

1 Identity of *Bhavana annandalei*

2

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

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24 **Abstract**

25 *Bhavana annandalei* Hora 1920, is resurrected from the synonymy of *B. australis* (Jerdon  
26 1849) based on examination of freshly collected topotypic specimens. The two species can  
27 be distinguished by a combination of morphological, morphometric and meristic characters,  
28 and by genetic distance using mitochondrial *cox1* gene. The distribution of *B. annandalei* is  
29 restricted to the river systems draining the Agasthyamalai hills, below the Shencottah Gap in  
30 southern Western Ghats.

31

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

33

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

42 Hora's (1920) description of *B. annandalei* was however, based on a single adult  
43 female specimen collected by Dr. Annandale from Travancore. Though Hora (1920) seemed  
44 to have access to additional juvenile specimens collected by Captain Sewell from the Nilgiris  
45 (Cherambadi) and Wayanad (Nellimunda, Mananthavady and near Vythiri), he did not  
46 examine them or provide other details. Subsequently, Hora (1937; p8) extended the  
47 distribution of the species to Mysore, based on four specimens collected by M.S Bhimachar

48 from a stream between Kottigehar and Balehonnur (erstwhile Mysore State = current day  
49 Tunga River System in Karnataka). No details of the specimens were provided.

50 In his review on 'Homalopterid fishes from Peninsular India', Hora (1941)  
51 synonymized *B. annandalei* with *B. australis*, after examining specimens from throughout its  
52 distribution range including Kallar/South Travancore (current day Vamanapuram River,  
53 Kerala); Pampadumpara/North Travancore (current day Periyar River, Kerala); Sethumadai  
54 Hills/ Mysore (current day Anamalai hills near Pollachi, Tamil Nadu); and Kottigehar/Mysore  
55 (current day Tunga River, Karnataka), and realizing that his description of *B. annandalei* was  
56 based mainly on immature specimens. This synonymy was subsequently adopted by Menon  
57 (1987) in his review of the homalopterid loaches of India, but without examining the type  
58 (or fresh topotypes) of *B. annandalei*, or the topotypes of *B. australis*. Later workers  
59 followed this synonymy and considered *Bhavana* to be monotypic (Talwar & Jhingran 1991;  
60 Menon 1999, Kottelat 2012).

61 Given their hill-stream adaptations (Hora 1920, 1937, 1941), and the fact that the  
62 type locality of *B. annandalei* (Tenmalai) and *B. australis* (Walayar) are at least 300km apart  
63 and separated by two significant biogeographic barriers - the Palghat Gap and the  
64 Shencottah Gap (see Anoop et al. 2018), it is highly unlikely that the two are conspecific.  
65 Collection of fresh topotypic specimens of both *B. australis* and *B. annandalei* and detailed  
66 examination and comparison of their biometrics, and genetic distance analysis based on the  
67 mitochondrial *cox1* gene, revealed that the two species are clearly distinct. We therefore  
68 resurrect *Bhavana annandalei* Hora 1920, from the synonymy of *B. australis* (Jerdon 1849)  
69 and provide notes on the distribution range of this species.

70 Six specimens of putative topotypic *Bhavana annandalei* were collected from  
71 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 ***Bhavana 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

96 **Diagnosis.** *Bhavana annandalei* is distinguished from its only known congener *B. australis*  
97 by a combination of characters including: low density and sparsely distributed tubercles on  
98 dorsal surface of head, especially on operculum, (vs. high density of tubercles on dorsal  
99 surface of head and operculum) (Image 3); eyes dorsally positioned (vs. dorso-laterally  
100 positioned) (Image 3); gape of mouth comparatively farther from snout tip, as a result the  
101 rostral barbels reaching anterior border of upper lip, (vs. gape of mouth closer to snout tip,  
102 and rostral barbels reaching posterior border of upper lip) (Image 3); rostral flaps between  
103 the rostral barbels fleshier (vs. less fleshier) (Image 3); fewer post dorsal scales (34–36 vs.  
104 38–41); fewer scales above the lateral line (11–12 vs. 14–15); and caudal peduncle stout  
105 with its depth to width ratio 1.8–2.3 (vs. laterally compressed caudal peduncle with depth to  
106 width ratio 2.8–3.6).

