# Geographic and environmental variation in *Bryconops* sp. cf. *melanurus* (Ostariophysi: Characidae) from the Brazilian Pantanal

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**Abstract** Morphometric analyses of 220 specimens of a characid, *Bryconops* sp. cf. *melanurus*, from the Brazilian Pantanal were used to describe allometric growth in that species and determine whether specimens from highland habitats were more streamlined than those from lowland habitats. Relative warp analysis of 14 landmarks and principal component analysis of 28 interlandmark distances returned complementary results. The increased streamlining of the highland specimens is highly consistent with known inductive effects of high water velocity on fish phenotypes. Genetic differentiation and inductive effects of temperature variation are also potential explanations of the observed phenotypic differentiation.

**Key words** Ecomorphology · Morphometrics · Polymorphism · South America

Individuals of single fish species that inhabit different geographic regions or are exposed to different environmental conditions frequently exhibit different phenotypes (Gould and Johnston, 1972; Brett, 1979; Schlichting and Pigliucci, 1996). Among the many environmental factors that can induce intraspecific variation in fishes, the effects of differences in temperature (Hubbs, 1922; Barlow, 1961; Beacham, 1990), water velocity (Claytor et al., 1991; McLaughlin and Grant, 1994; Imre et al., 2002), and microhabitat (Lundberg and Stager, 1985; Layzer and Clady, 1987; O'Reilly and Horn, 2004) are among the best documented. Despite extensive research on the structure and cause of phenotypic variation in fishes from the Northern Hemisphere (most foregoing citations), there have been fewer studies in fishes from South American freshwaters (but see Lundberg and Stager, 1985; Wimberger, 1992; Fink and Machado-Allison, 2001; Langerhans et al., 2003). Many South American species are known from only a handful of collections, and even widespread species may be poorly collected or rare at any given locality. Therefore, large series of South American conspecifics from a variety of localities and habitats may provide uncommon opportunities to examine the geographic and environmental structure of phenotypic variation.

Between 24 August and 14 September 1998, we collected a large series of an undescribed characid fish species, *Bryconops* sp. cf. *melanurus*, from 23 localities in the world's largest wetland, the Pantanal of Mato Grosso do Sul, Brazil (Fig. 1). *Bryconops* sp. cf. *melanurus* from the Pantanal belongs to a monophyletic group including *B. melanurus* (Bloch), *B. inpai* (Knöppel et al.), and *B. affinis* (Günther) (Chernoff and Machado-Allison, 1999).

*Bryconops* sp. cf. *melanurus* and *Bryconops melanurus* are distinguished from the other members of this group by the coloration of the caudal fin. The caudal fins of *Bryconops* sp. cf. *melanurus* and *B. melanurus* have a central dark stripe that is lacking in all other species of *Bryconops*. *Bryconops melanurus* has a clearly defined stripe that occupies the central rays of the caudal fin with clear areas above and below the stripe (Chernoff et al., 1994: fig. 2). In the species from the Pantanal, the caudal fin stripe extends well up onto the fin rays of the dorsal lobe, and in larger specimens almost the entire dorsal lobe of the caudal fin is darkened. *Bryconops*. sp. cf. *melanurus* and *B. melanurus* also differ in the thickness of the lateral stripe, the anteroposterior position of the pelvic-fin insertion, and the degree of denticulation of the gill rakers of the first pharyngeal arch. The two species also inhabit nonoverlapping geographic ranges, with *B. melanurus* occurring in the Guyanas (Chernoff et al., 1994).

Because this sample of *Bryconops* sp. cf. *melanurus* was collected from a variety of highland and lowland stream habitats, it provided an excellent opportunity to study intraspecific phenotypic variation in the context of environmental and geographic variation. Our objectives in this study were (1) to quantify the phenotypic variation of

*Bryconops* sp. cf. *melanurus*, and (2) to determine whether geographic and/or environmental categories defined phenotypically differentiated groups in this sample of this species.

## Materials and Methods

*Specimens examined.*—After excluding badly contorted individuals, we measured 220 specimens of *Bryconops* sp. cf. *melanurus* ranging from 20 mm to 79 mm standard length, collected from 23 sampling localities. These specimens represent a complete postlarval ontogenetic series. They are catalogued in The Field Museum of Natural History (FMNH 108397–108419). Specific locality information, including longitude and latitude, is available at http://fm1.fieldmuseum.org/collections/search.cgi?dest=fish. To our knowledge, no other examples of this species exist in collections.

*Classification of specimens.*—The 23 sampling localities were classified into five geographic regions: Anhumas River, lower Negro River, middle Negro River, Miranda River, and Taboco River (Fig. 1, Table 1). These five regions were recognized on the bases of hydrologic separation among rivers, geographic separation among the sampling localities, differences in the structure of the fish and riparian plant communities, and differences in geology and soil type (Willink et al., 2000). Collection localities in the Anhumas River and middle and lower Negro River all lay within the Pantanal wetlands at low elevations. Exact elevations were not recorded, but the Pantanal wetlands are known to be between 80 and 180 m above sea level (Assine and Soares, 2004) and the sampling localities were situated well within the main topographic depression that forms the Pantanal. Therefore, the collection localities in the Negro and Anhumas Rivers are at the lower end of the range of elevations cited by Assine and Soares (2004), approximately 100 m above sea level. Conversely, the collection localities in the Miranda and Taboco Rivers lie in the highlands that surround the Pantanal wetlands. These highland localities are at 200 m above sea level or higher (Assine and Soares, 2004) and occur in a region characterized by cerrado vegetation or semideciduous forest as opposed to wetland vegetation.

