CAPTIVE BREEDING OBSERVATIONS SUPPORT THE VALIDITY OF
A RECENTLY DESCRIBED CICHLID SPECIES IN
LAKE APOYO, NICARAGUA

Ronald G. Oldfield

ABSTRACT.—*Amphilophus citrinellus*, *A. zaliosus*, and a recently described species, *A. astorquii*, were held in a large aquarium and allowed to mate. The species mated assortatively. Observations were made of the breeding fish and their offspring, and of additional fish raised in the laboratory, including *A. labiatus*. *Amphilophus astorquii* were distinguishable from *A. zaliosus* by having a less elongate lateral spot, deeper body, and a more rounded snout, and were distinct from *A. citrinellus* and *A. labiatus* by having black breeding coloration, a continuous lateral stripe, a more elongate lateral spot, and a shallower body. Characters shared between *A. astorquii* and *A. zaliosus*, but absent in *A. citrinellus* and *A. labiatus*, including black breeding coloration, an elongate lateral spot, a continuous lateral stripe, and a relatively shallow body suggest recent common ancestry and intra-lacustrine speciation.

Key words: *Amphilophus*, crater lake, evolution, mate choice, speciation.

INTRODUCTION

The Midas cichlid species complex is a group of cryptic species from the Great Lakes and volcanic crater lakes of Nicaragua (Villa, 1976), with the type species of the genus, *Amphilophus citrinellus* (Günther, 1864; Fig. 1), from Lake Nicaragua. Populations in some of the lakes appear to have recently evolved into multiple species. In 1976 Barlow & Munsey described the arrow cichlid, *Amphilophus zaliosus*, as a species endemic to Lake Apoyo, and determined that it was more similar to sympatrically occurring *A. cf. citrinellus* than to its two congeners, *A. citrinellus* and *A. labiatus*, in other lakes, suggesting that *A. zaliosus* arose from *A. cf. citrinellus* within Lake Apoyo. Villa (1982), however, did not accept that *A. zaliosus* was a valid species. Despite the position taken by Villa (1982), laboratory experiments provided additional evidence that *A. zaliosus* was a distinct species: both *A. zaliosus* and *A. cf. citrinellus* from Lake Apoyo preferred to mate with conspecifics when given a choice between their own species or the other (Baylis, 1976), and the specific status of *A. zaliosus* is now recognized (Reis *et al.*, 2004). More recently, Barluenga *et al.* (2006a) used genetic and morphometric methods in an attempt to demonstrate sympatric
speciation in Lake Apoyo by showing that *A. zaliosus* arose from *A. citrinellus* after the introduction of the latter species from nearby Lake Nicaragua.

There has been recent evidence that additional cryptic *Amphilophus* species might exist in Nicaragua. Stauffer & McKay (2002) described three new species from Lake Xiloa and McKay et al. (2002) suggested that three more might also exist in Lake Apoyo. McKay et al. (2002) provided photographs and microsatellite allele frequencies for the putative Lake Apoyo species but no characters were presented and the species were not described. Barluenga et al. (2006a; 2006b) provided no indication that so many species might exist in Lake Apoyo (Schliewen et al., 2006), and only this year were the three additional species finally described (Stauffer et al. 2008): *A. astorquii*, *A. flaveolus*, and *A. chancho*. The descriptions were based on morphological and color characters in wild specimens. However, Central American cichlids are well known for phenotypic plasticity (Meyer, 1987), and the role of plasticity in producing the diagnostic characters of each putative species is currently unknown. In addition, Stauffer et al. (2008) stated that the three species mate assortatively in the lake, but no data were provided.