107

108 **Description.** Morphometric and meristic data are provided in Table 1 and Table 2,  
109 respectively. General body form as per Image 1a and Image 2a. Head details as in Image 3a,  
110 c.

111 Body elongate, depressed anteriorly, compressed laterally posteriorly; dorsal profile  
112 convex, deepest at dorsal-fin origin. Body wider than its depth at dorsal-fin origin, deeper  
113 than wide at anus. Head small, rounded, less than one-fourth of standard length; depressed,  
114 longer than broad, with minute sparsely distributed indistinct tubercles on dorsal surface of  
115 head. Eyes small, dorsally positioned, not visible from underside of head. Snout pointed in  
116 lateral view, round in dorsal view. Nostrils positioned dorso-laterally, closer to anterior  
117 border of eye than to snout tip, anterior nostril situated inside a skin flap covering the  
118 posterior nostrils. Mouth inferior. Lips fleshy. Gape of mouth less than three times  
119 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 *cox1* gene.

153

154 **Distribution.** *Bhavana 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 *Bhavana* 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.** *Bhavana 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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214 **TABLE 1.** Morphometric data of *Bhavana 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                            | <i>Bhavana annandalei</i> |            | <i>Bhavana 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-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  |

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218 **Table 2.** Meristic data of *Bhavana 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                   | <i>Bhavana annandalei</i>                 | <i>Bhavana australis</i>                            |
|------------------------------|---|---|
| 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),<br>67+3 (1) | 65+3 (2), 65+4 (1), 66+3 (1), 68+3 (1),<br>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)                                      |

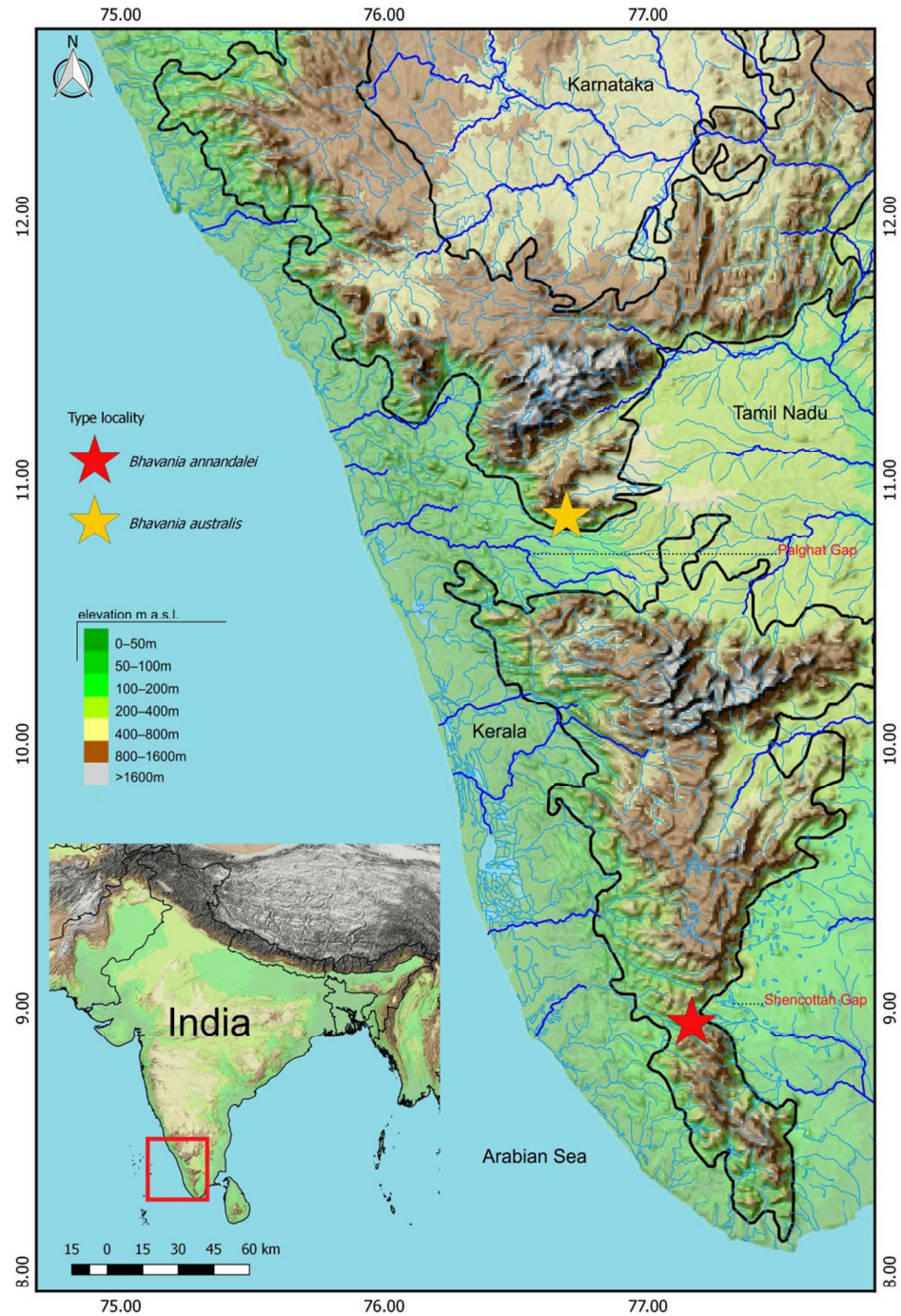
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224 **Figure 1.** Collection localities of putative topotypes of *Bhavana annandalei* and *B. australis*.

