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, morphometric and meristic characters, and by genetic distance using mitochondrial *cox1* gene. The distribution of *B. annandalei* is restricted to the river systems draining the Agasthyamalai hills, below the Shencottah Gap in southern Western Ghats.

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

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. 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).

Given their hill-stream adaptations (Hora 1920, 1937, 1941), 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 *cox*1 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 Tenmala (Kallada River), Kerala, and six specimens of putative topotypic *B.*

72 *australis* were collected from near the Kavarakund falls, upstream of Malampuzha
73 Reservoir, Kerala, India (Fig. 1). Specimens collected in the current study are in the museum
74 collection of the Kerala University of Fisheries and Ocean Studies (KUFOS), Kochi, India.
75 Morphometric measurements were taken for 37 characters (measured to the nearest
76 0.1mm using digital callipers) and meristic values were determined for 10 characters using a
77 stereo-zoom microscope (Table 1 and 2). For statistical analysis of morphometric data,
78 subunits of body were taken as percentage of standard length and subunits of head were
79 taken as percentage of head length. Principal component analysis (PCA) was performed to
80 check whether the two species formed distinct clusters in multivariate space using
81 correlation matrix. Null hypothesis that the clusters are not significantly different from each
82 other was tested using Analysis of similarities (ANOSIM) employing Euclidian distances and
83 9999 permutations. Statistical analysis was performed in PAST 4.02 (Hammer et al. 2001).
84 Genetic sequences of mitochondrial partial cytochrome oxidase subunit 1 (*cox1*) were
85 obtained from our related study (Sidharthan et al., In Review). Gene sequences were aligned
86 using MUSCLE (Edgar 2004) and raw genetic distance was estimated using MEGA 7 (Kumar
87 et al. 2016).
88
89 ***Bhavania annandalei* Hora 1920**
90 (Image 1, 2 and 3)
91
92 **Materials examined.** KUFOS.19.AS.BH.02.1-6, 6 ex., 07.02.2019, 8.945 N & 77.158 E, 32.7–
93 37.6 mm SL, Palaruvi falls, Tenmala, Kallada River, Kerala, India, coll. Arya Sidharthan, E.S.
94 Abhijith, and George Joseph.
95

Diagnosis. *Bhavania annandalei* is distinguished from its only known congener *B. australis* by a combination of characters including: 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); eyes dorsally positioned (vs. dorso-laterally positioned) (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).

Description. Morphometric and meristic data 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, depressed anteriorly, compressed laterally 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, dorsally positioned, not visible from underside of head. Snout pointed in lateral view, round in dorsal view. Nostrils positioned dorso-laterally, 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 than three times maximum head width. Barbels three pairs, two rostral: outer rostral barbels shorter than

120 inner ones; one pair of maxillary barbels, situated slightly anterior to the angle of mouth.
121 Three fleshy rostral flaps interspaced between rostral barbels. Gill opening small, restricted
122 above the base of the pectoral fin.

123 Body with scales except chest and belly. Lateral line complete, with 68–72 small
124 scales. Caudal peduncle slender, its length almost three times its depth. Dorsal-fin
125 originating slightly behind the pelvic-fin origin, closer to tip of snout than to caudal-fin base;
126 with two unbranched followed by seven branched and a simple ray. Pectoral fin elongated,
127 longer than head, with six unbranched, followed by ten branched and a simple ray. Pelvic-fin
128 length almost equal to head length; fin origin closer to snout tip than to end of caudal
129 peduncle, its posterior end not reaching anus, with two unbranched and eight branched
130 rays. Anal fin with two unbranched and five branched rays. Caudal fin forked, with 19
131 principal rays.

132

133 **Colouration**: In life (Image 1a), body is chestnut brown on dorsal and lateral sides, creamish-
134 white on chest and belly; 3–4 prominent broad dark brown ventral bands; 2 broad ventral
135 bands on the dorsal-fin base. There are 3 black-coloured bands on the dorsal fin, 6–7 bands
136 on the pectoral, 3 bands on the pelvic, 1–2 bands on the anal, and 4 bands across the caudal
137 fin.