The current report focuses on one of the putative species from Lake Apoyo, *Amphilophus astorquii*. An experiment was designed to (1) test for assortative mating in this species, and (2) eliminate the potential for phenotypic plasticity in order to identify genetic differences in morphology and coloration between *A. astorquii* and other *Amphilophus* species. *Amphilophus astorquii*, *A. citrinellus*, and *A. zaliosus* were allowed to mate in a common-garden-type captive environment. This environment allowed freedom of mate choice among individuals, and eliminated environmental factors as a cause for differences observed among offspring of different parentage. Observations focused on behavior, coloration, and body shape, and were interpreted in the context of characters reported by Barlow & Munsey (1976). Those authors diagnosed *A. zaliosus* as differing from *A. cf. citrinellus* by possessing a long, shallow body and a lateral spot that is notably longer than deep. In addition, when *A. zaliosus* displays the ‘stripe’ color pattern (Barlow, 1976), the central lateral spot is often fused with the other lateral spots that form the stripe, whereas in *A. cf. citrinellus* the spots are in a row and appear as a stripe, but are not connected to one another (Barlow & Munsey, 1976).

**MATERIALS AND METHODS**

In 2005, adult males and females of three putative *Amphilophus* species (Fig. 2) were placed into a large aquarium (113 l: 244 x 61 x 76 cm) set up with natural gravel, rocks, and wood to create a heterogeneous and complex environment (Fig. 3). Fish included one male and one female *A. astorquii* caught in Lake Apoyo, two male and two female oligomelanic *A. citrinellus* second generation captive bred from wild stock obtained from Lake Nicaragua (the type locality of this species), two males and one female *A. zaliosus* second generation captive-bred from wild stock obtained from Lake Apoyo, and one female wild-caught in Lake Apoyo. The *A. astorquii* were identified independently by Kenneth McKay and then by Jeffrey McCrary, two of the authors that initially proposed it to be a distinct species.

Observations were made of the mating behavior and coloration of the adults. Offspring were observed alive and then were preserved and compared with additional *Amphilophus* specimens on hand at the University of Michigan Museum of Zoology. Only *A. astorquii* and *A. citrinellus*...
successes fully reproduced in the experimental environment, but \textit{A. zaliosus} (second generation captive-bred from parents caught in Lake Apoyo) and \textit{A. labiatus} (second generation captive-bred from parents caught in Lake Nicaragua) were raised in the laboratory in additional aquaria, which allowed comparisons among juveniles of all four species after developing under similar conditions. Data were gathered from \textit{Amphilophus} specimens cataloged in the UMMZ collection for additional comparisons.

Lateral stripe and central lateral spot were examined on all specimens. Lateral stripe was considered continuous on an individual if melanophores connected any two adjacent lateral spots. Statistical analyses were performed to help characterize the shape of the central lateral spot within each sample. A sign test was used to determine if the spot was generally long or generally deep by assessing whether the number of individuals in which the spot was longer than deep (or deeper than long) was greater than that expected by chance. Spot length was not equal to spot depth in any individual. To determine if the lateral spot was significantly longer than it was deep (or deeper than it was long), a paired t-test was used to compare spot lengths with spot depths within each sample. Data were not analyzed if specimens were not exhibiting the 'stripe' color pattern (Barlow, 1976), or if there was an insufficient sample size.

Statistical comparisons of color pattern and body depth across samples were made among captive raised juveniles in order to minimize environmental differences that might influence morphological development (Barlow, 1976; Meyer, 1987; Stauffer et al., 1995). Relative length of the lateral spot was calculated by two different methods. The first was the ratio of spot length/spot depth. The second was spot length/standard length (SL). For each measure, relative spot length was compared among species using a one-way ANOVA with Tamhane post-hoc analysis to account for unequal variances. Relative body depth was calculated as the ratio of body depth at the deepest vertical plane /SL, and was compared among species using a one-way ANOVA with Tukey HSD post-hoc analysis. Alpha was set at 0.05 for all analyses.

**RESULTS**

Each fish paired with a mate of its own species. One of the most notable observations was the breeding coloration of \textit{Amphilophus astorquii} and \textit{A. zalisosus}. Both species changed from the typical barred coloration (Barlow, 1976) to solid black (Fig. 4).

The \textit{Amphilophus astorquii} pair and one pair of \textit{A. citrinellus} successfully produced offspring. Two weeks after the pair of \textit{A. astorquii} produced their
second brood, the neighboring pair of *A. citrinellus* also produced a brood. The *A. citrinellus* offspring began to intermingle freely with the *A. astorquii* offspring and the two eventually formed a single social unit. Even at small size (2-3 cm SL), the *A. astorquii* were visually distinguishable from the Lake Nicaragua *A. citrinellus* (Fig. 5).

