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Title: Morphological Characterization of *Badis* Species (Teleostei: Badidae) from Nepal

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I characterized morphological variation among 172 *Badis* sp. specimens (Teleostei: Badidae), collected from across Nepal and previously identified at *Badis badis*, to investigate whether any represented an undescribed species. Size-corrected principal component analysis (PCA), cluster analysis (CA), and discriminant analysis (DA) revealed four significantly different groups. The first group was characterized by shallower body depth, shorter pelvic to anal distance, and lack of a cleithral blotch. It was identified as *Badis andrewraoi*, previously unknown from Nepal, yet represented in my sample by 19 specimens from small rivers in the southeastern part of the country. Distribution of the remaining groups did not correspond to a zoogeographic hypothesis relating to major river drainages. Instead, morphological differences among these groups was better explained by allometric shifts. I provide information on the distribution and ecology of *B. andrewraoi* and *B. badis* in Nepal.

Keywords: Allometry, morphology, Asia, fish, *Badis badis*, *Badis andrewraoi*, Taxonomy, Anabantiformes

Morphological Characterization of *Badis* Species (Teleostei: Badidae) from Nepal

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Preface

I have formatted this thesis for submission to the journal *Zootaxa*.

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Introduction

The teleost genus *Badis* Bleeker has gained many recognized species recently, growing from four in 1957 to 24 in 2016. The genus was established by Bleeker in 1854 with a single species, *Badis badis* (Hamilton) in the family Nandidae. Regional variation within *B. badis* led Day (1875) to distinguish three forms that are now recognized as species—*B. badis*, *B. assamensis* Ahl, and *B. ruber* Schreitmüller (Kullander & Britz 2002). Description of *B. siamensis* Klausewitz in 1957 brought the species count to four. Family Badidae was erected by Barlow *et al.* in 1968, but no new species of *Badis* were recognized until Kullander & Britz (2002) revised the genus and added another eight—*B. blosyrus* Kullander & Britz, *B. chittagongis* Kullander & Britz, *B. corycaeus* Kullander & Britz, *B. ferrarisi* Kullander & Britz, *B. kanabos* Kullander & Britz, *B. khwae* Kullander & Britz, *B. kyar* Kullander & Britz, and *B. pyema* Kullander & Britz. Twelve more species of *Badis* have been described since that revision—*B. tuivaiei* Vishwanath & Shanta, *B. dibruensis* Geetakumari & Vishwanath, *B. juergenschmidti* Schindler & Linke, *B. singenensis* Geetakumari & Kadu, *B. triocellus* Khynriam & Sen, *B. britzi* Dahanukar, Kumkar, Katwate & Raghavan, *B. andrewraoi* Valdesalici & van der Voort, *B. autumnum* Valdesalici & van der Voort, *B. kyanos* Valdesalici & van der Voort, *B. soraya* Valdesalici & van der Voort, *B. laspiophilus* Valdesalici & van der Voort, and *B. pancharatnaensis* Basumatary, Choudhury, Baishya, Sarma & Vishwanath.

Badids (encompassing the genera *Badis* and *Dario* Kullander & Britz) are distributed in India, Myanmar, Bangladesh, Nepal, Thailand, Bhutan, Pakistan, and China (Ruber *et al.* 2004), with the largest number of species in India and Myanmar (Valdesalici & van der Voort 2015). *Badis badis*, as currently defined, is widely

distributed in Pakistan, Nepal, India, Bhutan, and Bangladesh; however, given that many populations formerly classified as *B. badis* have been recognized as species (e.g., Kullander and Britz 2002, Geetakumari & Vishwanath 2010, Khynriam & Sen 2011, Basumatary *et al.* 2016), it is likely that more species remain to be discovered.

Historically, only *B. badis* was known from Nepal, leading me to ask whether other *Badis* species might exist there. I analyzed morphology, meristic characters, and color patterns of museum specimens collected in Nepal, all of which had been previously identified as *B. badis*, to assess their identifications and morphological variation within and among the three major river drainages of the country—the Karnali, Gandaki, and Koshi, west to east, respectively—all of them tributaries of the Ganges River in India.

Materials and methods

Material examined

I examined 172 specimens from Nepal, identified as *B. badis*, from 32 lots on loan from four museums as follows: 12 specimens from California Academy of Sciences, San Francisco, California (CAS); one specimen from the United States National Museum of Natural History, Smithsonian Institution, Washington D.C. (USNM); 26 specimens from Oklahoma State University, Department of Zoology, Stillwater, Oklahoma (OSUS); and 133 specimens from the University of Kansas Biodiversity Institute, Lawrence, Kansas (KU). For each lot, lot number, number of specimens (in parentheses), standard length minima and maxima, and locality, including latitude/longitude coordinates (in parentheses; obtained from museum records for 164 specimens, and others estimated using Google Earth®) were as follows: CAS 50183 (1), 26.2 mm, Chitwan Valley,

Khageri River, Gandaki River drainage; CAS 50206 (3), 26.0–34.7 mm, Chitwan Valley, Khoriamohan in Someswar Hills, Gandaki River drainage; CAS 50236 (1), 24.6 mm, Chitwan Valley, in Churia Hills, Gandaki River drainage; CAS 50293 (5), 13.9–17.3 mm, Chitwan Valley, Gandaki River drainage (27.551254°, 84.174043°); CAS 50386 (2), 23.2–23.5 mm, farm pond, 1–2 km E of Kalaiya, Gandaki River drainage (27.022616°, 85.025161°); USNM 274795 (1), 29.5 mm, Chitwan National Park, Gandaki River drainage; OSUS 15576 (3), 25.1–37.8 mm, Dhungre River, Sauraha, Gandaki River drainage (27.59556°, 84.48139°); OSUS 15664 (2), 11.1–12.3 mm, Narayani River, Kharkhadeghat, Gandaki River drainage (27.70167°, 84.33972°); OSUS 15784 (2), 23.2–24.7 mm, Khageri River, Gandaki River drainage (27.63278°, 84.48917°); OSUS 15950 (5), 33.7–38.4 mm, Khageri River, Gandaki River drainage; OSUS 16866 (3), 16.1–19.8 mm, Narayani River at Binai River confluence, Gandaki River drainage (27.54917°, 83.92389°); OSUS 16966 (1), 23.7 mm, Narayani River, near Rapti River confluence, Gandaki River drainage; OSUS 17058 (9), 21.4–28.9 mm, borrow pits E of Koshi Barrage, Koshi River drainage; OSUS 17396 (1), 40.6 mm, Narayani River, near Rapti River confluence, Gandaki River drainage; KU 28514 (3), 27.3–35.9 mm, upstream from Koshi River Barrage, Koshi River drainage (26.5249996°, 86.9349976°); KU 28569 (5), 18.9–27.3 mm, downstream from Koshi River Barrage, Koshi River drainage (26.5182991°, 86.9266968°); KU 28590 (6), 17.2–28.5 mm, associated seepage at Koshi Tappu Wildlife Refuge, Koshi River drainage (26.6233006°, 87.0333023°); KU 28609 (33), 18.9–38.6 mm, seepage at Bhantabari, Koshi River drainage (26.5249996°, 86.9732971°); KU 28670 (14), 19.8–39.9 mm, Brahamadev, Karnali River drainage (29.0816994°, 80.1417007°); KU 29126 (5), 27.6–35.0 mm, NE of Karkharbhitta, Mechi

