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Shape variation in surface and cave populations of the armoured catfishes *Ancistrus* (Siluriformes: Loricariidae) from the São Domingos karst area, upper Tocantins River, Brazil

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Body shape of four distinct populations of *Ancistrus cryptophthalmus* from different caves in the São Domingos karst area and the local epigean (surface) *Ancistrus* species were compared using geometric morphometrics. Angélica and Bezerra catfishes were closer to each other and successively to the studied sample of epigean catfishes than to Passa Três and São Vicente I catfishes. Greater similarity between catfishes from the Angélica-Bezerra cave system to epigean catfishes than to other cave catfishes may represent a plesiomorphic feature. On the other hand, the present analyses allowed the distinction between Passa Três and São Vicente I catfishes, which inhabited the same cave system. Topographic isolation due to the presence of several waterfalls interposed between the stream reaches where these catfishes live may explain such morphological differentiation. Also, the presence of a waterfall downstream of the reach where the Angélica sample was obtained may have contributed to the partial isolation of these catfishes from those living in Bezerra Cave. Differences in population sizes probably influenced the degrees of morphological differentiation observed among the cave populations. The mosaic distribution of character states indicated that the four populations of *A. cryptophthalmus* have evolved rather independently, but the continuous variation suggests a recent connection between these populations, perhaps with a limited gene flow. Considering other features such as reduction of eyes and pigmentation, and changes in body shape, a separate species status for the epigean and hypogean *Ancistrus* species from the São Domingos karst area has been maintained.

Key words: *Ancistrus*; cave catfishes; geometric morphometrics; Neotropical.

INTRODUCTION

The Neotropical region is remarkable for its rich hypogean (subterranean) ichthyofauna, with several troglobitic (exclusively subterranean) species in Mexico, Caribbean islands, Venezuela, Ecuador, Peru, Bolivia and Brazil. The great majority of hypogean fishes from South America are siluriforms belonging

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to the Heptapteridae (several genera), Trichomycteridae (*Trichomycterus*, *Ituglanis*) or Loricariidae (*Ancistrus*) (Trajano, 1997; Weber *et al*., 1998). Three cave species of *Ancistrus* have been described from Brazil (*A. cryptophthalmus* Reis, 1987 and *A. formoso* Sabino & Trajano, 1997) to Venezuela (*A. galani* Perez & Viloria, 1994). *Ancistrus galani* and *A. formoso* occur in one cave system each, present relatively low population densities, and are specialized troglobites showing very reduced eyes and pigmentation. In contrast, *A. cryptophthalmus* occupies two different cave systems, showing high population densities and variability regarding development of eyes and pigmentation, both of which are less reduced than in the other two species.

The occurrence of representatives of the typically grazing loricariids (Power, 1990; Buck & Sazima, 1995) in caves is rather unexpected in view of the absence of green organisms in this environment. The successful colonization of caves by *Ancistrus* catfishes is probably enabled by the adoption of a detritivorous diet (E. Trajano, pers. obs.). In addition, nocturnal habits associated with photophobic behaviour (Trajano & Souza, 1994) in *Ancistrus* catfishes would also favour the hypogean life.

In the course of previous studies on *A. cryptophthalmus* in the São Domingos karst area, State of Goiás, central Brazil, some morphological and behavioural differences were detected between cave populations, from Angélica, Bezerra to Passa Três Caves (Trajano, 2001; Bessa & Trajano, 2002). Fish from Passa Três Cave, the type locality for the species, present the most reduced eyes but less reduced pigmentation, and the weaker photophobia and hiding habits among these populations. Angélica and Bezerra populations have less reduced eyes, which are variable in size, show stronger photophobia and hiding habits, and pigmentation is more reduced in the Angélica population. In addition, differences in general body shape between catfishes from Angélica and from Passa Três were evident after hundreds were captured and examined for a population study (Trajano, 2001).

Such differences have raised interesting questions about the origin and relationships among the cave populations, and between them and the epigean (surface) *Ancistrus* species found in the same area. Are the cave populations the result of independent colonization events with subsequent parallel evolution in the subterranean habitat or, on the contrary, are they the result of a single colonization followed by differentiation in distinct caves? How connected are all these populations? Do they represent a single species or not?

In this study, a morphological approach to these questions was used, as a guideline for further investigations, including genetic studies. The morphological features of four cave populations of *A. cryptophthalmus* (the three previously known populations plus one recently discovered in the São Vicente I Cave) were compared to the epigean *Ancistrus* species, based on two-dimensional landmark methods. Geometric morphometrics allows visualization of differences in general body shape through spline diagrams, and provides a size-related measurement isometrically independent of shape (Bookstein, 1991; Rohlf, 1993). Rohlf & Marcus (1993) give an overview of the field of geometric morphometrics, while Marcus *et al.* (1996) include several examples of applications.

Landmark morphometrics is a set of techniques with a higher power of discrimination based on shape than other morphometric methods that have been used to investigate the relationships among cave fish populations, such as traditional morphometrics (Pavan 1945; Trajano & Britski, 1992; Weber, 1996) and truss geometric procedures (Burr *et al*., 2001). One of its main advantages is to allow visualization of shape changes through diagrams. Landmark morphometrics must be regarded as complementary to molecular techniques that have been used increasingly for the study of cave fishes (Cobolli-Sbordoni *et al*., 1996; Borowsky & Mertz, 2001; Espinasa & Borowsky, 2001; Strecker *et al*., 2003).