The coloration of the *Amphilopus astorquii* offspring was different than that of *A. citrinellus* offspring. The background color of *A. astorquii* was dull gray, while *A. citrinellus* had a slight yellow tinge to the body and red tinge to the dorsal and anal fins. In the large experimental aquarium, the juveniles of both species maintained the “lateral stripe” color pattern (Barlow, 1976), in
which only the central lateral spot of each vertical bar are darkened, and these spots together form a horizontal stripe along the side of the body. All the lateral spots connected to form a continuous stripe in *A. astorquii*, but the spots were slightly separated in *A. citrinellus*, resulting in a broken stripe. When 12 *A. astorquii* were removed from the large aquarium and individually isolated in smaller aquaria the lateral stripe became broken as the area between the spots lightened. When comparisons were made to additional museum specimens, the only other species observed with a continuous lateral stripe was *A. zaliosus*. All individuals of all other species displayed broken stripes (Table 1).

The large central lateral spot also differed between the *Amphilophus astorquii* and *A. citrinellus* offspring. In *A. astorquii* the spot was always longer than it
Table 1. Descriptive data of *Amphilophus* specimens of diverse origins and some of their color characters. Lateral stripe was considered continuous on an individual if there were melanophores connecting any two lateral spots. To determine if the lateral spot was generally longer than it was deep (or deeper than long), the number of individuals in which it was longer (or deeper) was compared to that expected by chance. To determine if the lateral spot was significantly longer than it was deep, the two sets of values were compared with a paired t-test. Data were not analyzed (NA) if specimens were not exhibiting the ‘stripe’ color pattern (Barlow 1976), or if there was an insufficient sample size.

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Wild or Captive-bred</th>
<th>n</th>
<th>SL (mean ±SD, mm)</th>
<th>Lat. stripe</th>
<th>Lat. spot generally longer than deep?</th>
<th>Lat. spot signif. longer or deeper?</th>
<th>UMMZ #</th>
</tr>
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<tr>
<td><em>A. astorquii</em></td>
<td>L. Apoyo C</td>
<td>21</td>
<td>62.0±6.1 solid</td>
<td>21 longer, p&lt;0.001</td>
<td>long, p&lt;0.001</td>
<td>248482</td>
<td></td>
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</tr>
<tr>
<td><em>A. cf. citrinellus</em></td>
<td>L. Apoyo W</td>
<td>1</td>
<td>99.5 broken</td>
<td>0 longer</td>
<td>deep, p=NA</td>
<td>245110</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. citrinellus</em></td>
<td>L. Nicaragua C</td>
<td>22</td>
<td>53.2±5.8 broken</td>
<td>1 longer, p&lt;0.001</td>
<td>deep, p&lt;0.001</td>
<td>248841</td>
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<td></td>
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<tr>
<td><em>A. citrinellus</em></td>
<td>Costa Rica C</td>
<td>6</td>
<td>116.6±9.9 broken</td>
<td>0 longer, p&lt;0.001</td>
<td>deep, p&lt;0.004</td>
<td>248483</td>
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</tr>
<tr>
<td><em>A. citrinellus</em></td>
<td>Costa Rica W</td>
<td>3</td>
<td>52.3±15.4 NA</td>
<td>0 longer, p=NA</td>
<td>deep, p&lt;0.001</td>
<td>180647</td>
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<td>L. Managua W</td>
<td>5</td>
<td>72.8±26.3 broken</td>
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<td>deep, p=0.085</td>
<td>180617</td>
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<tr>
<td><em>A. cf. citrinellus</em></td>
<td>L. Xiloa W</td>
<td>4</td>
<td>129.6±4.5 broken</td>
<td>1 longer, p=NA</td>
<td>NA</td>
<td>181827</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. zaliosus</em></td>
<td>L. Apoyo W</td>
<td>5</td>
<td>101.9±26.3 solid</td>
<td>5 longer, p&lt;0.063</td>
<td>long, p&lt;0.055</td>
<td>234807, 234808, 245109</td>
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<tr>
<td><em>A. zaliosus</em></td>
<td>L. Apoyo C</td>
<td>7</td>
<td>64.7±19.0 solid</td>
<td>7 longer, p=0.016</td>
<td>long, p&lt;0.001</td>
<td>247666</td>
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</tr>
<tr>
<td><em>A. labiatus</em></td>
<td>L. Nicaragua C</td>
<td>12</td>
<td>56.4±7.2 broken</td>
<td>7 longer, p=0.774</td>
<td>square, p=0.682</td>
<td>248480</td>
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</tr>
</tbody>
</table>

