Comparative ecology and phylogeography in East African cichlid fishes

Inauguraldissertation
zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel
von

Adrian	**Indermaur**
(Vorname, ausgeschrieben)	(Familienname)

Berneck	**St. Gallen**
(Heimat)	(Kanton oder Land)

Basel, 2016

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät

auf Antrag von

Prof. Dr. Walter Salzburger, Dr. Ulrich Schliewen

(Mitglieder des Dissertationskomitees: Fakultätsverantwortliche/r, Dissertationsleiter/in, Korreferent/in)

Basel, den 25.03.2014

(Datum der Genehmigung durch die Fakultät)

Prof. Dr. Jörg Schibler

Dekanin/Dekan
(Name der/des amtierenden Dekanin/Dekans einsetzten)

Für meine Familie,
mit unendlichem Dank und in ewiger Liebe!

Contents

Introduction

"I have never met an animal or organism that was not interesting, but some stand out as special; cichlid fishes are right up there."

George Barlow 2002, The Cichlid Fishes: Nature's Grand Experiment In Evolution

The study of biodiversity, i.e. the manifold forms of live, has been a major subject of human innate curiosity not only since Darwin's times but also for centuries before. It has on the one hand always been one of the great goals of humanity to catalog and describe the entire natural world in all its wonder and on the other hand humans have and do greatly profit in many ways from a complete knowledge of life with all its dangers as well as benefits. With Darwin and Wallace's first large advances in the field (Darwin & Wallace 1858), which in the case of Darwin was summarized so effectively in 'The Origin' (1859), it has become increasingly important not only to describe biodiversity but to investigate the dynamics underlying this diversity in a broad sense and to identify the exact processes in natural systems that lead to the vivid world we see today.
By observing the species assemblages in our natural world and its ecological properties one pattern stands out in specific. A large number of lineages seem to be diversifying or have diversified in a very short amount of time along trajectories of ecological adaptation. This results in a great number of ecologically diverse and species rich groups.
Since almost two decades these speciation outbursts, the so-called adaptive radiations, have been put forward as a major reason for a large portion of the diversity we see today (Schluter 2000). Adaptive radiations are indisputably a very complex process with many factors to consider. They are, however, not separable from the concept of ecological speciation by the means of natural selection (Schluter 2000). Hence the concept of convergent evolution (McGhee 2007), which states that when different organisms independently evolve similar morphological or behavioral traits as a result of similar ecological selection regimes, was put forward as an essential indicator of the 'adaptiveness' of respective species differences and/or similarities (Osborn 1902).
In the exceptionally species rich and eco-morphologically highly diverse assemblages of the East African Rift lakes (Salzburger 2009), the paradox was put forward that competitive ecological exclusion (Gause 1934) of converging species seems to require a temporal and special separation (allopatry) of the different lineages in order for them to coexist (Mayr 1984). Recent phylogenetic framework and molecular dating (e.g. Verheyen et al. 2003) seem to indicate though that in fact many lineages formed very rapidly thus not allowing the avoidance of competitive exclusion.

These questions are addressed in the first part of my thesis (part one: Comparative Ecology) where in **Chapter 1 ("Convergent evolution within an adaptive radiation of cichlid fishes")** we investigated ecologically based convergence within the Lake Tanganyika cichlid radiation.
Chapter 2 ("The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes") is a project where we investigated not an entire radiation but the convergent occurrence of a conspicuous trait which is thought to be highly adaptive, the thick lipped phenotype of cichlid fishes which is found across several lineages and large parts of their distribution. In this case the focus of the

study was to characterize the degree convergence in morphological and ecological traits as well as genetic developmental pathways.
Considering the strong connectivity of convergent evolution with the ecological properties of a habitat mediated by natural selection it is apparently crucial to study ecological parameters of habitats connected by convergent phenotypes. Such a study is described in **Chapter 3 (Depth-dependent abundance of Midas Cichlid fish (*Amphilophus spp.*) in two Nicaraguan crater lakes)** where we characterized effective population sizes by means of transect methods in order to compare two lakes exhibiting convergent phenotypes of cichlid fishes.
Outside of large continuous habitats such as the Great Lakes of the African Rift valley where many species co-occur and compete, as discussed above, detailed knowledge of the geographic distribution of species on a solid phylogenetic the background is crucial to the understanding of diversity in more structured habitats such as non-continuous wood land or river systems.