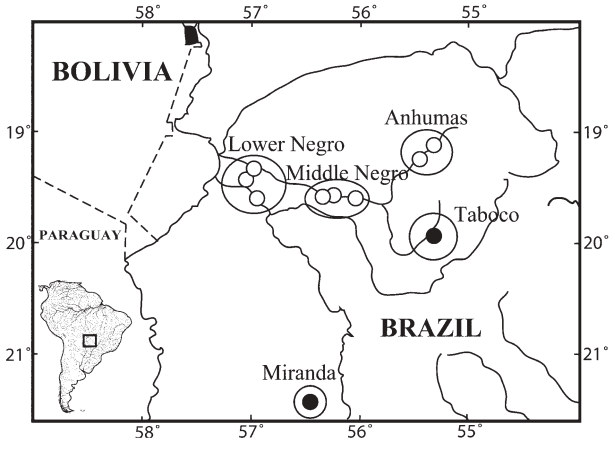


**Fig. 1.** Collection map for the Pantanal. *Open circles*, lowland localities; *filled circles*, highland localities. *Some symbols in the lowland regions* represent multiple collection localities

We also used the published locality information (Willink et al., 2000) to assign each sampling locality to one of four habitat types: river channels, backwaters, swamps, and springs (Table 1). The river channel category includes main channels as well as localities near the banks of main channels. The backwater category includes backwaters, sloughs, and lagoons. The swamp category represents muddy, blackwater marshes with abundant submerged vegetation. The spring category represents narrow streams with swift, clear water running over bedrock and sand. The two specimens in FMNH 108412 were not classified in a habitat type and were excluded from the habitat-based analysis because of ambiguity in the published habitat information (Willink et al., 2000).

*Landmarks and data collection.*—To quantify the phenotypic variation of *Bryconops* sp. cf. *melanurus*, we used relative warp analysis (RWA) (Bookstein, 1989, 1991; Rohlf, 1993) on 14 digitized landmarks per specimen (Fig. 2) and principal components analysis (PCA) on 28 interlandmark measurements (Fig. 2; Table 2). Our choice of landmarks mirrors studies of other *Bryconops* species (Machado-Allison et al., 1996; Chernoff and Machado-Allison, 1999).

**Table 1.** Number of specimens collected from each geographic region and environmental category, including all examined *Bryconops* sp. cf. *melanurus* minus a small number of contorted specimens

| | River channel | Backwater | Swamp | Spring | Ambiguous | Total |
|---|---|---|---|---|---|---|
| Lowland | | | | | | |
| Anhumas | 1 | 35 | — | — | — | 36 |
| Lower Negro | 37 | 56 | 1 | — | 2 | 96 |
| Middle Negro | 47 | 10 | 4 | — | — | 61 |
| Highland | | | | | | |
| Miranda | 15 | — | — | — | — | 15 |
| Taboco | — | — | — | 12 | — | 12 |
| Total | 100 | 101 | 5 | 12 | 2 | 220 |

**Table 2.** Eigenvalues and eigenvector loadings for PC1–4

| | | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|---|
| Eigenvalue | | 2.133 | 0.022 | 0.011 | 0.007 |
| Percent Eigenvalue | | 97.98 | 1.02 | 0.49 | 0.31 |
| LM | Distance | Eigenvector loadings | | | |
| | | PC1 | PC2 | PC3 | PC4 |
| 1, 6 | SL | 0.185 | –0.018 | –0.082 | 0.026 |
| 1, 2 | DORHEAD | 0.143 | 0.046 | 0.046 | 0.069 |
| 1, 3 | PREDORS | 0.172 | 0.020 | 0.022 | 0.005 |
| 1, 10 | PREPECT | 0.165 | 0.112 | 0.063 | 0.115 |
| 1, 9 | PREPELV | 0.173 | 0.028 | 0.032 | 0.042 |
| 1, 8 | PREANAL | 0.184 | 0.006 | 0.016 | –0.015 |
| 3, 9 | BDEPTH | 0.221 | –0.059 | 0.091 | –0.246[a] |
| 3, 4 | DBASE | 0.211 | –0.153 | 0.202[a] | –0.377[a] |
| 7, 8 | ABASE | 0.201 | 0.012 | –0.018 | 0.036 |
| 4, 5 | INTERDOR | 0.195 | 0.001 | –0.082 | 0.075 |
| 6, 7 | CPEDLENG | 0.193 | –0.167 | –0.656[a] | 0.149 |
| 5, 7 | AD_ATERM | 0.212 | –0.073 | 0.019 | –0.220[a] |
| 4, 8 | DTER_AOR | 0.227 | –0.062 | 0.026 | –0.191 |
| 4, 9 | DTER_P2O | 0.218 | –0.066 | 0.088 | –0.280[a] |
| 3, 8 | DOR_AOR | 0.225 | –0.077 | 0.045 | –0.205[a] |
| 5, 8 | AD_AORIG | 0.207 | –0.032 | –0.015 | –0.089 |
| 5, 6 | AD_HYP | 0.198 | –0.136 | –0.527[a] | 0.136 |
| 4, 7 | DTER_ATE | 0.202 | –0.001 | –0.043 | 0.034 |
| 3, 10 | DORIG_P1 | 0.197 | –0.054 | 0.018 | –0.117 |
| 9, 10 | P1_P2 | 0.183 | –0.070 | –0.006 | –0.025 |
| 1, 14 | HEAD | 0.156 | 0.128 | 0.114 | 0.148 |
| 12, 13 | EYE | 0.151 | –0.033 | 0.220[a] | 0.269[a] |
| 1, 12 | SNOUT | 0.153 | 0.913[a] | –0.168 | –0.144 |
| 1, 11 | JAW | 0.177 | 0.152 | 0.130 | 0.232[a] |
| 13, 14 | POSTORB | 0.163 | 0.005 | 0.130 | 0.150 |
| 11, 12 | MAX_AORB | 0.190 | –0.089 | 0.224[a] | 0.413[a] |
| 11, 13 | MAX_PORB | 0.182 | 0.009 | 0.183 | 0.372[a] |
| 11, 9 | MAX_P2 | 0.172 | –0.026 | –0.012 | 0.002 |

LM column indicates landmarks used to calculate each distance (see Fig. 2)

[a] Loadings with absolute magnitude greater than 0.20

We inserted size 0000 insect pins into each specimen under a dissecting microscope to mark the precise location of 13 of the landmarks. We did not pin the tip of the snout.