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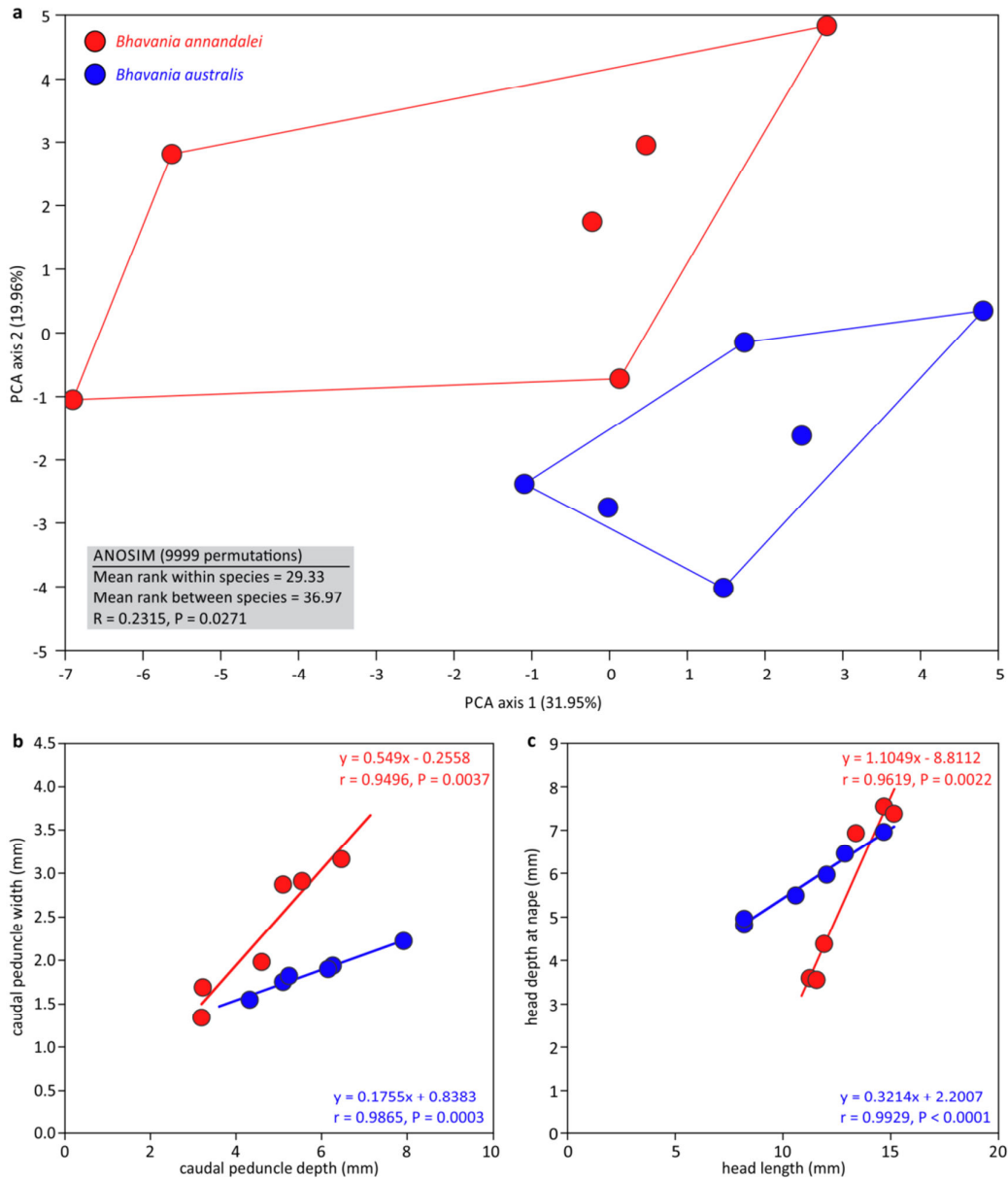