In part two of my thesis I combine different studies dealing with combination of distributional patterns, patterns of phylogenetic relationships and ecological factors. The cichlid fishes of the east African rivers have become increasingly important in the understanding of large-scale relations of African cichlid fishes (Wagner et al. 2012, Koblmüller et al. 2012, Loh et al. 2012)
In **Chapter 4 ("Back to Tanganyika: a case of a recent immigration into a species-flock of East African cichlid fishes")** we investigate a recently discovered dispersal event of a modern cichlid lineage (*Haplochromis spp.*) across major watershed barriers in Eastern Africa.
Along the same lines in **Chapter 6 ("Admixture between divergent mitochondrial lineages and greater phenotypic variation in a basal haplochromine cichlid fish from Lake Chila, Zambia")** we investigate phylogeographic history of a basal haplochormine clade (genus: Pseudocrenilabrus) with detailed investigation of a case of hybridization of two distinct lineages combined with the ecological opportunity of a new habitat (colonization of a lake).
In **Chapter 5 ("Divergence between lake and stream habitats in an East African cichlid fish")** we investigate the degree of ecological divergence of a riverine cichlid species, which also occurs in pure lake habitats (*Astatotilapia burtoni*). Genetic, morphological and ecological diversity is assessed in four different replicated affluent river systems of Lake Tanganyika.
Morphological diversity within natural populations is the crucial prerequisite for natural selection to act on and to enable ecological adaptive evolution. A special case of such morphological variation where the beneficial natural symmetry is broken is the mouth asymmetry of the scale eating cichlids of Lake Tanganyika, which was the main topic of the third part of my thesis. Here we have an intensely studied and highly discussed system apparently providing textbook examples of many biological principals such as frequency dependent selection (Hori 1993). Nevertheless many crucial aspects are still missing to the full understanding of the dynamics within system. In **Chapter 7 ("A field based assessment of attack strategies and feeding success in the scale eating cichlid fish *Perissodus microlepis* (Perciformes)")** I conducted a field experiment to investigate the correlation of mouth asymmetry with attack strategies and the feeding performance of mixed morph as opposed to uniform populations.

References

- Darwin, C.R. and Wallace, A.R. 1858. On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection. J Proc Lin Soc Lond 3: 46-50.

- Darwin, C.R. 2001. The Origin of Species Vol. XI. The Harvard Classics. New York: P.F. Collier & Son, 1909–14; Bartleby.com.

- Gause, G.F. 1934. The struggle for existence. Baltimore, Williams and Wilkins Co.

- Koblmüller, S., Katongo, C., Phiri, H. and Sturmbauer, C. 2012. Past Connection of the Upper Reaches of a Lake Tanganyika Tributary with the Upper Congo Drainage Suggested by Genetic Data of Riverine Cichlid Fishes. African Zoology, 47(1), 182–186. doi:10.3377/004.047.0115

- Loh, Y. H. E., Bezault, E., Muenzel, F. M., Roberts, R. B., Swofford, R., Barluenga, M. et al. 2013. Origins of Shared Genetic Variation in African Cichlids. Molecular Biology and Evolution, 30(4), 906–917. doi:10.1093/molbev/mss326

- Mayr, E. 1984. Evolution of fish species flocks: a commentary.

- McGhee, G.R. 2007. The geometry of evolution: adaptive landscapes and theoretical morphospaces. Cambridge, UK ; New York, Cambridge University Press.

- Osborn, H.F. 1902. The law of adaptive radiation. American Naturalist 36: 353-363.

- Salzburger, W. 2009. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. Molecular Ecology 18: 169-185.

- Schwarzer, J., Swartz, E. R., Vreven, E., Snoeks, J., Cotterill, F. P. D., Misof, B. and Schliewen, U. K. 2012. Repeated trans-watershed hybridization among haplochromine cichlids (Cichlidae) was triggered by Neogene landscape evolution. Proceedings of the Royal Society B: Biological Sciences, 279(1746), 4389–4398.

-Schluter, D. 2000. The Ecology of Adaptive Radiation. New York, Oxford University Press.

- Verheyen, E., Salzburger W., Snoeks J. and Meyer A. 2003. Origin of the Superflock of Cichlid Fishes from Lake Victoria, East Africa. Science, 300(5617), 325–329. doi:10.1126/science.1080699

part one:

Comparative Ecology

Chapter 1

Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

Moritz Muschick, **Adrian Indermaur** and Walter Salzburger

Current Biology (2012)

AI helped with the sample collection, sample processing, sequencing, data analyses and drafting of the manuscript.

Current Biology 22, 2362–2368, December 18, 2012 ©2012 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2012.10.048

Report

Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

Moritz Muschick,[1] Adrian Indermaur,[1] and Walter Salzburger[1,*]
[1]Zoological Institute, University of Basel, 4051 Basel, Switzerland

Summary

The recurrent evolution of convergent forms is a widespread phenomenon in adaptive radiations (e.g., [1–9]). For example, similar ecotypes of anoles lizards have evolved on different islands of the Caribbean [2, 6], benthic-limnetic species pairs of stickleback fish emerged repeatedly in postglacial lakes [1, 3], equivalent sets of spider ecomorphs have arisen on Hawaiian islands [7, 8], and a whole set of convergent species pairs of cichlid fishes evolved in East African Lakes Malawi and Tanganyika [10, 11]. In all these cases, convergent phenotypes originated in geographic isolation from each other. Recent theoretical models, however, predict that convergence should be common within species-rich communities [12, 13], such as species assemblages resulting from adaptive radiations. Here, we present the most extensive quantitative analysis to date of an adaptive radiation of cichlid fishes, discovering multiple instances of convergence in body and trophic morphology. Moreover, we show that convergent morphologies are associated with adaptations to specific habitats and resources and that Lake Tanganyika's cichlid communities are characterized by the sympatric occurrence of convergent forms. This prevalent coexistence of distantly related yet ecomorphologically similar species offers an explanation for the greatly elevated species numbers in cichlid species flocks.

