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Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex

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Cichlid fishes are known for their adaptive radiations with prolific speciation, but also for their substantial intraspecific polymorphism and phenotypic plasticity. The *Amphilophus citrinellus* species complex from lakes of Nicaragua has been studied extensively as an example of trophic and colour polymorphism and possible sympatric speciation. We use the methods of geometric morphometrics to investigate variation in body shape in this species complex. There is significant shape variation between the widespread *A. citrinellus* and the more locally distributed *A. labiatus* and *A. zaliosus*, which shows patterns that are consistent with the taxonomic descriptions of those taxa. Contrary to the expectations from models of adaptive character divergence, the shape differences between species in analyses restricted to populations occurring syntopically are smaller than the corresponding differences computed from samples pooled over all locations. Within *A. citrinellus*, there is considerable variation among lakes as well as between the alternative colour and trophic morphs, suggesting local differentiation based on genetic and ecophenotypic mechanisms.

ADDITIONAL KEYWORDS: canonical variate analysis – landmarks – pharyngeal jaws – phenotypic plasticity – Procrustes method – size – thin plate spline.

INTRODUCTION

Variation in body shape can reflect ecological and behavioural differences. Because water is a dense medium, body shape particularly affects behavioural performance characteristics in fishes and other aquatic organisms (Webb, 1984). The body shape of fishes can therefore be expected to be of particular ecological and evolutionary relevance. Accordingly, morphometric studies can provide useful information on the evolution of fishes (Schluter, 1993; Klingenberg & Ekau, 1996; Walker, 1997; Caldecutt & Adams, 1998; Douglas *et al.*, 2001; Rüber & Adams, 2001; Hulsey & Wainwright, 2002).

Fishes of the family Cichlidae have long been known for their spectacular adaptive radiation and phenotypic plasticity, and have therefore served as a model for evolutionary and ecological studies (e.g. Fryer & Iles, 1972; Barlow, 2000). The species complex of the Midas cichlid *Amphilophus citrinellus* (Günther) and related taxa occurring in Nicaraguan lakes has attracted considerable interest for its colour polymorphism (Barlow, 1976), trophic polymorphism (Meyer, 1989, 1990a,b) and as a possible example of incipient sympatric speciation (Barlow & Munsey, 1976; McKaye, 1980; Meyer, 1990a; Wilson, Noack-Kunnmann & Meyer, 2000; McKaye *et al.*, 2002; Stauffer & McKaye, 2002). Coloration appears to have a polygenic basis (Barlow, 1976), and assortative mating according to colour has been observed (Barlow, 1976; McKaye & Barlow, 1976), leading to genetic structuring of populations according to colour morphs (Wilson *et al.*, 2000). The trophic polymorphism consists of differences in the pharyngeal jaw apparatus between a papilliform morph which eats soft food and a molariform morph equipped for crushing hard food items such as mollusc shells (Meyer, 1989, 1990a,b). There appears to be no genetic differentiation between trophic morphs

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within populations of *A. citrinellus* (Wilson *et al*., 2000), but sympatric species tend to differ in diet and the associated pharyngeal jaw morphology (McKaye *et al*., 2002). In other cichlid species, phenotypic plasticity plays an important role for similar polymorphisms of pharyngeal jaws (Greenwood, 1965) or other feeding structures (Meyer, 1987; Wimberger, 1991; Bouton, Witte & van Alphen, 2002). Because the frequency of colour and trophic morphs in *A. citrinellus* varies among lakes and with water depth (Barlow, 1976; McKaye & Barlow, 1976), there is also considerable potential for interaction of these genetic and environmental factors.

In Nicaraguan lakes, *A. citrinellus* occurs with several other closely related species (Barlow & Munsey, 1976; McKaye *et al*., 2002; Stauffer & McKaye, 2002). Here, we compare *A. citrinellus* with *A. labiatus* (Günther) and *A. zaliosus* (Barlow) (Fig. 1; Barlow & Munsey, 1976). *A. citrinellus* is the most widespread of the three species, *A. labiatus* is restricted to the large Lakes Managua and Nicaragua, and *A. zaliosus* is endemic to Lake Apoyo, a small crater lake, where it coexists with other putative species of the complex (Fig. 2; Barlow, 1976; McKaye *et al*., 2002). All the species of the *A. citrinellus* complex may be of relatively recent common origin (Barlow, 1976; McKaye *et al*., 2002). If ecological factors played a role in speciation (e.g. Schluter, 2000), then one would expect local character divergence, with syntopic populations of different species being more divergent compared with populations living separately (Barlow & Munsey, 1976). Other possibilities are introgressive hybridization (Barlow & Munsey, 1976), ecophenotypic variation or local adaptive differentiation, in which case syntopic populations of two species should be more similar due to local gene exchange or geographical variation in environmental conditions. Because these scenarios yield different predictions for morphological similarity, they can be studied with morphometric methods. Here, we use the methods of geometric morphometrics (Bookstein, 1991; Dryden & Mardia, 1998) to analyse body shape variation among populations and among alternative colour and trophic morphs of this species complex from different lakes in Nicaragua.

