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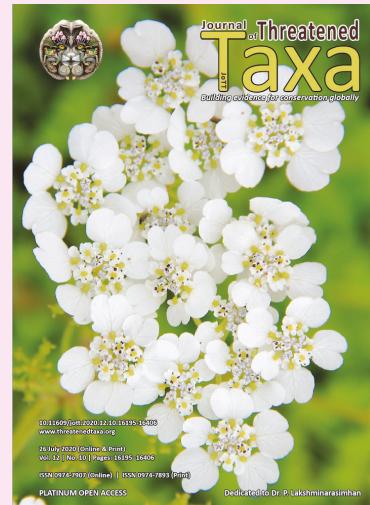
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THE IDENTITY AND DISTRIBUTION OF *BHAVANIA ANNANDALEI* HORA, 1920 (CYPRINIFORMES: BALITORIDAE), A HILLSTREAM LOACH ENDEMIC TO THE WESTERN GHATS OF INDIA

Remya L. Sundar, V.K. Anoop, Arya Sidharthan, Neelesh Dahanukar & Rajeev Raghavan

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The identity and distribution of *Bhavania annandalei* Hora, 1920 (Cypriniformes: Balitoridae), a hillstream loach endemic to the Western Ghats of India

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Abstract: *Bhavania annandalei* Hora 1920, is resurrected from the synonymy of *B. australis* (Jerdon, 1849) based on examination of freshly collected topotypic specimens. The two species can be distinguished by a combination of morphological characters including low, dense, and sparsely distributed tubercles on dorsal surface of head and operculum, rostral barbels reaching anterior border of upper lip, rostral flaps between the rostral barbels fleshier, 11–12 scale rows above the lateral line, and caudal peduncle stout with its depth to width ratio less than 2.5. The two species formed significantly distinct clusters in multivariate space. Further, the two species have a raw genetic distance of 6.4% in the mitochondrial cytochrome oxidase subunit 1 gene. The distribution of *B. annandalei* is restricted to the river systems draining the Agasthyamalai Hills, below the Shencottah Gap in southern Western Ghats, while *B. australis* occurs in rivers north of the Shencottah Gap.

Keywords: Agasthyamalai, Cobitoidea, Kerala, mountain loach, synonymy.

Editor: Anonymity requested.

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Author details: REMYA L. SUNDAR, is interested in taxonomy, diversity and distribution of freshwater and estuarine fishes of Kerala. V.K. ANOOP is interested in the systematics and biogeography of loaches of the Western Ghats. ARYA SIDHARTHAN is interested in molecular ecology and phylogeography of balitorid loaches of the Western Ghats. NEELESH DAHANUKAR is interested in molecular phylogenetics and biogeography of freshwater fishes of the Western Ghats. RAJEEV RAGHAVAN is interested in conservation of aquatic biodiversity of Western Ghats with special reference to freshwater fishes.

Author contribution: RR, ND and AS conceived the study; RLS, VKA and AS carried out the field surveys and laboratory studies; all authors equally contributed to the writing of the manuscript.

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INTRODUCTION

The hillstream loach *Bhavania annandalei* was described by Hora (1920; p203) from Tenmalai, erstwhile Travancore State (= current day southern Kerala), and suggested that the species occurs throughout the southern Western Ghats in the Nilgiris, Malabar, and Travancore. Hora (1920) diagnosed *B. annandalei* from its only known congener, *B. australis* (Jerdon, 1849) (type locality: Walliar Jungle = Walayar), by a combination of characters; the most prominent of which included a broad snout (vs. pointed), interrupted lower lip (vs. continuous), caudal-lobes equal (vs. lower lobe longer), and presence of a pair of papillae on the lower lip (vs. absence).

Hora's (1920) description of *B. annandalei* was however, based on a single adult female specimen collected by Dr. Annandale from Travancore, Kerala. Though, Hora (1920) seemed to have access to additional juvenile specimens collected by Captain Sewell from the Nilgiris (Cherambadi) and Wayanad (Nellimunda, Mananthavady, and near Vythiri), he did not examine them or provide other details. Subsequently, Hora (1937; p8) extended the distribution of the species to Mysore, based on four specimens collected by M.S. Bhimachar from a stream between Kottigehar and Balehonnur (erstwhile Mysore State = current day Tunga River System in Karnataka). No details of the specimens were provided.