MATERIALS AND METHODS

STUDY LOCALITIES

Ancistrus cryptophthalmus has been recorded from caves belonging to two cave systems, in São Domingos, State of Goiás: Angélica and Bezerra Caves, forming the Angélica-Bezerra cave system, and São Vicente I and Passa Três Caves, part of São Vicente cave system (Fig. 1). In spite of intensive collecting efforts, troglobitic armoured catfishes were not found in any other cave in the São Domingos karst area. An epigean species of *Ancistrus*, probably new (S. Fisch-Muller, pers. comm.), is widespread in the surface streams of the São Domingos karst area. The Bezerra and Angélica streams run westwards and, after passing through the homonymous caves, they unite at their resurgence (outlet) ends. They then join the São Vicente River *c*. 3 km south-west from the cave resurgence. The São Vicente River is a tributary to the Paranã River, one of the main streams forming the Tocantins River, in the Amazon Basin. There are epigean reaches both upstream and downstream of the Angélica and Bezerra Caves. The stream conduit inside the Angélica Cave is *c*. 8 km long, width varying from 5 to 10 m and depths from 0·5 to >2 + m. In most of its extension, it is a fast-flowing stream running

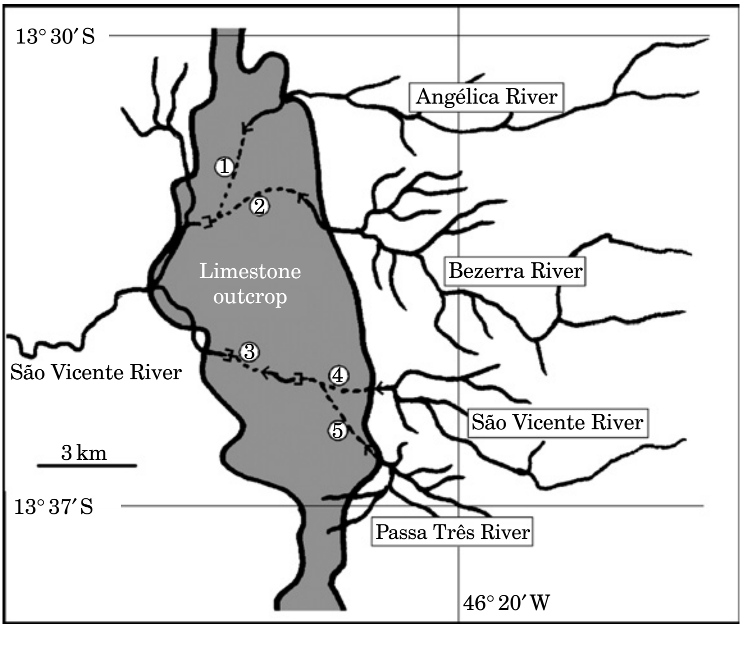

FIG. 1. São Domingos karst area (part), north-eastern State of Goiás, Central Brazil. 1, Angélica Cave; 2, Bezerra Cave; 3, São Vicente II Cave; 4, São Vicente I Cave; 5, Passa Três Cave.

over a rocky substratum (boulders, gravel and sand). The Bezerra Cave stream conduit is also 8 km long, but the mean width is half that of Angélica.

Passa Três Cave is a tributary conduit to the São Vicente I Cave, formed by a lower order stream. After a short epigean course, the Passa Três stream sinks into the Passa Três Cave and, after a conduit *c.* 2 km long, it joins directly to São Vicente I Cave through a 3·5 m waterfall (in the dry season) *c.* 3·5 km from the São Vicente I sinkhole. Mean stream width in the Passa Três Cave is *c.* 1·0 m. The São Vicente I stream gallery, *c.* 9 km long, is generally similar in dimensions and habitat types to that in the Angélica Cave, except for the occurrence of several high and very strong waterfalls in the first 3 km inside the São Vicente I.

Specimens of *A. cryptophthalmus* were observed throughout Passa Três Cave, but their density visibly decreased after the first 400 m towards the São Vicente I Cave. In São Vicente I Cave, they have been found and collected in the first 500 m, upstream of a high and particularly violent waterfall. Their occurrence downstream of this point, in the direction of the junction with the Passa Três conduit, was not confirmed. Inside Angélica Cave, armoured catfishes also concentrate upstream, attaining high population densities of 0·9 individuals m^{-2} (Trajano, 2001) in the first 2 km. No individuals were seen in the last 2 km upstream of the Angélica resurgence but, according to a local guide, these catfishes are occasionally seen there. Catfishes were not seen downstream inside Bezerra Cave, in the last 1 km towards the stream resurgence from the cave.

The epigean undescribed species of *Ancistrus* is commonly found in reaches of the Angélica, Bezerra and São Vicente streams (Fig. 1), usually occurring up to the cave entrances. No physical barrier seems to exist between the cave and epigean populations, which present a typically parapatric distribution. Therefore, some interbreeding between *A. cryptophthalmus* and the epigean *Ancistrus* sp. cannot be ruled out. On the other hand, no epigean *Ancistrus* was found upstream of the Passa Três Cave, and the *A. crytophthalmus* population inhabiting this conduit seems to be isolated upstream.

Epigean *Ancistrus* sp. have also been observed (and collected) in streams of the São Domingos karst area to the south of the São Vicente system, such as the Palmeiras and São Bernardo epigean reaches (13° 49′ S; 46° 21′ W).

COLLECTION OF SPECIMENS AND DATA ANALYSIS

Ancistrus cryptophthalmus specimens were hand-netted in the four cave localities during the dry seasons (between April and September) of 1999, 2000 and 2001. On the same occasions, specimens of the undescribed species of *Ancistrus* were collected in several epigean streams in the São Domingos karst area (Angélica, Bezerra, Palmeiras and São Bernardo streams), using seines and electrofishing. Immediately after capture, all catfishes were killed by over-anaesthesia in benzocaine solution and fixed with 4% formaldehyde. The fishes were subsequently preserved in 70% ethanol.

The analysis encompassed 33 specimens of *A. cryptophthalmus* (Appendix) from the Angélica Cave, 24 of which were adults (>40·0 mm standard length, L_S, which is the minimum size of females with developed gonads, according to Trajano, 2001), sample size range 35·7–56·7 mm L_S; 13 specimens from the Bezerra Cave, including 11 adults, size range 36·3–56·7 mm L_S; 20 adult specimens from the São Vicente I, size range 40·9–56·6 mm L_S; 13 specimens from the Passa Três Cave, including 10 adults, sample size range 39·5–59·0 mm L_S. Size distribution in these samples corresponds to the best represented size classes. Larger, less frequent size classes (60–70 mm L_S; one extreme case of 78·0 mm L_S) were not represented, as well as juveniles <35·0 mm L_S. For comparison, 28 specimens of the epigean undescribed species of *Ancistrus* were also studied (Appendix), most collected in epigean reaches of the Angélica and Bezerra streams and some from the São Bernardo to Palmeira streams. The sample size range, 32·9–47·7 mm L_S, reflected the generally smaller sizes of epigean specimens.