Fig. 3. The experimental aquarium in which three *Amphilophus* species (*A. citrinellus*, *A. astorquii*, and *A. zaliosus* – including those individuals pictured in Figs. 2a, 2b, and 2c) were placed and allowed to form breeding pairs and reproduce.

was deep. In *A. citrinellus*, the spot was almost always deeper than long (Table 1). When comparisons were made to additional specimens, all had deep lateral spots, except *A. zaliosus*, which had an elongate lateral spot, and *A. labiatus*, in which the spot was square in shape (Table 1). When spot shape was compared quantitatively among captive raised juveniles of four *Amphilophus* species (Fig.
6) it was found to be more elongate in *A. astorquii* than in *A. citrinellus* (p < 0.001) and *A. labiatus* (p < 0.001), in both measures of relative length (spot length/spot depth and spot length/SL), and less elongate than in *A. zaliosus* in one measure (spot length/depth: p < 0.05), but not significantly different than *A. zaliosus* in the other (spot length/SL: p > 0.05) (Table 2).

Body depth in *Amphilophus astorquii* was significantly less than in captive-bred *A. citrinellus* (p < 0.001) and *A. labiatus* (p < 0.001), but was greater than in *A. zaliosus* (p < 0.001) of similar size (Table 2). In general appearance, the snout of *A. astorquii* seemed to be more rounded than the pointed snouts of *A. labiatus* (as well as having smaller lips than *A. labiatus*) and *A. zaliosus*, and slightly more rounded than in *A. citrinellus*.

**DISCUSSION**

*Amphilophus astorquii* possesses characters relating to breeding coloration, lateral stripe, lateral spot, body depth, snout shape, and lip size that distinguish it from *A. citrinellus, A. zaliosus*, and *A. labiatus* (Table 3). These characters were apparent in juveniles that were raised in captivity under similar environmental conditions, indicating that genetic factors contribute to their expression.

*Amphilophus astorquii* was distinguished from *A. citrinellus* from Lake Nicaragua. The offspring of *A. astorquii* had a more continuous lateral stripe, a more elongate lateral spot, a shallower body, and different background coloration than *A. citrinellus* offspring. In addition, *A. astorquii* had black breeding coloration whereas normal-morph *A. citrinellus* are known to have barred breeding coloration with a deep lateral spot (Barlow & Munsey, 1976 Fig. 2; Villa, 1976 Fig. 3). The two species each chose conspecific mates in the experimental aquarium.

*Amphilophus astorquii* was also distinguishable from *A. zaliosus*, by having a less elongate lateral spot, a deeper body, and a more rounded snout. *Amphilophus astorquii* mated assortatively from *A. zaliosus*, suggesting that mate choice may be a factor in reproductive isolation of the two species in their natural environment.

*Amphilophus astorquii* was also distinct from *A. labiatus*, which had a broken lateral stripe, a more square lateral spot, a deeper body, and larger lips.

*Amphilophus astorquii* shares characters with *A. zaliosus* that are typically not present in other *Amphilophus* species. Generally, in normally-colored morphs (those with barred rather than oligomelanic coloration) of *Amphilophus* species the bars become darker and the background color lighter during spawning, resulting in a very distinct, high contrast pattern (Barlow, 1976). Although *A. astorquii* (Fig. 1) and *A. zaliosus* (Konings, 1989 p. 215; McKay *et al.*, 2002 Fig. 20; Heigns, 2004 p. 26) have coloration typical of other *Amphilophus* cichlids when they are not breeding, they are the only two cichlids in Lake Apoyo that turn black when breeding. Observation of black breeding coloration in the laboratory is consistent with previous field (McKay *et al.*, 2002 Figs. 11, 12, 21) and captive (Heijns, 2002 p. 15) photographs of *A. zaliosus* and field observations of *A. astorquii* (McKay *et al.*, 2002 Figs. 16, 17; Stauffer *et al.*, 2002 Fig. 20; Heigns, 2004 p. 26).
Amphilophus citrinellus male exhibiting barred coloration typically observed during breeding in normal-morph (non-oligomelanic) individuals. (This individual was not used in the current experiment.) (b) *A. astorquii* female in the experimental aquarium, exhibiting solid black breeding coloration, which was also observed in the male.

