

Supplementary Table 2 | Mismatch analysis parameters. μ is the expansion parameter (95% consistence intervals are given in parentheses); μ_b and μ_h are the mutation parameters before and after the expansion respectively; SSD is the test of the validity of a stepwise expansion model based on the sum of square deviations between the observed and the expected mismatch; the significance of the test is estimated with a parametric bootstrap approach, and the same method is used to test the significance of the Raggedness index (probability values: ^{ns} $p > 0.05$, $*p \leq 0.05$, $p < 0.001$). Applying a commonly used molecular clock calibration for cichlid control region¹⁵, the demographic expansion of *A. citrinellus* in Lake Apoyo correlates with the maximum estimated age of the lake of ~23,000 years (see ref. 15 for further details), while the major demographic expansion of *A. zaliosus* appears to have occurred in less than half that time.**

	Mean no. of differences	μ	μ_b	μ_h	SSD	Raggedness index
<i>A. citrinellus</i>	2.193	2.27(0.77-3.79)	0.15	2139.32	0.001 ^{ns}	0.025 ^{ns}
<i>A. zaliosus</i>	0.615	0.57(0.00-1.07)	0.13	1354.94	0.013 ^{ns}	0.169 ^{ns}

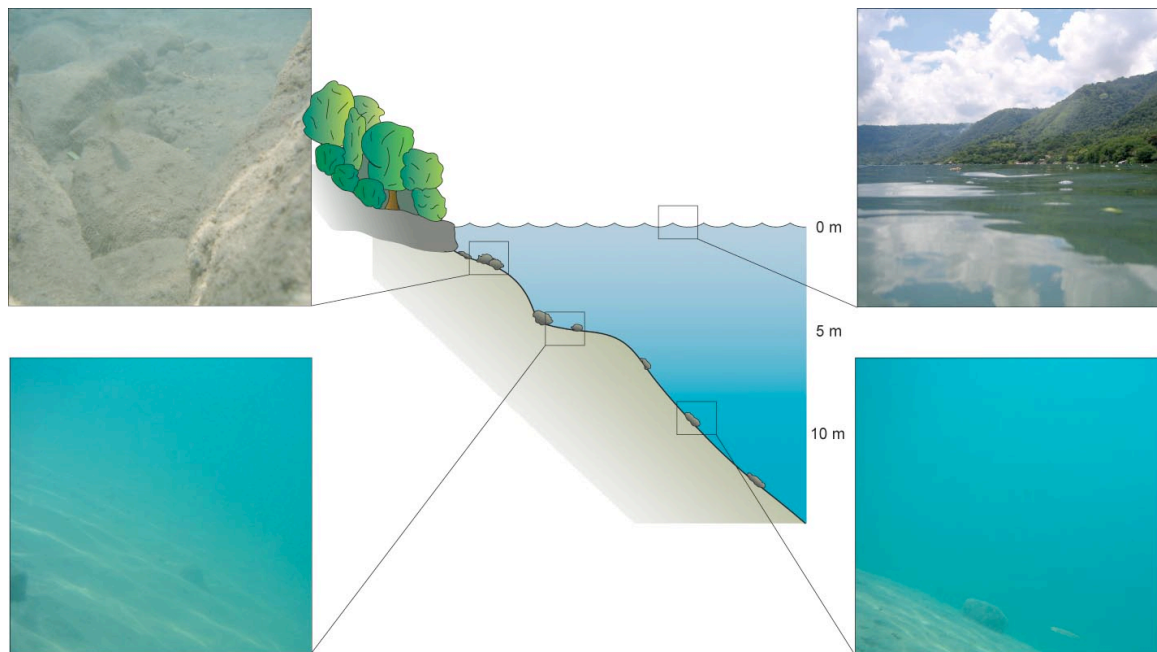
Supplementary Table 3 | Number of alleles, observed (H_o) and expected (H_e) heterozygosities at every microsatellite locus, number of mitochondrial alleles for each population and number of polymorphic loci in AFLP. Number of private alleles are given in parentheses. Asterisks indicate loci with a significant deviation from Hardy Weinberg equilibrium after Bonferroni correction.