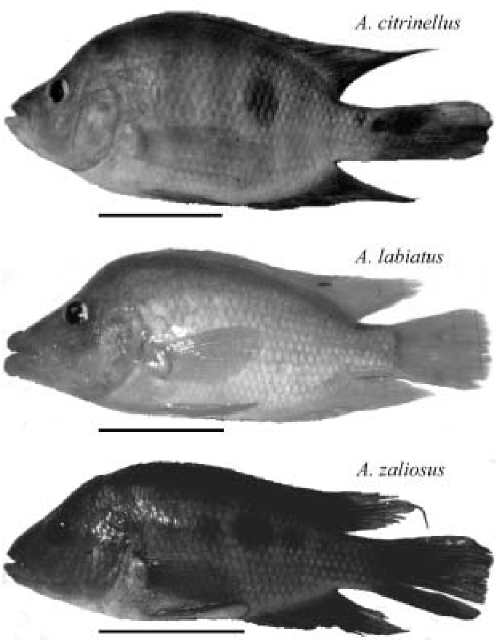

Figure 1. Typical specimens of the three species of the *Amphilophus citrinellus* complex. The specimens of *A. citrinellus* and *A. zaliosus* are of the normal colour morph, whereas the specimen of *A. labiatus* is of the gold morph. Scale bars = 5 cm.

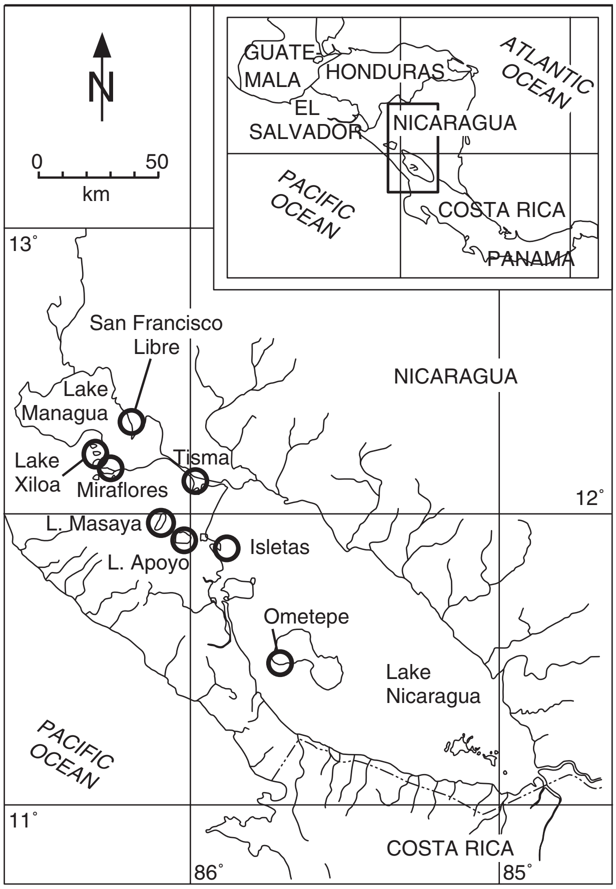

Figure 2. Map of the sampling locations. *Amphilophus citrinellus* is widespread in the Nicaraguan lakes, whereas *A. labiatus* occurs only in the large lakes and *A. zaliosus* is endemic to Lake Apoyo (Barlow & Munsey, 1976).

MATERIAL AND METHODS

SPECIMENS AND DATA COLLECTION

Fish were caught in February 2001 in the following locations in Nicaragua (Fig. 2): Lake Nicaragua (two locations: Ometepe and Isletas), Lake Managua (two locations: San Francisco Libre, Miraflores), a pond near Tisma near the Tipitapa River, Lake Xiloa, Lake Masaya and Lake Apoyo. Lakes Nicaragua and Managua are large lakes draining into the Atlantic ocean, and the pond near Tisma is situated between these two lakes and is seasonally connected to them. In contrast, Lakes Apoyo, Masaya, and Xiloa are small, isolated crater lakes. The samples from Lakes Nicaragua and Managua and from Tisma were acquired from local fishermen, whereas those from the crater lakes were collected specifically for this study (M.B). All fish were caught with gill nets, but the mesh size was not the same, and therefore the size distributions differed among locations.

Each fish was placed on a measuring board with 1-cm gradations and photographed in side view. The photographic slides were later scanned and landmark positions were determined from the digital images. Thirteen landmarks were digitized, of which two (anterior and posterior margin of the eye) were used to compute a single landmark at the centre of the eye. The analyses therefore consider 12 landmarks (Fig. 3), recording the position of the mouth and eye (landmarks 1 and 2), the positions of the bases of the pectoral and pelvic fins (landmarks 4–6), the positions of the dorsal and anal fin bases (landmarks 3, 7, 8 and 10), and the shape of the caudal peduncle (landmarks 8–12). Specimens that were photographed with their mouth and opercles wide open and specimens with opened abdominal cavity were excluded from the analyses. The sizes of the final samples are given in Table 1.

All specimens were identified to species according to the characters given in the taxonomic descriptions (Barlow & Munsey, 1976) and scored for their colour and pharyngeal jaw morphs. We distinguished the normal and gold colour morphs: the normal colour is grey or brown with dark bars or spots, whereas the gold morph is whitish to yellow, orange or red and lacks the dark markings (here, the gold morph includes all brightly coloured types described by Barlow, 1976). The pharyngeal jaws were classified as papilliform or molariform: papilliform pharyngeal jaws have numerous slender teeth, whereas the molariform pharyngeal jaws have fewer, thicker teeth and are more robust overall (Meyer, 1990a,b).

MORPHOMETRIC AND STATISTICAL ANALYSIS

All analyses characterized body shape with the methods of geometric morphometrics (Bookstein, 1991; Dryden & Mardia, 1998). The first step of each analysis is a least-squares Procrustes superimposition (Dryden & Mardia, 1998) to extract shape co-ordinates for use in further statistical analyses. The Procrustes method separates shape from size and eliminates variation in the position and orientation of specimens. Specimens are first scaled to unit centroid size, which is a measure of spread of the landmarks around their centre of gravity (centroid). The scaled configurations are then superimposed to have the centroid in common, rotated to optimal fit according to a least-squares criterion, and projected to a linear shape tangent space (for details, see Rohlf & Slice, 1990; Dryden & Mardia, 1998; Rohlf, 1999). The resulting shape co-ordinates can be analysed with the tools of multivariate statistics (e.g. Mardia, Kent & Bibby, 1979; Flury, 1997).