Results and Discussion

Adaptive radiation, the rapid evolution of a multitude of species from a common ancestor as a consequence of their adaptation to various ecological niches, is thought to be responsible for much of the morphological and ecological diversity on earth [4, 9]. Interestingly, parallel adaptive radiations of the same group of organisms frequently produce convergent forms [1–9], which is commonly understood as the result of independent adaptations to similar ecological conditions [3, 4, 14, 15]. Convergence in morphology and behavior is typically observed between species that evolved in geographic isolation [2, 3, 7, 10]. Theoretical models, on the other hand, predict that convergence should also be common within species-rich communities [12, 13], thus challenging the standard ecological premises that closely related species should be ecologically similar [16, 17] and that two species cannot coexist in the same niche [18]. Such models suggest that there is an alternative strategy for enabling stable coexistence than to be sufficiently distinct: to be sufficiently similar. According to these models, convergent evolution actually appears to be characteristic in "species-saturated communities" [12] and to occur when the number of species exceeds the number of available niches [13], as is probably the case in the exceptionally diverse species flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi, and Tanganyika.

Against this background we explore the cichlid fish assemblage of Lake Tanganyika (LT) (Figure 1A) and provide what is to date the most thorough examination of a cichlid adaptive radiation. Our integrative study combines molecular phylogenetic, geometric morphometric, and diet analyses in a data set of more than a thousand specimens from 71 species (see Table S1 available online and Experimental Procedures). Our morphological comparisons focus on two ecologically highly relevant characters, overall body shape and the shape of the lower pharyngeal jaw bone (LPJ). The LPJ is the central unit of the pharyngeal jaw apparatus, which is a second set of tooth-bearing jaws in the pharynx used to process food [11, 22] (Movie S1). Finally, we use carbon and nitrogen stable isotope ratios as proxy for trophic ecology—in combination with stomach and gut content analyses.

We first present a robust phylogenetic framework for the species flock (Figure 1B), which largely agrees with previous studies [19, 20]. When clustering the species according to body and LPJ shape, the phylogenetic structure vanishes (Figures 2A and 2C), indicating that the shape of these traits is largely uncoupled from the phylogenetic background of a species. All larger cichlid tribes are broken up into two or more body and LPJ shape clusters, and the different tribes overlap in morphospace (Figures S1A and S1B). A large fraction of the sister taxa are not each other's closest ally in the morphological cluster analyses, and the cluster trees based on shape data are incongruent with the molecular phylogeny (body shape: Δ -lnL = 2885.87; Δ tree length = 1059; $P_{SH} < 0.001$; $P_{KH} < 0.001$; LPJ shape: Δ -lnL = 3709.20; Δ tree length = 1484; $P_{SH} < 0.001$; $P_{KH} < 0.001$). Instead of correlating with phylogeny, species that are morphologically alike are, in general, more similar in trophic ecology (Figures 2 and S1). This integrated analysis leads to two main observations. First, species from distinct clades are grouped into the same morphoclusters, whereas sister-species are often quite distinct morphologically (Figure S2); this suggests prevalent convergence in body and LPJ shape within the cichlid species flock of LT. Second, there appears to be a strong link between (trophic) morphology and ecology in LT cichlids; this suggests that, just like in other cases of convergent evolution, natural selection is the driving force in the evolution of convergent forms [1, 5, 15, 23]. In the following, we provide examples for convergent species and quantify convergence in sympatry in the cichlid species flock of LT.