We suspended each pinned specimen approximately 1 cm above the surface of a Hewlett-Packard Scanjet ADF flatbed scanner and captured images as 600 dpi TIFF files without smoothing or sharpening. Color and contrast were adjusted to improve clarity. Trials with graph paper at the same height above the surface indicate that the imaging error of this method is ≤0.003 mm.

Landmarks were digitized using TPSDIG ver. 1.20 (Rohlf, 1998a). We tested precision by digitizing five specimens five times each and calculating the error variance at each landmark for each specimen. Error variances range from 0.05 $mm^2$ to 0.12 $mm^2$ and are much less than the variance among specimens.

We transformed the 28 distance variables to natural logs (ln) to render their variances independent of their means and linearize allometries (Bookstein et al., 1985; Sokal and Rohlf, 1995). We computed the principal components (PCs) from the variance–covariance matrix of ln distances in Statistica BASIC (StatSoft, 2002). Landmark configurations were scaled to unit centroid size (Bookstein et al., 1985) and aligned to their consensus with Generalized Procrustes Analysis (GPA) (Rohlf and Slice, 1990; Dryden and Mardia, 1998) in TPSRELW ver. 1.18 (Rohlf, 1998b). Relative warps (RWs) were calculated from aligned specimens ($\alpha = 0$) with TPSRELW (Rohlf, 1998b). Regression of distance in tangent space on Procrustes distance in TPSSMALL ver. 1.19 (Rohlf, 1998c), indicated that shape variation in this dataset is small enough to be linear in the tangent plane.

*Statistical analyses*.—To determine whether geographic and/or environmental categories defined phenotypically differentiated groups in this sample of *Bryconops* sp. cf. *melanurus*, we used a series of analyses of covariance (ANCOVA) and multivariate analyses of variance

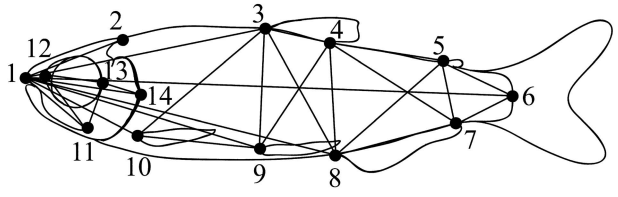


**Fig. 2.** Landmarks and distances used in morphometric analysis: (*1*) tip of the snout; (*2*) posteriormost tip of supraoccipital crest; (*3*) dorsal-fin origin, marked at the anterior junction of the first ray with the dorsal midline; (*4*) posterior end of the dorsal-fin base, marked at the junction of the last fin ray with the dorsal midline; (*5*) anterior junction of the adipose fin with the dorsal midline; (*6*) posterior margin of hypural plate, marked along the body midline; (*7*) posterior end of anal-fin base, marked at the junction of the last anal ray with the ventral midline; (*8*) anterior end of the anal-fin base, marked at the junction of the first anal ray with the ventral midline; (*9*) left pelvic-fin insertion; (*10*) left pectoral-fin insertion; (*11*) posterior tip of the left maxilla; (*12*) anteriormost point along bony margin of the orbit; (*13*) posteriormost point along bony margin of the orbit; (*14*) posteriormost point of the opercle

(MANOVA) partitioned by region or habitat. These analyses were carried out on selected sets of eigenvectors or relative warps with nonequivalent eigenvalues or singular values as determined by Anderson's test for equivalency of eigenvalues (Anderson, 1963; Morrison, 1990:336). Because such sets of eigenvectors describe the unique major axes of elliptical distributions, they contain most or all biologically meaningful variation.

We analyzed the environmental and geographic structure of allometric PCs and RWs by analyses of covariance (ANCOVAs) using ln centroid size as the covariate. Allometric PCs and RWs were identified by their significant correlation with centroid size (see Results). We also compared PCs to a vector of perfect isometry (Anderson, 1963; Morrison, 1990:337) to distinguish allometric shape change from pure size change. Where overall ANCOVA comparisons were significant, we calculated a series of pairwise ANCOVAs post hoc to evaluate the significance of each possible pairwise comparison between regions or habitats, thereby determining which pairs of categories contained specimens with significantly different growth allometries. By analyzing allometric components separately from nonallometric components, we avoided diagnosing shape differences among samples that differed only in the size of individuals.

We analyzed the environmental and geographic structure of the nonallometric PC and RW scores with a series of one-way MANOVAs, a technique commonly used in the analysis of categorized morphometric data (Zelditch et al., 2004). Multiway MANOVA cannot be applied to this dataset, because not all habitat types occur in all regions and the precise amount of variance explained by each factor cannot be calculated (Zelditch et al., 2004). Where overall MANOVA comparisons were significant, we calculated Tukey honest significant differences, unequal *N* (HSD) post hoc to evaluate the significance of each possible pairwise comparison between regions or habitats. The post hoc tests determined which pairs of regions or habitats contained specimens with significantly different mean body shapes in this sample of *Bryconops* sp. cf. *melanurus*.

To confirm that the difference between highland (Miranda and Taboco) and lowland (Anhumas and lower and middle Negro) regions obtained by the multivariate tests (see Results) reflected significant differences in the univariate distances, we performed univariate ANCOVAs with ln centroid size as the covariate on the 14 ln-transformed interlandmark distances (50%) that most strongly influenced the specimen scores on the PCs and RWs that differed between elevations. We identified the 14 distances in the following manner: for the allometric PC1 we chose the distances corresponding to the most allometric loadings, namely those greater than 0.21 or less than 0.16. For the nonallometric PC4 we chose all measurements with loadings of absolute magnitude 0.20 or greater. For RW1 and RW3 we compared the deformation grids corresponding to the endpoints of the observed variation, determined qualitatively which landmarks differed most between the extremes, and chose the distances linking those landmarks. All distances contributing to at least one significant PC or RW were included in the univariate ANCOVAs. To ensure that we compared populations of similar size distributions, we removed the 156 smallest specimens from the lowland group (Anhumas, upper and middle Negro) and the single smallest specimen from the highland group (Miranda, Taboco). The exclusion of these specimens equalized the centroid size means of the subgroups at 72.9 $mm^2$ and approximately equalized the centroid size ranges (highland range, 57.0–95.2 $mm^2$; lowland range, 64.6–102.0 $mm^2$). We calculated the significance of each univariate ANCOVA and tested for homogeneity of variances (Levene's test) and slopes. We also calculated the mean and standard deviation of each distance for both subgroups.

