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Evidence for sympatric speciation?

Arising from: M. Barluenga, K. N. Stölting, W. Salzburger, M. Muschick & A. Meyer *Nature* **439**, 719–723 (2006)

Sympatric speciation is difficult to demonstrate in nature and remains a hotly debated issue. Barluenga *et al.*[1] present a case of putative sympatric speciation for two cichlid species in the Nicaraguan crater lake Apoyo, but they overlook or reinterpret some key published information on the system. Although sympatric speciation is possible in theory[2,3], we show here that, when this information is taken into account, the results of Barluenga *et al.*[1] do not provide conclusive evidence for sympatric speciation: this is because the null hypothesis of multiple invasion with introgression cannot be rejected.

Figure 2a in Barluenga *et al.*[1] is a modified version of Fig. 3 in Barluenga and Meyer[4] (compared in Fig. 1), in which the central mitochondrial (mt) DNA haplotype, from which the Lake Apoyo haplotypes are derived, is shared by two species from Lake Nicaragua, *Amphilophus labiatus* and *A. citrinellus*, at similar frequencies, and is also found in different members of the *A. citrinellus* complex from other lakes. These results therefore do not allow any conclusion about which of these species is ancestral to the mitochondrial lineage of Lake Apoyo cichlids. It follows that the restriction by Barluenga *et al.*[1] of their subsequent analyses of nuclear DNA markers to only *A. citrinellus* is unjustified.

Mitochondrial monophyly of the Lake Apoyo specimens, as indicated by the mtDNA haplotype network of Barluenga *et al.*[1] (which is based on a single point mutation), does not imply monophyly of a species pair that can exchange genes. Multiple colonization with introgression is frequently associated with mitochondrial monophyly too, as shown for sympatric stickleback species and other sympatric fish[5–7], and in fact the nuclear genetic data of Barluenga *et al.*[1] support this scenario. In all analyses of variation at selectively neutral nuclear genes, *A. citrinellus* of Lake Apoyo is much closer to *A. citrinellus* of Lake Nicaragua than is *A. zaliosus*. This seems incompatible with sympatric speciation from one founder, which, by definition, leaves both daughter species equally related to their common ancestor when measured over many neutral loci.

Their factorial correspondence analysis (FCA) shows that *A. citrinellus* from Lake Apoyo is genetically intermediate between *A. citrinellus* from Lake Nicaragua and *A. zaliosus* from Lake Apoyo (see Fig. 2d of ref. 1); this is exactly as would be predicted if the Lake Apoyo population of *A. citrinellus* was the result of a second wave of colonization from Lake Nicaragua that was followed by introgression from the population that had resulted from the first colonization — namely *A. zaliosus*. Consistent with this, their FCA and bayesian population-assignment tests both reveal nuclear genotypes from Lake Nicaragua and admixed genotypes in the sample of *A. citrinellus* from Lake Apoyo but not in *A. zaliosus*, and FCA and bayesian population-assignment tests indicate introgression between these two species (Fig. 2d and Fig. 3 in ref. 1).

The claim by Barluenga *et al.*[1] of monophyly of Lake Apoyo cichlids is also weakened by their incomplete sampling of all the species in the lake. This lake contains four or five distinct and assortatively mating *Amphilophus* forms, referred to as *A. cf. citrinellus*, *A. zaliosus*, *A.* "Apoyo amarillo", *A.* "chancho" and *A.* "short", for which genetic and phenotypic information is available[8], although they have not been formally described. Barluenga *et al.*[1] acknowledge this work[8] but mention only two species, rather than the four or five that are found in Lake Apoyo; also, several other described species of the *A. citrinellus* species complex are known to exist in the area[9]. Recognition and analysis of the species-level diversity of the system is a prerequisite for reconstructing the evolutionary history of speciation. However, it is unclear whether any of these other species