*Amphilophus zaliosus* (Barlow & Munsey, 1976 Fig. 5, 6; Heijns, 2004 p. 26) and *A. astorquii* are also the only *Amphilophus* species known to have elongate lateral spots. Dark colored individuals which may represent additional species have been photographed in other Nicaraguan crater lakes (Barlow & Munsey, 1976 Figs. 3, 4; Stauffer & McKay, 2002 Fig. 14; Heijns, 2005b), but they appear to have deep lateral spots (McKay et al., 2002 Fig. 22; Heijns, 2005a p. 12). Like *A. zaliosus*, *A. astorquii* in the current experiment had a shallower body than *A. citrinellus* or *A. labiatus*. Barlow & Munsey (1976) considered a continuous lateral stripe to be a distinguishing feature of *A. zaliosus*, but *A. astorquii* also shares this character.
The co-occurrence of black breeding coloration, an elongate lateral spot, and a continuous lateral stripe in *Amphilophus astorquii* and *A. zaliosus*, but the absence of these characters in *A. citrinellus* and *A. labiatus*, and the observation that these differences are maintained under controlled laboratory conditions, suggests genetic similarity and common ancestry between *A. astorquii* and *A. zaliosus* within Lake Apoyo. This is not consistent with McKaye *et al.* (2002) who found microsatellite allele frequencies of *A. astorquii* to be most similar to *A. flaveolus*.

The characters discussed above may also be valuable in distinguishing *Amphilophus astorquii* from the other Lake Apoyo *Amphilophus* species described by Stauffer *et al.* (2008). *Amphilophus astorquii* may turn black when mating but most other *Amphilophus* species do not. *Amphilophus chancho* exhibit a barred reproductive color pattern both in Lake Apoyo (McKaye *et al.*, 2002 Figs. 13 and 14; Heijns, 2005a, 2005b; Stauffer *et al.*, 2008) and in captivity (CJ Exotics, 2006a). Captive-bred juvenile *A. chancho* have a background color of tan-gold (CJ Exotics, 2006b) rather than gray, which was observed in *A. astorquii*. The *A. astorquii* in the current study had an elongate lateral spot, while most other *Amphilophus* species in Lake Apoyo have deep lateral spots (Barlow, 1976 Fig. 5; Barlow & Munsey, 1976 Fig. 2). *Amphilophus chancho* have deep lateral spots both in nature (McKaye *et al.*, 2002 Figs. 13 & 14; Heijns, 2005a p. 9) and captivity (CJ Exotics, 2006a). However, whereas the lateral stripe is typically broken in *Amphilophus* species except *A. zaliosus* and *A. astorquii*, *A. chancho* has been photographed with both broken (McKaye *et al.*, 2002 Figs. 13, 14), and continuous stripes (CJ Exotics, 2006a).

*Amphilophus astorquii* also differs from *A. flaveolus*; the latter species exhibits a barred breeding color pattern in Lake Apoyo (McKaye *et al.*, 2002 Fig.
Fig. 5. Live juveniles hatched and photographed in the experimental aquarium: (a) *Amphilophus citrinellus*, exhibiting broken lateral stripe, deep central lateral spot, deep body, and slight yellow tinge to the body, (b) *A. astorquii*, exhibiting continuous lateral stripe, elongate central lateral spot, relatively shallow body, and drab gray body color.

18; Stauffer *et al.*, 2008). Whereas *A. astorquii* has an elongate lateral spot, *A. flaveolus* has a deep lateral spot both in nature (McKaye *et al.*, 2002 Fig. 18), and captivity (Heijns, 2005a p. 8). The continuous lateral stripe also seems to distinguish *A. astorquii* from *A. flaveolus*, for which published photos have only indicated a broken stripe (McKaye *et al.*, 2002 Fig. 18).