138

139 **Morphometric analysis.** In the morphometric analysis, using size-adjusted characters, the
140 two species clustered separately on the first two PCA axes (Fig. 2a). The clusters were
141 significantly different from each other (ANOSIM, 9999 permutations, R = 0.2315, P = 0.0271)
142 indicating that the species formed distinct clusters in multivariate space. While length-
143 length relationships for most characters showed similar trends for both the species, there

144 were two relationships that showed marked differences. Length-length relationship
145 between caudal peduncle depth and width (Fig. 2b) suggested that caudal peduncle width
146 increased rapidly with increasing depth of the caudal peduncle in the case of *B. annandalei*
147 as compared to *B. australis*. Similarly, length-length relationship between head length and
148 head depth at nape (Fig. 2c) suggested that head depth increased rapidly with increasing
149 head length in the case of *B. annandalei* as compared to *B. australis*.
150
151 **Genetic analysis.** Topotypic *B. annandalei* (MT002520) differs from putative topotypic *B.*
152 *australis* (MT002518) with a raw genetic distance of 6.4% in the *cox*1 gene.
153
154 **Distribution**. *Bhavania annandalei* is known with certainty from the Kallada, Vamanapuram
155 and Neyyar River systems in southern Kerala, India. These river systems drain the western
156 slopes of the Agasthyamalai hill ranges, south of the Shencottah Gap. It is highly likely that
157 the species also occurs on the eastern slopes of the Agasthyamalai hills particularly in the
158 Tambaraparini River system in Tamil Nadu, but detailed surveys and voucher specimens are
159 required to confirm this.
160
161 **Remarks.** The density of chromatophores in *Bhavania* is likely to be dependent on the
162 micro-habitat as well as the colour and type of substratum it inhabits. Other ecological
163 factors that may influence body colour are forest/canopy cover, intensity of light, water
164 flow and water temperature (V.K. Anoop Pers. Observ.). This is reflected in the different
165 body colours shown by the two species in different habitats and locations (see Image 1), an
166 observation which was also made by Hora (1941).
167

168 **Comparative material.** *Bhavania australis*, KUFOS.19.AS.BH.01.1-6, 6ex., 13.04.2019,
169 10.8636 N & 76.6904 E, 46.4–58.8 mm SL, near Kavarakund falls, upstream of Malampuzha
170 Reservoir, Kerala, India, coll. M.R. Ramprasanth.
171
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178 the Kerala State Forest and Wildlife Department to VKA and AS.
179
180

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214 **TABLE 1.** Morphometric data of *Bhavania annandalei* (KUFOS.19.AS.BH.02.1-6, n = 6) and *B.*

215 *australis* (KUFOS.19.AS.BH.01.1-6, n = 6) putative topotypes.

216

Characters	*Bhavania annandalei*		*Bhavania australis*	
	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-supraoccipital 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

217

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

221

Characters	*Bhavania annandalei*	*Bhavania australis*
Pectoral-fin rays	6+10+I (6)	6+9+I (1), 6+10 (1), 6+10+I (4)
Dorsal-fin rays	ii+7+I (6)	ii+7 (3), ii+7+I (3)
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)
Predorsal 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)
Scale above the lateral line	11 (2), 12 (4)	14 (4), 15 (2)
Scale below the lateral line	9 (2), 10 (4)	10 (3), 11 (3)