A WV-CP410 Panasonic video camera with Nikon lens connected to a ComputerEyes frame-grabber was employed to capture images of the 107 specimens in dorsal view, horizontally aligned. Morphometric data were acquired as homologous two-dimensional landmark co-ordinates on the dorsal surface of head and trunk using the software tpsDig, version 1·37 (Rohlf, 2003*a*). Fifteen landmarks (Fig. 2) were digitized per specimen in dorsal view: (1) snout tip; (2) right lateral margin of snout where crossed by a line perpendicular to the midbody line, situated halfway from the snout tip to the anterior nareal rim; (3) same as (2), left side; (4) midpoint in anterior margin of right nostril; (5) same as (4), left side; (6) midpoint in anterior margin of right orbit; (7) midpoint in posterior margin of right orbit; (8) posterior tip of right opercle; (9) same as (6), left side; (10) same as (7), left side; (11) same as (8), left side; (12) posterior tip of supraoccipital bone process; (13) origin of first dorsal-fin ray; (14) origin of adipose-fin spine; (15) posterior margin of last dorsal-series plate at midline, right before the first procurrent caudal-fin ray.

To avoid inflation of d.f. related to symmetrical forms, the means of the left and right landmarks were used, after aligning through the sagittal line, represented in only one (left) side with 10 landmarks for all statistical analysis. To facilitate visualization, however, both sides in splines and vector diagrams were used. Co-ordinates were aligned through general procrustes alignment (GPA) using the software Relative Warps, version 1·31 (Rohlf, 2003*b*). The method of relative warps (RW) (Bookstein, 1991; Rohlf, 1993) computes a consensus configuration (least-squares procrustes average configuration) based on the landmark co-ordinates of all specimens. Shape differences between this consensus landmark configuration and each individual specimen were obtained and used to compute the matrix of partial warp scores. Relative warp scores were computed by principal components analysis (PCA) of the covariance matrix of the partial warps scores, with the α parameter set to -1, since preliminary data analysis indicated that structures delimited by very close landmarks (for example, the eyes) are of great importance in morphological discrimination between epigean and subterranean catfishes. This value for the α parameter gives greater weight to partial warps in all smaller spatial scales (Rohlf, 1993). The uniform shape component, calculated by the linearized procrustes superimposition method (Bookstein, 1996), was included in the matrix of partial warps scores.

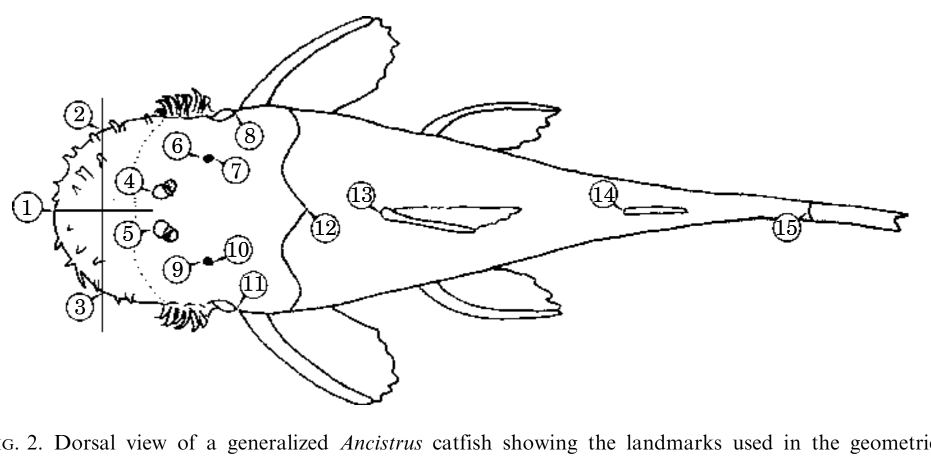

FIG. 2. Dorsal view of a generalized *Ancistrus* catfish showing the landmarks used in the geometric morphometric analyses [1, snout tip; 2, right lateral margin of snout where crossed by a line perpendicular to the midbody line, situated halfway from the snout tip to the anterior nareal rim; 3, same as 2 (left side); 4, midpoint in anterior margin of right nostril; 5, same as 4 (left side); 6, midpoint in anterior margin of right orbit; 7, midpoint in posterior margin of right orbit; 8, posterior tip of right opercle; 9, same as 6 (left side); 10, same as 7 (left side); 11, same as 8 (left side); 12, posterior tip of supraoccipital bone process; 13, origin of first dorsal-fin ray; 14, origin of adipose-fin spine; 15, posterior margin of last dorsal-series plate at midline, right before the first procurrent caudal-fin ray].

The matrix of partial warp scores (the weight matrix, *W*) was used in a canonical variates analysis, in order to confirm patterns already suggested by the relative warp scores. Mahalanobis D2 distances were used to compute an unweighted pair-group method with arithmetic mean (UPGMA) phenogram showing the relationships among samples based on shape differences. The software tpsRegr version 1·28 (Rohlf, 2003*c*) was used to regress shape changes over the first and second canonical variates axes, using vectors to depict the deformation in shape corresponding to the extremities of the ordination. Classification of individuals from each cave was also undertaken by the discriminant function.

The average shape for each sample was also computed, using the Morpheus *et al.* software (Slice, 1994/99) to obtain thin-plate spline diagrams representing deformation of different pairs of configurations, including the epigean and all other populations and some others that showed a greater amount of shape changes.

Size was assessed by centroid size, computed as the square root of the sum of squared distances between the landmarks and the centroid of the configuration (Bookstein, 1991), and compared among samples by ANOVA. Relationship between size and shape was examined plotting centroid size *v.* the first relative warp. SAS version 8·02 was used in all statistical analyses.

Eyes represent, together with the melanic pigmentation, the characters most widely affected by regressive evolution in troglobitic organisms. In view of the great intra- and interpopulational variation observed for this character in the presently studied populations, it was analysed by the regression of eye diameter on head length.