In his review on 'Homalopterid fishes from Peninsular India', Hora (1941) synonymized *B. annandalei* with *B. australis*, after examining specimens from throughout its distribution range including Kallar/South Travancore (current day Vamanapuram River, Kerala); Pampadumpara/North Travancore (current day Periyar River, Kerala); Sethumadai Hills/Mysore (current day Anamalai hills near Pollachi, Tamil Nadu); and Kottigehar/Mysore (current day Tunga River, Karnataka), and realizing that his description of *B. annandalei* was based mainly on immature specimens. This synonymy was subsequently adopted by Menon (1987) in his review of the homalopterid loaches of India, but without examining the type (or fresh topotypes) of *B. annandalei*, or the topotypes of *B. australis*. Later workers followed this synonymy and considered *Bhavania* to be monotypic (Talwar & Jhingran 1991; Menon 1999; Kottelat 2012). '*Bhavania arunachalensis*', described by Nath et al. (2007) from Naodhing drainage in Arunachal Pradesh, is considered to be a 'species inquirenda et incertae sedis' (i.e., doubtful identity and uncertain placement) (Kottelat 2012), and is most likely a species of the genus

Balitora (see Fricke et al. 2020).

Given their hill-stream adaptations (widespread paired fins, flattened ventral surfaces with body suckers and rasping mouths on their ventral surface allowing them to firmly grasp rock or gravel surfaces necessary in the mountain torrents) (Chen 1980; Kottelat 2012), and the fact that the type locality of *B. annandalei* (Tenmalai) and *B. australis* (Walayar) are at least 300km apart and separated by two significant biogeographic barriers - the Palghat Gap and the Shencottah Gap (see Anoop et al. 2018), it is highly unlikely that the two are conspecific. Collection of fresh topotypic specimens of both *B. australis* and *B. annandalei* and detailed examination and comparison of their biometrics, and genetic distance analysis based on the mitochondrial cox1 gene, revealed that the two species are clearly distinct. We, therefore, resurrect *Bhavania annandalei* Hora, 1920, from the synonymy of *B. australis* (Jerdon, 1849) and provide notes on the distribution range of this species.

Six specimens of putative topotypic *Bhavania annandalei* were collected from Palaruvi falls at Tenmalai (Kallada River), Kerala, and six specimens of putative topotypic *B. australis* were collected from near the Kavarakund falls, upstream of Malampuzha Reservoir, Kerala, India (Fig. 1). Samples were collected using a hand net/scoop net during early morning hours, fixed in 10% formalin and transferred to 70% ethanol for permanent voucher storage in the museum collections of the Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, India. Gill tissues were obtained from fresh specimens and preserved in absolute ethanol. Morphometric measurements were taken for 37 characters (measured to the nearest 0.1mm using digital calliper) and meristic values were determined for 10 characters using a stereo-zoom microscope. For meristic counts, values in parenthesis after the count represent its frequency. For fin ray counts, unbranched fin rays are expressed as small roman numerals. For pectoral fin, fin rays are provided as padded fin rays + branched fin rays + unbranched fin rays. For statistical analysis of morphometric data, subunits of body were taken as percentage of standard length and subunits of head were taken as percentage of head length. Principal component analysis (PCA) was performed to check whether the two species formed distinct clusters in multivariate space using correlation matrix. Null hypothesis that the clusters are not significantly different from each other was tested using analysis of similarities (ANOSIM) employing Euclidian distances and 9999 permutations. Statistical analysis was performed in PAST 4.02 (Hammer et al. 2001). Genetic sequences