River drainage (26.645°, 88.1667°); KU 29197 (5), 26.9–34.3 mm, Lohandra River, Belbari, India's Fulahar (Mahananda) River drainage (26.6599998°, 87.4116974°); KU 29358 (3), 20.9–27.0 mm, oxbow lake 1 km E of Kamalpur, Koshi River drainage (26.6883°, 86.96°); KU 29394 (4), 18.3–21.9 mm, east of Bardiya National Park headquarters, Karnali River drainage (28.4517002°, 81.2450027°); KU 40368 (4), 20.1–25.5 mm, Mechi River at Karkarbhitta, at bridge crossing at border with India, Mechi River drainage (26.64597222°, 88.16230556°); KU 40409 (1), 22.3 mm, Biring River at highway, Mechi River drainage (26.64183333°, 87.93730556°); KU 40417 (2), 25.7–30.4 mm, Chisang River at gravel mine along highway, Fulahar River drainage (26.65825°, 87.48847222°); KU 40426 (22), 18.6–32.2 mm, Kesaliya River, E of Belbari on highway, Fulahar River drainage (26.65880556°, 87.44638889°); KU 40436 (3), 16.9–35.4 mm, Lohandra River, Belbari, Fulahar River drainage (26.66288889°, 87.40813889°); KU 40500 (16), 20.2–29.3 mm, Ambasa River, highway between Chisapani and Babai, Karnali River drainage (28.50036111°, 81.32327778°); KU 40586 (1), 32.1 mm, Rapti River upstream from confluence with Lothar, on border of Chitwan National Park, Gandaki River drainage (27.56255556°, 84.70475°); KU 40602 (2), 25.7–28.6 mm, Khair Khola at bridge crossing highway east of Ratnanagar, Gandaki River drainage (27.61841667°, 84.53266667°); and KU 40648 (4), 15.8–29.5 mm, tributary of Rapti River, Chitwan National Park, Gandaki River drainage (27.57241667°, 84.49638889°).

Methods

Methods were modeled after Kullander & Britz (2002). I used nine morphometric lengths, 12 meristic characters, and three color patterns, for a total of 24 variables.

Morphometric characters were measured using digital calipers (Mitutoyo Corporation, Model No. CD-6” CS, Japan) to the nearest 0.1 mm on the left side of the specimen unless damaged, and were converted to proportions of standard length for descriptive statistics. The nine length measurements included head length, snout length, orbital diameter, interorbital width, upper jaw length, lower jaw length, body depth, pelvic fin length, and pelvic to anal distance. Meristic characters were dorsal fin spines, dorsal fin rays, anal fin rays, pectoral fin rays, scales in a lateral row, anterior lateral line scales, posterior lateral line scales, gill rakers, dentary cranial pores, lachrymal cranial pores, abdominal vertebrae, and caudal vertebrae. Color patterns were vertical bars, postorbital stripe, and cleithral blotch, which were coded as number of vertical bars, number of scales along the length of the postorbital stripe, and presence or absence of a cleithral blotch. A dissecting microscope (Carl Zeiss, Stemi DV4, Göttingen, Germany) was used to examine specimens. I set aside two specimens and re-measured them after every 15 new specimens to prevent distortion of data resulting from potential gradual change in measuring technique (Strauss & Bond 1990).

Habitat characteristics were summarized from data for 10 sites in Nepal where 73 *Badis* sp. specimens were collected by D. Edds in 1984–85 and 1996. Total acidity (mg/l), total alkalinity (mg/l), total hardness (mg/l $CaCO_3$), pH, CO_2 (mg/l), and dissolved oxygen (DO) (mg/l) were measured with a Hach kit model AL-36B (Hach Co., Loveland, Colorado, USA). Additional habitat characteristics, available for four sites, were as follows: altitude above sea level, estimated from a topographic map in 1984–85 and measured with a Garmin GPS in 1996; current speed, measured with a Gurley pygmy meter no. 625 (Gurley Precision Instruments, Troy, New York, USA); water depth and

water clarity, gauged with a Secchi disk mounted on a calibrated pole; substrate composition, assessed visually using a modified Wentworth scale (Cummins 1962) by estimating percent mud (ca. 0.1 mm diameter), sand (0.1–2 mm), gravel (2–16 mm), pebble (16–64 mm), cobble (64–256 mm), and boulder (≥256 mm); and percent coverage by filamentous algae and submerged, floating, or emergent vegetation, estimated visually.

Data analysis

I used SAS® software (SAS Institute Inc. 2010), for all data analyses. I standardized morphometric data for size-dependent variation using the allometric method of Elliott *et al.* (1995): $M_s = M_o (L_s/L_o)^b$, where M_s is the standardized measurement, M_o is the original measurement, L_s is the arithmetic mean standard length for all fish from all samples, L_o is the standard length of the individual fish, and b is the slope of the regression of log M_o on log L_o using all fish. Log or square root transformations were applied when they improved skewness or kurtosis. Normality of distributions of pectoral fin ray counts and counts of scales in a lateral row improved when they were log-transformed, whereas snout length and dorsal fin rays improved from square root transformation. For any specimen with at least one damaged character (n = 68 specimens, 129/4128 potential values), mean values for the population (n = 172) were used as placeholders in SAS (Note that no individuals stood out in subsequent analyses involving such placeholders).

Principal component analysis (PCA) was employed to reduce dimensionality and highlight important mensural, meristic, and color pattern characters through significant principal component (PC) loadings (Rao 1964, Kullander & Britz 2002). PCA uses an

orthogonal transformation to convert potentially correlated variables into a set of linearly uncorrelated variables. Morphometric characters were run separately from meristic and color pattern characters in two separate PCAs because measurements and counts are on a different scale. The morphometric PCA was run using a covariance matrix of standardized data while meristic PCA (including color pattern) was run using a correlation matrix. Criteria used for determining the number of significant PCs included eigenvalues ≥ 1, scree plot shape, and interpretability (SAS Institute Inc. 2010). I tested for differences in significant PC axes among drainages using analysis of variance (ANOVA) with sequential Bonferroni correction (Rice 1989) and post-hoc Tukey's tests. I used PCA scatter plots of individuals labeled according to their major river drainage to search for a relationship between morphological variation and geography.

Cluster analysis (CA) was performed using the scores for each specimen on separate morphometric PC1 axis and the meristic (including color patterns) PC1 and PC2 axes. To determine the number of significant clusters, I examined the cubic clustering criterion (CCC), pseudo F statistic, and pseudo T^2 statistic (SAS Institute Inc. 2010) by finding local "peaks" of the CCC and pseudo F combined with a small pseudo T^2 followed by a larger pseudo T^2 with the next cluster fusion (McGarigal *et al.* 2000, Summers *et al.* 2006). I used discriminant analysis (DA), with statistical significance assessed according to Wilk's lambda (λ), on the morphometric PC1 axis, the meristic PC1 and PC2 axes, and clusters from cluster analysis to cross-validate identifiability of the clusters. I used linear regression to investigate allometric growth (McGarigal *et al.* 2000, Kuzmanović *et al.* 2012, Ruiz-Campos *et al.* 2016).