Perhaps the most striking case of convergent evolution within LT's cichlid assemblage involves *Neolamprologus prochilus* and the enigmatic "*Ctenochromis*" *benthicola* (Figure 3A and indicated in bold in Figures 1 and 2). Both species occur sympatrically and are similar to a degree that even local fishermen, who otherwise ably distinguish species, consider them as one. In line with this, geometric morphometric analyses cluster them together, they have similar stable isotope signatures (Figures 2 and S1), and they show the same

*Correspondence: walter.salzburger@unibas.ch

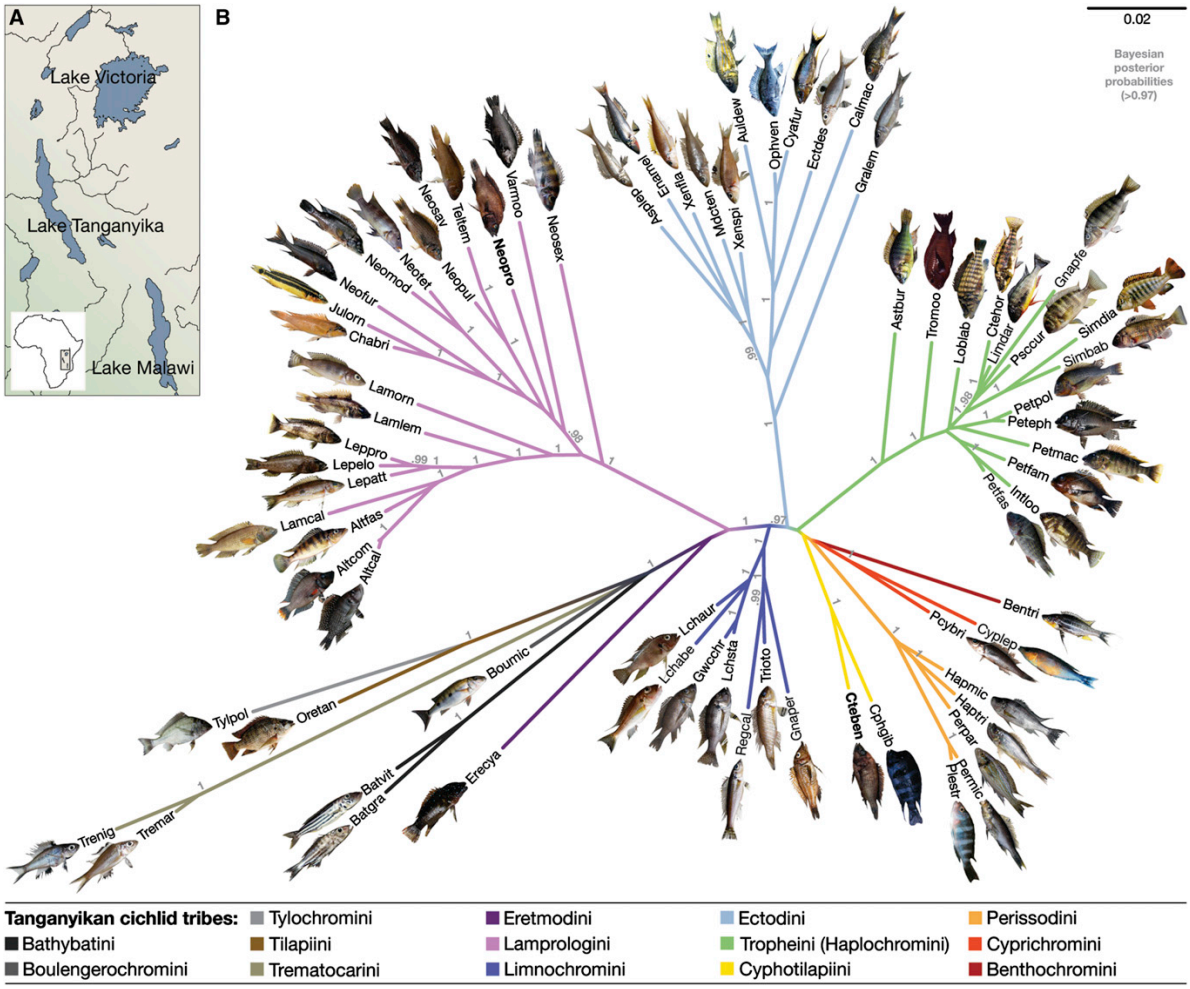


Figure 1. The Cichlid Species Flock of Lake Tanganyika

(A) Map of East Africa showing the three Great Lakes. Lake Tanganyika (LT) is the oldest lake in East Africa and, consequently, accommodates the genetically, morphologically, and ecologically most diverse cichlid species flock [11, 19].

(B) Maximum-likelihood phylogeny of the 71 Tanganyikan cichlid species in our core data set, based on two nuclear (*ednrb1*, *phpt1*) and one mitochondrial (ND2) marker (2,013 bp in total) and the GTR+G model of molecular evolution. Numbers above the branches depict Bayesian posterior probabilities >0.97. Full species names are given in Table S1; different colors denote the main cichlid lineages ("tribes"), some of which are likely to have undergone secondary subradiations [19–21]. Note that the cichlid adaptive radiations of Lakes Malawi and Victoria consist of one of these tribes only, the Haplochromini (the Tanganyikan representatives of which are often referred to as Tropheini) [21]. Our phylogeny confirms the monophyly of the tribes; at least seven genera are, however, paraphyletic, which already indicates convergence in traits used to classify them initially. For example, the putative haplochromine "*Ctenochromis*" *benthicola* (Cteben) emerges as a member of the Cyphotilapiini, whereas its congener, *C. horei* (Ctehor) remains within the Tropheini/Haplochromini. The other paraphyletic genera are *Gnathochromis* (Gna), *Lamprologus* (Lam), *Limnochromis* (Lch), *Neolamprologus* (Neo), *Perissodus* (Per), and *Petrochromis* (Pet). Images of the fishes were taken directly in the field.