Because of multiple uses of the same data, we used the sequential Bonferroni procedure (Rice, 1989) to correct the results from all tests of phenotypic difference at group-wide type I error rates of 5%. Significant *P* values are reported only in relation to the corrected critical values.

## Results

**Interlandmark distances.** Four principal components accounted for 99.8% of the variance among the interlandmark distances (Table 2) and included all variation that was distinguishable from error (eigenvalue equivalency of PC3,4, $\chi^2_{[2]} = 11.9$, $P < 0.050$). PC1 (98.0% of variance) had all loadings of the same sign and similar magnitudes and correlated perfectly with centroid size ($R = 1.00$). Anderson's (1963) test of isometry rejected PC1 as a vector of isometric growth ($P < 0.0010$). Therefore, PC1 was an ontogenetic trajectory describing allometric shape change linked to increases in size.

The allometric change described by PC1 (Table 2) included a proportionally greater increase in body depth (BDEPTH, DOR_AOR, and DTER_AOR) and smaller increase in the head measures (HEAD, EYE, SNOUT, JAW, and DORHEAD) with respect to increase in size. PC2–4

**Table 3.** Results from separate one-way analyses of covariance (ANCOVAs) of PC1 or RW1 with ln centroid size and multivariate analyses of variance (MANOVAs) of PC2–4 or RW2–3, partitioned by geography (region) and environment (habitat)

| Effect | Data | Wilks' λ | Rao's *R* | *F* | Homogeneity of slopes: *P* | Levene's test: *P* | *DF* 1 | *DF* 2 | *P* value | Critical value |
|---|---|---|---|---|---|---|---|---|---|---|
| Region | PC1 | — | — | 6.51 | 0.412 | <0.001[a] | 4 | 214 | <0.001[a] | 0.025 |
| | PC2–4 | 0.631 | 8.93 | — | — | — | 12 | 563 | <0.001[a] | 0.025 |
| | RW1 | — | — | 15.5 | 0.012[a] | <0.001[a] | 4 | 214 | <0.001[a] | 0.025 |
| | RW2–3 | 0.876 | 3.68 | — | — | — | 8 | 428 | <0.001[a] | 0.025 |
| Habitat | PC1 | — | — | 5.69 | 0.103 | 0.031[a] | 3 | 213 | <0.001[a] | 0.050 |
| | PC2–4 | 0.925 | 1.87 | — | — | — | 9 | 516 | 0.054 | 0.050 |
| | RW1 | — | — | 2.35 | <0.001[a] | 0.002[a] | 3 | 213 | 0.073 | 0.050 |
| | RW2–3 | 0.957 | 1.57 | — | — | — | 6 | 426 | 0.156 | 0.050 |

We performed separate sequential Bonferroni corrections for each dataset (PC1, PC2–4, RW1, and RW 2–3) at a group-wide type I error rate of 5% with two comparisons per dataset; corrected critical values appear in the final column

Determinations of the significance of the results of Levene's tests and tests of homogeneity of slopes are not Bonferroni corrected

[a] Significant *P* values

**Table 4.** Significance of post hoc pairwise ANCOVAs (PC1 and RW1) and HSD tests (PC2–4 and RW2–3) for differences among regions

| Comparison | PC1 | | | PC2 | PC3 | PC4 | RW1 | | | RW2 | RW3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *P* | Homogeneity of slopes: *P* | Levene's test: *P* | *P* | *P* | *P* | *P* | Homogeneity of slopes: *P* | Levene's test: *P* | *P* | *P* |
| Middle Negro × Lower Negro | 0.202 | 0.083 | <0.001[a] | 0.718 | 0.818 | 0.999 | 0.668 | 0.012[a] | <0.001[a] | 0.746 | 0.996 |
| Middle Negro × Anhumas | 0.002[a] | 0.748 | 0.001[a] | 0.910 | 0.908 | 0.042 | 0.404 | 0.356 | 0.004[a] | 1.00 | 0.889 |
| Middle Negro × Taboco | 0.002[a] | 0.425 | 0.013[a] | 0.369 | 0.991 | 0.026 | <0.001[a] | 0.108 | 0.006[a] | 0.981 | 0.391 |
| Middle Negro × Miranda | 0.089 | 0.650 | <0.001[a] | 0.132 | 0.634 | <0.001[a] | <0.001[a] | 0.177 | <0.001[a] | 1.00 | 0.014 |
| Lower Negro × Anhumas | 0.547 | 0.394 | 0.831 | 1.000 | 1.000 | 0.075 | 0.175 | 0.010[a] | 0.534 | 0.904 | 0.968 |
| Lower Negro × Taboco | 0.001[a] | 0.820 | 0.570 | 0.125 | 0.881 | 0.038 | 0.004[a] | 0.414 | 0.219 | 0.811 | 0.300 |
| Lower Negro × Miranda | 0.175 | 0.340 | 0.032[a] | 0.025 | 0.304 | <0.001[a] | <0.001[a] | 0.427 | 0.006[a] | 0.995 | 0.008[a] |
| Anhumas × Taboco | 0.162 | 0.514 | 0.315 | 0.143 | 0.870 | 0.667 | 0.288 | 0.043[a] | 0.129 | 0.978 | 0.144 |
| Anhumas × Miranda | 0.079 | 0.573 | 0.002[a] | 0.031 | 0.289 | 0.001[a] | <0.001[a] | 0.052 | 0.003[a] | 1.00 | 0.002[a] |
| Taboco × Miranda | 0.004[a] | 0.351 | 0.046[a] | 0.999 | 0.936 | 0.210 | 0.349 | 0.912 | 0.120 | 0.948 | 0.833 |

Each PC or RW was treated as an independent dataset with ten comparisons for the purposes of Bonferroni correction

Corrected critical values for the significant PC and RW comparisons range from 0.005 to 0.008

Determinations of the significance of the results of Levene's tests and tests of homogeneity of slopes are not Bonferroni corrected

[a] Significant *P* values after sequential Bonferroni correction for group-wide type I error rates of 5%

did not positively or negatively correlate with centroid size ($R = 0.00$) and represented only shape variation. Although the shape differences described by these three components accounted for a much smaller percentage of variance than the quadrupling of scale and associated shape change included in PC1, PC2–4 were unique axes. PC2 described variation in snout length (SNOUT) and PC3 described primarily variation in caudal peduncle length (CPEDLENG and AD_HYP). PC4 described variation in the length of the dorsal-fin base (DBASE), the length of the jaw (JAW, MAX_AORB, and MAX_PORB), the diameter of the eye (EYE), and the depth of the body (BDEPTH) (Table 2).