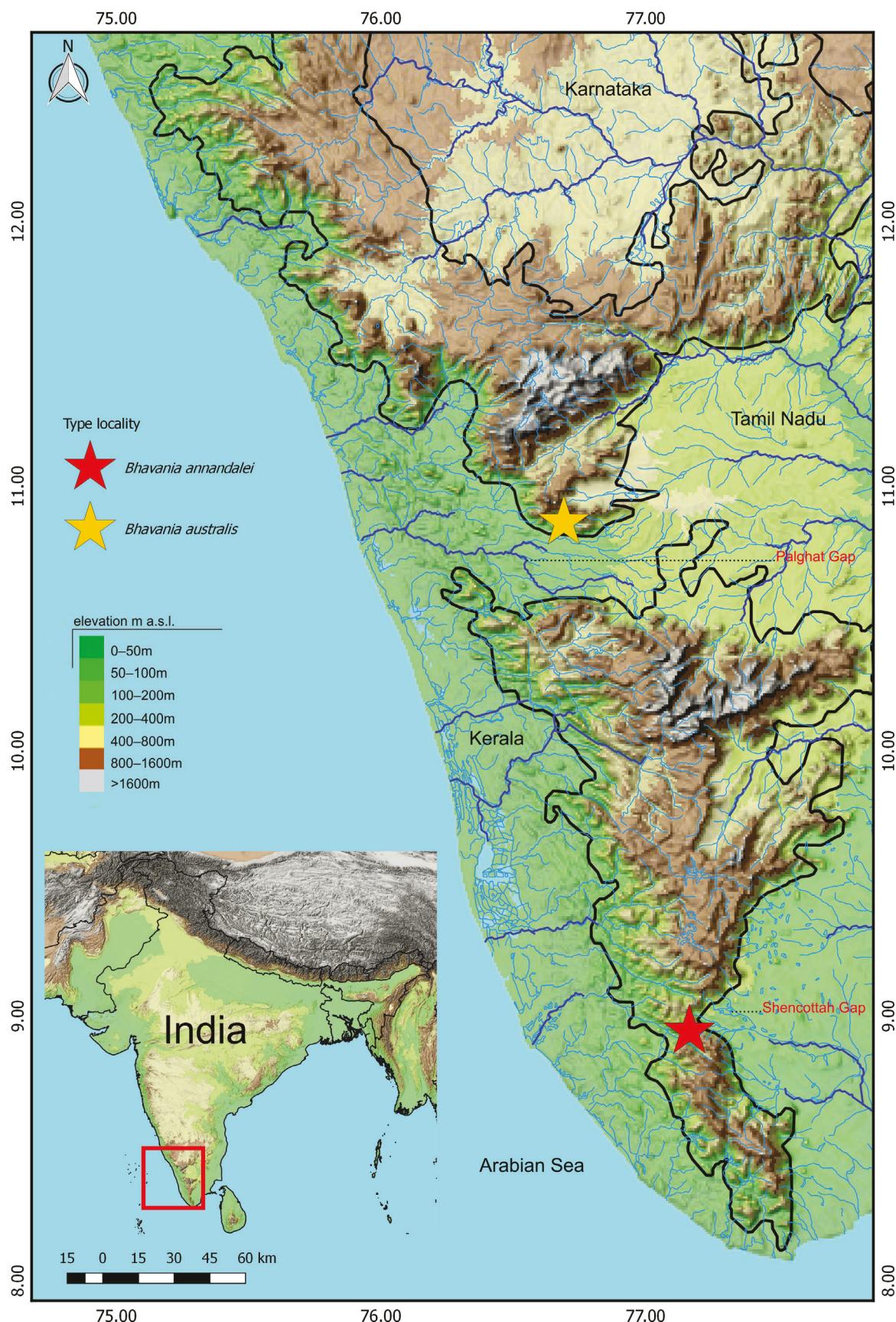


Figure 1. Collection localities of putative topotypes of *Bhavania annandalei* and *B. australis*.

of mitochondrial partial cytochrome oxidase subunit 1 (*cox1*) of topotypic *Bhavania annandalei* and *B. australis* were obtained from our ongoing study (Sidharthan et al. Unpublished). Additional sequences were downloaded from GenBank database. Gene sequences were aligned using MUSCLE (Edgar 2004) and raw genetic distance was estimated using MEGA 7 (Kumar et al. 2016). Data were partitioned into three codon positions of *cox1* gene. Partition analysis (Chernomor et al. 2016) and ModelFinder (Kalyaanamoothy et al. 2017) were used to find the right partitioning scheme and nucleotide substitution model for the partition scheme employing minimum Bayesian information criterion (BIC). Maximum likelihood (ML) analysis was performed in IQ Tree (Nguyen et al. 2015) with best partition scheme and ultrafast bootstrap support for 1,000 iterations (Hoang et al. 2018). Phylogenetic tree was edited in FigTree v1.4.2 (Rambaut 2009).

***Bhavania annandalei* Hora, 1920**
(Images 1–3)

Materials examined: KUFOS.19.AS.BH.02.1–6, 6 ex., 07.ii.2019, 8.945N & 77.158E, 32.7–37.6 mm SL, Palaruvi falls, Tenmala, Kallada River, Kerala, India, coll. Arya Sidharthan, E.S. Abhijith, & George Joseph.

Diagnosis. *Bhavania annandalei* is distinguished from its only known congener *B. australis* by a combination of characters: low density and sparsely distributed tubercles on dorsal surface of head, especially on operculum, (vs. high density of tubercles on dorsal surface of head and

operculum) (Image 3); gape of mouth comparatively farther from snout tip, as a result the rostral barbels reaching anterior border of upper lip, (vs. gape of mouth closer to snout tip, and rostral barbels reaching posterior border of upper lip) (Image 3); rostral flaps between the rostral barbels fleshier (vs. less fleshier) (Image 3); fewer post-dorsal scales (34–36 vs. 38–41); fewer scales above the lateral line (11–12 vs. 14–15); and caudal peduncle stout with its depth to width ratio 1.8–2.3 (vs. laterally compressed caudal peduncle with depth to width ratio 2.8–3.6). This species has a fin formula of D. ii+7+i; P. 6+10+i; V. ii+7–8; A. ii+5, and scale counts of LI. 65–67 and L.tr. 11–12/9–10.