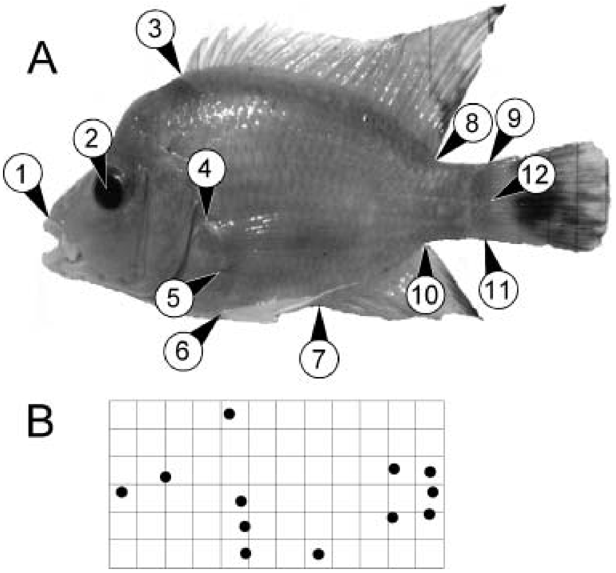

Figure 3. Landmarks used in this study (A) and overall average configuration for *A. citrinellus, A. labiatus* and *A. zaliosus* with the grid used to visualize shape changes in the further analyses (B). Landmark 1: mouth, at junction of premaxillary and ethmoid. Landmark 2: centre of eye. Landmark 3: anterior base of dorsal fin. Landmark 4: anterior base of pectoral fin (leading edge). Landmark 5: posterior base of pectoral fin. Landmark 6: anterior base of pelvic fin. Landmark 7: anterior base of anal fin. Landmark 8: posterior base of dorsal fin. Landmark 9: dorsal base of caudal fin. Landmark 10: posterior base of anal fin. Landmark 11: ventral base of caudal fin. Landmark 12: centre of base of caudal fin at lateral line.

Outliers in the shape data for the pooled samples were identified in a preliminary analysis using multivariate standard distance (Flury, 1997: 188 f) as the criterion. After removal of outliers, new analy-

Table 1. Sample sizes of the different species and locations by colour and trophic morphs

		Papilliform jaws		Molariform jaws	
Species	Location	Normal	Gold	Normal	Gold
A. citrinellus	Miraflores	58	32	—	—
	San Francisco Libre	15	12	1	1
	Tisma	20	24	1	—
	Isletas	13	4	22	10
	Ometepe	—	—	29	2
	Lake Xiloa	11	—	34	—
	Lake Masaya	60	1	—	—
	Lake Apoyo	6	—	1	—
A. labiatus	Isletas	28	54	—	—
A. zaliosus	Lake Apoyo	40	—	5	—

ses, including the Procrustes fit, were performed with the 484 specimens for which complete data were available. All computations for statistical analyses and graphical display of the results were carried out using routines written in Matlab for this study.

Assessment of allometry

Allometry is an important factor for shape variation in fishes (Loy, Cataudella & Corti, 1996; Loy *et al*., 1998; Reis, Zelditch & Fink, 1998) and can potentially confound analyses of group differences. We assessed the data for allometry by multivariate regression of shape on centroid size (e.g. Loy *et al*., 1996; Monteiro, 1999). Specimens were separated according to species, sampling location, and trophic and colour morphs. For all 16 such samples with more than ten specimens, allometry was tested by a permutation test with 10 000 iterations that simulated the null hypothesis of independence between size and shape by randomly exchanging the value for centroid size among individuals (e.g. Good, 1994; Edgington, 1995). Only one of the samples (*A. citrinellus* from Lake Masaya) showed a significant effect of size, one sample had a nominally significant effect that was clearly non-significant after sequential Bonferroni correction for multiple tests, and the remaining 15 samples showed no allometric effect whatsoever. The reason for this absence of within-sample allometry is probably the narrow size spectrum within each sample, which may be due to the strong size selectivity of gill nets. Even for the sample from Lake Masaya, size accounted for only 9% of shape variation (Procrustes variance; Klingenberg & McIntyre, 1998), and we therefore did not apply any allometric size corrections within samples.

Because the size distributions differed between samples, we also examined whether there were any allometric effects on shape differences among samples. Size accounted for only 6% of shape variation, and the permutation test indicated that allometric effects were not statistically significant ($P = 0.45$). Therefore, no correction for size differences among locations was required.

Analysis of shape differences among groups

To visualize the species differences, we used transformation grids produced with the thin-plate spline interpolation technique (e.g. Bookstein, 1991: 316–338; Dryden & Mardia, 1998: 205–222). Transformation grids show the shape change from a grid with square cells superimposed onto the average landmark configuration (Fig. 3B) to a grid that is deformed to fit a target configuration. The initial comparison used the average shape of all species as the starting configuration, and each species mean as a target shape in turn. For better visualization, the differences were exaggerated five-fold. Because the sample sizes for the three described species in our data set differed widely (with many more specimens of *A. citrinellus* than of the other taxa), we computed the overall average shape from the three species means to give each species the same weight. All statistical analyses, however, were based on the individual specimens included in the respective analysis, and Procrustes tangent projections use the mean of all specimens (Rohlf, 1999).