RESULTS

The first three RW (with $\alpha = -1$) comprised almost 80% of the total variance (RW1 = 61·90%, RW2 = 10·64% and RW3 = 6·89%). Plots of the first *v.* the second relative warp (Fig. 3) show a tendency towards separation between the studied populations, from the Passa Três population to the unnamed epigean species in the first RW axis, with Angélica and Bezerra populations in an

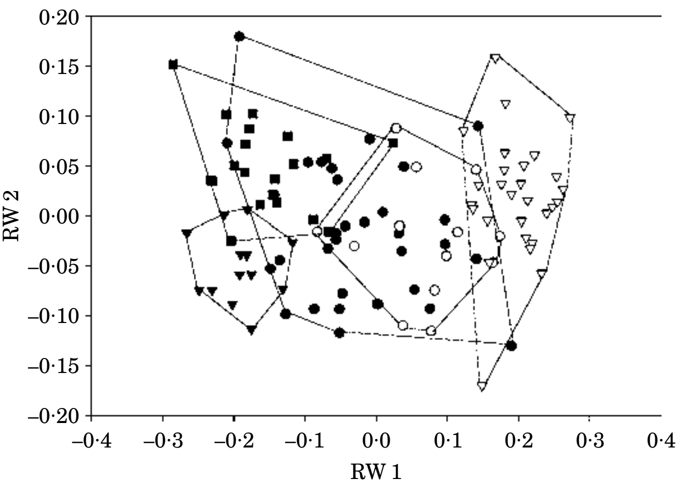

FIG. 3. Bivariate plots of relative warp scores for individual *Ancistrus* specimens [*Ancistrus cryptophthalmus* from the Angélica Cave (●), Bezerra Cave (○), São Vicente I Cave (■), Passa Três Cave (▼) and for the epigean *Ancistrus* species (▽)] aligned by general procrustes alignment (GPA).

intermediate position. In the second RW axis there is a slight trend towards separation of the São Vicente I population.

The first two canonical axes were responsible for 82% of the observed variation. The Mahalanobis D2 distances are given in Table I. The *F*-value is significant ($P < 0{\cdot}001$) for each paired comparison. The scores of individuals in the first and second canonical variates axes (Fig. 4), confirmed the trends suggested by the RW scores. Along the first axis, the epigean population discriminates from all others, showing a small superimposition with the Angélica population. The latter and Bezerra populations are also partially superimposed, while the São Vicente I and Passa Três populations are well separated from all of them. Along the second axis, the São Vicente I population is also separated from all other populations.

The classification matrix according to the discriminant function is given in Table II. Based on this function, 92% of cases were classified correctly, *i.e.* they were attributed to the populations to which they actually belonged. The following percentages were found for the correct classification of the individuals per population: Angélica, 79%; Bezerra, 92%; epigean, 96%; Passa Três, 100%; São Vicente I, 100%. It is noteworthy that only two misclassifications referred to pairs of populations in different stream drainages (Angélica-Bezerra *v.* São Vicente), *i.e.* 98% of individuals were correctly classified according to their stream drainage (microbasin).

Shape changes associated with the extreme values of each one of these axes are depicted using vectors in Fig. 5. The first axis in Fig. 5 (epigean $\rightarrow$ Angélica + Bezerra $\rightarrow$ São Vicente I $\rightarrow$ Passa Três) corresponds to a decrease of eye size, decrease of head size (both in width and length), narrowing of the snout, and elongation of the body between the dorsal fin and the base of the caudal fin. The second axis (epigean + Angélica + Bezerra + Passa Três $\rightarrow$ São Vicente I) corresponds to a widening of the head, including some separation of the nostrils and eyes, and shortening of the caudal region, between the origins of the adipose and caudal fins.

There were significant differences in size among the populations (ANOVA, $F_{4,102}$, $P < 0{\cdot}001$), and Tukey's *a posteriori* indicated that differences were between the São Vicente I and all other populations. A box-plot representing the distribution of centroid sizes, shows that centroid size in the São Vicente I population is smaller than in all the others (Fig. 6). The relationship between size and shape, represented by the first RW *v.* the centroid size (Fig. 7), indicates that

TABLE I. Mahalanobis D 2 distances (above diagonal) and their corresponding *F*-values (below) computed among the five studied epigean and cave populations of *Ancistrus* based on the partial warp matrix. All values are significant at $P = 0{\cdot}001$

Population	Angélica	Bezerra	Epigean	Passa Três	São Vicente I
Angélica	0·00	12·04	15·13	28·10	18·75
Bezerra	5·98	0·00	18·71	31·89	21·92
Epigean	12·22	8·86	0·00	61·67	37·73
Passa Três	13·97	11·05	29·19	0·00	23·42
São Vicente I	12·45	9·21	23·46	9·84	0·00

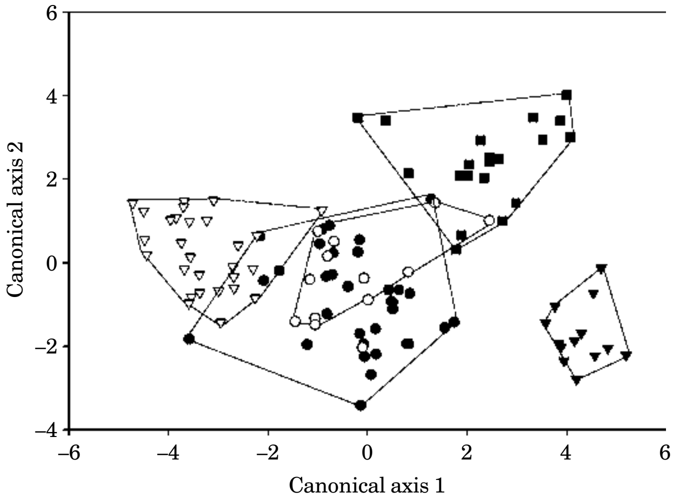

FIG. 4. Canonical variate scores of first and second canonical variate analysis based on partial warps plus the uniform component for each studied *Ancistrus* population (see Fig. 3).

the main separation among the *Ancistrus* populations described by the first RW is not isometrically correlated with size.

Regressions of eye diameter on head length are shown in Fig. 8. There is a continuous variation of eye size in the studied populations but, unlike the sequence observed for general body shape, direction of clinal variation was epigean → Bezerra → Angélica → São Vicente I → Passa Três. A third, distinct sequence of clinal variation was found for degree of pigmentation: epigean (light black to brownish) → Bezerra (brownish to light brownish) → Passa Três (light brownish to dark yellow-greenish) → São Vicente I and Angélica (light brownish to light yellow). In this case, the largest variation was observed for the São Vicente I and Angélica populations, which included a range of pigmentation types, from those that were as heavily pigmented as the specimens from the Bezerra Cave to the less pigmented specimens among *A. cryptophthalmus*. The great majority were intermediate, but less pigmented than Passa Três specimens.

