

are included in the *A. cf. citrinellus* sample of Barluenga *et al.*¹. Their morphological analysis does not justify the authors' conclusions about the number of morphologically differentiated taxa, because even *A. zaliosus* and *A. citrinellus* broadly overlap in morphospace (Fig. 4b of ref. 1).

Because Barluenga *et al.*¹ exclude *A. labiatus* and overlook the phenotypic and taxonomical complexity of the *A. citrinellus* complex, their microsatellite-based phylogenetic inferences (see their supplementary Fig. 3a, b) cannot show monophyly of their two species in Lake Apoyo. These phylograms are based on allele frequencies for which the authors have simply pooled samples into *A. zaliosus* and *A. cf. citrinellus* "Apoyo".

In conclusion, the intermediate nuclear-genetic position of the Lake Apoyo *A. citrinellus* population between *A. zaliosus* and *A. citrinellus* from Lake Nicaragua is incompatible with sympatric speciation. Instead, it indicates that two invasions occurred, followed by introgressive hybridization and fixation of one mitochondrial

haplotype — as in other fish species⁷. The close proximity of Lake Apoyo and Lake Nicaragua makes this easily possible.

Because *A. citrinellus* and *A. labiatus* in Lake Nicaragua are hardly distinguishable at microsatellite loci⁴ and their mtDNA sequences are indistinguishable, we do not yet know whether these colonizations involved two waves of one of these species, or one of each. It will be necessary to test these alternatives, and to determine whether genetic similarity of the Lake Apoyo endemics is due to secondary introgression or shared ancestry^{7,10,11}.

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Barluenga *et al.* reply

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We reported a case of sympatric speciation in the Nicaraguan Midas cichlid species complex¹. Schlieven *et al.*² question the interpretation of aspects of our data, but their proposed alternative scenario of multiple colonization and hybridization is considerably less parsimonious, contains some inconsistencies, and is incompatible with the available evidence.

Amphilophus labiatus is not a sister species of the Lake Apoyo *Amphilophus* fauna³. The central haplotype in Fig. 2 of ref. 1 indeed contains specimens of *A. labiatus* and *A. citrinellus*; this figure, as indicated¹, is a simplified version of our earlier one³. However, we have shown that *A. labiatus* is more distantly related to the monophyletic Lake Apoyo assemblage than is *A. citrinellus* from Lake Nicaragua³ (*Nature* did not permit us to show additional analyses or figures to this effect). This is also supported by morphometrics⁴ and the absence of *A. labiatus* from Lake Apoyo. Our microsatellite, mitochondrial (mt) DNA and amplified fragment-length polymorphism (AFLP) analyses¹ confirm that *A. zaliosus* and *A. citrinellus* from Lake Apoyo are each other's closest relatives.

There is no evidence to support the assertion by Schlieven *et al.*² that *A. citrinellus* of Lake Apoyo is closer to *A. citrinellus* of Lake Nicaragua than is *A. zaliosus*. Factorial correspondence analysis does not either, as it illustrates present but not past genetic distances (in fact, any ancestral population should be equidistant from all of its descendants). Similarly,

with only three potential cases in the more than 120 individuals included from Lake Apoyo (as determined by the analyses using Structure software; see Fig 3 in ref. 1), introgression is very rare in *A. citrinellus* — if it exists at all ($P < 80\%$), as determined by the Structure analysis. The argument by Schlieven *et al.*² for secondary introgression from Lake Nicaragua into Lake Apoyo is based on a single specimen, which is unlikely to be an introgressant as it contains alleles of the genomes of all three populations, which is likely to be an artefact of the analysis. The monophyly of Lake Apoyo's *Amphilophus* species and the complete endemism of its mtDNA haplotypes argue against secondary colonization.

Instead, the analyses all indicate that *A. zaliosus* evolved sympatrically from *A. citrinellus* within Lake Apoyo. We showed that *A. zaliosus* is only about half as old as *A. citrinellus* from Lake Apoyo¹ (note that *A. citrinellus* from Lake Apoyo carries only a subset of the global *A. citrinellus* microsatellite alleles and that *A. zaliosus* carries only about half of the Lake Apoyo *A. citrinellus* alleles). These genetic data therefore rule out the alternative scenario proposed by Schlieven *et al.*, in which *A. citrinellus* entered Lake Apoyo in a second wave of colonization after *A. zaliosus*.

We do not believe that our sampling of the taxonomic diversity in Lake Apoyo was inadequate. Our Lake Apoyo data set does include morphs that others⁵ call "chanchó", "short" and

"amarillo". These morphotypes have never been formally described as species, no voucher specimens and no phenotypic or meristic information is available (only some photographs), and no experimental or observational data have been published that would support assortative mating. A previous genetic analysis⁵ based on three microsatellites yielded inconclusive results. Our own, much more detailed, analyses¹ find, so far, evidence for only two genetically discernable units of *Amphilophus* in Lake Apoyo — *A. zaliosus* and *A. citrinellus*. The seeming overlap in morphospace between the two Lake Apoyo species is due only to the two-dimensional projection of a multidimensional plot.

In summary, we maintain that the data fully support our original interpretations, whereas Schlieven *et al.*² propose a much less likely scenario that is not supported by the available data.

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