As a statistical test for the presence of overall differences among species and trophic or colour morphs, we used Wilk's lambda (Mardia *et al*., 1979: 82–84), which is equivalent to a test of the group effect in a one-way MANOVA. Pairwise comparisons of specific groups were based on Procrustes distance, the standard measure for the magnitude of shape differences used in geometric morphometrics (e.g. Bookstein, 1996). Procrustes distance is an absolute measure of the degree of shape difference between two configurations, and therefore does not depend on factors like the variation within samples. Procrustes distance is closely approximated by Euclidean distance in Procrustes tangent space, which can be computed as the square root of the sum of squared differences between the two means for all co-ordinates. We used permutation methods (Good, 1994; Edgington, 1995) to test against the null hypothesis of no mean difference between the samples.

To assess the variation between groups, we used canonical variate analysis (CVA; Mardia *et al.* 1979: 338–348). Also called canonical discriminant analysis, CVA is a widespread technique for assessing and displaying variation among groups relative to the variation within the groups (for more detailed explanations, see Albrecht, 1980; Campbell & Atchley, 1981). The method successively extracts axes (canonical variates, CVs) from multidimensional space that have the greatest ratio of among-group to within-group variance. Therefore, plots of the first few CVs are optimal displays of differences among groups. These plots are scaled relative to the pooled estimate of within-group variation.

To display the shape features associated with the CVs, we used thin-plate spline interpolation (e.g. Bookstein, 1991: 316–338; Dryden & Mardia, 1998: 205–222) to produce transformation grids that show the transformation from a grid with square cells superimposed onto the average landmark configuration (Fig. 3B) to a configuration shifted up or down along the respective CV axis by an arbitrary amount (0.06 Procrustes units).

Assessment of clustering in the data

In one case, the samples of Lake Apoyo, where no differences in body shape between named species could be found, we used a parametric bootstrap test based on *k*-means clustering to examine whether there was any evidence of clustering in the data (for a more detailed description, see Klingenberg *et al.*, 2001: 789). This test simulates homogeneous random data as a multivariate normal distribution with a covariance matrix that has the same eigenvalues as in the original data set, and applies the *k*-means clustering method (Krzanowski & Marriott, 1995: 80) to find clusters maximizing the between-group differences relative to the within-group variation. If clustering in the data is real, the ratio of between- to within-group variance should be greater in the real data than in the simulated random data sets. We conducted 10 000 random simulations for this test.

RESULTS

DIFFERENCES AMONG SPECIES

The three species considered here differed significantly in their average body shapes (Wilk's lambda = 0.29; $P < 0.001$). The Procrustes distance between *A. citrinellus* and *A. labiatus* was 0.038, the one between *A. citrinellus* and *A. zaliosus* was 0.047, and the one between *A. labiatus* and *A. zaliosus* was 0.050 ($P < 0.0001$ for permutation tests for all three pairwise comparisons).

The body shape differences from the overall mean showed characteristic features for each of the three species (Fig. 4). *A. citrinellus* had a higher body than the other two species, and its transformation grid was therefore characterized by a relative dorsoventral expansion and anterior–posterior contraction. The anterior–posterior contraction was particularly strong in the trunk region, noticeably stronger, for example, than in the gill region between the eye and the pectoral and pelvic fin bases. The caudal peduncle of *A. citrinellus* tapered toward the posterior and its central section (landmark 12) did not extend as far posteriorly as in the other two species (in Fig. 4, the 'notching' at landmark 12 is due to the exaggeration of the shape differences). Overall, *A. citrinellus* had a large head and a high body shape tapering posteriorly and a trunk region shortened in comparison to the other two species. In contrast, both other species showed various degrees of dorsoventral compression and anterior–posterior elongation, and a dorsoventral expansion of the posterior part of the caudal peduncle. In *A. labiatus*, the elongation was most pronounced in the head, whereas the trunk was relatively short. The relatively long distance

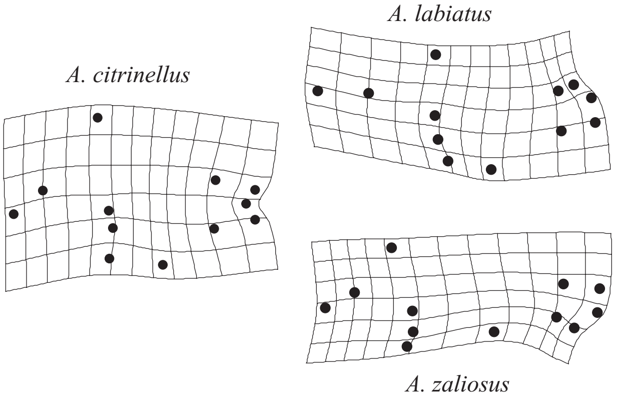

Figure 4. Shape changes from the overall average of *A. citrinellus, A. labiatus* and *A. zaliosus* to the shape means of the three species. The transformation grids show the deformations from the overall average, where all grid cells are square (Fig. 3B). The shape differences have been exaggerated five-fold for better visualization.

between landmarks 1 and 2 indicated an elongated snout in *A. labiatus*. The base of the pelvic fin was set behind the base of the pectoral fin, in relative proximity to the anterior base of the anal fin. The dorsal contour of the caudal peduncle was shorter than the ventral edge. In summary, *A. labiatus* had an elongated head that was enlarged relative to the trunk. In contrast, the distinctive feature of the body shape of *A. zaliosus* was the strongly elongated trunk but shortened and relatively small head. The base of the anal fin was relatively short, and the ventral contour of the caudal peduncle was shorter than the dorsal contour.

