	3.45	2.42	2.68	3.73	3.80	3.53	3.35
Body width at anal fin origin (mm)	4.83	3.08	2.84	3.23	2.57	2.64	2.15	2.26	2.93	2.84	2.80	2.52
Pre dorsal distance (mm)	16.58	13.07	12.07	12.62	10.53	10.88	10.85	10.48	11.87	11.8	12.05	10.26
Dorsal to hypural distance (mm)	15.97	12.64	11.67	12.54	9.93	9.78	8.26	8.99	11.02	10.66	10.68	9.97
Prepelvic distance (mm)	16.02	12.83	12.33	12.86	11.19	11.09	11.01	10.72	12.56	11.26	12.41	10.41
Preanal distance (mm)	23.55	18.32	16.75	17.71	14.38	15.53	13.94	14.6	16.59	15.41	16.40	14.11
Prepectoral distance (mm)	9.46	8.10	7.23	7.84	6.27	6.74	6.43	6.68	7.42	6.90	7.21	6.22
Dorsal fin length (mm)	8.21	6.87	6.23	6.89	5.46	5.61	4.56	5.30	5.75	5.80	5.84	5.49
Dorsal fin spine length (mm)	5.88	4.57	3.74	4.72	3.46	3.54	3.62	3.22	4.13	3.86	4.28	3.58
Length of dorsal fin base (mm)	6.14	4.97	4.28	4.86	3.21	3.62	2.33	2.38	4.20	4.08	4.03	3.27
Pectoral fin length (mm)	6.93	5.03	4.37	4.95	3.63	3.84	3.32	3.27	4.31	3.82	3.94	3.70
Anal fin depth (mm)	6.23	5.14	4.33	5.11	3.73	3.76	3.31	3.39	4.21	4.18	4.23	3.80
Caudal peduncle length (mm)	6.47	4.64	4.53	4.85	4.06	4.09	4.02	3.74	4.42	4.16	4.15	4.01
Caudal peduncle depth (mm)	4.92	3.84	3.64	3.87	2.97	3.15	2.71	2.35	3.51	3.41	3.29	2.91

Appendix B. Morphometry of specimens collected from Sanguem, Goa.