Results

The morphometric PC1 axis accounted for 84.6% of the total morphometric variance, whereas the meristic PC1 and PC2 axes accounted for 24.9% (13.2% and 11.7%, respectively) of total meristic variance. The highest loadings on the meristic PC1 axis were for absence of cleithral blotch (-0.46), number of anal fin rays (0.42), and number of pectoral fin rays (0.42), whereas the highest loadings on the meristic PC2 axis were for number of anterior lateral line scales (0.43), number of posterior lateral line scales (0.40), number of scales in the postorbital stripe (-0.37), and number of abdominal vertebrae (0.36) (Table 1). Morphometric PC1's highest loadings were for body depth (0.59), pelvic to anal distance (0.58), and pelvic fin length (0.41) (Table 1).

CA (Figure 1) yielded four statistically significant clusters. All three of the PCs entered DA as significant ($p < 0.0001$). Wilks lambda (λ) was 0.176 ($p < 0.0001$), indicating significant overall differences among the four designated clusters. The percentage of individuals that were correctly classified by DA was 87.2% for cluster 4, 88.2% for cluster 2, 100% for cluster 1, and 100% for cluster 3.

A scatter plot of individuals on the meristic PC1 axis versus the morphometric PC1 axis (Figure 2) showed that CA cluster 2 separated from clusters 1, 3, and 4 mostly according to the meristic PC1 axis. As demonstrated by PC axis loadings (Table 1), this separation of group 1 from the others was based on lack of a cleithral blotch and having shallower body depth and longer pelvic to anal fin distance. Comparison of mean values of morphometrics and modal values of meristic and color patterns of cluster 2 specimens to other *Badis* species that lack a cleithral blotch (*B. autumnum*, *B. assamensis*, *B. blosyrus*, *B. britzi*, *B. corycaeus*, *B. kyanos*, *B. kyar*, *B. laspiophilus*, *B. pyema*, *B.*

singenensis, and *B. triocellus*) showed that CA cluster 2 represents a *Badis* species not previously recorded from Nepal, which was subsequently identified as *B. andrewraoi* (Figure 3). I found 15 specimens of *B. andrewraoi* in five museum lots composed exclusively of that species—KU 29126, KU 40368, KU 40409, KU 40417, and KU 40436. One lot, KU 29197, had four *B. andrewraoi* (now KU 41396) and one *B. badis*. The six lots containing *B. andrewraoi* were from four sites in extreme southeastern Nepal—two in Morang District (Lohandra and Chisang rivers) and two in Jhapa District (Mechi River)—all tributaries of the Fulahar (Manananda) River in India.

To investigate variation among the remaining 153 specimens, I restandardized and transformed the data, removing cleithral blotch as a variable because all remaining specimens had a cleithral blotch, and reran PCA, CA, and DA. The morphometric PC1 and meristic PC1 axes met the criteria for significance, with morphometric PC1 accounting for 85.4% of the total morphometric variance and meristic PC1 accounting for 13.2% of the meristic variance. The highest loadings on the meristic PC1 axis were for pectoral fin rays (0.46) and anterior lateral line scales (0.38), and for the morphometric PC1 axis they were body depth (0.60) and pelvic to anal distance (0.59) (Table 2). Three significant clusters were produced by CA (Figure 4), referred to here as clusters 1, 3, and 4 because membership of the clusters changed only slightly from the analysis including *B. andrewraoi*. Clusters 1, 3, and 4 were discriminated using DA ($\lambda = 0.286$, $p < 0.0001$), with the lowest correct classification being 91.9% for cluster 4 and the other two clusters having 100% correct classification.

Comparison of mean values of morphometrics and modal values of meristic and color patterns for these 153 specimens with those other *Badis* species with a cleithral

blotch (*B. chittagongis*, *B. dibruensis*, *B. ferrarisi*, *B. juergenschmidti*, *B. kanobos*, *B. khwae*, *B. pancharatnaensis*, *B. ruber*, *B. siamensis*, *B. soraya*, *B. tuivaiei*) showed them to be consistent with identification as *B. badis* (Figure 5). In addition to having a cleithral blotch, the *B. badis* sample (n = 153) exhibited greater range of variation than the *B. andrewraoi* sample (n = 19) for most morphometric (Table 3) and meristic (Table 4) characters.

To further investigate separation among clusters 1, 3, and 4, I used ANOVA and Tukey's tests to test for mean differences among river drainages. ANOVA of scores on the morphometric PC1 axis indicated significant difference ($F = 5.90$, $df = 2$, $p = 0.003$) between major river drainages, and Tukey's tests ($p < 0.05$) showed differences between the Koshi and Gandaki river drainages. Scores on the meristic PC1 axis revealed a significant difference ($F = 6.26$, $df = 2$, $p = 0.002$), and Tukey's tests ($p < 0.05$) showed differences between the Koshi and Karnali rivers. Despite these differences between major river basins, the 153 specimens did not group separately by drainage when plotted according to their scores on the PC axes (Figure 6). Moreover, regression of individual morphometric PC1 scores on standard length revealed a strong and significant relationship ($R^2 = 0.92$, $p < 0.0001$; Figure 8), and sizes of individuals in CA clusters 1, 3, and 4 overlapped only slightly. Together, these results show that the discontinuities among clusters 1, 3, and 4 in PC space (Figure 7) relate mainly to overall body size and to correlated differences in shape resulting from allometric shifts. Small body size (as in cluster 1) was characterized by shallower body depth and shorter pelvic to anal distance, medium body size (as in cluster 4) was characterized by a larger range of pectoral fin rays, scales in lateral row, and anterior and posterior lateral line scale count, and large

body size (as in cluster 3) was characterized by deeper body depth and longer pelvic to anal distance (Table 2).

Distribution

Among the 32 lots of *Badis* spp. collected during surveys from west to east in Nepal, *B. andrewraoi* was found only in small rivers in the Mechi/Mahananda/Fulahar drainages in the southeast, whereas *B. badis* occured west of those in all three major river drainages (Figure 9). *Badis andrewraoi* occurred at four locations, one of them (26.64597222°, 88.16230556°) being only 20 km from the species' type locality in the Mahananda River in India (Valdesalici & van der Voort 2015). *Badis badis* occurred in the lowland Tarai (and Inner Tarai) of Nepal's Karnali, Gandaki, and Koshi river drainages, at ≤ 213 meters above sea level.

Badis andrewraoi and *B. badis* occurred syntopically at one site—Lohandra River at Belbari, Morang District (26.6599998°, 87.4116974°), an indirect tributary of the Fulahar (Mahananda) River in India. Syntopy for badids has been documented previously only by Kullander & Britz (2002), who reported *B. badis* with the following species: *B. assamensis* in Assam, India, Brahmaputra River drainage; *B. kanabos*, *B. blosyrus*, and *Dario dario* Kullander & Britz in Assam, India, Kokrajhar District, Brahmaputra River drainage; and *B. kyar*, *B. corycaeus*, and *Dario hysginon* Kullander & Britz in Myitkyina District, Myanmar, Irrawaddy River drainage.