Loci	<i>A. zalius</i> (n = 38)	<i>A. citrinellus</i> (n = 82)	Other lakes (n = 502)	Total
Acit1				
No. Alleles	2	4	8(3)	8
H_o	0.143	0.072	0.572*	
H_e	0.208	0.085	0.629	
Acit2				
No. Alleles	7	13	29(14)	29
H_o	0.618	0.639	0.894*	
H_e	0.655	0.712	0.921	
Acit3				
No. Alleles	16	14	26(6)	26
H_o	0.806	0.845	0.867	
H_e	0.881	0.900	0.923	
Acit4				
No. Alleles	6	7	17(9)	17
H_o	0.500	0.507*	0.771*	
H_e	0.573	0.708	0.853	
Acit6				
No. Alleles	1	1	2(1)	2
H_o	-	-	0.070	
H_e	-	-	0.071	
TmoM7				
No. Alleles	5(1)	6(1)	12(6)	14
H_o	0.581	0.409*	0.544	
H_e	0.555	0.651	0.593	
Unh002				
No. Alleles	5	10	27(16)	27
H_o	0.688	0.686	0.839*	
H_e	0.627	0.772	0.907	
Unh011				
No. Alleles	3	4(1)	5(2)	6
H_o	0.735	0.486	0.637*	
H_e	0.627	0.563	0.644	
Unh012				
No. Alleles	4	13	27(13)	27
H_o	0.400*	0.629	0.878*	
H_e	0.459	0.687	0.934	
Unh013				
No. Alleles	6	10	26(14)	26
H_o	0.800	0.479	0.792*	
H_e	0.751	0.565	0.877	
mtHaplotypes	4(1)	21(18)	165(165)	187
AFLP polymorphic sites	226	223	-	-

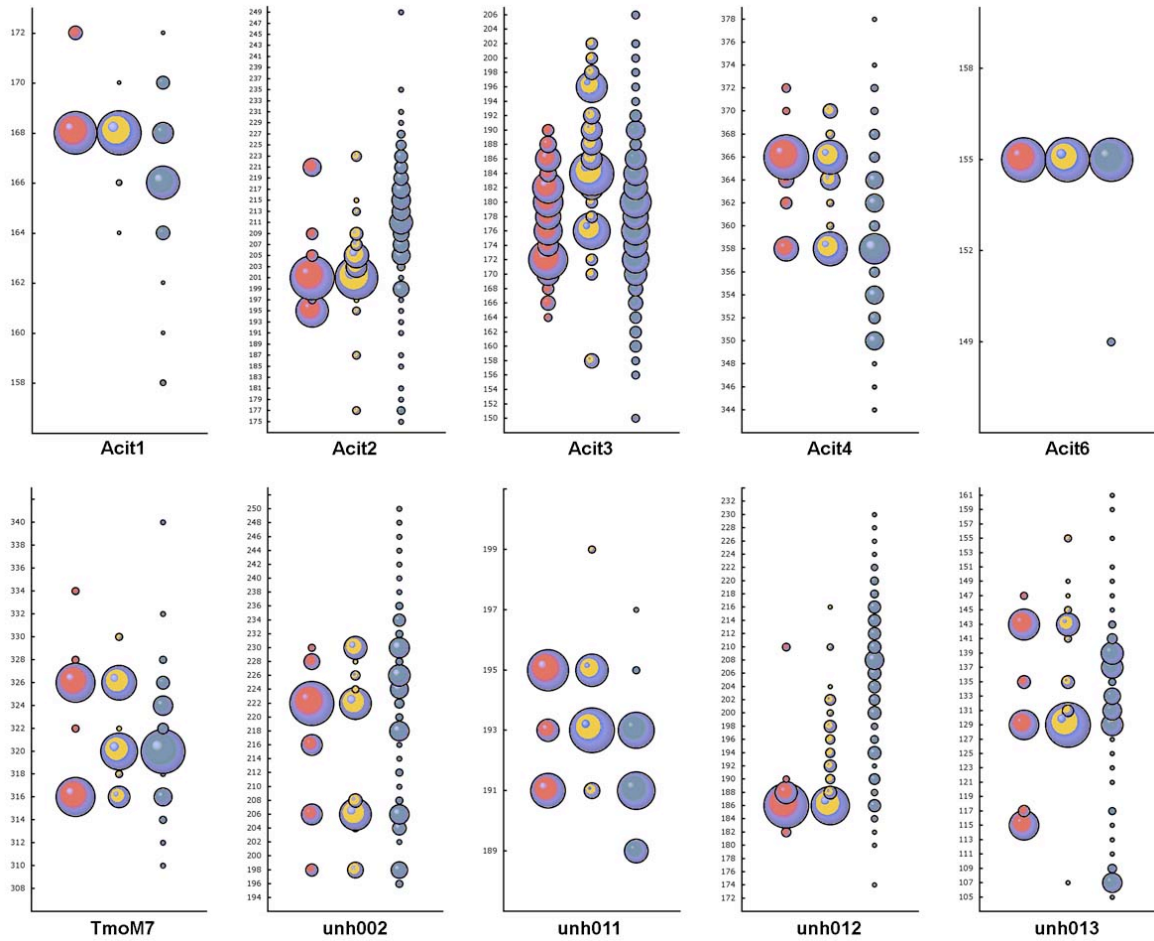
Supplementary Table 4 | Pairwise genetic distances. Using 10 microsatellite loci we calculated the pairwise F-statistics with ARLEQUIN among sampling localities within and between the two *Amphilophus* species in Crater Lake Apoyo (after Bonferroni; ^{ns} $p > 0.05$, $*p \leq 0.05$, $p < 0.001$). While all interspecific comparisons are highly significant proving complete reproductive isolation between the two species, all intraspecific tests reveal that there is no genetic structure at all within each species from different localities around the lake (see also Supplementary Fig. 4). This result is further corroborated by a maximum likelihood based analysis of migration rates, which estimated a high rate of migrants per generation between the sampling localities (average number of migrants: *A. citrinellus* = 22.8; *A. zaliosus* = 15.3). The extensive levels of gene flow within the respective species clearly demonstrate panmixia. The sympatric occurrence of both species throughout the lake together with the high numbers of migrants between distant sampling sites rule out the possibility of (micro-) allopatric or parapatric speciation scenarios.**