Description: Morphometric and meristic data of *Bhavania annandalei* are provided in Table 1 and Table 2, respectively. General body form as per Image 1a and Image 2a. Head details as in Image 3a, c.

Body elongate, dorso-ventrally depressed anteriorly, laterally compressed posteriorly; dorsal profile convex, deepest at dorsal-fin origin. Body wider than its depth at dorsal-fin origin, deeper than wide at anus. Head small, rounded, less than one-fourth of standard length; depressed, longer than broad, with minute sparsely distributed indistinct tubercles on dorsal surface of head. Eyes small, dorso-laterally positioned, not visible from underside of head. Snout pointed in lateral view, round in dorsal view. Nostrils positioned dorsally, closer to anterior border of eye than to snout tip, anterior nostril situated inside a skin flap covering the posterior nostrils. Mouth inferior. Lips fleshy. Gape of mouth less



Image 1. Putative topotypes: a—*Bhavania annandalei* | b—*B. australis* in life (specimens not preserved).

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Table 1. Morphometric data of *Bhavania annandalei* (KUFOS.19.AS.BH.02.1-6, n=6) and *B. australis* (KUFOS.19.AS.BH.01.1-6, n=6) putative topotypes.

Characters	<i>Bhavania annandalei</i>		<i>Bhavania australis</i>	
	Mean (sd)	Range	Mean (sd)	Range
Total length (mm)	62.3 (18.2)	40.2–85.8	76.1 (10.6)	62.4–90.2
Standard length (SL, mm)	50.8 (14.5)	33.2–70.2	62.4 (8.8)	51.6–74.2
Head length (HL, mm)	11.1 (2.6)	8.2–14.7	13.0 (1.7)	11.3–15.2
% SL				
Head length	22.2 (1.6)	20.9–24.7	20.9 (0.9)	19.8–21.9
Pre-pectoral length	18.0 (0.9)	17.2–19.7	18.2 (0.9)	17.1–19.4
Pre-dorsal length	49.8 (1.9)	46.7–52.1	47.3 (1.8)	44.5–49.4
Pre-pelvic length	44.0 (1.1)	42.4–45.1	44.3 (1.5)	42.0–46.6
Pre-vent length	70.3 (2.5)	67.4–73.2	69.6 (1.6)	67.7–71.6
Pre-anal fin length	79.1 (1.8)	77.1–81.6	78.7 (1.6)	77.3–81.8
Origin of pelvic fin to anus distance	29.3 (1.5)	26.6–30.7	28.7 (3.1)	26.2–34.4
Anal fin to anus distance	9.2 (1.3)	7.6–11.0	9.9 (0.6)	9.1–10.4
Post dorsal length	44.3 (1.2)	42.9–46.1	44.7 (1.0)	43.6–46.2
Body depth at dorsal fin origin	14.3 (0.8)	12.9–15.2	13.2 (0.6)	12.0–13.8
Body width dorsal fin origin	17.7 (1.2)	15.5–18.8	18.5 (0.7)	17.4–19.3
Height of dorsal fin	19.2 (1.2)	16.9–20.0	20.1 (0.9)	19.1–21.7
Dorsal-fin base length	11.9 (0.7)	11.1–12.7	12.0 (0.4)	11.3–12.4
Body depth at anal fin origin	11.4 (0.5)	10.6–11.9	11.0 (0.5)	10.4–11.9
Body width at anal fin origin	7.0 (0.7)	6.2–7.9	6.9 (0.6)	5.9–7.6
Length of upper caudal lobe	20.7 (1.4)	18.4–22.0	20.6 (1.9)	19.0–24.1
Length of lower caudal lobe	24.3 (1.5)	22.3–26.1	22.2 (1.4)	20.8–24.7
Length of median caudal rays	17.6 (1.4)	15.2–19.2	16.2 (0.7)	15.3–16.7
Anal fin length	14.1 (0.9)	12.8–15.5	15.5 (0.5)	14.9–16.3
Anal fin base length	7.0 (0.7)	6.4–8.3	7.2 (0.5)	6.6–7.9
Pelvic fin length	22.5 (1.3)	21.3–24.8	22.8 (0.8)	21.6–23.9
Pectoral fin length	26.8 (1.7)	24.4–29.7	26.4 (1.2)	24.2–27.3
Length of caudal peduncle	13.3 (1.8)	11.7–15.9	14.2 (0.7)	13.1–15.1
Caudal peduncle depth	9.3 (0.3)	8.9–9.7	9.3 (0.8)	8.4–10.7
Caudal peduncle width	4.5 (0.5)	3.9–5.1	3.0 (0.1)	2.8–3.2
% HL				
Snout-supra-occipital distance	93.3 (5.6)	86.7–101.0	100.6 (5.4)	94.2–107.5
Gape of mouth	23.8 (3.3)	19.7–26.9	29.6 (3.2)	25.5–35.3
Head depth at eye	41.2 (2.1)	39.3–44.9	42.6 (3.2)	37.9–45.9
Head width at eye	75.3 (5.5)	68.3–80.9	83.4 (6.5)	75.7–93.0
Head depth at nape	52.9 (5.3)	47.3–60.3	41.8 (9.8)	30.7–51.6
Snout length	57.6 (5.3)	51.4–64.9	58.6 (2.8)	56.2–63.3
Maximum head width	83.0 (9.0)	71.1–95.8	88.9 (4.3)	84.1–94.3
Eye diameter	20.3 (2.8)	16.5–23.7	17.5 (1.2)	15.3–18.8
Interorbital width	35.7 (4.8)	30.6–42.9	39.0 (4.1)	33.2–45.0
Internarial width	27.3 (2.2)	24.2–30.3	29.9 (2.6)	26.1–33.7