The CVA of the pooled samples also showed similar differences among species. Although species average shapes were clearly distinct, the 90% equal frequency ellipses indicated considerable overlap in the scatter of data (Fig. 5). The CV1 set *A. citrinellus* apart from the other two species, and was associated with the relative height of the body and the tapering of the caudal peduncle. The CV2 was primarily a contrast of *A. labiatus* vs. *A. zaliosus*, corresponding to differences in the relative length of the trunk, the arrangement of the pelvic fins, and the shape of the caudal peduncle.

Local variation between A. citrinellus and A. labiatus in Lake Nicaragua

The picture became more complex when the differentiation among named species was examined locally. All specimens of *A. labiatus* in our study were caught at the location of Isletas, in Lake Nicaragua, where this taxon was found syntopically with *A. citrinellus*. Accordingly, we conducted an analysis of body shape variation just in this location. An analysis distinguishing species as well as the trophic and colour morphs indicated that variation among groups was just statistically significant (Wilk's lambda = 0.35; $P = 0.046$). The corresponding CVA indicated that variation among the different morphs of *A. citrinellus* appeared to be at least as important as the differences between nominal species (Fig. 6). Indeed the Procrustes distance between the average shapes for the pooled morphs of both species was 0.018 and was marginally non-significant in the permutation test ($P = 0.056$). This distance was smaller than that for the pooled samples from all locations, indicating that the body shape of *A. citrinellus* at Isletas was more similar to *A. labiatus* than it was to the average shape of *A. citrinellus* from all sampling locations.

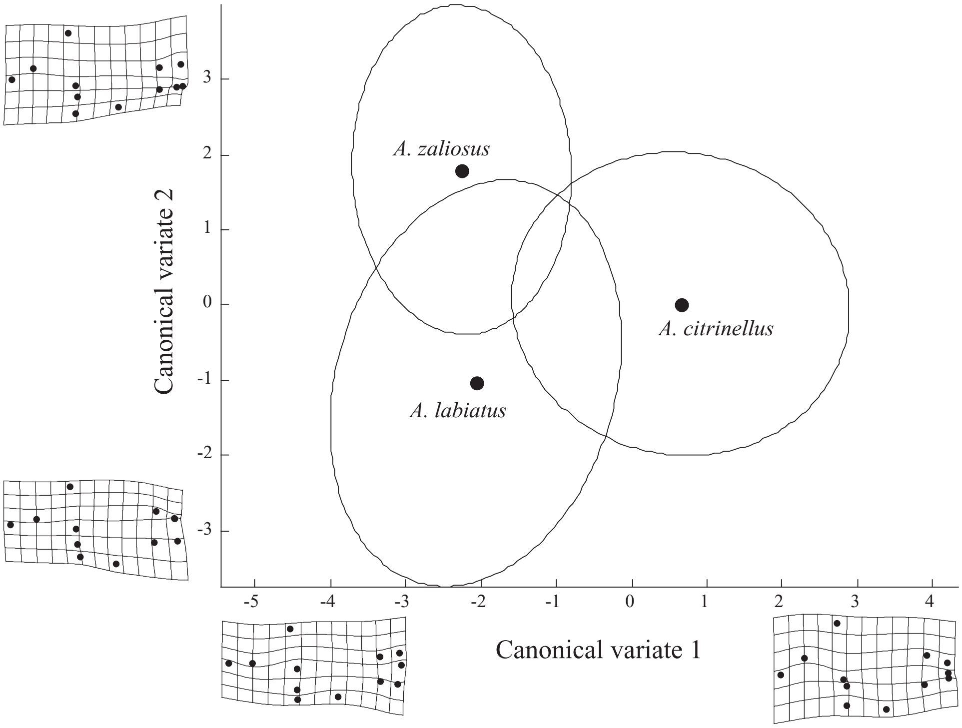

Figure 5. Canonical variate (CV) analysis of variation among *A. citrinellus, A. labiatus* and *A. zaliosus*. The plot shows the species averages (pooled samples of all locations) and the 90% equal frequency ellipses for the scatter of data points. The transformation grids show the shape changes from the overall mean associated with each CV axis (by an arbitrary amount of 0.06 Procrustes units in positive and negative directions). In the diagram showing the shape transformation for CV2 with negative sign, landmarks 9 and 12 are superimposed.