Characters	BNHS FWF 63	BNHS FWF 64	BNHS FWF 65	BNHS FWF 66	BNHS FWF 67	BNHS FWF 68	BNHS FWF 69	BNHS FWF 70	WILD- 13- PIS-044	WILD- 13- PIS-045	ZSI- WRC P/3567	ZSI- WRC P/3567
Total Length (mm)	50.38	50.42	45.14	42.80	36.51	35.63	37.85	40.65	49.96	39.04	45.85	33.99
Standard Length (mm)	39.29	39.66	34.32	32.25	27.90	27.18	29.13	31.20	38.69	29.82	35.14	26.30
Head length (mm)	10.90	12.04	10.08	9.97	8.06	7.87	9.06	9.47	11.14	9.03	10.53	8.53
Head depth (mm)	10.05	10.09	8.29	7.81	6.90	6.63	7.25	7.95	9.81	7.02	8.73	5.96
Head width (mm)	6.36	6.31	5.33	4.77	4.34	4.33	4.47	5.18	6.29	4.74	5.61	4.00
Snout length (mm)	3.54	3.65	2.87	2.94	2.34	2.38	3.05	2.39	3.29	2.63	3.31	2.44
Eye diameter (mm)	3.26	3.40	2.97	2.92	2.41	2.64	2.42	3.51	3.20	2.84	3.10	2.42
Inter orbital width (mm)	3.93	3.99	3.53	3.50	3.06	3.12	3.05	2.05	3.92	3.23	3.80	2.83
Body depth (mm)	17.34	17.61	14.19	12.98	10.91	11.11	10.78	11.17	16.72	11.69	15.36	9.62
Body width at dorsal fin origin (mm)	6.95	7.01	5.62	4.94	4.90	4.31	4.44	4.88	6.85	4.74	5.97	3.83
Body width at anal fin origin (mm)	4.90	4.95	4.12	3.89	3.17	3.32	3.40	3.09	4.82	3.69	4.37	2.88
Pre dorsal distance (mm)	20.48	21.73	17.60	17.32	14.59	14.80	15.12	14.69	20.25	15.60	18.92	14.01
Dorsal to hypural distance (mm)	18.91	18.41	16.53	14.91	13.99	13.06	13.94	13.49	18.27	14.26	16.66	12.57
Prepelvic distance (mm)	19.10	20.72	17.11	16.54	14.1	13.43	15.41	13.50	18.79	14.99	17.92	14.17
Preanal distance (mm)	27.98	29.28	24.59	24.24	19.83	19.36	21.41	20.81	28.05	20.90	25.99	20.07
Prepectoral distance (mm)	10.04	11.90	9.76	9.79	7.87	7.45	8.53	8.76	10.42	8.88	10.13	7.42
Dorsal fin length (mm)	10.40	10.43	8.54	9.72	7.36	7.85	7.92	7.52	10.12	8.31	9.62	6.62
Dorsal fin spine length (mm)	7.66	7.62	5.98	6.65	5.28	5.18	5.74	5.24	7.13	5.62	6.77	5.16
Length of dorsal fin base (mm)	7.55	7.52	6.45	6.55	5.31	5.07	4.81	5.19	7.43	5.81	7.12	4.28
Pectoral fin length (mm)	7.93	8.75	7.27	7.09	6.42	6.24	6.17	3.13	7.90	6.47	8.27	5.30
Anal fin depth (mm)	7.43	6.58	6.63	6.98	5.42	5.33	5.16	4.73	7.44	6.21	7.04	4.65
Caudal peduncle length (mm)	6.26	6.62	5.96	5.82	5.27	4.94	5.80	5.10	6.02	5.81	6.14	4.55
Caudal peduncle depth (mm)	5.96	6.09	5.32	4.92	4.18	4.03	4.19	4.62	5.83	4.46	5.43	3.82

Appendix C. Morphometry of specimens collected from Karwar, Karnataka.

Characters	ZSI-WRC P/3572	ZSI-WRC P/3571	ZSI-WRC P/3571	ZSI-WRC P/3571
Total Length (mm)	Caudal fin damaged	Caudal fin damaged	Caudal fin damaged	Caudal fin damaged
Standard Length (mm)	37.28	36.1	31.76	30.73
Head length (mm)	11.33	11.95	8.95	8.68
Head depth (mm)	8.16	7.92	7.47	7.31
Head width (mm)	5.97	5.97	5.23	5
Snout length (mm)	4.01	4.03	2.84	2.6
Eye diameter (mm)	3.32	3.43	3.1	2.65
Inter orbital width (mm)	4.08	4.05	3.72	3.17
Body depth (mm)	15.66	14.6	13.42	12.01
Body width at dorsal fin origin (mm)	5.55	4.93	4.73	4.45
Body width at anal fin origin (mm)	3.11	3.38	3.16	2.65
Pre dorsal distance (mm)	19.94	20	16.54	14.16
Dorsal to hypural distance (mm)	20.51	17.6	16.22	16.57
Prepelvic distance (mm)	18.12	18.25	15.38	14.49
Preanal distance (mm)	26.8	26.21	22.18	21.46
Prepectoral distance (mm)	11.75	12.23	10	8.68
Dorsal fin length (mm)	9.23	10.76	8.75	damaged
Dorsal fin spine length (mm)	5.96	6.69	6.44	damaged
Length of dorsal fin base (mm)	6.47	6.71	6.42	6.24
Pectoral fin length (mm)	7.16	7.52	8.58	6.28
Anal fin depth (mm)	7.1	6.98	5.92	6.55
Caudal peduncle length (mm)	5.6	6.34	5.1	4.67
Caudal peduncle depth (mm)	5.78	5.69	5.15	5.14

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