227 **Figure 2.** Morphometric analysis. (a) Principal Component Analysis scatter plot of factor  
228 scores and ANOSIM statistics. (b) Linear regression between caudal peduncle depth and  
229 width. (c) Linear regression between head length and head depth at nape.

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232



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

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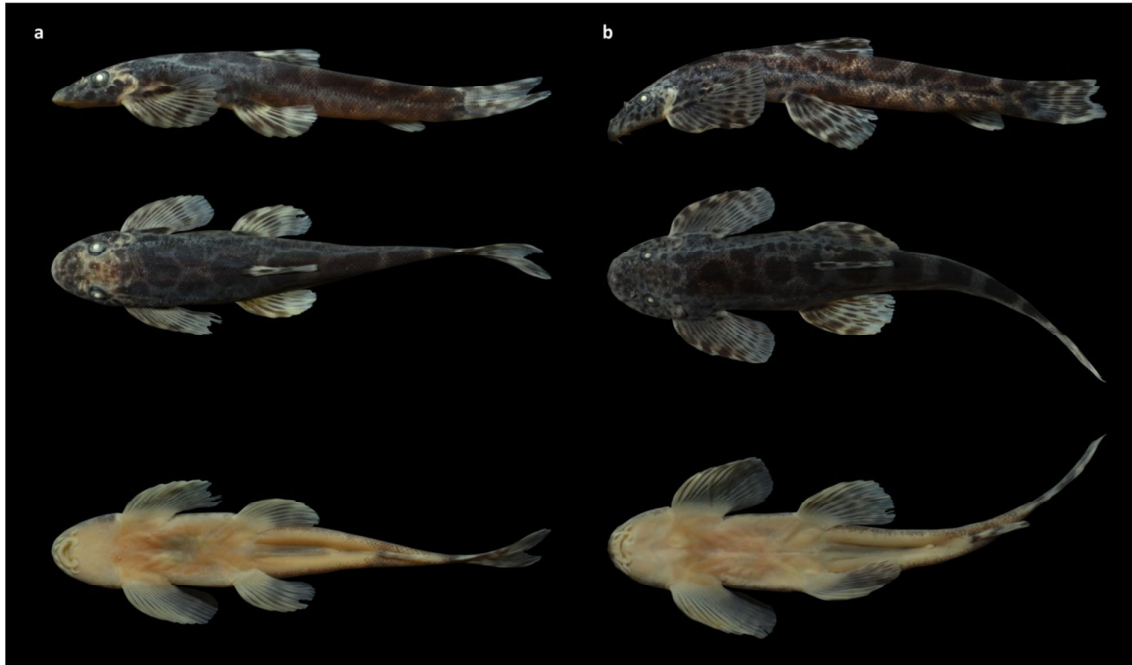


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239 **Image 2.** Dorsal, lateral and ventral images of putative topotypes of (a) *Bhavana annandalei*  
240 and (b) *B. australis*.  
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245 **Image 3.** Dorsal and ventral view of head. (a, c) *Bhavana annandalei* and (b, d) *B. australis*.

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