Table 2. Meristic data of *Bhavania australis* (KUFOS.19.AS.BH.01.1-6, n=6), and *B. annandalei* (KUFOS.19.AS.BH.01.1-6, n=6) putative topotypes. Numbers in parenthesis indicate frequency of character state in the materials examined.

Characters	<i>Bhavania annandalei</i>	<i>Bhavania australis</i>
Dorsal-fin rays	ii+7+i (6)	ii+7 (3), ii+7+i (3)
Pectoral-fin rays	6+10+i (6)	6+9+i (1), 6+10 (1), 6+10+i (4)
Pelvic-fin rays	ii+7 (2), ii+8 (4)	ii+7 (4), ii+7+i (2)
Anal-fin rays	ii+5 (6)	ii+5 (4), ii+5+i (1); ii+6 (1)
Caudal-fin rays	19 (6)	19 (6)
Lateral line scales	65+4 (2), 66+3 (1), 67+3 (2), 67+3 (1)	65+3 (2), 65+4 (1), 66+3 (1), 68+3 (1), 69+3 (1)
Pre dorsal scales	29 (1), 30 (2), 31 (3)	28 (3), 29 (2), 30 (1)
Post dorsal scales	34 (3), 35 (2), 36 (1)	38 (1), 39 (2), 40 (2), 41 (1)
Scales above lateral line	11 (2), 12 (4)	14 (4), 15 (2)
Scales below lateral line	9 (2), 10 (4)	10 (3), 11 (3)