After sequential Bonferroni correction, ANCOVA of the PC1 scores with centroid size indicated significant differences among regions ($P < 0.025$) and habitats ($P < 0.05$) (Table 3). Neither comparison rejected homogeneity of slopes but both rejected homogeneity of variances. Because ANCOVA is known to be robust to deviation from homogeneity of variances (Sokal and Rohlf, 1995), the significance of Levene's test in this and other ANCOVAs did not invalidate the overall result. Pairwise ANCOVAs among regions revealed differences between the Taboco region and all regions except the Anhumas (Table 4). There was also a significant difference between the middle Negro and Anhumas regions. All post hoc ANCOVAs for regional differences on PC1 satisfied the homogeneity of slopes assumption, although variances were frequently not homogeneous. Pairwise ANCOVAs on PC1 among habitats revealed differences between the sample from the spring habitat and those from all other habitats (Table 5). Due to heterogeneity of slopes, the significant difference obtained by pairwise comparison between springs and swamps may not indicate a consistent shape difference across all stages of ontogeny.

MANOVAs on the scores from PC2–4 indicated significant differences among regions ($P \leq 0.025$) but not among habitats (Table 3). The post hoc HSD tests identified significant differences on PC4 between the fish from the Miranda River and fish from the upper and middle Negro River and the Anhumas River but not the Taboco River (Fig. 3, Table 4).

**Landmark configurations.** Anderson's (1963) test rejected equivalency of singular values for RW1–3 ($\chi^2_{[3]} = 30.7$, $P < 0.05$) but failed to reject equivalency among the higher numbered RWs. Therefore RW1–3 contained all the variation among landmark configurations that was distinguishable from error. Together, RW1–3 summarized 62.8% of total shape variation, with 38.6% in RW1, 15.4% in RW2, and 8.9% in RW3.

Figure 4 illustrates the range of variation described by RW1–3. Each image in Fig. 4 represents an extreme of shape

**Table 5.** Significance of post hoc pairwise ANCOVAs on PC1 for differences among habitats

| Comparison | *P* | Homogeneity of slopes: *P* | Levene's test: *P* |
|---|---|---|---|
| Channels × backwaters | 0.061 | 0.596 | 0.132 |
| Channels × springs | <0.001[a] | 0.629 | 0.228 |
| Channels × swamps | 0.560 | 0.035[a] | 0.116 |
| Backwaters × springs | 0.006[a] | 0.468 | 0.028[a] |
| Backwaters × swamps | 0.156 | 0.010[a] | 0.018[a] |
| Springs × swamps | 0.005[a] | 0.011[a] | 0.090 |

Corrected critical values for the significant PC and RW comparisons range from 0.008 to 0.013

Determinations of the significance of the results of Levene's tests and tests of homogeneity of slopes are not Bonferroni corrected

[a] Significant *P* values after sequential Bonferroni correction for group-wide type I error rates of 5%

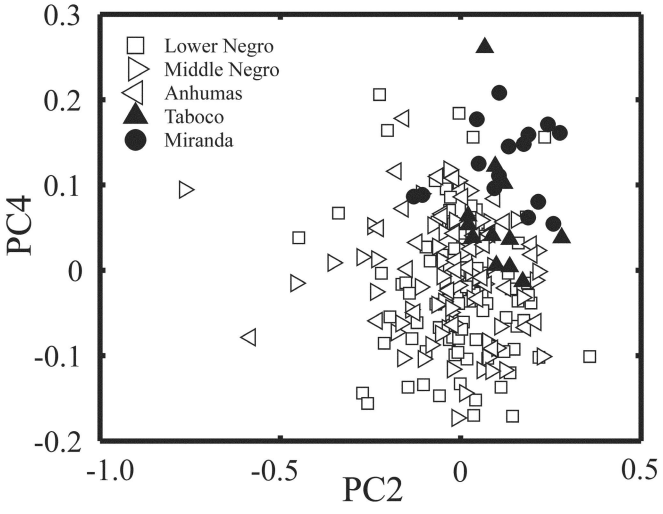


**Fig. 3.** Scatterplot of PC2 against PC4, categorized by region. Specimens from highland localities (Miranda, Taboco) appear as *filled symbols*; those from lowland localities (Anhumas, lower Negro, middle Negro) appear as *open symbols*