General shape changes between pairs of *Ancistrus* populations, represented by thin-plate splines, are illustrated in Fig. 9. The phenogram computed using Mahalanobis distances (Fig. 10) reveals the greater similarity in body shape

TABLE II. *Post hoc* classification matrix of cases according to the discriminant function

Population	Angélica	Bezerra	Epigean	Passa Três	São Vicente I	Per cent correct
Angélica	26	3	3	0	1	79
Bezerra	0	12	0	0	1	92
Epigean	0	1	27	0	0	96
Passa Três	0	0	0	13	0	100
São Vicente I	0	0	0	0	20	100
Total	26	16	30	13	22	92

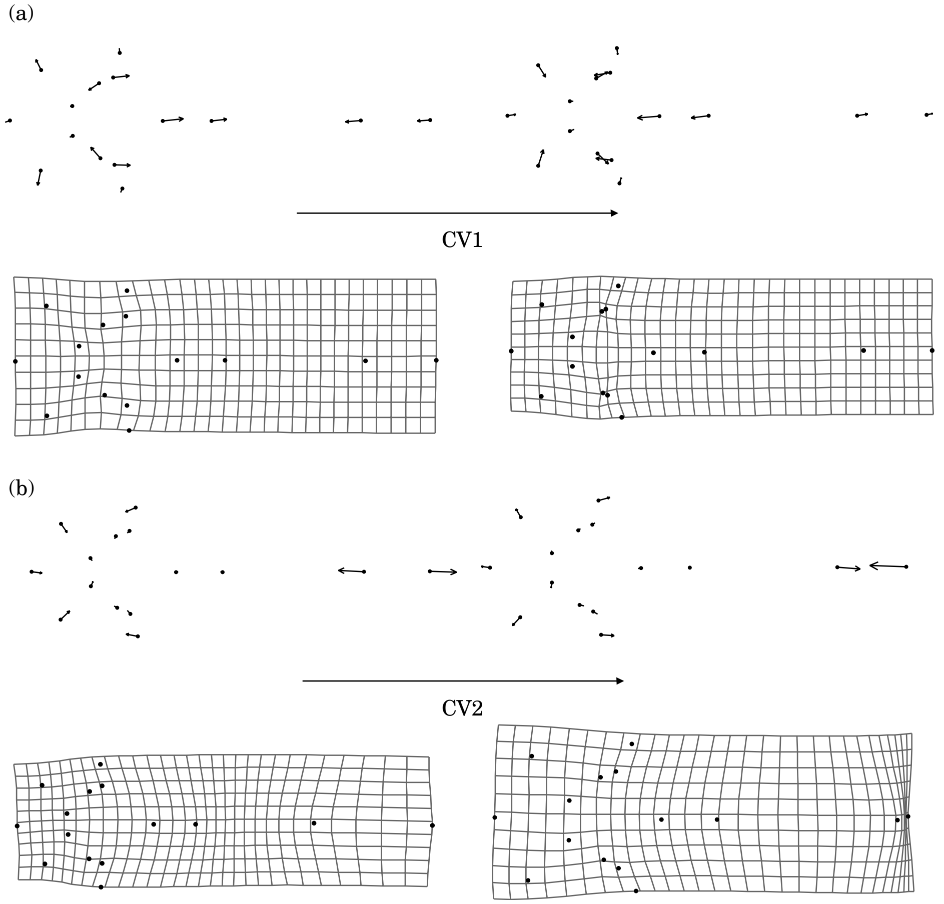

FIG. 5. Shape variation in epigean and cave *Ancistrus* from São Domingos karst area resulting from regression of the partial warp matrix over (a) first and (b) second canonical variates. •, the reference form; →, shape changes associated with each axis.

between Angélica and Bezerra populations on one hand, and São Vicente I and Passa Três populations on the other, corresponding to the two separate cave systems where these populations are found. According to this phenogram, the studied epigean *Ancistrus* are closer to those from the Angélica-Bezerra cave system than to those from the São Vicente cave system as regards to general body shape.

DISCUSSION

The advantages of the landmark morphometrics approach over traditional morphometrics, providing a more robust method to detect differences in body shape among populations and species, was demonstrated by Kassam *et al.* (2003). These authors compared species belonging to two genera of cichlids previously thought to be ecological equivalents in Lakes Malawi and

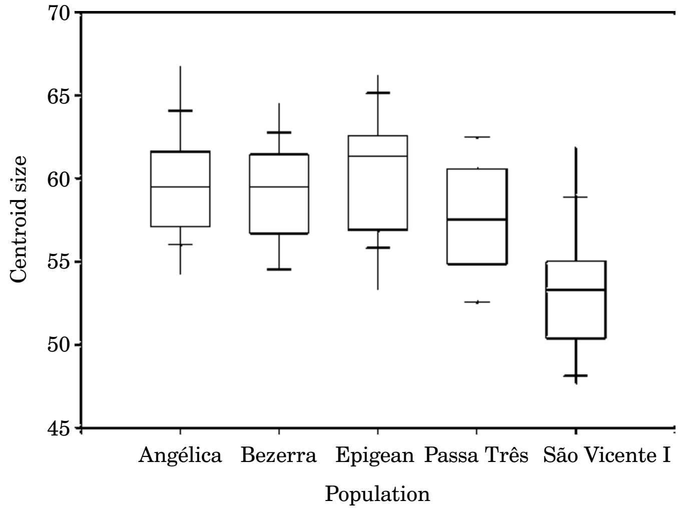

FIG. 6. Box-plot of centroid size for each studied sample showing first and third quartiles, median and extremes.

Tanganyika respectively, and were able to find differences in morphotypes, which did not support the equivalence hypothesis. The important contribution of this relatively new approach for evolutionary studies is also made clear in the present analysis, which corresponds to the first application of landmark morphometry to the study of subterranean fish populations and their comparison with close epigean relatives.