Ecology

Habitat characteristics for *B. andrewraoi* were summarized from data for two sites that accounted for nine specimens, and for *B. badis* from data for eight sites at which 64

specimens were collected by D. Edds in Nepal in 1984–85 and 1996 (Table 5). It is important to note that sampling at different sites often occurred in different seasons.

Four *B. badis* sites had additional habitat measures. Mean (SD) habitat characteristics, weighted by number of specimens per site for these four sites were as follows: altitude above sea level 205 (7.1) m, current speed 0.0015 (0.0) m sec^{-1}, water depth 74 (0.5) cm, and water clarity 20 (0.0) cm. Substrate composition for these four *B. badis* sites were as follows: 1) 10% mud, 5% sand, 40% gravel, 30% stone, 15% rubble, 5% boulder; 2) 90% sand, 10% mud; 3) 75% mud, 25% sand; and 4) 100% mud. Aquatic vegetation coverage at these sites were as follows: 1) 50% filamentous algae; 2) 20% filamentous algae, 3) 5% submerged, 5% floating; 2% emergent; and 4) 20% submerged, 5% filamentous algae.

Species associated syntopically with *B. andrewraoi* included *Barilius barila* (Hamilton), *B. bendelisis* (Hamilton), *Opsarius barna* (Hamilton), *Pethia conchonius* (Hamilton), *Lepidocephalichthys guntea* (Hamilton), *Aplocheilus panchax* (Hamilton), and *Channa orientalis* Bloch & Schneider. Species collected at *B. badis* sites included *Notopterus notopterus* (Pallas), *Barilius shacra* (Hamilton), *Danio rerio* (Hamilton), *Devario devario* (Hamilton), *Opsarius barna*, *Pethia conchonius*, *Rasbora daniconius* (Hamilton), *Glossogobius giuris* (Hamilton), *Parambassis baculis* (Hamilton), *Xenentodon cancila* (Hamilton), *Channa orientalis*, *Nandus nandus* (Hamilton), and *Leiodon cutcutia* (Hamilton).

Discussion

Morphological analysis of 172 specimens previously identified as *B. badis* from Nepal recovered four significant groups. One group was identified as *B. andrewraoi*—a

species previously unrecorded in Nepal—representing a westward range extension for that species. Its morphology and distribution are unique compared to other *Badis* species, including others that lack a cleithral blotch (Valdesalici & van der Voort 2015). The *B. andrewraoi* specimens were captured in southeastern Nepal; additional work is needed to document the species' range limits. The type locality of *B. andrewraoi* is in the Mahananda River drainage of Darjeeling District, West Bengal, India (Valdesalici & van der Voort 2015). Two Nepalese lots of *B. andrewraoi* (KU 29126 and KU 40368) were collected in the Mechi River, Mahananda River drainage, roughly 20 km from the type locality, but one lot (KU 41396) was collected in the Lohandra River, Mahananda River drainage, an additional 70 km west.

The three remaining groupings of specimens according to their morphological and meristic variation reflected allometric growth of *B. badis*. Despite standardizing morphometric data to remove size and use only shape related to each quantified character (Elliott *et al*. 1995), these three clusters showed allometric shifts in body size. Similar body size patterns have been demonstrated for other fishes, with Klingenberg & Froese (1991) mathematically demonstrating allometric growth patterns even after size correction in gadiforms and pleuronectiforms, and Kuhajda *et al.* (2007) reporting allometric growth using size-corrected PCA in hatchery-reared sturgeons.

ANOVA and Tukey's tests for 153 *B. badis* specimens showed a significant difference between the Koshi and Gandaki river drainages on morphometric PC1 and a significant difference between the Koshi and Karnali river drainages on meristic PC1. However, the ellipses seen in Figure 6 show that there is broad overlap in morphometric variation among major drainages. Sampling of the different drainages was conducted in

different seasons, thus it seems likely that seasonal patterns of reproduction and growth, rather than drainage-specific intrinsic differences in morphology might explain these results and the differences between clusters 1 and 3 (Figure 7). Cluster 1 individuals, the predominantly smaller specimens, were collected during the pre-monsoon season in the Gandaki River drainage (17 of 32 Gandaki specimens, 53.1%) and cluster 3, the predominantly larger specimens, were collected during the post-monsoon season in the Koshi River drainage (36 of 87 Koshi specimens, 41.4%). Eleven of the 12 members in cluster 1 were from the Gandaki River drainage, and 12 of the 16 members in cluster 3 were collected in the Koshi River drainage. Overall size variation appeared to be continuous (Figure 8), thus separation into size-related clusters might relate to absolute size thresholds at which important allometic shifts occur, or might be biologically unimportant and simply an outcome of uneven representation by our sample (153 specimens) of the range of what was actually continuous variation.

I was unable to distinguish the sex of most individuals, especially smaller specimens (≤ 25 mm, n = 72). Kullander & Britz (2002) reported that *B. badis* males have a more elevated predorsal contour and a non-inflated abdomen and that females have generally fainter fin pigmentation and shorter pelvic, soft dorsal, and anal fins; however, these authors stated that most of their specimens had no external sexually dimorphic characters. My inability to consistently identify sex led me to exclude that character from analysis. Future studies could search for consistent ways to separate sexes of *Badis* specimens.

Previous authors have not described *B. badis* habitat in detail (e.g. “found in clear streams in the lowlands”; Hamilton 1822, Day 1875, Shaw & Shebbeare 1937). In Nepal,

B. badis habitat has been described only as “lowland rivers and backwaters” (Edds 1986a, b). Habitat descriptions for *B. andrewraoi* are likewise very general, such as “from a medium-sized river with a sand substrate” (Valdesalici & van der Voort 2015). My data provide additional details of habitat conditions for *B. badis* and *B. andrewraoi* in Nepal. *Badis badis* was found in lowland streams, commonly with fine substrate (e.g., mud or mud/sand mixture) and little vegetative cover. *Badis andrewraoi* was collected from similar habitat, with some, potentially seasonal, differences in water chemistry. However, I had limited habitat data taken once per location and at different times of the year; thus, they may not represent true average habitat condition for these species. Future work should include more comprehensive characterization of habitat for *B. andrewraoi* and *B. badis*.

The morphological, distributional, and habitat data for *B. andrewraoi* and *B. badis* presented here should help facilitate future research on these and other species of the genus. Future studies should compare *B. badis* through genetic analysis. Ruber *et al.* (2004) examined genetic differences among 33 individuals representing 13 badid species and found evidence to support the *Badis* species groups defined by Kullander & Britz (2002) which had been assigned based on allopatric distributions as follows: *B. assamensis* group, *B. badis* group, *B. corycaeus* group, *B. ruber* group, and *B. kyar*. Detailed genetic analyses could reveal one or more undescribed species among populations currently identified as *B. badis* in Nepal, Pakistan, India, Bhutan, or Bangladesh.