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

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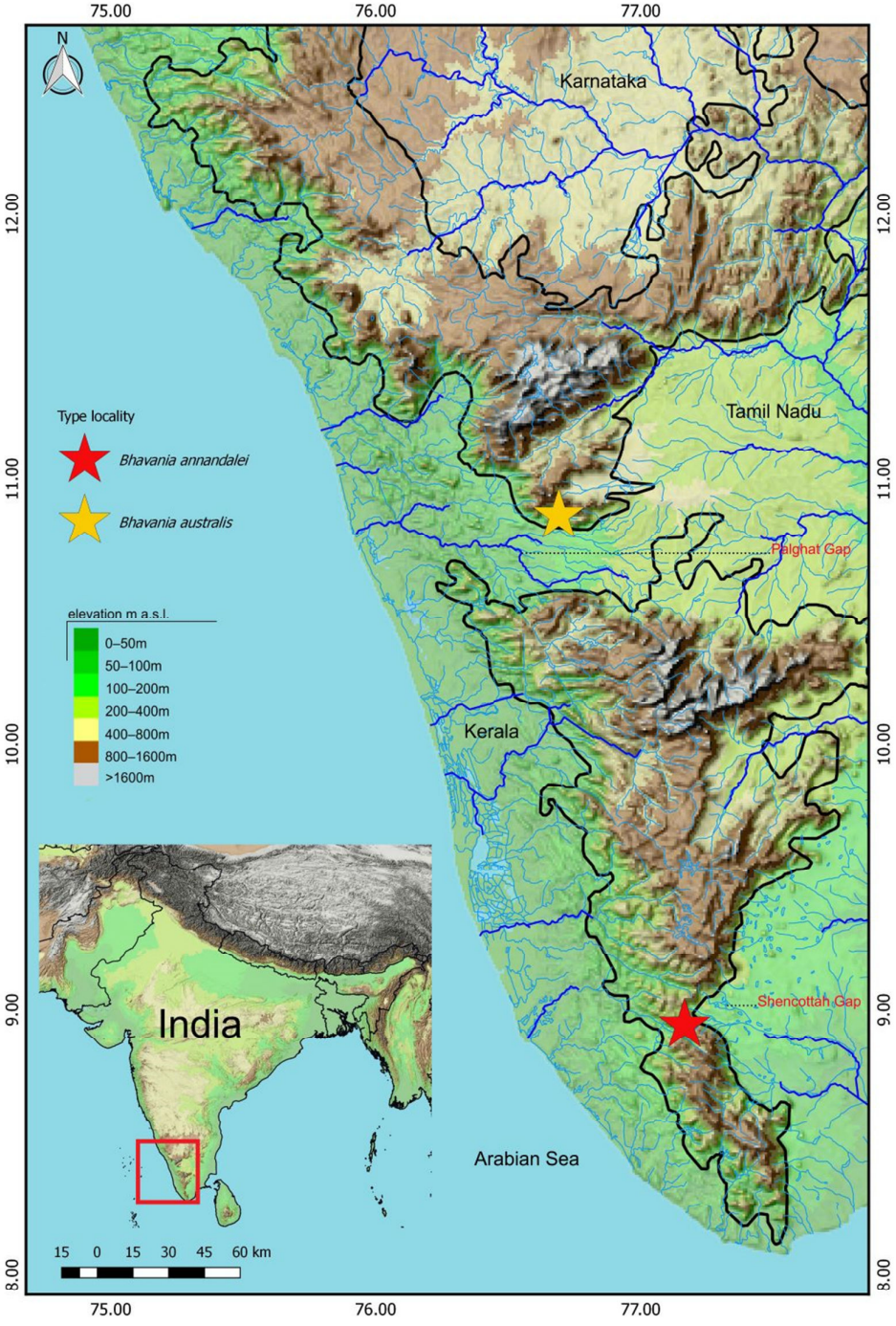