stomach contents, namely remnants of the endemic shrimp *Limnocaridina* sp. (Figure 3A). Yet, whereas *N. prochilus* belongs to the Lamprologini, "*C.*" *benthicola*—formerly considered a Haplochromini and congener of *C. horei*—now emerges as a member of the Cyphotilapiini (Figure 1B). Pairwise genetic distances of 10.6% and 1.4% in the mitochondrial and nuclear DNA, respectively, suggest that the two species are separated by several million years of independent evolution, which lies in the range of the eye-catching convergent species pairs observed between Lakes Tanganyika and Malawi [10]. But cichlids do not only resemble other endemic cichlids. The rare *Baileychromis centropomoides*, for example, is very similar in overall body shape to an endemic *Lates* sp. (Figures 3B and S3).

To quantify convergence in the LT cichlid species flock, we plotted relative morphological distance against phylogenetic distance for each pair of species and compared it to simulations of trait evolution (Figure 4A). Applying a conservative threshold (see Experimental Procedures), we identify 122 and 132 species pairs that are convergent in body and LPJ shape, respectively, which is about five times more than predicted by the models. Importantly, more than three quarters of these convergent species pairs overlap in habitat and depth distribution (Table S2), and they show a significantly greater

Current Biology Vol 22 No 24
2364

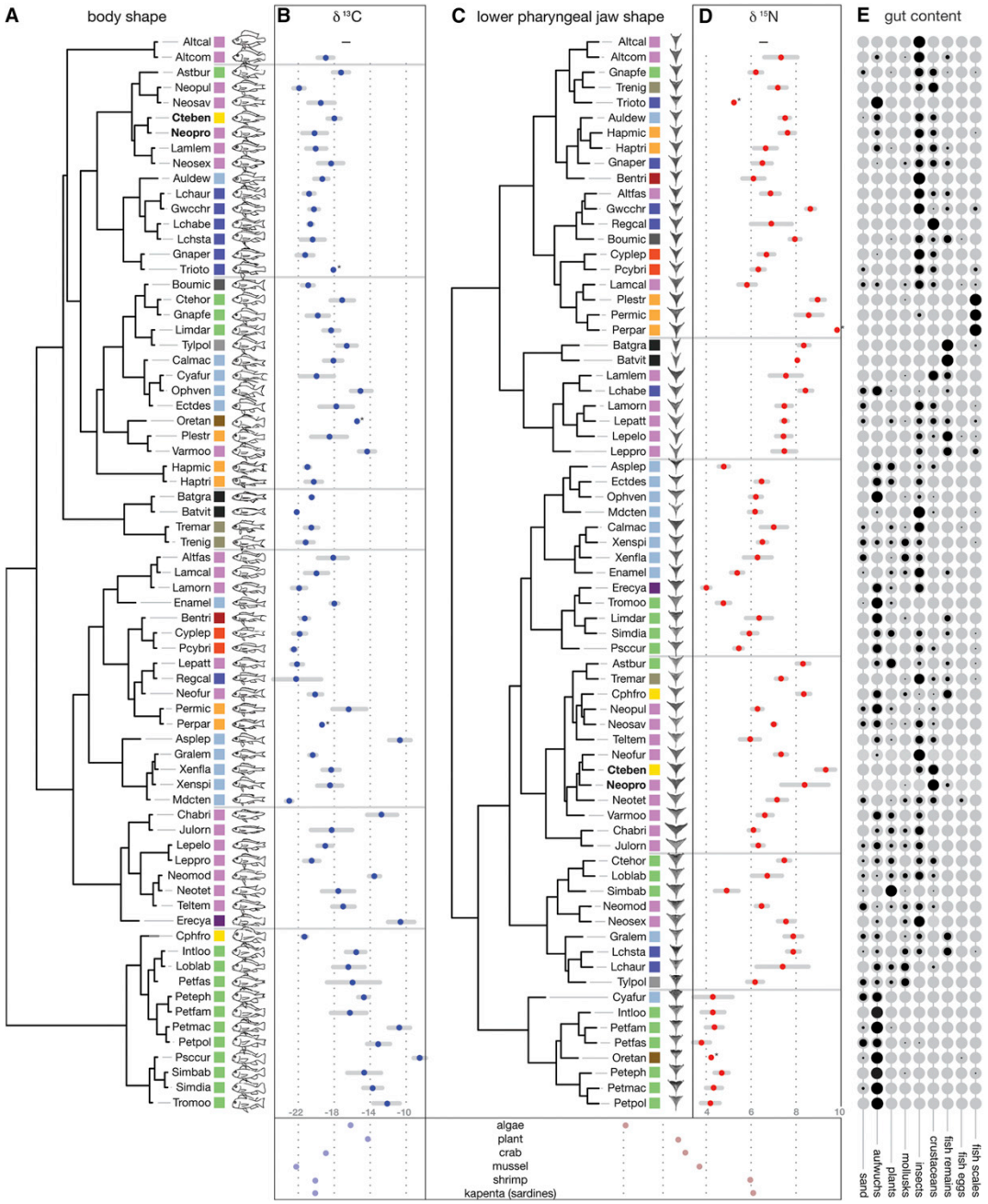


Figure 2. Ecomorphological Diversity in Cichlids from Lake Tanganyika

(A) Cluster analysis on the basis of 17 homologous landmarks on body shape.