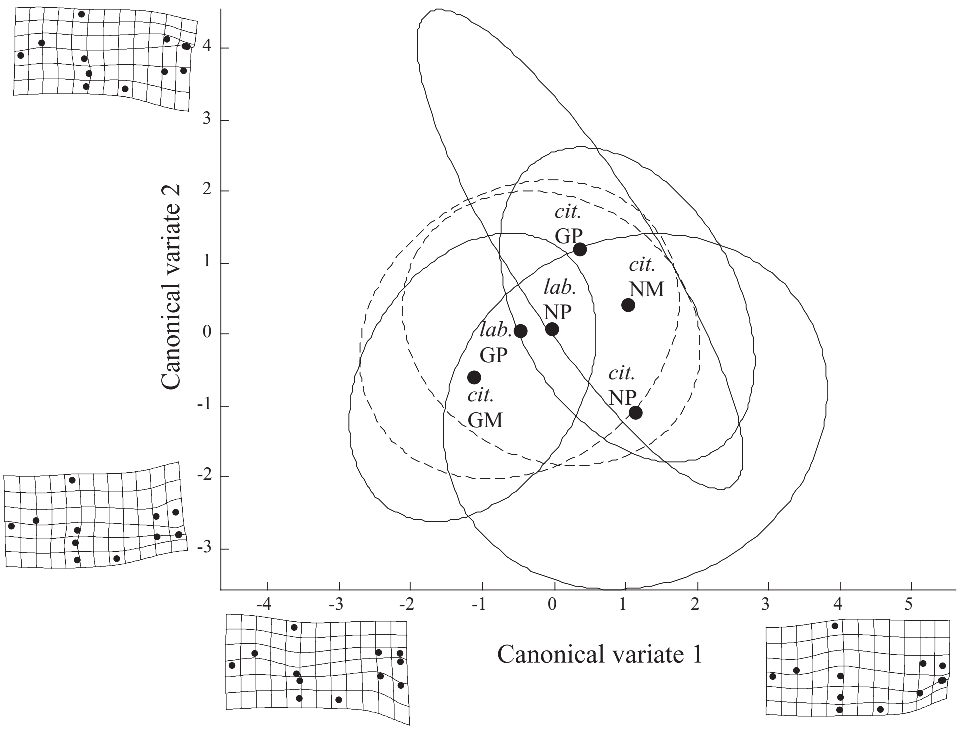

Figure 6. Variation between *A. citrinellus* and *A. labiatus* and among their trophic and colour morphs in syntopy. The data used is the sample from Isletas (Lake Nicaragua), where both species were collected. The 90% confidence ellipses are drawn with solid lines for *A. citrinellus* and with dashed lines for *A. labiatus*. *cit.*, *A. citrinellus*; *lab.*, *A. labiatus*; N, normal colour; G, gold colour; P, papilliform pharyngeal jaw; M, molariform pharyngeal jaw. In the diagrams showing the shape transformations for CV2, landmark 12 is superimposed on landmark 9 (for positive sign) and on landmark 11 (for negative sign).

Local variation between A. citrinellus and A. zaliosus in Lake Apoyo

The local analysis in Lake Apoyo, the only locality where *A. citrinellus* was found syntopically with *A. zaliosus*, was difficult because only seven specimens of *A. citrinellus* were caught (Table 1). The Procrustes distance between the two taxa in this local comparison was 0.026 and not statistically significant in the permutation test ($P = 0.16$). Ordinations by principal component analysis (not shown) did not reveal any apparent clustering of data points. Clustering enforced with the *k*-means clustering method resulted in a partitioning of specimens that was unrelated to the initial identification. To evaluate whether there was any clustering in the data, we ran a parametric bootstrap test, which indicated that the data from Lake Apoyo did not exceed the degree of clustering expected in homogeneous random data ($P = 0.91$). Therefore, our samples did not indicate any shape differences between the two species.

DIFFERENCES WITHIN *A. CITRINELLUS*

Geographic variation

There was considerable differentiation among collection sites in *A. citrinellus* (Wilk's lambda = 0.059, $P < 0.001$; colour and trophic morphs pooled within each location). The first CV primarily separated the three crater lakes (Lakes Apoyo, Masaya, and Xiloa) from the system of the large lakes (Fig. 7). The shape changes associated with the CV1 indicated that fish from the crater lakes tended to have a narrower base of the pectoral fin and a more strongly tapering caudal peduncle than did those from the large lakes. The CV2 separated the two large lakes and, in parallel, the crater lakes, and it more or less corresponded to a geographical direction from north-west to south-east, except for the sample from Tisma, which did not correspond to its position between Lakes Managua and Nicaragua. This CV was associated with a pronounced dorsoventral shift of the eye relative to the mouth, a dorsoventral compression or extension of the pectoral and pelvic fin bases, and variation in the shape of the caudal peduncle.

Variation among the trophic and colour morphs

The trophic and colour morphs clearly differed in their average shapes (Wilk's lambda = 0.45, $P < 0.001$), but there was extensive overlap between them (Fig. 8). The CV1 means of the groups with papilliform jaws

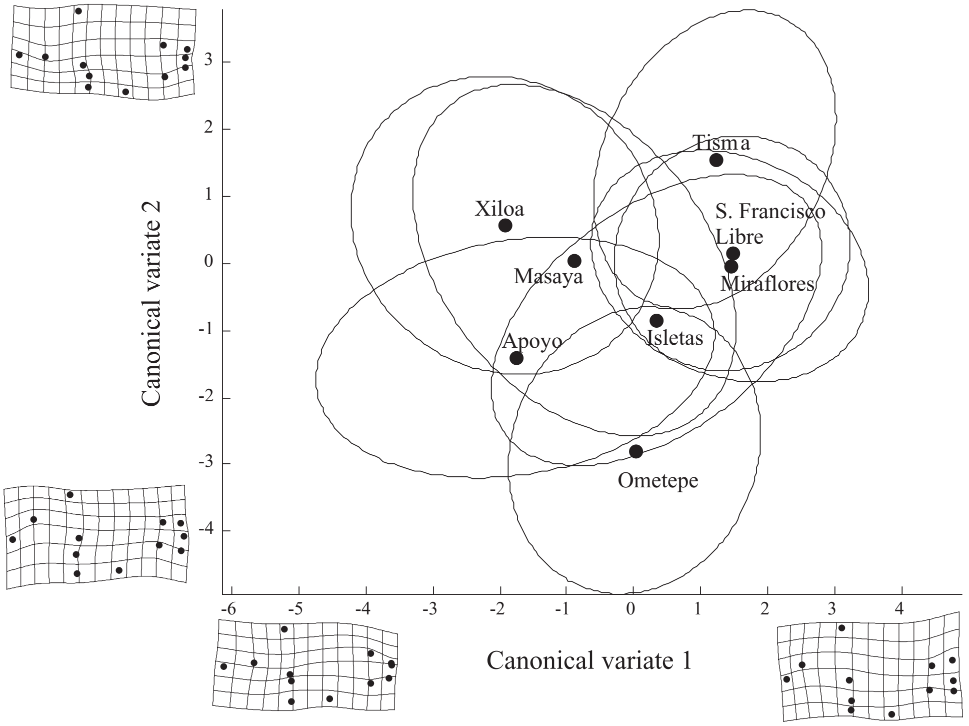

Figure 7. Shape variation among locations in *A. citrinellus*. The plot shows averages and 90% equal frequency ellipses from analyses of the pooled trophic and colour morphs at each location. The locations Miraflores and San Francisco Libre are at Lake Managua, and Ometepe and Isletas are at Lake Nicaragua, while Tisma belongs to the river system seasonally connecting them. Lakes Apoyo, Masaya, and Xiloa are isolated crater lakes.