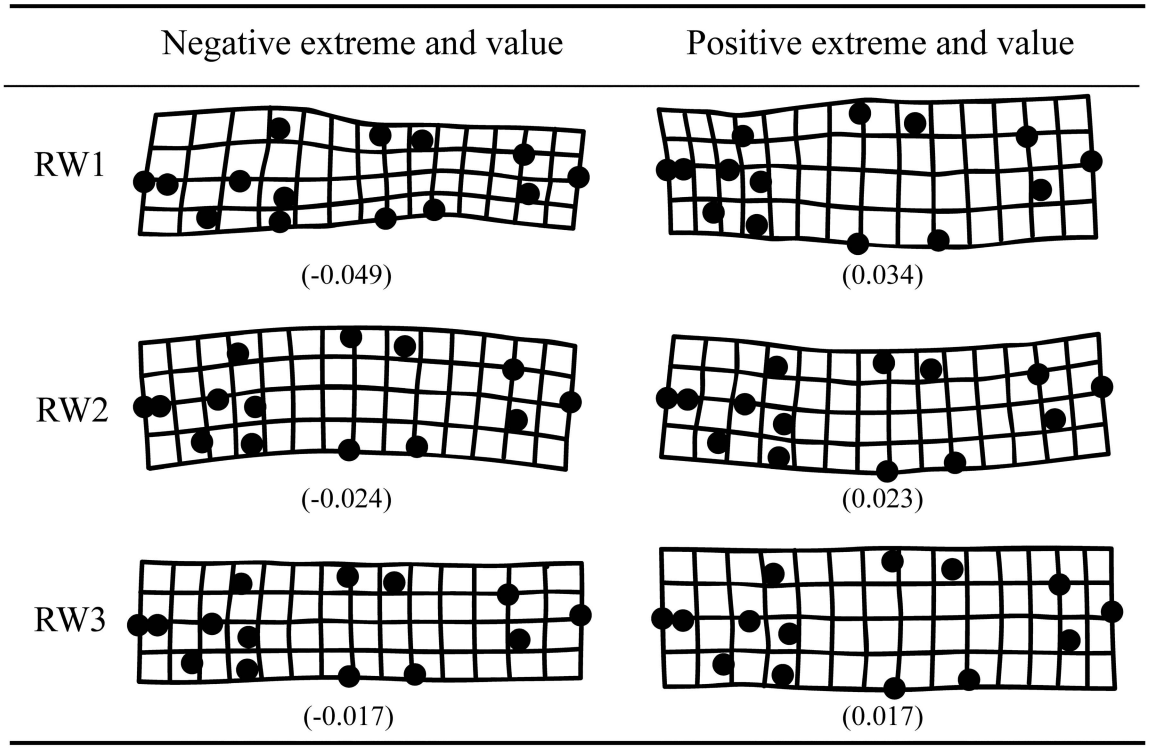


**Fig. 4.** Visualization of the range of variation described by RW1–3. Images represent the coordinate positions associated with the approximate positive and negative observed extremes of the distribution of warp scores, excluding outliers. Values indicate the diagrammed eigenvector scores

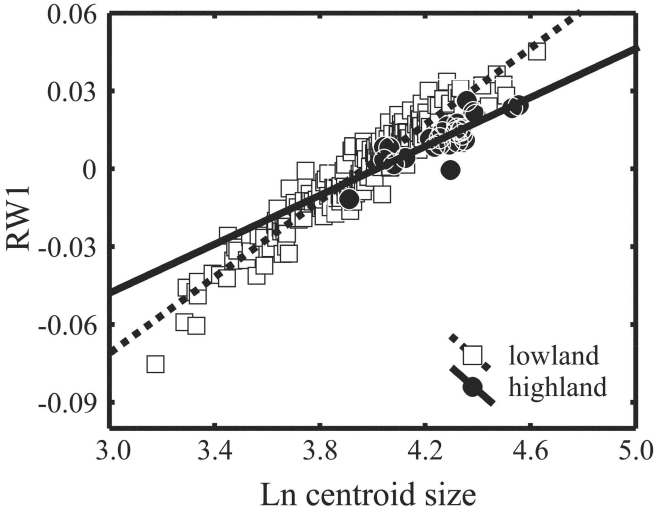


**Fig. 5.** Scatterplot of RW1 against ln centroid size showing different positive allometries for highland (*black circles*, $n = 27$) and lowland (*white squares*, $n = 193$) subgroups. Trendlines indicate linear regressions and are continued beyond the data for visual clarity only

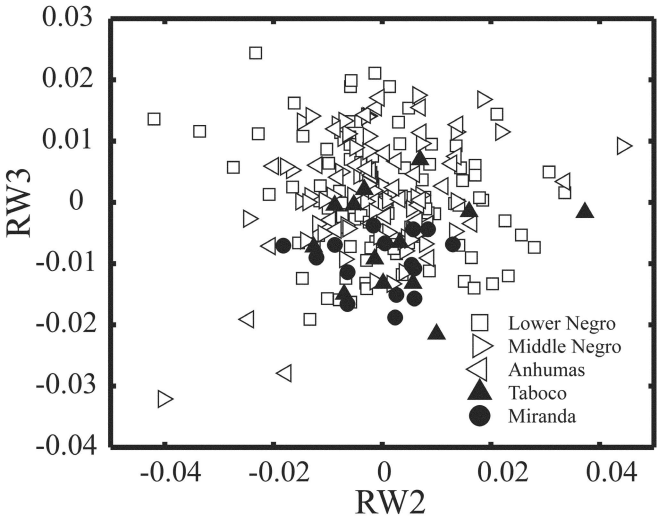


**Fig. 6.** Scatterplot of RW2 against RW3, categorized by region. Specimens from highland localities (Miranda, Taboco) appear as *filled symbols*; those from lowland localities (Anhumas, lower Negro, middle Negro) appear as *open symbols*

variation along its respective vector. Landmarks that changed position greatly from the negative to positive extreme of a warp influenced a specimen's score on that relative warp strongly. Although pure scaling relationships were removed during relative warp analysis, RW1 correlated with ln centroid size ($R = 0.94$) and was allometric (Fig. 5). As values (and centroid sizes) increased along RW1, the body became larger and deeper, and there was a decrease in the length of the head relative to body size (Fig. 4). These changes are similar to the allometry described by PC1. No other warp scores correlated significantly with ln centroid size ($R = 0.00$). RW2 contained variation in the dorsal profile; fish with negative scores had a steeper forehead, higher dorsal-fin bases, and lower anal-fin insertions than did positive-scoring specimens. RW2 also included variation in the position of the maxilla's posterior end and the adipose fin. On RW3, fish with negative scores had shallower bodies, longer caudal peduncles, and shorter dorsal-fin bases than did fish with positive scores.