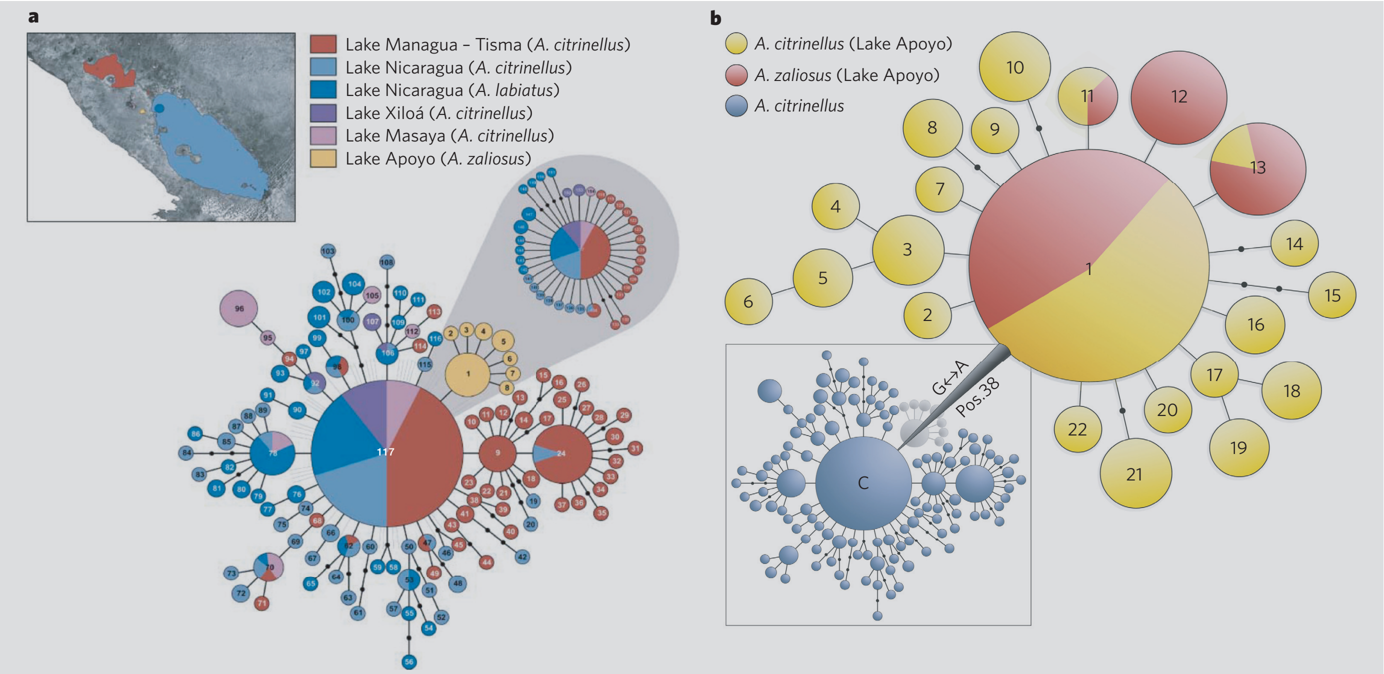

Figure 1 | Contrasting information in two identical haplotype networks of Nicaragua cichlids. a, The mitochondrial haplotype ancestral to Lake Apoyo (haplotype 117 in Fig. 3 of Barluenga *et al.*[4]; reproduced with permission) is shared between *Amphilophus. citrinellus* from five different lakes and *A. labiatus* from Lake Nicaragua. **b**, Haplotype C in Fig. 2a of Barluenga *et al.*[1] is identical to the haplotype 117 in **a**. The central haplotype networks (blue in ref. 1; blue, pink and red in ref. 4) are identical, except that all haplotypes assigned to *A. labiatus* in ref. 4 have been assigned to *A. citrinellus* in ref. 1. Note also the difference in *A. zaliosus* haplotype counts (four versus eight) in the two figures.

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 species[7]. 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 loci[4] 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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1. Barluenga, M., Stölting, K. N., Salzburger, W., Muschick, M. & Meyer, A. *Nature* **439,** 719–723 (2006).
2. Dieckmann, U., Doebeli, M., Metz, H. & Tautz, D. *Adaptive Speciation* (Cambridge Univ. Press, Cambridge, UK, 2004).
3. Gavrilets, S. *Fitness Landscapes and the Origin of Species.* Monographs in Population Biology 41 (Princeton University Press, Princeton, New Jersey, 2004).
4. Barluenga, M. & Meyer, A. *Mol. Ecol.* **13,** 2061–2076 (2004).
5. Taylor, E. B. & McPhail, J. D. *Proc. R. Soc. Lond. B* **267,** 2375–2384 (2000).
6. Bernatchez, L. *et al. Can. J. Fish. Aqu. Sci.* **52,** 179–185 (1995).
7. Seehausen, O. *Trends Evol. Ecol.* **19,** 198–207 (2004).
8. McKaye, K. R. *et al. Cuadernos de Investigación de la UCA* **12,** 19–47 (2002).
9. Stauffer, J. R. & McKaye, K. R. & Stauffer, J. R. *Cuadernos de Investigación de la UCA* **12,** 1–18 (2002).
10. Machado, C. A. *et al. Mol. Biol. Evol.* **19,** 472–488 (2002).
11. Schliewen, U. K. & Klee, B. *Front. Zool.* **1,** doi: 10.1186/1742-9994-1-5 (2004).

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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 complex[1]. Schliewen *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* fauna[3]. The central haplotype in Fig. 2 of ref. 1 indeed contains specimens of *A. labiatus* and *A. citrinellus*; this figure, as indicated[1], is a simplified version of our earlier one[3]. However, we have shown that *A. labiatus* is more distantly related to the monophyletic Lake Apoyo assemblage than is *A. citrinellus* from Lake Nicaragua[3] (*Nature* did not permit us to show additional analyses or figures to this effect). This is also supported by morphometrics[4] and the absence of *A. labiatus* from Lake Apoyo. Our microsatellite, mitochondrial (mt) DNA and amplified fragment-length polymorphism (AFLP) analyses[1] 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 Schliewen *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 Schliewen *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 Apoyo[1] (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 Schliewen *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 others[5] call "chancho", "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 analysis[5] based on three microsatellites yielded inconclusive results. Our own, much more detailed, analyses[1] 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 Schliewen *et al.*[2] propose a much less likely scenario that is not supported by the available data.

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1. Barluenga, M., Stolting, K. N., Salzburger, W., Muschick, M. & Meyer, A. *Nature* **439,** 719–723 (2006).
2. Schliewen, U. K., Kocher, T. D., McKaye, K. R., Seehausen, O. & Tautz, D. *Nature* **444,** doi:10.1038/nature05419 (2006).
3. Barluenga, M. & Meyer, A. *Mol. Ecol.* **13,** 2061–2076 (2004).
4. Klingenberg, C. P., Barluenga, M. & Meyer, A. *Biol. J. Linn. Soc.* **80,** 397–408 (2003).
5. McKaye, K. R. *et al. Cuadernos de Investigación de la UCA* **12,** 19–47 (2002).

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