Intra-lacustrine speciation may have occurred in Lake Xiloa (McKaye *et al.*, 2002) and Lake Apoyo (Barluenga *et al.*, 2006), but molecular analyses have not conclusively demonstrated such an event (Schliewen *et al.*, 2006; Barluenga *et al.*, 2006b). The mate-choice experiments employed by Baylis (1976) showed that *Amphilophus zaliosus* was reproductively isolated from sympatric *Amophilopus cf. citrinellus* and strongly supported Barlow & Munsey’s (1976) conclusion that *A. zaliosus* was a distinct species. The assortative mating observed in the current experiment similarly supports the proposal by McKaye *et al.* (2002) that *A. astorquii* is another distinct species in Lake Apoyo.
Observations on Amphilophus

Table 2. Relative lateral spot length and body depth among juvenile Amphilophus species captive-raised in the laboratory at the UMMZ (mean±SD). Relative lateral spot length was calculated by two methods. Spot length/spot depth in A. astorquii was significantly different than in each of the other species. Spot length/SL in A. astorquii was significantly different than each of the other species, except A. zaliosus. Relative body depth was significantly different between each pair of species.

<table>
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<tr>
<th>Species</th>
<th>Locality</th>
<th>Wild/ Captive</th>
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<th>Lateral spot length/depth</th>
<th>Body depth/SL</th>
<th>UMMZ #</th>
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<tbody>
<tr>
<td>A. astorquii</td>
<td>L. Apoyo</td>
<td>C</td>
<td>21</td>
<td>1.27±0.10</td>
<td>0.39±0.01</td>
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</tr>
<tr>
<td>A. citrinellus</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<tr>
<td>A. zaliosus</td>
<td></td>
<td></td>
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<td>&lt; 0.05</td>
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<td>A. labiatus</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
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<td>L. Nicaragua</td>
<td>C</td>
<td>22</td>
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<td>0.44±0.01</td>
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<td>L. Apoyo</td>
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<td>7</td>
<td>1.59±0.22</td>
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<td>A. labiatus</td>
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<td>1.01±0.08</td>
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</table>

Table 3. Summary of characters compared among the four species listed in Table 2. For breeding coloration: 0 = barred, 1 = solid black; lateral stripe: 0 = broken, 1 = solid; lateral spot: 0 = not elongate, 1 = elongate; body depth: 0 = relatively deep, 1 = relatively narrow; snout shape: 0 = rounded, 1 = pointed; lip size: 0 = small, 1 = large.

<table>
<thead>
<tr>
<th>Species</th>
<th>breeding coloration</th>
<th>lateral stripe</th>
<th>lateral spot</th>
<th>body depth</th>
<th>snout shape</th>
<th>lip size</th>
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<td>1</td>
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<td>A. citrinellus</td>
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</table>

Larger-scale mate choice tests similar to those performed by Baylis (1976) may confirm that the species discussed by Stauffer & McKaye (2002) and McKaye et al. (2002) in Lake Xiloa and Lake Apoyo are reproductively isolated at the prezygotic, ethological level. A phylogenetic analysis of behavioral and morphological characters might then successfully provide a historical context within which to interpret the evolution of Amphilophus species, whereas genetic approaches have yet been unconvincing (Schliewen et al., 2006). The diversity being revealed in these two lakes begs the question of whether similar levels of diversity might exist in cichlids in other Nicaraguan Lakes.

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Fig. 6. Preserved juveniles of (a) *Amphilophus citrinellus*, exhibiting broken lateral stripe, deep central lateral spot, and deep body, (b) *A. astorquii*, exhibiting continuous lateral stripe, elongate central lateral spot, and relatively shallow body. Scale is in cm.

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Fig. 6 (continued). Preserved juveniles of (c) *A. zaliosus*, exhibiting characters similar to those observed in *A. astorquii*, and (d) *A. labiatus*, with a broken lateral stripe, square-shaped central lateral spot, relatively deep body, and large lips. Scale is in cm.