After sequential Bonferroni correction, ANCOVAs on the RW1 scores indicated significant differences among regions ($P < 0.025$) but not among habitats (Table 3). It should be noted that both overall comparisons failed to meet the assumptions of homogeneity of slopes and variances and therefore may not indicate consistent shape differences across all stages of ontogeny. However, post hoc ANCOVAs revealed significant differences that separated the Miranda fish from all regions in the lowlands (middle and lower Negro, Anhumas) and the Taboco fish from those in the lower and middle Negro (Table 4) on RW1. All significant pairwise ANCOVAs of RW1 satisfied the assumption of homogeneity of slopes and can be considered statistically valid.

MANOVA comparisons among the scores on RW2–3 (Table 3) uncovered significant differences among regions ($P < 0.025$). There was no significant effect of habitat. After sequential Bonferroni correction, HSD results showed that RW3 contained significant differences between the Miranda and the lower Negro and Anhumas regions (Fig. 6, Table 4). The comparison between the Miranda and middle Negro region was very nearly significant ($P = 0.0140$).

**Univariate ANCOVAs.** The foregoing results suggested phenotypic differentiation between specimens from highland (Miranda, Taboco) and lowland (Anhumas, lower and middle Negro) regions (see Discussion). The post hoc tests (Table 4) identified 14 interlandmark distances (Table 6) that distinguished the highland population from the lowland population. The ANCOVA results for comparisons of each of these original distances, ln transformed and categorized by elevation and using ln centroid size as a covariate, appear in Table 6. Twelve of the distances were significantly different between elevations after sequential Bonferroni correction, although the distance from the anal-fin terminus to the rear of the hypural plate (CPEDLENG) and the diameter of the eye (EYE) were not. All variances were homogeneous, and parallelism was rejected in only 2 of the 14 comparisons.

## Discussion

**Regional variation.** Morphometric analyses suggested separation of specimens of *Bryconops* sp. cf. *melanurus* into two major regional groups: those collected in the highlands (Miranda and Taboco Rivers) and those collected in the lowlands (lower Negro, middle Negro and Anhumas Rivers). Eight of 21 pairwise post hoc tests between the Miranda River and one of the lowland regions identified a significant morphological difference, and 4 of 21 pairwise post hoc tests distinguished the Taboco River specimens

**Table 6.** ANCOVA results for comparisons among highland and lowland populations of fourteen ln transformed interlandmark distances (in mm), with tests of homogeneity of variances and slopes

| Distance | ANCOVA: *P* | Levene's test: *P* | Homogeneity of slopes: *P* | Highland: mean and SD | Lowland: mean and SD |
|---|---|---|---|---|---|
| Body depth and streamlining (dorsal to ventral measures) | | | | | |
| BDEPTH | <0.001[a] | 0.773 | 0.002[a] | 15.5 ± 2.0 | 16.3 ± 2.4 |
| DOR_AOR | <0.001[a] | 0.976 | 0.092 | 17.8 ± 2.5 | 18.5 ± 2.7 |
| DTER_AOR | <0.001[a] | 0.952 | 0.174 | 14.1 ± 2.0 | 14.6 ± 2.1 |
| DTER_P2O | <0.001[a] | 0.836 | 0.004[a] | 15.8 ± 2.0 | 16.8 ± 2.4 |
| AD_ATERM | <0.001[a] | 0.957 | 0.246 | 6.8 ± 0.9 | 7.2 ± 1.0 |
| Head and eye size | | | | | |
| HEAD | 0.011[a] | 0.986 | 0.489 | 15.4 ± 1.7 | 15.0 ± 1.6 |
| DORHEAD | 0.002[a] | 0.805 | 0.963 | 14.4 ± 1.5 | 14.0 ± 1.4 |
| EYE | 0.128 | 0.809 | 0.899 | 7.5 ± 0.8 | 7.4 ± 0.7 |
| Jaw length | | | | | |
| JAW | <0.001[a] | 0.836 | 0.653 | 9.7 ± 1.1 | 8.9 ± 1.0 |
| MAX_AORB | <0.001[a] | 0.462 | 0.377 | 7.6 ± 1.0 | 7.2 ± 0.8 |
| MAX_PORB | 0.009[a] | 0.333 | 0.353 | 6.4 ± 0.9 | 6.2 ± 0.8 |
| Length of caudal peduncle | | | | | |
| AD_HYP | 0.012[a] | 0.772 | 0.800 | 9.4 ± 1.2 | 9.1 ± 1.1 |
| CPEDLENG | 0.190 | 0.958 | 0.947 | 8.1 ± 1.1 | 7.9 ± 1.0 |
| Length of dorsal-fin base | | | | | |
| DBASE | <0.001[a] | 0.995 | 0.187 | 7.1 ± 0.9 | 7.4 ± 1.0 |

The smallest 156 specimens from the lowland group and the single smallest highland specimen were removed to equalize the mean centroid sizes at 72.9 $mm^2$

$n$ = 26 (highland) and $n$ = 37 (lowland); both subgroups have approximately equal size ranges (highland range = 57.0–95.2 $mm^2$, lowland range 64.6–102.0 $mm^2$)

Sequential Bonferroni-corrected critical values for the significant ANCOVA results range from 0.004 to 0.017

Means and standard deviations (mm) are given for both groups

[a]Significant *P* values

from a group of lowland specimens (Table 4). Conversely, only 2 of 28 post hoc tests between regions at similar elevations identified significant morphological differences (middle Negro × Anhumas and Taboco × Miranda on PC1). As a further test of the interelevational differences, we performed ANCOVAs between all highland and lowland fish on PC1 and RW1 and MANOVAs on PC2–4 and RW2–3. Three of the 4 tests gave significant results (PC1: $P = 0.547$ n.s.; PC2–4: $P < 0.001$; RW1: $P < 0.001$; RW2–3: $P < 0.001$).

Although some fish from the lowland regions were as streamlined as typical highland specimens (Figs. 3, 6), on average, fish from the Miranda and Taboco Rivers had the most streamlined bodies, longest caudal peduncles, and longest maxillae of any specimens in this study, with the streamlining reaching its greatest extent in the sample from the Miranda River (Figs. 3, 6; Table 4). The exaggeration of the streamlining in the Miranda fish appeared to be the source of the single significantly different post hoc comparison (PC1) between the Miranda and Taboco specimens (Table 4). At common centroid sizes, the highland specimens had shallower bodies (PC1, PC4, RW1, and RW3), larger heads (PC1 and RW1), longer maxillae (RW1 and PC4), longer caudal peduncles (RW3), and shorter dorsal-fin bases (PC4) than did the lowland specimens.