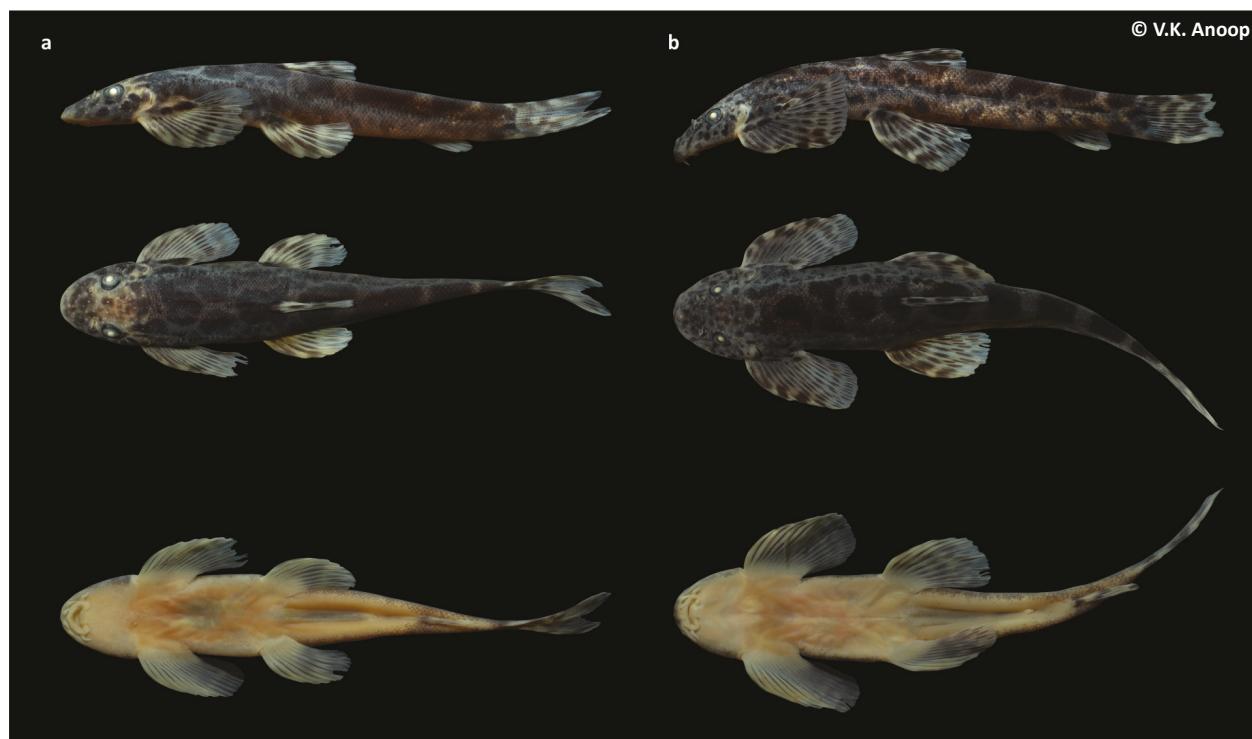


Image 2. Dorsal, lateral, and ventral images of putative topotypes: a—*Bhavania annandalei* | b—*B. australis*.

than three times maximum head width. Barbels three pairs, two rostral: outer rostral barbels shorter than inner ones; one pair of maxillary barbels, situated slightly anterior to the angle of mouth. Three fleshy rostral flaps interspaced between rostral barbels. Gill opening small, restricted above the base of the pectoral fin.

Body with scales except chest and belly. Lateral line complete, with 68–72 small scales. Caudal peduncle slender, its length almost three times its depth. Dorsal-fin originating slightly behind the pelvic-fin origin,

closer to tip of snout than to caudal-fin base; with two unbranched, followed by seven branched and a simple ray. Pectoral fin elongated, longer than head, with six unbranched, followed by 10 branched and a simple ray. Pelvic-fin length almost equal to head length; fin origin closer to snout tip than to end of caudal peduncle, its posterior end not reaching anus, with two unbranched and eight branched rays. Anal fin with two unbranched and five branched rays. Caudal fin forked, with 19 principal rays.