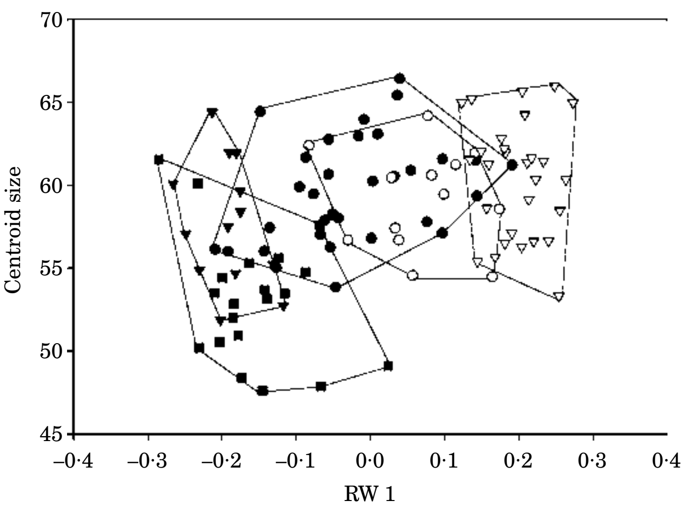

FIG. 7. Bivariate plot of scores for relative warp 1 *v*. centroid size for each studied *Ancistrus* population (see Fig. 3).

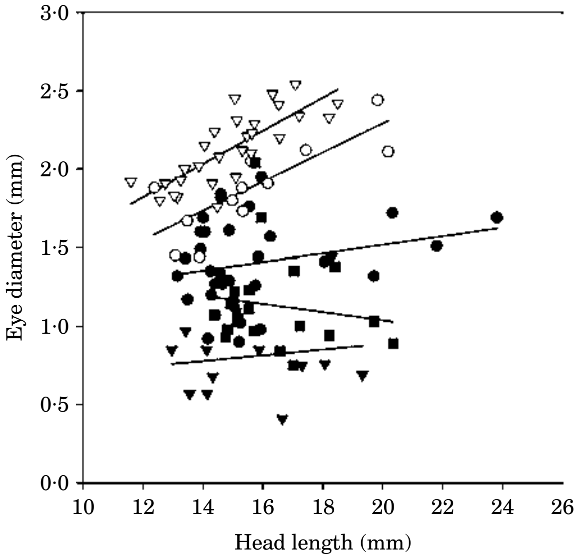

FIG. 8. Bivariate plot of head length and eye diameter for individual *Ancistrus* specimens for each studied population. The curves were fitted by: Angélica (●), $y = 0{\cdot}97 + 0{\cdot}27x$; Bezerra (○), $y = 0{\cdot}43 + 0{\cdot}093x$; São Vicente (■), $y = 1{\cdot}56 - 0{\cdot}026x$; Passa Três (▼), $y = 0{\cdot}52 + 0{\cdot}018x$; epigean (▽) $y = 0{\cdot}55 + 0{\cdot}106x$.

Although there is no phylogenetic hypothesis proposed for the group of species including *A. cryptophthalmus* and the epigean *Ancistrus* sp. from the São Domingos karst area, their current parapatric distribution makes it reasonable to accept, as a working hypothesis, a sister-group relationship between them. This is more parsimonious than to suppose that the cave populations derived from another *Ancistrus* species previously living in the area, which became locally extinct, being replaced by the one currently found in the epigean streams. On the other hand, the mosaic distribution of character states concerning general body shape, and development of eyes and pigmentation among the four studied cave populations indicates a certain degree of isolation, with some independent differentiation in these populations.

As regards to general body shape, Angélica and Bezerra catfishes are closest to each other (as expected, because they inhabit the same cave system) and then closer to the studied sample of epigean catfish than to Passa Três and São Vicente I catfishes. As hypotheses for future testing, the greater similarity between *A. cryptophthalmus* from the Angélica-Bezerra system and the studied samples of *Ancistrus* sp. (collected in epigean reaches of the Angélica and Bezerra streams) may correspond to a plesiomorphic feature (*i.e.* retention of a generalized body shape in *A. cryptophthalmus* from Angélica and Bezerra caves), or reflect a higher phylogenetic proximity between the cave and the epigean catfishes living in the Angélica-Bezerra drainage.

Except for the Angélica population, >90% of individuals of each sample were correctly assigned to their original populations using the discriminant function, and 98% were assigned to the correct stream drainage. This indicates that body

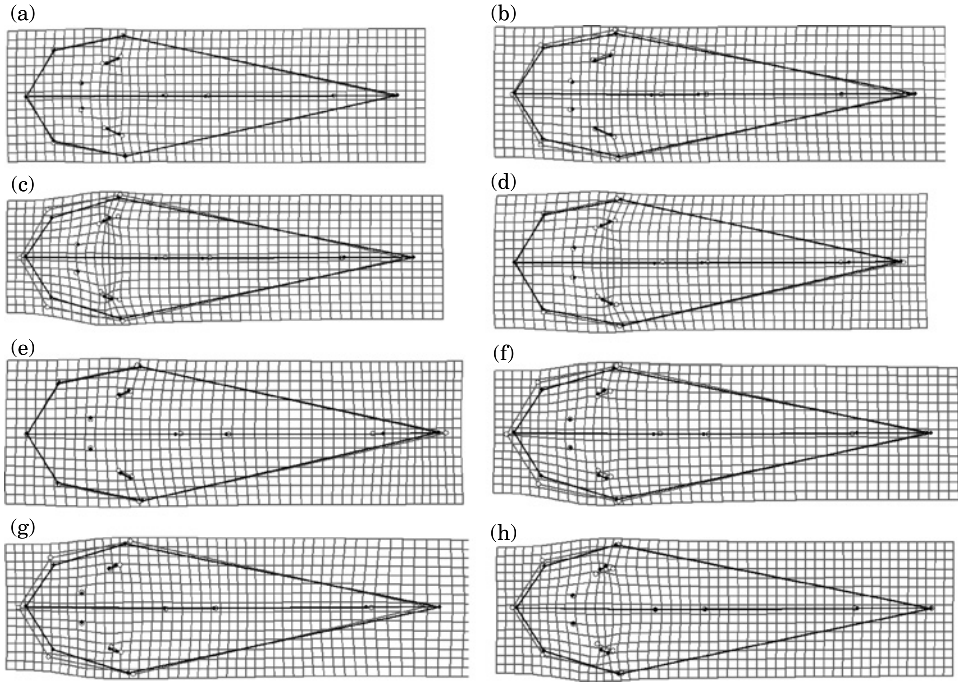

FIG. 9. Thin-plate spline diagrams showing deformations of a chosen reference (○) on a target (●) configuration. (a) Epigean on Angélica, (b) epigean on Bezerra, (c) epigean on Passa Três, (d) epigean on São Vicente, (e) Angélica on São Vicente, (f) Angélica on Passa Três, (g) São Vicente on Passa Três and (h) Bezerra on Passa Três.