	<i>A. citrinellus</i> (I)	<i>A. citrinellus</i> (II)	<i>A. citrinellus</i> (III)	<i>A. citrinellus</i> (IV)	<i>A. zaliosus</i> (II)	<i>A. zaliosus</i> (III)	<i>A. zaliosus</i> (IV)
<i>A. citrinellus</i> (I)	0						
<i>A. citrinellus</i> (II)	0.014 ^{ns}	0					
<i>A. citrinellus</i> (III)	0.000 ^{ns}	0.012 ^{ns}	0				
<i>A. citrinellus</i> (IV)	0.000 ^{ns}	0.002 ^{ns}	0.000 ^{ns}	0			
<i>A. zaliosus</i> (II)	0.095**	0.083**	0.093**	0.057**	0		
<i>A. zaliosus</i> (III)	0.146**	0.127**	0.152**	0.096**	0.000 ^{ns}	0	
<i>A. zaliosus</i> (IV)	0.110**	0.080*	0.124**	0.070*	0.000 ^{ns}	0.002 ^{ns}	0

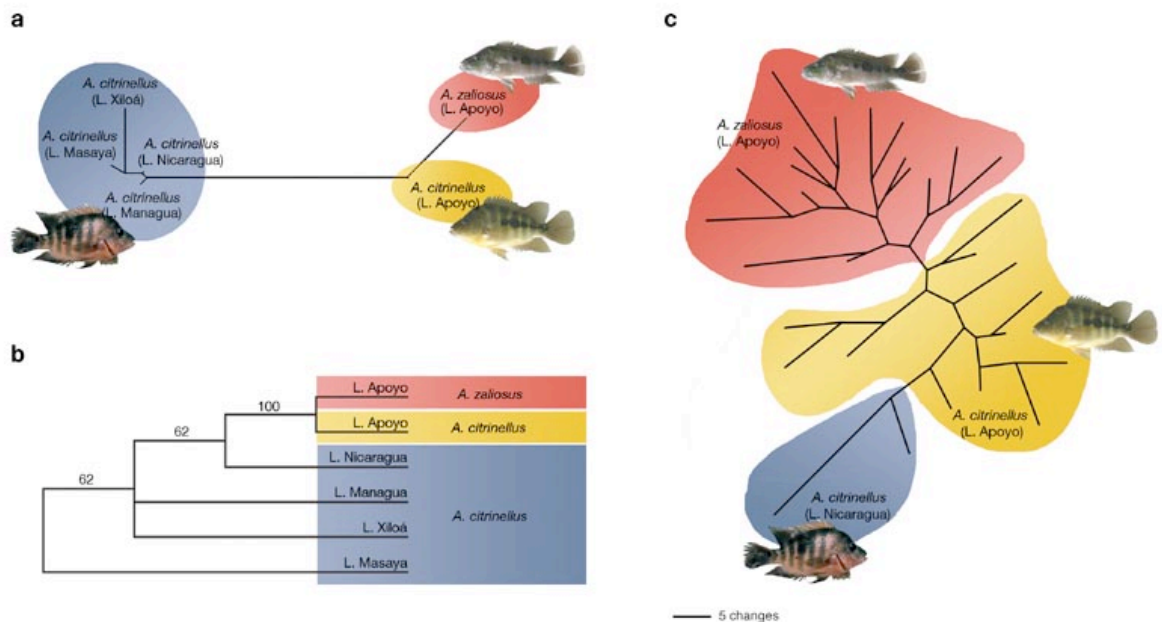
Supplementary Figure 1 | Physical structure of Crater Lake Apoyo. Crater Lake Apoyo is a conical volcanic caldera characterized by steep cliffs. The shore of the lake (0~1 m) is made up of rocks overlaid with sand and biofilm (see underwater photo on the upper left). The bottom of the lake is covered with sand, with volcanic rocks scattered throughout (see underwater photos in the lower row). The benthic species *A. citrinellus* typically occurs near the substrate in shallow areas (<30m), and the limnetic species *A. zaliosus* is most abundant just offshore where the shore drops off more rapidly¹³ (pers. obs.), although it is also observed at the surface in the center of Lake Apoyo where *A. citrinellus* is rarely caught. Despite behavioral differences, such as escape behavior¹³, the two species occur sympatrically over the sandy bottom throughout the entire lake, and we even found individuals of both species foraging in close proximity on rocks near the shore, in about 1 m depth. These observations suggest that the ranges of single individuals are relatively large.



Supplementary Figure 2 | Microsatellite allele distributions. Each circle shows one allele and its size represents the frequency in the respective population (red: *A. zaliosus*, yellow: *A. citrinellus* in L. Apoyo; blue: *A. citrinellus* in other lakes in Nicaragua) for ten microsatellite loci.



Supplementary Figure 3 | Nuclear marker phylogenies. **a**, Microsatellite based population tree calculated with CONTML applying a Brownian motion approximation. **b**, Microsatellite based population tree calculated with POPULATIONS applying the Cavalli-Sforza and Edwards method. Numbers above the branches are bootstrap values (10,000 replicates). **c**, Neighbor-joining phylogeny based on AFLP data. All nuclear markers clearly support the monophyly of the Lake Apoyo Midas Cichlids. The Arrow Cichlid (*A. zaliosus*) is derived from Lake Apoyo's Midas Cichlid (*A. citrinellus*) fauna rendering the ancestral species *A. citrinellus* paraphyletic.



Supplementary Figure 4 | Bayesian inference of population structure within each of the Crater Lake Apoyo cichlid species. The analyses of population structure with STRUCTURE of individuals of the Midas Cichlid (*A. citrinellus*) (a) and the Arrow Cichlid (*A. zalius*) (b) from four different localities of Lake Apoyo (roman numerals, see Figure 1b), based on 10 microsatellite loci, revealed the complete absence of genetic differentiation throughout the lake.