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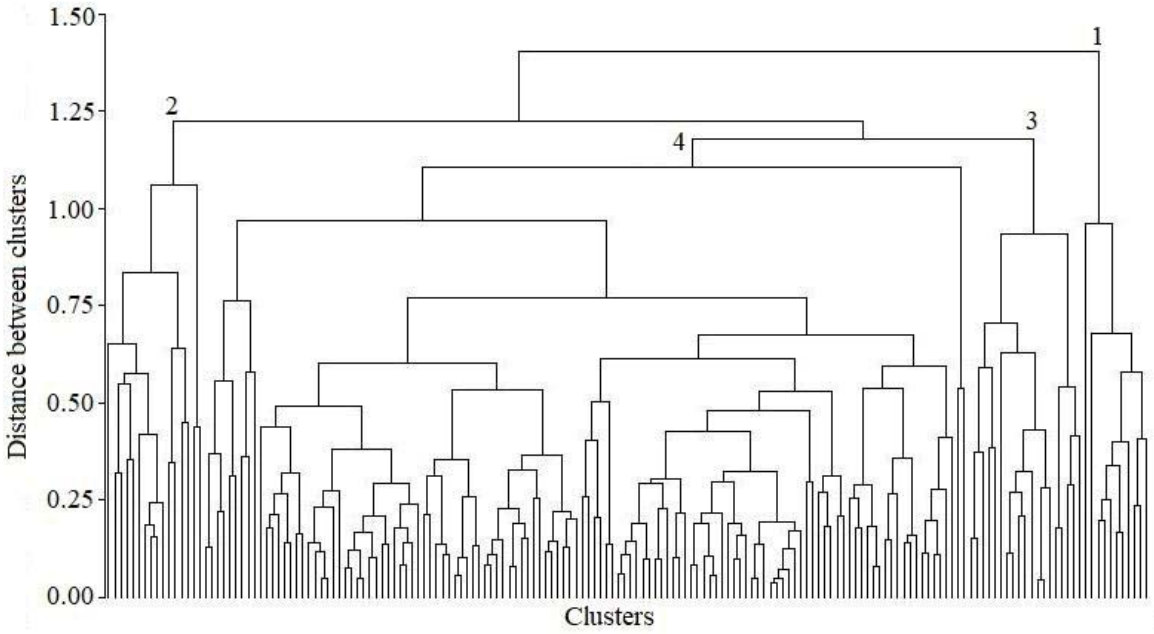

Figure 1. Results of cluster analysis showing morphological similarity of 172 specimens of *Badis* species from Nepal, with four significant clusters labeled.

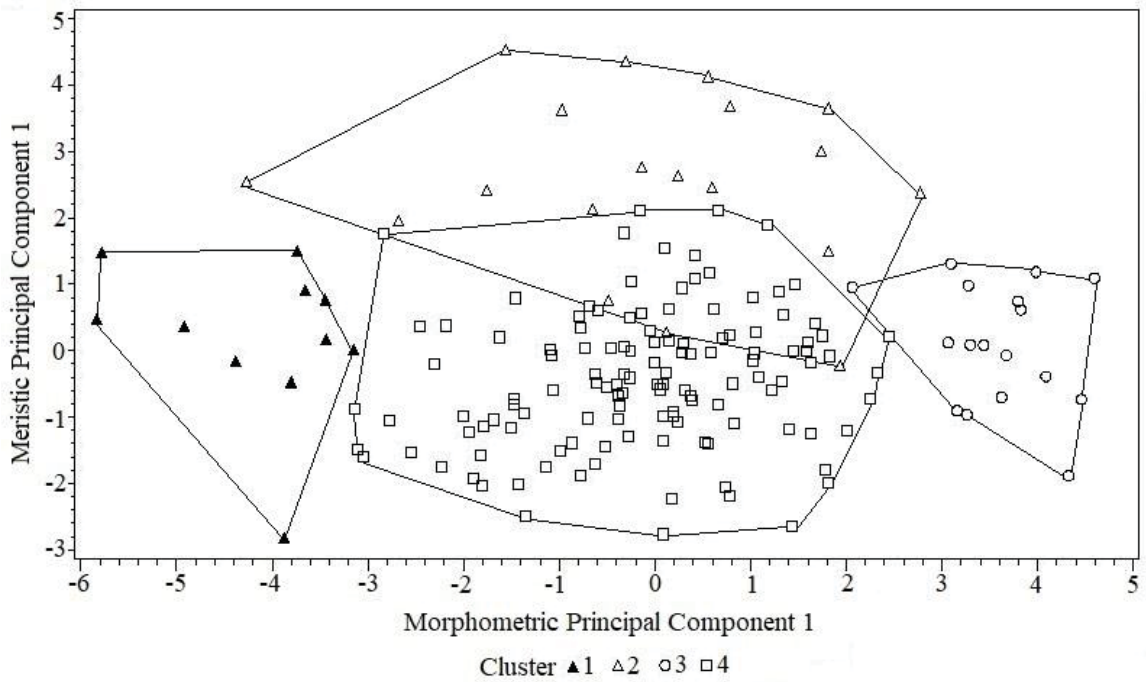

Figure 2. Scatter plot of individual scores on meristic PC1 versus morphometric PC1, showing separation of CA cluster 2 from clusters 1, 3, and 4 on y-axis (n = 172).

Figure 3. *Badis andrewraoi* (KU 40417, SL = 30.4 mm). Photo provided by University of Kansas Biodiversity Institute.

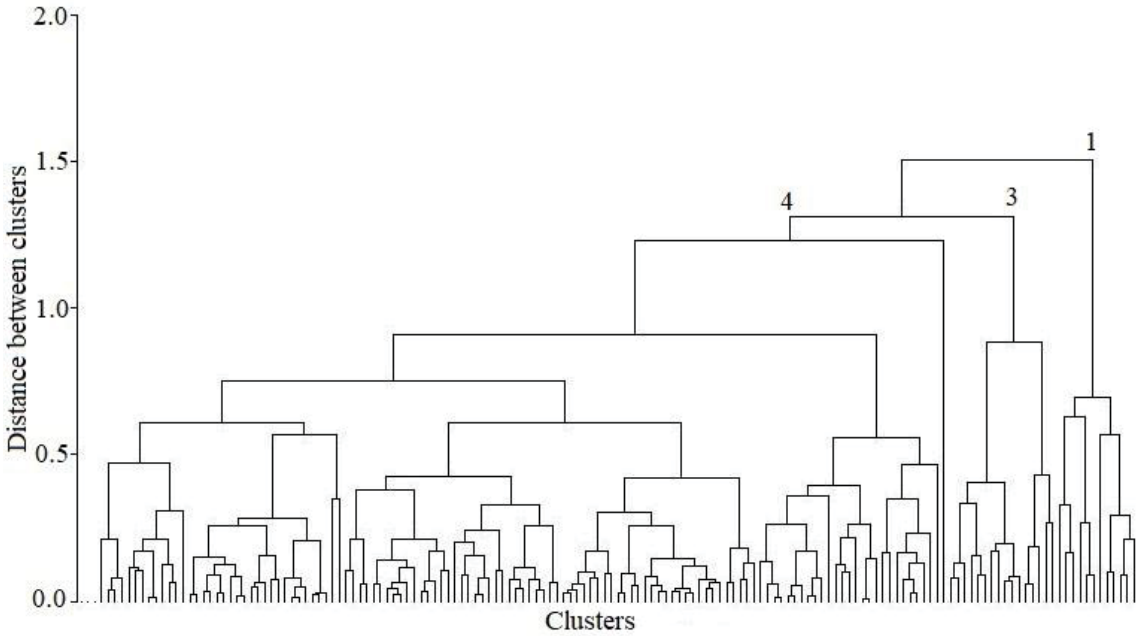

Figure 4. Results of cluster analysis showing morphological similarity of 153 specimens of *B. badis* from Nepal, with three significant clusters labeled 1, 3, and 4.