were lower compared with the means of the groups with molariform jaws. The greater CV1 in molariforms was associated with an increase in the relative size of the head, and particularly its branchial region, since the pectoral and pelvic fin bases were moved ventrally relative to the snout, eye, and dorsal fin base. However, there was no simple correspondence between trophic morph and body shape, because this difference was affected strongly by colour morph (Fig. 8). First, the fish of the normal colour morph with papilliform jaws had intermediate CV1 scores between the gold-coloured papilliforms and the molariforms. Second, the CV2 clearly set apart the gold-coloured molariforms from the other three groups.

The distinct shape of gold-coloured fish with molariform jaws is particularly apparent in the graph of differences of each morph from the overall mean of all four groups (Fig. 9). The difference involved a shortening and forward movement of the dorsal contour of the caudal peduncle and a weaker forward shift of the anterior base of the dorsal fin; as a result, the vertical lines of the transformation grid were bent forward on the dorsal side. There were also some traits that the molariform morphs of both colours had in common: the base of the pelvic fin was set at a greater distance ventrally and slightly anterior to the base of the pectoral fin, resulting in a deeper ventral part of the head and branchial region compared with the papilliform morph. There were no apparent shape features that would consistently distinguish the normal from the gold-coloured morph.

DISCUSSION

This study has revealed a variety of patterns of body shape variation in the *A. citrinellus* species complex. Not only did the three named species differ in shape (Fig. 4), but *A. citrinellus*, the species sampled most extensively, also showed ample geographic variation (Fig. 7) and differentiation among its colour and trophic morphs (Fig. 9). This structure of variation among populations and alternative morphs is consistent with previous studies of morphometric variation (Meyer, 1990b) and molecular markers (Wilson *et al.*, 2000).

The species differences are consistent with the qualitative descriptions and ratios of distance measurements published in a detailed taxonomic treatment of the group (Barlow & Munsey, 1976). Our analyses confirmed the characterization of *A. citrinellus* as having a deeper body and steeper forehead than the other two species considered here (Fig. 4), and they particularly

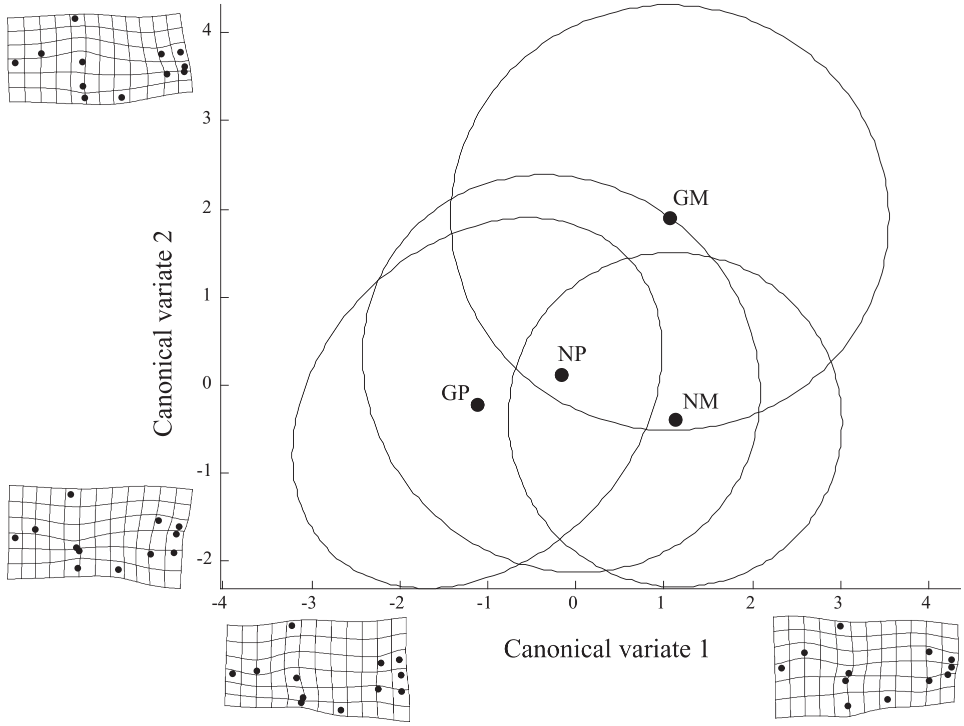

Figure 8. Variation among trophic and colour morphs in *A. citrinellus*. Canonical variates were computed from samples of the four combinations of colour and trophic morphs pooled across locations. N, normal colour; G, gold colour; P, papilliform pharyngeal jaws; M, molariform pharyngeal jaws.