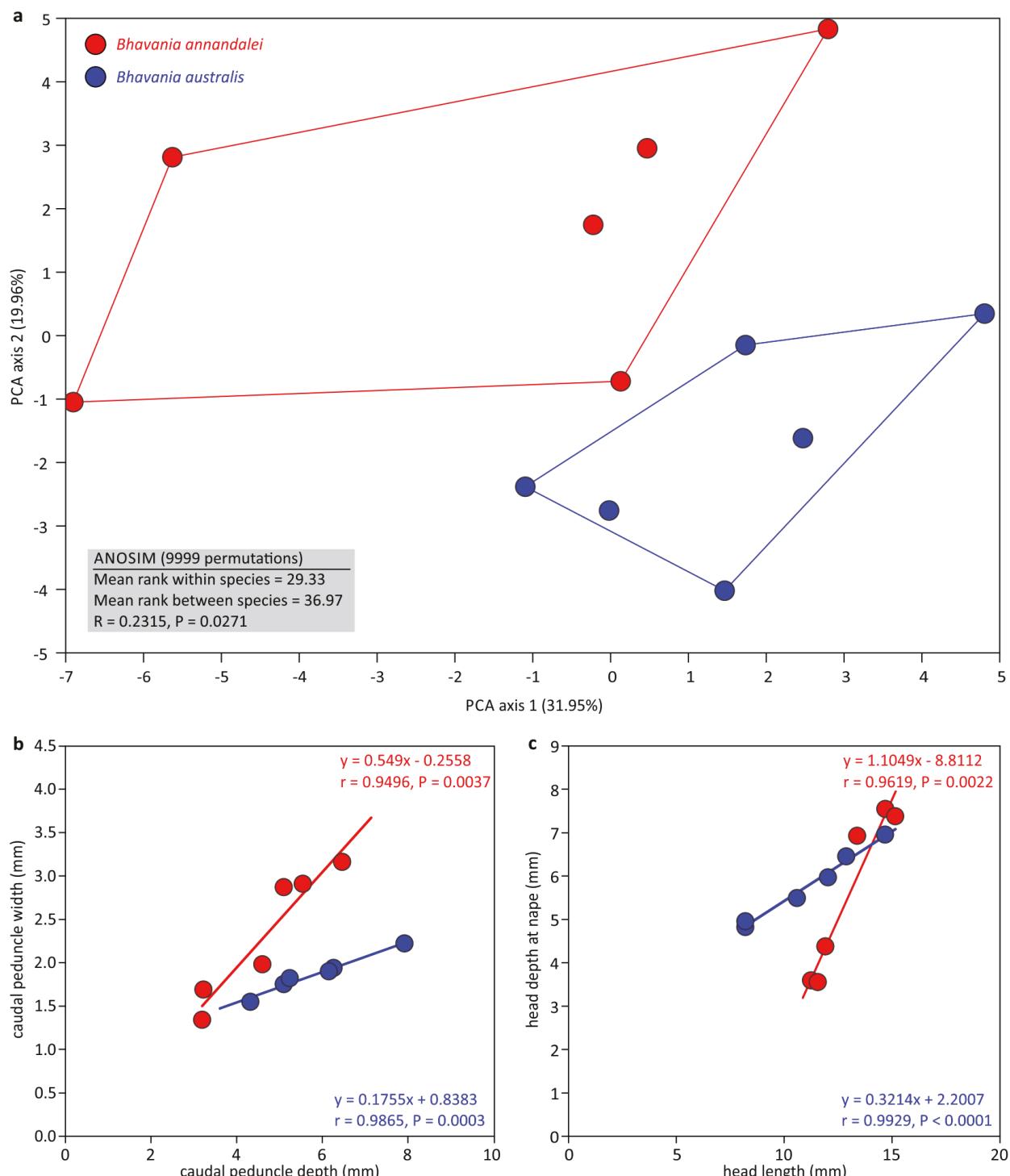


Figure 2. Morphometric analysis: a—Principal Component Analysis scatter plot of factor scores and ANOSIM statistics | b—Linear regression between caudal peduncle depth and width | c—Linear regression between head length and head depth at nape.

Colouration: In life (Image 1a), body is chestnut brown on dorsal and lateral sides, creamish-white on chest and belly; 3–4 prominent broad dark brown ventral bands; two broad ventral bands on the dorsal-fin base. There are three black-coloured bands on the

dorsal fin, 6–7 bands on the pectoral, three bands on the pelvic, 1–2 bands on the anal, and four bands across the caudal fin.

Morphometric analysis: In the morphometric analysis, using all the size-adjusted characters (Table 1),



Image 3. Dorsal and ventral view of head: a, c—*Bhavania annandalei* | b, d—*B. australis*.

the two species clustered separately on the first two PCA axes (Fig. 2a). The clusters were significantly different from each other (ANOSIM, 9999 permutations, $R = 0.2315$, $P = 0.0271$) indicating that the species formed distinct clusters in multivariate space. While length-length relationships for most characters showed similar trends for both the species, there were two relationships

that showed marked differences. Length-length relationship between caudal peduncle depth and width (Fig. 2b) suggested that width increased rapidly with increasing depth in the case of *B. annandalei* compared to *B. australis*. Similarly, length-length relationship between head length and head depth at nape (Fig. 2c) suggested that head depth increased rapidly with