(B) $\delta^{13}C$ stable isotope signatures.
(C) Cluster analysis on the basis of eight homologous and six sliding landmarks on the lower pharyngeal jaw bone.
(D) $\delta^{15}N$ stable isotope signatures.
(E) Results from the stomach and gut content analyses (in volume %).
Outlines in (A) are based on real photographs; images in (C) are taken from dissected LPJs (see Table S1 for details). The main morphoclusters are separated by gray lines, and the tribes are colored as in Figure 1. Colored dots in (B) and (D) represent average values; gray bars indicate 95% confidence limits of a t distribution. * marks species with too small a sample size, so that 95% confidence intervals were not calculated. The ratio between the rare isotope ^{13}C to ^{12}C (the $\delta^{13}C$ value) indicates the primary carbon source, which may vary between macrohabitats (e.g., benthic versus pelagic), whereas the $\delta^{15}N$ value (^{15}N to ^{14}N) serves as proxy for the relative trophic level of an organism. Accordingly, in LT cichlids, $\delta^{13}C$ values correlate with body shape clusters ($F = 2.66$, $p < 0.005$), whereas $\delta^{15}N$ values correlate with LPJ shape ($F = 4.03$, $p < 0.005$). Note that each trophic level is separated by approximately 3.4‰ in $\delta^{15}N$ from the one below. To facilitate comparisons, we also included average stable isotope values for some plant and animal species from LT (see box at the bottom).

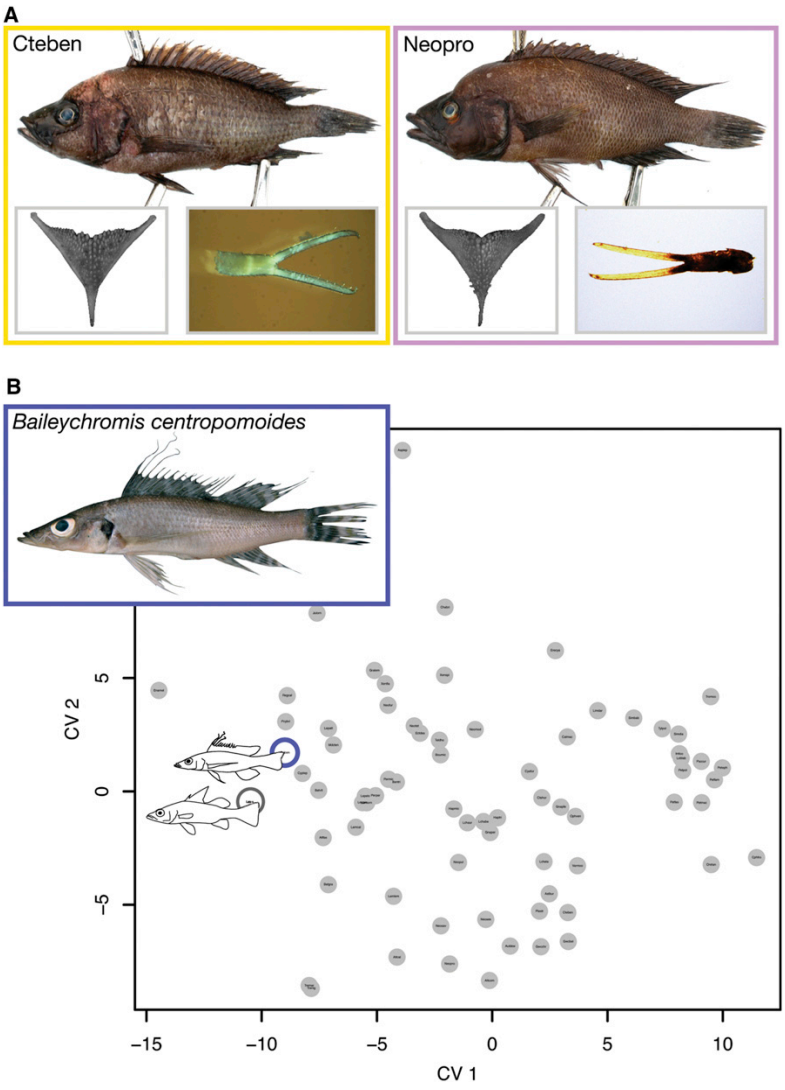


Figure 3. The Curious Cases of Convergent Evolution between "*Ctenochromis*" *benthicola* and *Neolamprologus prochilus* and between *Baileychromis centropomoides* and *Lates* sp.