Figure 5. *Badis badis* (KU 28609, SL = 33.3 mm). Photo provided by University of Kansas Biodiversity Institute.

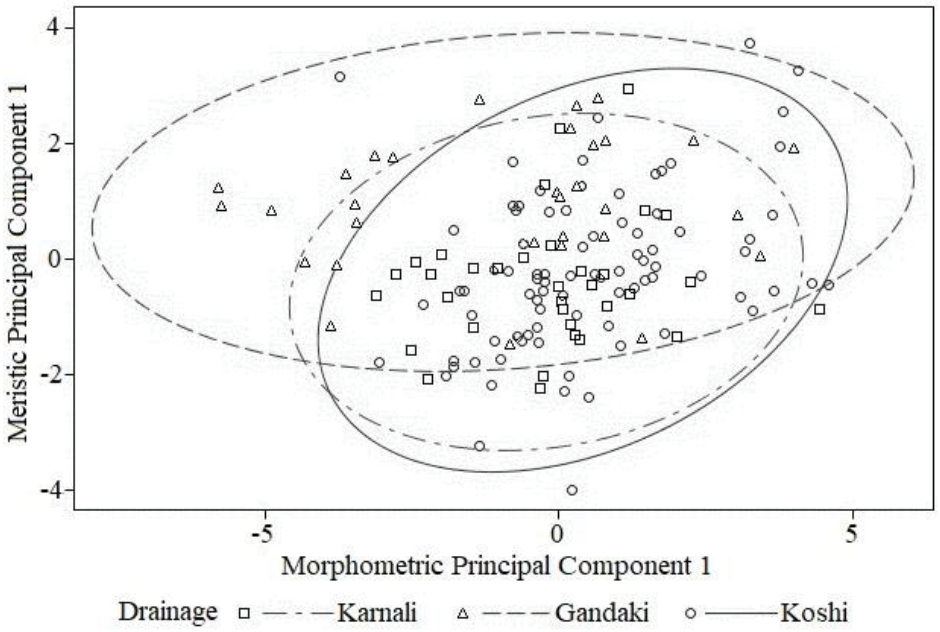

Figure 6. Scatter plot of individual scores on meristic PC1 versus morphometric PC1 for Nepal *B. badis* (n = 153), with 95% prediction ellipses for major river drainages, west to east.

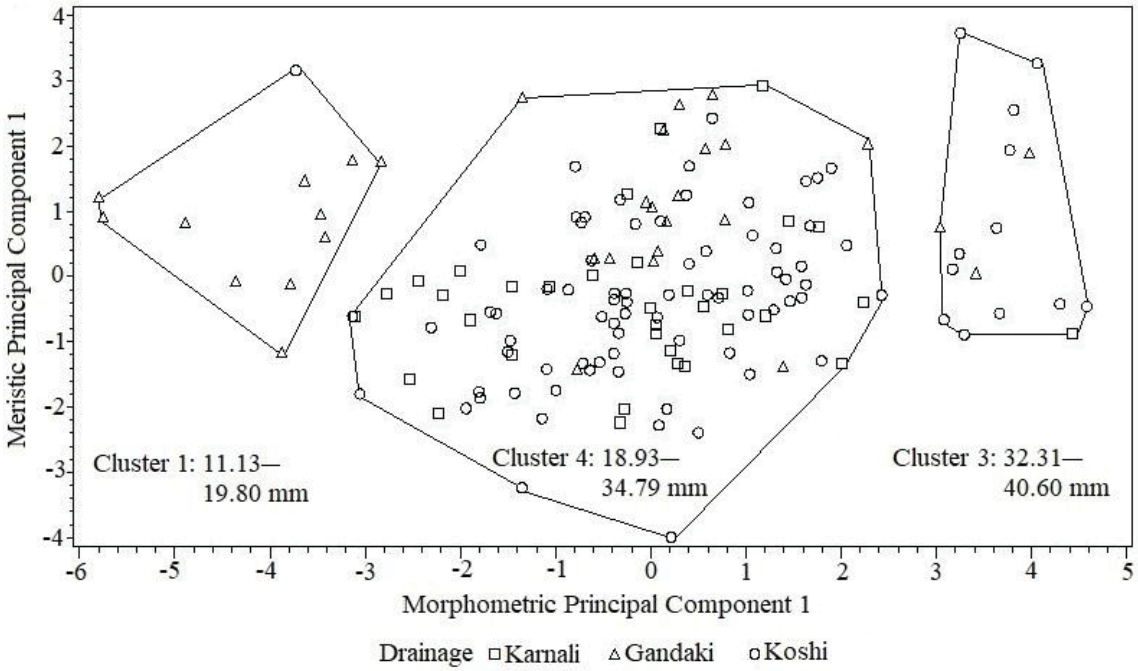

Figure 7. Scatter plot of individual scores on meristic PC1 versus morphometric PC1 for Nepal *B. badis* (n = 153) separated by cluster. Note differences in size among clusters. Symbols correspond to distribution within major river drainages.