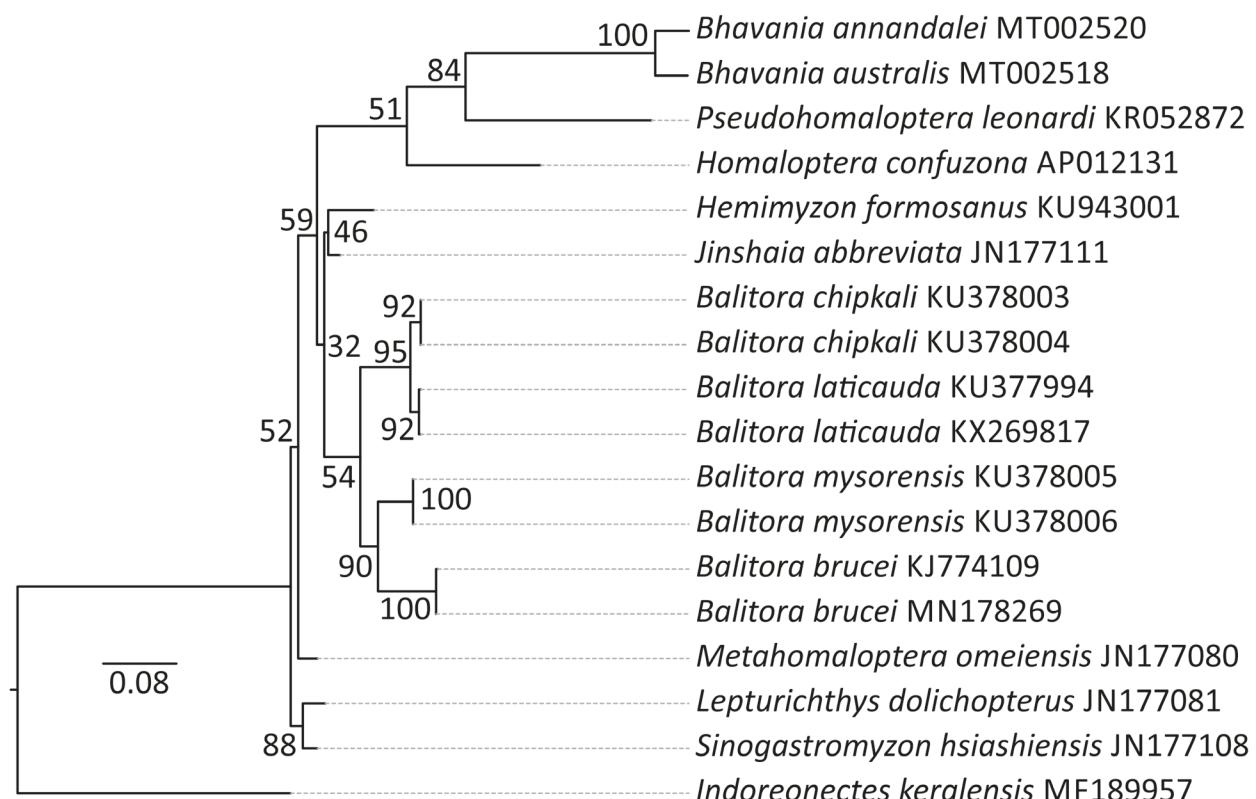


Figure 3. Maximum likelihood phylogenetic tree based on mitochondrial cytochrome oxidase subunit 1 gene using best partition scheme and model selection ($\ln L$ of consensus tree = -2631.97). *Indoreonectes keralensis* (Nemacheilidae) is used as an outgroup. Values along the nodes are percentage bootstraps based on 1,000 iterations.

increasing head length in the case of *B. annandalei* compared to *B. australis*.

Genetic analysis: Partition analysis and model selection identified separate nucleotide substitution models for all three codon positions, TN \neq I for first codon, F81+ F for second codon, TN+F+G4 for third codon position of *cox1* gene ($BIC = 5570.419$, $\ln L = -2633.96$, $df = 47$). Maximum likelihood phylogenetic tree based on best partition scheme and model selection (Fig. 3) recovered *Bhavania annandalei* and *B. australis* as a clade sister to Southeast Asian congeners of Balitoridae. Topotypic *B. annandalei* (MT002520) differed from topotypic *B. australis* (MT002518) with a raw genetic distance of 6.4% in the *cox1* gene.

Distribution: *Bhavania annandalei* is known with certainty from the Kallada, Vamanapuram, and Neyyar river systems in southern Kerala, India. These river systems drain the western slopes of the Agasthyamalai Hill ranges, south of the Shencottah Gap. It is highly likely that the species also occurs on the eastern slopes of the Agasthyamalai Hills particularly in the Tambaraparani River system in Tamil Nadu, but detailed surveys and voucher specimens are required to confirm this. In

this context, we believe that previous records of *B. australis* from several tributaries of the Tambaraparani, Manimuthar, and Chittar draining the eastern slopes of the Agasthyamalai (Johnson & Arunachalam 2009), could most likely represent *B. annandalei*.

Remarks: The density of chromatophores in *Bhavania* is likely to be dependent on the micro-habitat as well as the colour and type of substratum it inhabits. Other ecological factors that may influence body colour are forest/canopy cover, intensity of light, turbidity, water flow and water temperature (V.K. Anoop pers. obs. 2018 and 2019). This is reflected in the different body colours shown by the two species in different habitats and locations (see Image 1), an observation which was also made by Hora (1941).

Comparative material: *Bhavania australis*, KUFOS.19.AS.BH.01.1–6, 6ex., 13.iv.2019, 10.8636N & 76.6904E, 46.4–58.8 mm SL, near Kavarakund falls, upstream of Malampuzha Reservoir, Kerala, India, coll. M.R. Ramprasant.

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