227 **Figure 2.** Morphometric analysis. (a) Principal Component Analysis scatter plot of factor
228 scores and ANOSIM statistics. (b) Linear regression between caudal peduncle depth and
229 width. (c) Linear regression between head length and head depth at nape.
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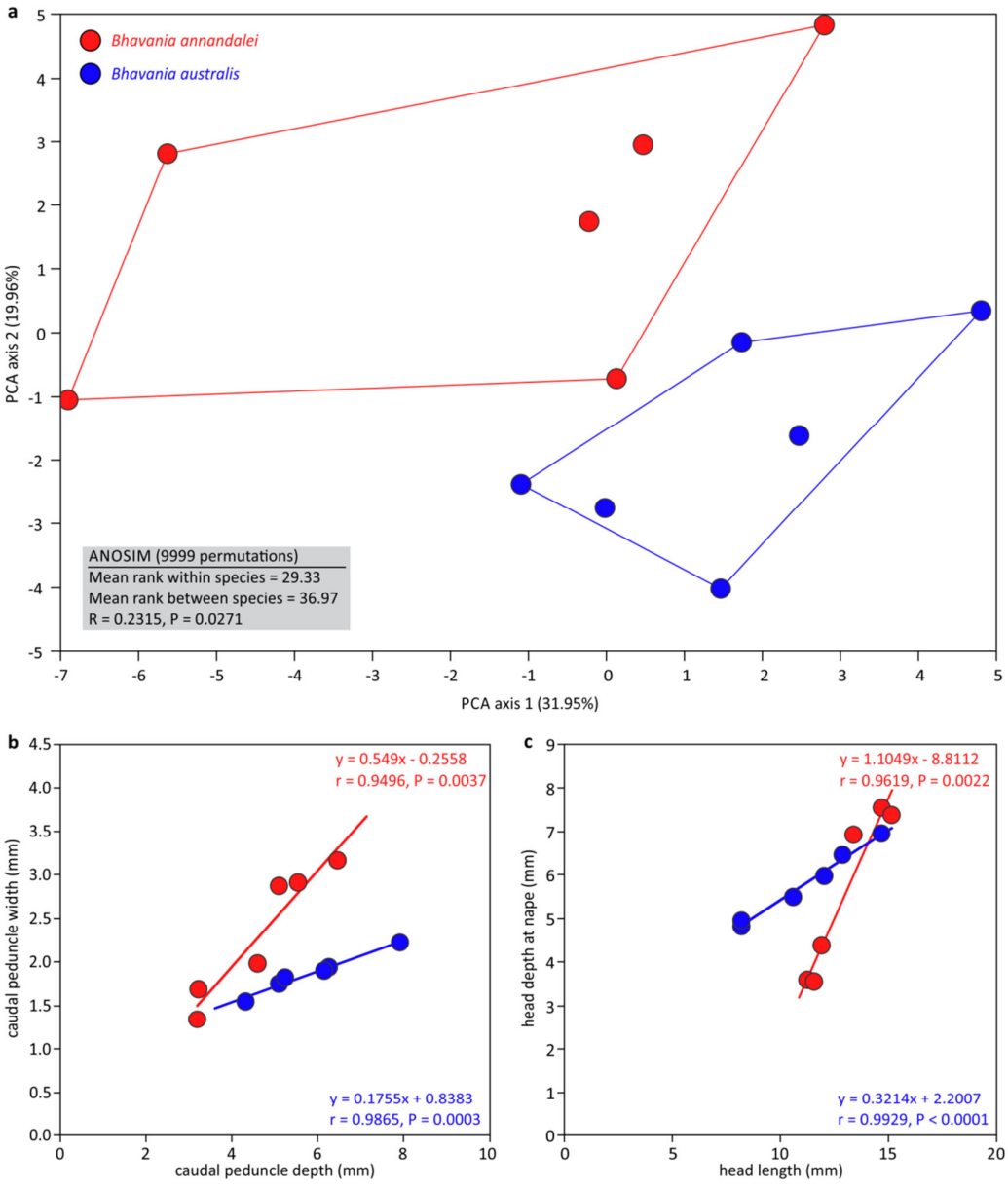
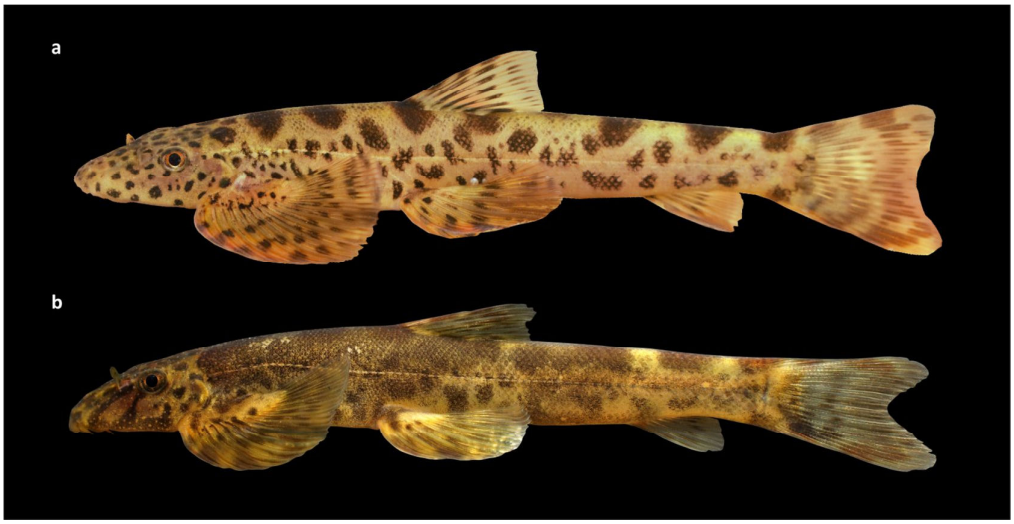