(A) "*C.*" *benthicola* (Cteben) and *N. prochilus* (Neopro) are phylogenetically distinct (Figure 1) but show great similarities in morphology and in stable isotope signatures (Figure 2). For each species, the LPJ and a pincer of the freshwater shrimp *Limnocaridina* sp. (found in the stomach of the respective specimen) is shown.

(B) Canonical variates analysis showing that *B. centropomoides* is morphologically similar to *Lates* sp. endemic to LT (*B. centropomoides* shows the by far smallest Procrustes distance to *Lates*; see Figure S3). Each dot represents a species. Note that *Lates* used to be classified in the family Centropomidae until recently, which is where the species name for *Baileychromis* is derived from.

overlap in diet compared to random species pairs ($p < 0.05$ for body shape; $p < 0.0001$ for LPJ shape). These results demonstrate that cichlid communities within LT are characterized by the sympatric occurrence of convergent forms and that convergence is particularly prevalent in trophic morphology.

We then performed disparity-through-time (DTT) analyses to reconstruct convergent evolution along the evolutionary history of the species flock. The DTT analysis uncovers a large overlap in body morphology between the subclades emerging in the progress of the radiation (Figure 4B). The DTT plots on the basis of LPJ shape reveal that phases of larger subclade overlap are punctuated by a phase of neutral-like disparity. Overall, there is a strong signal of convergent evolution, which is unlikely to be explained by varying rates of speciation or of morphological evolution, because both have been shown to be rather constant in the cichlid adaptive radiation of LT [20, 25] (Figure S4). The DTT analyses thus suggest that convergent evolution in body and LPJ shape occurred throughout the time course of the radiation.

A large proportion of phenotypic differentiation in LT's cichlid assemblage occurred along only a few principal axes in morphospace (Figure 4C), which reflect adaptations to specific habitats and feeding regimes. For body shape, we detect divergence and convergence in the relative body height, which generally correlates with a pelagic or benthic lifestyle, respectively; the relative sizes of the head and trunk; the sizes of mouth and eye; and the position of the mouth. The divergent and convergent features of the LPJ involve its relative length and width (affecting lever ratios), the relative size and position of the posterior horns (important muscle attachment sites), and the shape of the toothed area. Interestingly, the DTT trajectory for LPJ shape largely coincides with the trajectory of the stable isotope data (Figure 4B), underpinning synchronized differentiation in both an important trophic character (the pharyngeal jaw apparatus) and the trophic niche (as approximated by stable isotopes). This once more confirms a strong link between morphology and ecology in LT cichlids.

In comparison with other renowned examples of adaptive radiation, the situation in LT is unique in its richness of convergent forms that evolved in situ and that coexist in the same habitats (Figures 2, 3, and 4). But what has triggered convergent evolution within the species flock of cichlids in LT? One possibility is that convergent evolution is a feature of advanced adaptive radiations, such as the LT cichlid species flock, which constitutes the relatively oldest cichlid radiation of the East African lakes. Representatives of distant lineages that independently adapt to the same habitat and the resources therein later in the radiation might then already be sufficiently distinct in certain life-history traits to enable coexistence. In the convergent species pair *N. prochilus* and "*C.*" *benthicola* (Figure 3), for example, the former is a substrate spawner, whereas the latter is a mouthbrooder. Convergence