Results of the univariate ANCOVAs on absolute distances (Table 6) confirmed the foregoing interpretation of the multivariate tests. Because the slopes of their regressions on centroid size were homogeneous (Table 6), the interelevational differences in the lengths of the head (HEAD and DORHEAD), jaw (JAW, MAX_AORB, and MAX_PORB), caudal peduncle (AD_HYP), and dorsal-fin base (DBASE) length existed over the size ranges that we measured. Dissimilar slopes for two of the five cross-body measures (BDEPTH and DTER_P2O) suggested that some differences in body depth were caused by different growth trajectories in the highlands and lowlands and were only apparent in adult fish. These allometric differences were best summarized by RW1, in which the lowland fish were more highly allometric than the highland fish (Fig. 5).

**Habitat variation.** We obtained a significant habitat effect from only the ANCOVA of PC1 scores (Table 3). Post hoc pairwise tests of PC1 among habitat classes revealed that fish in the spring sample exhibited a different mean phenotype from that of the specimens from channels, backwaters, and swamps (Table 5). The comparison with the swamp fish was of dubious significance for reasons of heterogeneity of slopes and the small sample size ($n$ = 5) of

swamp fish. Because fish were found in springs only in the Taboco River, this result was fully congruent with the post hoc ANCOVAs among regions along PC1, which suggested that fish from the Taboco River exhibited a distinctive morphology. Because spring habitats were found only in the Taboco region (highlands), it was impossible to determine whether the distinctiveness of the spring fish in the Taboco was a result of regional or elevational differences, or whether a unique environmental feature of spring habitats also contributed to phenotypic differentiation.

**Potential sources of variation.** Because of very unequal sample sizes and the lack of multiple collection localities in some regions, it is possible that the phenotypic differences recognized among the available specimens of *Bryconops* sp. cf. *melanurus* did not reflect the true phenotypic structure of natural populations. The significant difference between the highland and lowland populations in this study was based upon a comparatively small series of adult specimens from only two highland localities, and the distinctiveness of the spring fish was based upon an even smaller series from a single locality. Conclusive demonstration of a phenotypic difference between populations of *Bryconops* sp. cf. *melanurus* would require the addition of many additional specimens at a wide range of body sizes from multiple localities.

Assuming that the apparent phenotypic differentiation among populations of *Bryconops* sp. cf. *melanurus* inhabiting different regions and habitats was not the result of sparse sampling, there are at least two potential, nonexclusive explanations. Phenotypic differences could have been generated by genetic differences among populations, or environmental conditions associated with changes in elevation or habitat may have influenced the ontogeny and postjuvenile morphology of *Bryconops* sp. cf. *melanurus.* No genetic data exist for *Bryconops* sp. cf. *melanurus*, and the effect of genetic differentiation on phenotypic differentiation cannot currently be investigated. However, enough information about environmental differences between the highlands and lowlands in the Pantanal exists to identify several possible environmental drivers of phenotypic variation.

The most likely environmental correlates of changes in elevation are changes in water temperature and velocity. Although water velocity was not measured during the collecting expedition, currents were observed to be much faster in the headwaters of the Pantanal than anywhere in the lowlands (Willink et al., 2000). Typical headwaters included springs and narrow, swift streams with exposed cobbles or bedrock along the stream bottom, whereas the lowland collection localities included wide meandering channels, swamps, and muddy backwaters. There is also evidence from the unpublished field notes that streams were cooler in the headwaters of the Pantanal. The mean water temperature at highland localities throughout the Pantanal was 19.8° ± 2.2°C ($n$ = 11), while the mean lowland water temperature was 21.7° ± 2.2°C ($n$ = 26), a mean difference of about 2°C.

Many field and experimental studies have demonstrated that development in swift water can induce more streamlined bodies and narrower caudal peduncles in fishes (Claytor et al., 1991; McLaughlin and Grant, 1994; Imre et al., 2002). Streamlining theoretically improves performance and reduces drag during the sustained swimming required of individuals living in fast water (Webb, 1984; Bisson et al., 1988). Slender *Bryconops* sp. cf. *melanurus* with shallower bodies (DOR_AOR, DTER_AOR, and AD_ATERM) and longer caudal peduncles (AD_HYP) were found in highland regions characterized by swift, narrow streams and springs, whereas deeper-bodied fish with shorter caudal peduncles lived in the lowland regions, characterized by larger rivers, backwaters, and swamps. Similarly, Langerhans et al. (2003) found that samples of the congeneric *Bryconops caudomaculatus* collected from swift river channels were more fusiform than those collected from lagoons. Because the phenotypic differences among highland and lowland specimens of *Bryconops* sp. cf. *melanurus* mirrored the differences known to exist within other species living in swift and slow water, variation in water velocity may have induced the shape differences observed between highland and lowland samples.

Temperature variation is also known to induce change in fish phenotypes, but the more streamlined bodies (DOR_AOR, DTER_AOR, AD_ATERM, and AD_HYP) and longer maxillae (JAW, MAX_AORB, and MAX_PORB) observed in the highland specimens of *Bryconops* sp. cf. *melanurus* (Table 6) do not represent the typical phenotypic responses of other species to development in colder water, such as shorter heads, deeper bodies, smaller eyes, shorter maxillae, and attenuated fins (Chernoff, 1982; Beacham, 1990; Leslie and Grant, 1994). If these results are general, then temperature may be a less likely, although still possible, explanation for the interelevational phenotypic differences in *Bryconops* sp. cf. *melanurus.* Ultimately, exploration of the relative contributions of genotype and environment to phenotypic variation in *Bryconops* sp. cf. *melanurus* will require additional collecting efforts, field measurements of water velocity and temperature, genetic samples, and experimental manipulations of larval populations.

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