shape is a robust character to separate *Ancistrus* populations, especially for those living in different stream drainages (Angélica-Bezerra *v.* São Vicente micro-basins). It is also a good character, although less robust, to separate populations

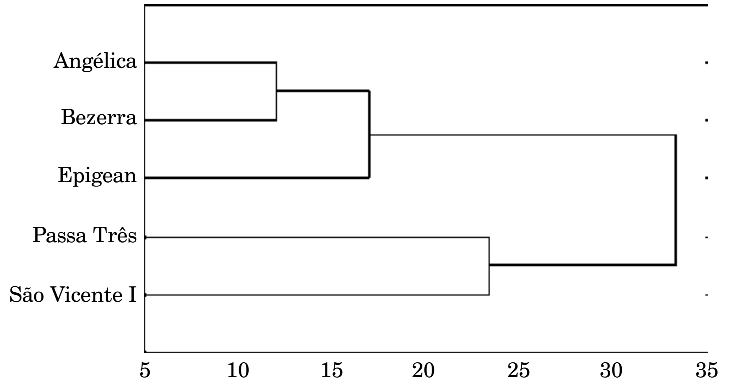

FIG. 10. UPGMA phenogram based on Mahalanobis distances between the studied populations of *Ancistrus* from the São Domingos karst area.

within stream drainages, including stream reaches inside caves, as indicated by the complete separation between São Vicente I and Passa Três samples.

The lower percentage of correct classifications observed for the Angélica sample (79%) is consistent with the higher variability in development of eyes and pigmentation in this species, with partial superposition with the other studied populations. Larger population sizes than observed for the catfishes in the São Vicente system (*c.* 8000 individuals in the studied 2000 m long reach inside Angélica Cave, in contrast with 1000 individuals in the 2000 m long Passa Três conduit; Trajano, 2001), associated with the possibility of some genetic interbreeding with epigean catfishes that are found adjacent to the cave entrance, may account for the low morphological differentiation and the high intrapopulational variation observed for body shape and eye development in the Angélica Cave.

It is noteworthy that the present analyses allowed the complete separation between Passa Três and São Vicente I catfishes. Topographic isolation due to the presence of several high and very strong waterfalls between the cave stream reaches where these catfishes live may explain the morphological differentiation between Passa Três and São Vicente I populations. In fact, the São Vicente I population corresponds to the population found upstream of the first high waterfall inside São Vicente I Cave, 500 m from the cave entrance and 3 km from the downstream connection with the Passa Três conduit. The small population size estimated for the Passa Três Cave may result in a level of genetic drift that produces faster evolutionary rates in that population, which would function as a peripheral isolate. The population size is not known for the studied São Vicente I population, but it may also correspond to a small population isolated from populations living in downstream reaches (such as the one from the Passa Três Cave) by the waterfall mentioned above. On the other hand, the closer morphological proximity between the São Vicente I and Passa Três catfishes than to the other studied populations is consistent with the hypothesis of a common ancestor based on the fact that they live in the same cave. Such a putative ancestor may have occupied the whole cave in the past, when it was more level and before alluvial erosion produced the waterfalls that split the population.

Despite the communication between Angélica and Bezerra Caves, which form a single cave system joined at their downstream ends, the presence of a waterfall downstream from where the Angélica sample was obtained may also contribute to partial isolation of the two studied populations, explaining the observed differences in body shape. Furthermore, apparently the Bezerra population is smaller than that of Angélica, thus predicting a higher divergence rate.

Differences in body shape may be related to habitat. Angélica and São Vicente I cave streams are characterized by strong currents in the sites where these catfishes concentrate and a water volume several times larger than that of the Passa Três cave stream. In epigean streams, *Ancistrus* individuals also concentrate in reaches with moderate to strong currents. The water currents are intermediate in the Bezerra Cave, where the catfishes show correspondingly an intermediate condition concerning snout width.

The studied cave populations appear to be undergoing a process of morphological differentiation. The observed variability indicates that this is a relatively recent process and that, at least in some cases (especially for Angélica Cave), genetic isolation from epigean population may not be complete.

Body shape, as indicated by the morphometric data, supports the separation between the epigean species and the different populations of *A. cryptophthalmus*. To avoid proliferation of taxonomic names, and in the absence of a phylogenetic analysis of *Ancistrus* taxa, a conservative position is adopted, maintaining a single species status for all cave populations together, separated from the epigean species (still unnamed).

A preliminary molecular study comparing *A. cryptophthalmus* from Angélica, Bezerra and Passa Três Caves and epigean *Ancistrus* sp. was done based on MtDNA (Moller & Parzefall, 2001). Low genetic diversity was observed for *A. cryptophthalmus*, and no evidence for multiple origins of the studied cave populations was found. Nevertheless, it is too soon to completely discard this possibility, and more data, comprising different genetic markers, are needed to test the single *v.* multiple origin hypotheses as well as the degree of genetic connectiveness between the cave populations.

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References

Bessa, E. & Trajano, E. (2002). Light reaction and cryptobiotic habits in armoured catfishes, genus *Ancistrus*, from caves in Central and Northwest Brazil (Siluriformes: Loricariidae). *Mémoires de Biospéologie* **28,** 29–37.

Bookstein, F. L. (1991). *Morphometric Tools for Landmark Data. Geometry and Biology*. New York: Cambridge University Press.

Bookstein, F. L. (1996). Standard formula for the uniform shape component in landmark data. In *Advances in Morphometrics* (Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice D. E., eds), pp. 153–168. New York: Plenum Press.