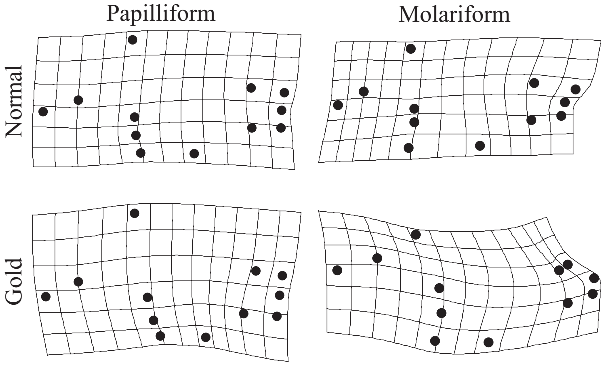

Figure 9. Body shape differences among the trophic and colour morphs of *A. citrinellus*. The shape changes were computed from the average shape of *A. citrinellus* to each morph, and have been amplified five-fold for better visibility.

underscored the great variability of this taxon, which may be a complex of multiple sibling species (McKaye *et al.*, 2002). Barlow & Munsey (1976) described *A. labiatus* as having a slender body and pointed head, in addition to the characteristically enlarged lips. Our analyses confirmed this by showing the head region to be expanded relative to the trunk, and add further aspects like the somewhat more deeply set eye and the pelvic fin base shifted posteriorly in relation to the pectoral fin base (Fig. 4). The relatively long snout of *A. labiatus*, along with its characteristic hypertrophied lips, may relate to the foraging habits of this species, which feeds with the mouth in contact with the substrate (Barlow & Munsey, 1976). Finally, the elongate body shape of *A. zaliosus* was used as one of the defining traits for the species (Barlow & Munsey, 1976), and the morphometric analysis located this elongation particularly in the trunk region, whereas the head was not found to be enlarged. This elongation may relate to the life style of *A. zaliosus*, which inhabits open water more than do the other species (Barlow & Munsey, 1976), and therefore parallels the differentiation between benthic and limnetic forms observed in other species complexes of freshwater fishes (Schluter, 1993) or differences between more benthic and more pelagic life styles in marine fishes (Klingenberg & Ekau, 1996).

The samples of the three species, with *A. citrinellus* samples pooled across different locations, had statistically different average shapes and could be separated with only moderate overlap (Fig. 5). Surprisingly, however, it was much more difficult to distinguish named species in samples from the same location. The Procrustes distances between species in these local comparisons were considerably smaller than they were for the overall comparison (0.018 and 0.038 for *A. citrinellus* against *A. labiatus*, 0.026 and 0.047 for *A. citrinellus* against *A. zaliosus*). The range of variation of *A. labiatus* was mostly contained within that of the different morphs of *A. citrinellus* caught at the same location (Isletas; Fig. 6), and the difference in mean shapes between the two species did not quite reach the conventional 5% significance level. In the sample from Lake Apoyo, the mean shapes of *A. citrinellus* and *A. zaliosus* could not be statistically distinguished at all, and there did not appear to be any clustering in the data. Continuous morphometric variation between *A. citrinellus* and *A. zaliosus* has also been found in a previous study (T. Hrbek, K. Parsons & A. Meyer, unpubl. data). Taken together, these analyses suggest that *A. labiatus* and *A. zaliosus* tend to have shapes that are more similar to the syntopic populations of *A. citrinellus* than they are to allotropic populations of that species.

This finding is contrary to the expectation of morphological divergence as it would be associated with a scenario whereby the named species have originated recently by sympatric speciation and ecological diversification (e.g. Schluter, 2000). In that case, one would expect greater differences between species where they occur syntopically because of selection favouring extreme phenotypes. Patterns consistent with this hypothesis have been reported for the *A. citrinellus* species complex (Barlow & Munsey, 1976). An alternative explanation more consistent with the morphometric data of our study would be introgressive hybridization, as has also been hypothesized for these taxa (Barlow & Munsey, 1976). Given the complex behavioural mechanisms of mate choice that produce the observed associative mating by colour morph (reviewed by Barlow, 1998), there may be possibilities for a rich structuring of partial reproductive isolation and introgression. Another possibility is that ecophenotypic effects or local adaptive evolution leads to similar body shape of syntopic populations in response to their shared environment. Detailed studies of molecular markers can contribute to resolve this issue (M. Barluenga & A. Meyer, unpubl. data), particularly when combined with ecological and behavioural data (McKaye *et al*., 2002).

Wilson *et al*. (2000) reported substantial genetic differentiation between lakes, and particularly for Lake Nicaragua it also showed significant genetic differentiation between the colour morphs, but not between the alternative pharyngeal jaw morphs. Our analyses showed that there are differences in body shape between the colour morphs of *A. citrinellus*, but that there is also a substantial effect of the pharyngeal morph (Figs 8, 9). The molariform morph differs from the papilliform morph primarily by an enlargement of the lower head region that houses the pharyngeal jaw apparatus, reflecting the hypertrophy of the pharyngeal jaws and associated musculature (e.g. Meyer, 1990b). This is consistent with the finding that the pharyngeal jaw polymorphism has consequences for many other structural features of the head and gill region in another cichlid species (Smits, Witte & van-Veen, 1996). It is more surprising that there appear to be substantial interactions between the effects of the colour and pharyngeal jaw morphs, that is, the effect of jaw morph on body shape also depends on colour (and vice versa). To unravel the mechanisms underlying this phenomenon, an experimental ‘common garden’ approach would be required.

Incipient sympatric speciation has been invoked for the colour morphs of *A. citrinellus* based on behavioural observations (McKaye, 1980), ecological considerations (Meyer, 1990a) and a study of molecular markers (Wilson *et al*., 2000). Sympatric speciation has also been hypothesized as a possible cause for the divergence of *A. citrinellus* and *A. zaliosus* in Lake Apoyo (Barlow & Munsey, 1976) and for a number of additional putative species in Lakes Apoyo and Xiloa (McKaye *et al*., 2002), some of which have been described as taxonomic species (Stauffer & McKaye, 2002). Our morphometric data do not provide support for the hypothesis of local speciation and character divergence, but they underscore the intricate nature of variation in this species complex.

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