233 **Image 1.** Putative topotypes of (a) *Bhavania annandalei,* and (b) *B. australis* in life
234 (specimens not preserved).
235



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239 **Image 2.** Dorsal, lateral and ventral images of putative topotypes of (a) *Bhavania annandalei*

240 and (b) *B. australis*.

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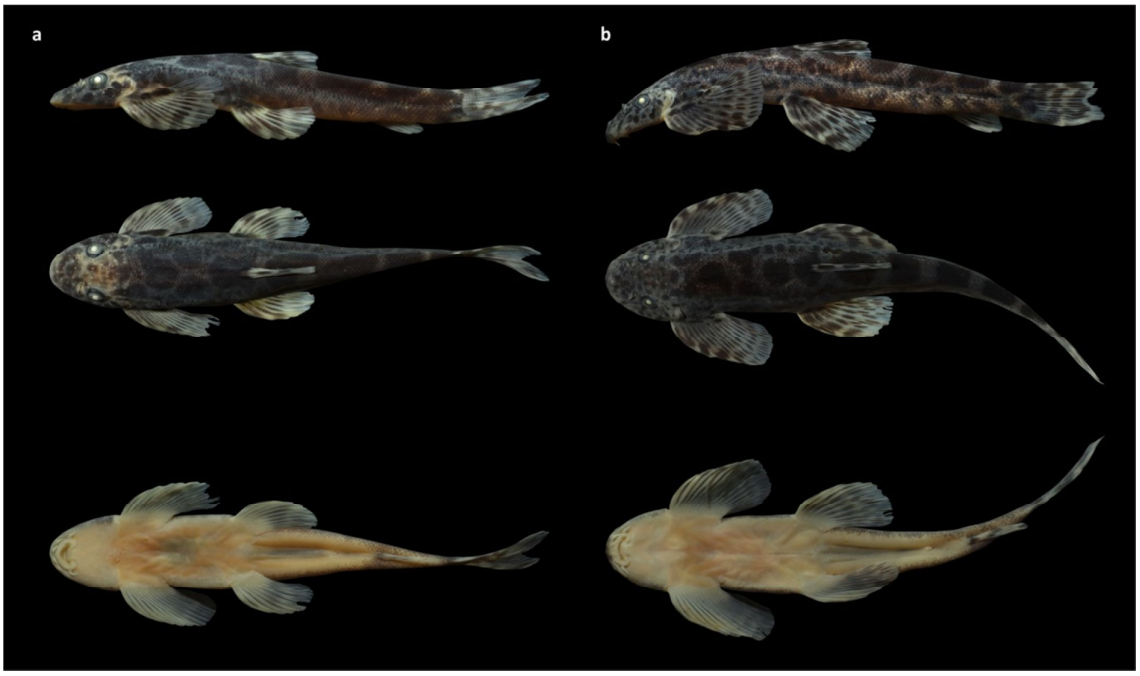

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245 **Image 3.** Dorsal and ventral view of head. (a, c) *Bhavania annandalei* and (b, d) *B. australis*.

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