Borowsky, R. B. & Mertz, L. (2001). Genetic differentiation among populations of the cave fish *Schistura oedipus* (Cypriniformes: Balitoridae). *Environmental Biology of Fishes* **62,** 225–231.

Buck, S. M. C. & Sazima, I. (1995). An assemblage of mailed catfishes (Loricariidae) in southeastern Brazil: distribution, activity, and feeding. *Ichthyological Explorations of Freshwaters* **6,** 325–332.

Burr, B. M., Adams, G. L., Krejca, J. K., Paul, R. J. & Warren, M. L., Jr. (2001). Troglomorphic sculpins of the *Cottus carolinae* species group in Perry County, Missouri: distribution, external morphology, and conservation status. *Environmental Biology of Fishes* **62,** 279–296.

Cobolli-Sbordoni, M., De Matthaeis, E., Mattoccia, M., Berti R. & Sbordoni, V. (1996). Genetic variability and differentiation of hypogean Cyprinid fishes from Somalia. *Journal of Zoological Systematics and Evolutionary Research* **34,** 75–84.

Espinasa, L. & Borowsky, R. B. (2001). Origins and relationship of cave populations of the blind Mexican tetra, *Astyanax fasciatus*, in the Sierra de El Abra. *Environmental Biology of Fishes* **62,** 233–237.

Kassam, D. D., Adams, D. C., Hori, M. & Yamaoka, K. (2003). Morphometric analysis on ecomorphologically equivalent cichlid species from Lakes Malawi and Tanganyika. *Journal of Zoology* **260,** 153–157.

Marcus, L. F., Corti, M., Loy, A., Naylor, G. J. P. & Slice, D. E. (Eds) (1996). *Advances in Morphometrics*. New York: Plenum Press.

Moller, D. & Parzefall, J. (2001). Single or multiple origin of the subterranean catfish *Ancistrus cryptophthalmus*. What we can learn from molecular data. In *XV International Symposium of Biospeleology-Abstracts* (Trajano, E. & Pinto-da-Rocha, R., eds), p. 38. São Paulo: Société Internationale de Biospéologie.

Pavan, C. (1945). Os peixes cegos das cavernas de Iporanga e a evolução. *Boletins da Faculdade de Filosofia, Ciências e Letras, Biologia Geral 6*, **79,** 1–104.

Power, M. E. (1990). Resource enhancement by indirect effects of grazers: armored catfish, algae, and sediment. *Ecology* **71,** 897–904.

Rohlf, F. J. (1993). Relative warp analysis and an example of its application to mosquito wings. In *Contributions to Morphometrics* (Marcus, L. F., Bello, E. & Garcia-Valdecasas, A., eds), pp. 131–159. Madrid: Museo Nacional Ciencias Naturales (CSIC).

Rohlf, F. J. (2003*a*). *TpsDig*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. (2003*b*). *Relative Warps*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. (2003*c*). *TpsRegr*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Rohlf, F. J. & Marcus, L. F. (1993). A revolution in morphometrics. *Trends in Ecology and Evolution* **8,** 129–132.

Slice, D. E. (1994/99). *Morpheus* et al. *Software for Morphometric Research*. Stony Brook, NY: Department of Ecology and Evolution, State University of New York at Stony Brook.

Strecker, U., Bernatchez, L. & Wilkens, H. (2003). Genetic divergence between cave and surface populations of *Astyanax* in Mexico (Characidae, Teleostei). *Molecular Ecology* **12,** 699–710.

Trajano, E. (1997). Synopsis of Brazilian troglomorphic fishes. *Mémoires de Biospéologie* **24,** 119–126.

Trajano, E. (2001). Habitat and population data of troglobitic armoured cave catfishes, *Ancistrus cryptophthalmus* Reis, 1987, from central Brazil (Siluriformes: Loricariidae). *Environmental Biology of Fishes* **62,** 195–200.

Trajano, E. & Britski, H. A. (1992). *Pimelodella kronei* (Ribeiro,1907) e seu sinônimo *Caecorhamdella brasiliensis* Borodin, 1927: morfologia externa, taxonomia e evolução (Teleostomi, Siluriformes). *Boletim de Zoologia*, (São Paulo) **12,** 53–89.

Trajano, E. & Souza, A. M. (1994). The behaviour of *Ancistrus cryptophthalmus*, an armoured blind catfish from caves of Central Brazil, with notes on syntopic *Trichomycterus* sp. (Siluriformes, Loricariidae, Trichomycteridae). *Mémoires de Biospéologie* **21,** 151–159.

Weber, A. (1996). Cave dwelling catfish populations of the genus *Rhamdia* (Pimelodidae, Siluroidei, Teleostei) in Mexico. *Mémoires de Biospéologie* **23,** 73–85.

Weber, A., Proudlove, G. S. & Nalbant, T. T. (1998). A – Morphology, systematic diversity, distribution, and ecology of stygobitic fishes. In *Encyclopaedia Biospeologica*, Tome II (Juberthie, C. & Decu, V., eds), pp. 1179–1190. Moulis: Société de Biospéologie.

Appendix

Material Studied

Ancistrus cryptophthalmus: MZUSP 79050 (10), MZUSP 79051 (4), MZUSP 79052 (5), MZUSP 79053 (4), MZUSP 79054 (2), MZUSP 79055 (3), MCP 29964 (5), Angélica River inside Angélica Cave, São Domingo, Goiás, Brazil; MZUSP 79056 (5), MZUSP 79057 (2), MZUSP 79058 (3), MCP 29965 (3), Bezerra River inside Bezerra Cave, São Domingo, Goiás, Brazil; MZUSP 79063 (17), MCP 29966 (3), São Vicente River inside São Vicente I Cave, São Domingo, Goiás, Brazil; MZUSP 79059 (1), MZUSP 79060 (4), MZUSP 79061 (2), MZUSP 79062 (3), MCP 29967 (3), Passa Três River inside Passa Três Cave, São Domingo, Goiás, Brazil.

Ancistrus sp: MZUSP 79064 (2), São Bernardo River, São Domingo, Goiás, Brazil; MZUSP 79065 (3), Angélica River, São Domingo, Goiás, Brazil; MZUSP 79066 (17), MCP 29968 (5), Bezarra River, São Domingo, Goiás, Brazil; MZUSP 79067 (1), São Bernardo River, São Domingo, Goiás, Brazil.