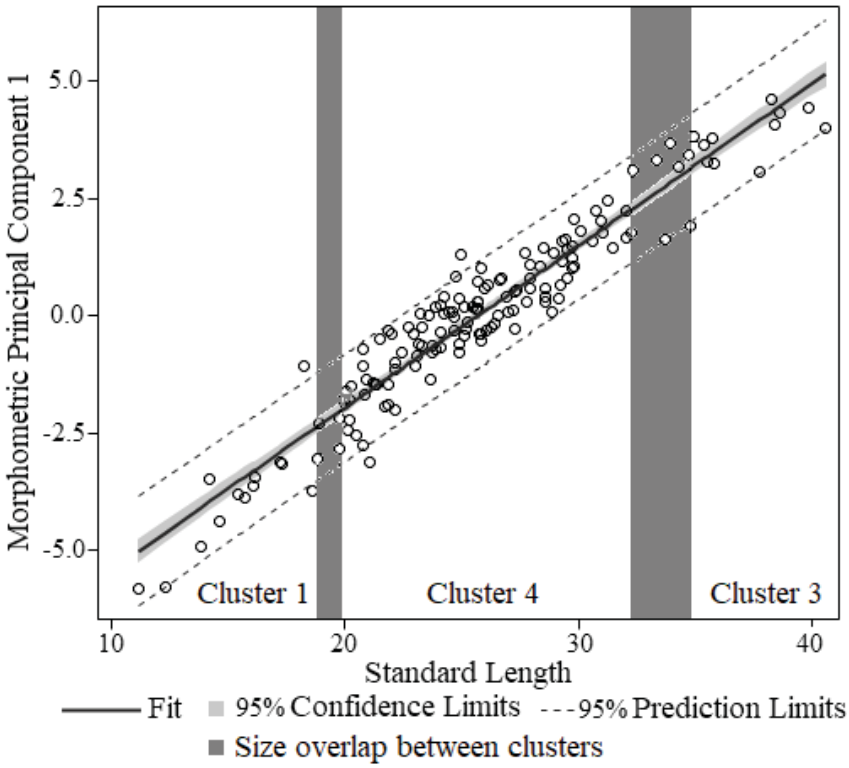

Figure 8. Regression ($p < 0.0001$, $R^2 = 0.92$) of morphometric PC1 versus standard length of *B. badis* specimens from Nepal (n = 153) showing allometric growth, with extent of cluster overlap indicated by width of vertical bars.

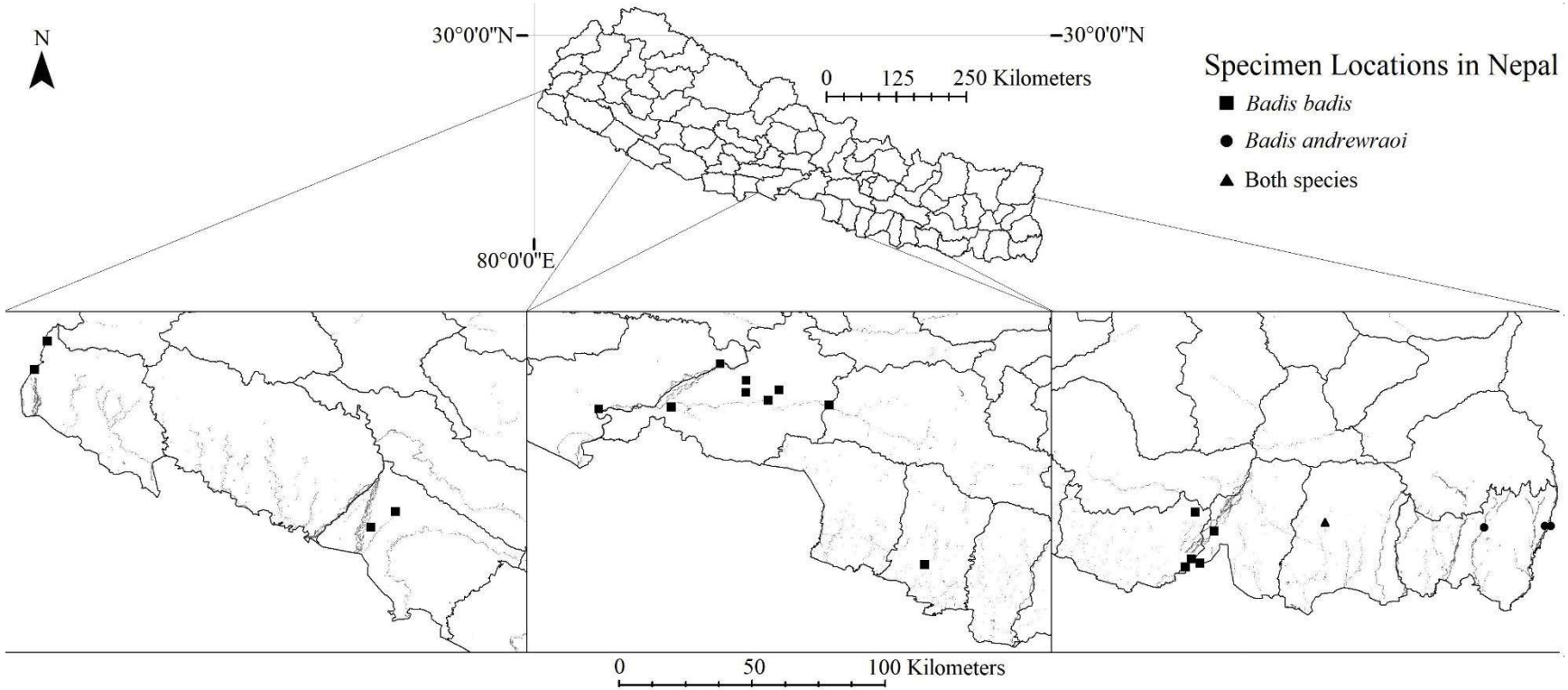

Figure 9. Collection localities for this study, including sites with only *B. badis*, only *B. andrewraoi*, and both species. Panels left to right show western Nepal, including Karnali River drainage; central Nepal, including Gandaki River drainage; and eastern Nepal, including Koshi and Mechi (Mahananda/Fulahar) river drainages.

Table 1. Loadings for PCA of 15 meristic or color pattern characters and nine morphometric characters for *Badis* specimens (n = 172) from Nepal.

Character	Meristic PC1	Meristic PC2
Dorsal fin spines	-0.36554	0.04913
Dorsal fin rays	0.35870	-0.03196
Anal fin rays	0.42293	-0.04934
Pectoral fin rays	0.41911	0.20585
Scales in lateral row	0.06638	0.27321
Anterior lateral line scales	0.10976	0.43146
Posterior lateral line scales	0.06578	0.39915
Gill rakers	0.04412	-0.30108
Dentary cranial pores	-0.06296	-0.11746
Lachrymal cranial pores	-0.10882	-0.14021
Abdominal vertebrae	-0.29372	0.36311
Caudal vertebrae	0.17105	0.17293
Vertical bars	0.05907	-0.26397
Scales in postorbital stripe	-0.11744	-0.37325
Cleithral blotch	-0.46028	0.19178
	Morphometric PC1	
Head length	0.27045	
Snout length	0.04229	
Orbital diameter	-0.00338	
Interorbital width	0.15473	
Upper jaw length	0.14394	
Lower jaw length	0.15852	
Body depth	0.59320	
Pelvic fin length	0.41053	
Pelvic anal distance	0.57866	

Table 2. Loadings for PCA of 14 merisitic or color pattern characters and nine morphometric characters for *B. badis* specimens (n = 153) from Nepal.

Character	Meristic PC1
Dorsal fin spines	-0.08728
Dorsal fin rays	0.16530
Anal fin rays	0.18492
Pectoral fin rays	0.45687
Scales in lateral row	0.36345
Anterior lateral line scales	0.38175
Posterior lateral line scales	0.33640
Gill rakers	-0.25004
Dentary cranial pores	0.01045
Lachrymal cranial pores	-0.08902
Abdominal vertebrae	0.21002
Caudal vertebrae	0.21416
Vertical bars	-0.20658
Scales in postorbital stripe	-0.35769
	Morphometric PC1
Head length	0.25988
Snout length	0.04020
Orbital diameter	-0.00572
Interorbital width	0.15735
Upper jaw length	0.13866
Lower jaw length	0.15154
Body depth	0.59760
Pelvic fin length	0.39791
Pelvic anal distance	0.59026

Table 3. Morphological comparison of Nepalese specimens of *B. andrewraoi* (n = 19) and *B. badis* (n = 153) as minimum, maximum, and mean (SD), expressed as a percentage of standard length (SL) or modal meristic count.

	B. andrewraoi		*B. badis*	
Morphometrics	Min–Max	Mean (SD)	Min–Max	Mean (SD)
Standard length (SL) in mm	16.92–35.39	27.76 (4.94)	11.13–40.60	25.70 (5.64)
As percentage of SL				
Head length	29.75–34.18	31.70 (1.31)	26.72–36.51	32.07 (1.83)
Snout length	5.03–9.04	7.10 (0.88)	5.01–8.64	6.86 (0.80)
Orbital diameter	8.56–11.23	9.64 (0.74)	8.34–13.98	10.68 (1.09)
Interorbital width	5.18–9.36	6.43 (1.03)	4.08–9.62	7.20 (0.98)
Upper jaw length	7.01–9.53	8.49 (0.70)	4.90–9.95	8.35 (1.08)
Lower jaw length	8.02–11.26	10.06 (0.80)	7.81–13.17	10.51 (1.14)
Body depth	26.52–32.33	28.57 (1.32)	26.62–35.02	31.67 (1.71)
Pelvic fin length	21.22–28.91	24.75 (1.84)	20.33–27.92	24.86 (1.36)
Pelvic to anal distance	29.73–32.36	30.99 (0.75)	26.83–37.05	32.12 (1.89)

Meristics	Min–Max	Mode (SD)	Min–Max	Mode (SD)
Dorsal fin spines	15–17	15 (0.60)	14–18	16 (0.79)
Dorsal fin rays	8–11	9 (0.69)	7–12	9 (0.86)
Anal fin rays	6–8	7 (0.60)	5–8	7 (0.54)
Pectoral fin rays	12–15	12 (0.91)	10–15	12 (0.72)
Scales in lateral row	24–29	26 (1.25)	24–33	27 (1.50)
Lateral line scales	16–22/1–4	21/4 (1.65/1.11)	12–24/0–7	20/3 (2.74/1.25)
Gill rakers	6–7	7 (0.36)	4–8	7 (0.54)
Dentary pores	3–4	3 (0.22)	3–4	3 (0.11)
Lachrymal cranial pores	3	3 (0.00)	2–4	3 (0.23)
Caudal vertebrae	13–15/12–15	14 (0.49)	13–15	14 (0.31)
Abdominal vertebrae	12–15	13 (0.68)	12–14	13 (0.50)
Vertical bars	11	11 (0.00)	9–12	11 (0.35)
Scales in postorbital stripe	3	3 (0.00)	1–5	3 (0.80)
Cleithral blotch	Absent	Absent	Present	Present

Table 4. Frequency distribution of meristic counts of *B. andrewraoi* (n = 19 unless damaged) and *B. badis* (n = 153 unless damaged) specimens from Nepal. An en dash (–) indicates that no specimen exhibited that count.

Species	n	Character													
		Dorsal fin spines/rays													
		14/9	15/7	15/8	15/9	15/10	15/11	16/7	16/8	16/9	16/10	16/12	17/7	17/8	17/9
B. andrewraoi	19	–	–	–	9	1	1	–	3	3	1	–	–	–	1
B. badis	150	3	2	2	13	3	–	6	20	36	10	1	5	24	18
		17/10	18/7	18/8	18/10										
		–	–	–	–										
		3	1	1	2										
		Anal fin rays													
		5	6	7	8										
B. andrewraoi	19	–	4	12	3										
B. badis	149	2	66	80	1										
		Pectoral fin rays													
		10	11	12	13	14	15								
B. andrewraoi	19	–	–	10	5	3	1								
B. badis	151	1	16	91	37	5	1								
		Scales in a lateral row													
		24	25	26	27	28	29	30	31	33					
B. andrewraoi	18	1	3	7	5	–	2	–	–	–					
B. badis	146	6	13	29	41	37	11	7	1	1					

		Lateral line scales													
		12/3	13/2	13/3	13/4	14/0	14/3	14/4	15/2	15/3	16/2	16/3	17/0	17/1	17/2
B. andrewraoi	17	–	–	–	–	–	–	–	–	–	1	–	–	1	–
B. badis	108	1	1	1	1	1	4	2	1	2	1	1	1	–	1

		17/3	17/4	17/6	18/0	18/1	18/2	18/3	18/4	19/2	19/3	19/4	19/5	20/2	20/3
		–	–	–	–	2	–	1	–	–	–	2	–	–	1
		2	1	1	3	–	2	6	3	5	3	2	2	3	11

		20/4	20/5	21/2	21/3	21/4	21/5	21/6	21/7	22/3	22/4	22/5	22/7	23/3	24/4
		2	–	1	2	3	–	–	–	–	–	–	–	–	–
		7	3	3	2	4	1	1	1	5	10	3	1	2	2

		Gill rakers			
		4	6	7	8
B. andrewraoi	19	–	3	16	–
B. badis	153	1	58	93	1

		Dentary cranial pores	
		3	4
B. andrewraoi	19	18	1
B. badis	153	151	2

		Lachrymal cranial pores		
		2	3	4
B. andrewraoi	19	–	19	–
B. badis	152	1	144	7

		Vertebrae (abdominal/caudal)							
		13/13	14/12	14/13	14/14	15/12	15/13	15/14	15/15
B. andrewraoi	19	4	1	5	8	–	–	–	1
B. badis	153	1	4	94	39	1	12	2	–

Table 5. Weighted average (SD) water chemistry parameters from two locations for *B. andrewraoi* (n = 9) and eight locations for *B. badis* (n = 64) from Nepal.

Parameter	*B. andrewraoi*	*B. badis*
Total acidity (mg/l)	45.6 (27.0)	11.5 (0.8)
Total alkalinity (mg/l)	41.0 (32.4)	37.2 (20.1)
Total hardness (mg/l $CaCO_3$)	105 (83.0)	120 (57.2)
pH	7.4 (0.5)	8.0 (0.0)
CO_2 (mg/l)	11.6 (7.9)	10.0 (1.6)
Dissolved oxygen (mg/l)	5.6 (1.6)	8.5 (1.1)

I, William Kenneth Blair, hereby submit this thesis/report to Emporia State University as partial fulfillment of the requirements for an advanced degree. I agree that the Library of the University may make it available to use in accordance with its regulations governing materials of this type. I further agree that quoting, photocopying, digitizing or other reproduction of this document is allowed for private study, scholarship (including teaching) and research purposes of a nonprofit nature. No copying which involves potential financial gain will be allowed without written permission of the author. I also agree to permit the Graduate School at Emporia State University to digitize and place this thesis in the ESU institutional repository.

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