Current Biology Vol 22 No 24
2366

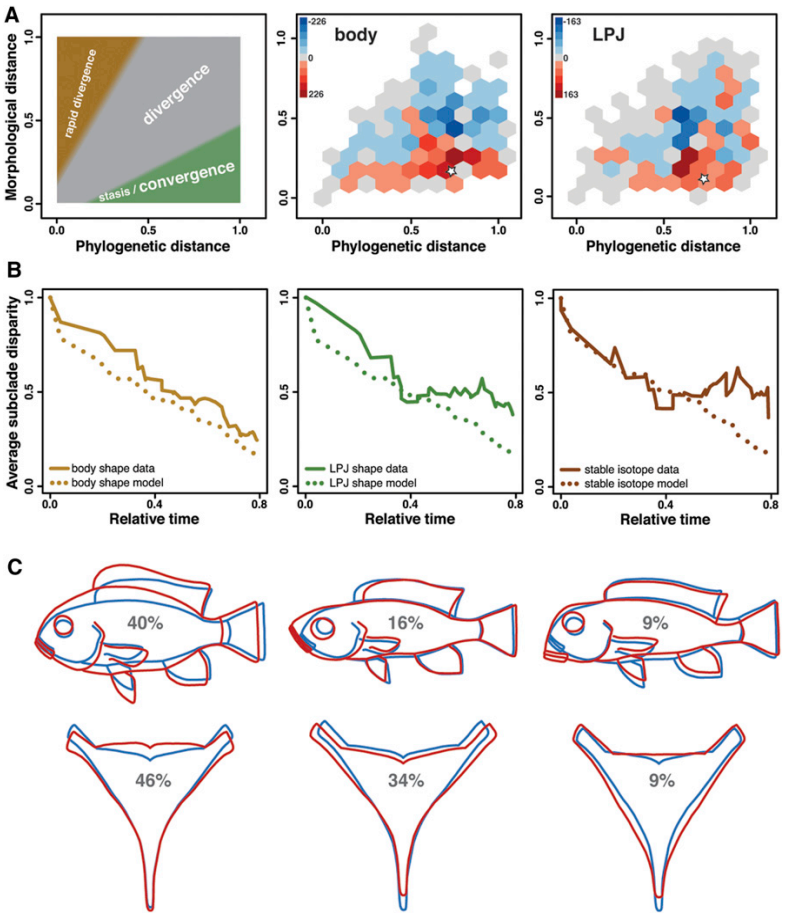


Figure 4. Convergence and Adaptive Disparity in the Cichlid Species Flock in Lake Tanganyika

(A) Pairwise distance-contrast plots showing the correlation between phylogenetic versus morphological distance. The expectation from neutral trait evolution ("divergence") is a correlation between morphological and phylogenetic distance. Species pairs with small morphological yet large phylogenetic distance are indicative of stasis (in cases where there are no intermediate species with distinct morphologies) or convergent evolution [24]. To assess the prevalence of convergent evolution in body and jaw shape, we contrasted the positions occupied by all pairwise comparisons (n = 2,485) with those resulting from a Brownian motion model of trait evolution. We binned the data points into hexagons, the colors of which reflect the differential abundance of observed versus model comparisons. Different shades of blue indicate that our data contained fewer comparisons than expected from the model, whereas shades of red indicate that there were more pairwise comparisons in the data. The latter are predominant in the area indicative for convergence. The white asterisk marks the convergent species pair "*Ctenochromis*" *benthicola* and *Neolamprologus prochilus* (see Figure 3).

(B) Disparity-through-time (DTT) plots showing the average disparity retained in subclades (for body shape and LPJ shape and stable isotopes). Here, DTT plots inform about the time course of ecomorphological evolution. Moving along the phylogeny (from the root to the tips), the relative disparity of subclades is calculated at each internal node, averaged, and plotted against evolutionary time. The observed data is compared to a scenario of trait evolution estimated under a Brownian motion model (dotted line) on the same phylogeny. In order to avoid the effects of "tip overdispersion" due to missing terminal taxa, the most recent 20% of the plots were omitted.

(C) Shape changes along axes, which account for most of the divergence in the LT cichlid radiation. Axes are derived from evolutionary principal component analyses for body (first, second, and fourth axis) and LPJ shape (first, second, and third axis). The relative variance explained by each axis is given in percent.

(and niche overlap) would then be the product of secondary subradiations within the main Tanganyikan tribes [19, 20] superimposed upon each other—a stage that other adaptive radiations might not yet have reached. This scenario seems unlikely, though, given that our DTT analyses reveal a signal of convergence that is constantly high throughout the radiation (Figure 4B). Also, empirical studies comparing various adaptive radiations [26] and theoretical work [27] revealed that diversity appears to be greatest in radiations of intermediate ages and to actually decrease toward later stages. A second possibility is that convergent species initially emerged in isolation—e.g., when LT was temporarily split into separate basins during extremely low lake stands [28]—and only became admixed at a later stage of their evolution. Again, this does not seem to be compatible with our DTT and LTT analyses, which revealed that the signal of divergence and convergence is rather constant throughout the radiation and not restricted to certain periods—e.g., of lake level low stands—only.

That morphological differentiation resulted in convergence in LT might better be explained by the limited number of niches and, hence, adaptive zones (compared to the number of species) that cichlids can invade within the lake [29]. Alternatively, there might be a limit in the number of possible morphologies that cichlids can produce, due to some sort of developmental or genetic constraint [14]. The main morphoclusters in body and LPJ shape (Figure 2) might reflect such constraints. Perhaps it is also a combination of the finite number of niches and morphologies that explains convergence within the adaptive radiation of LT cichlids.

In any case, convergence in ecologically relevant traits within a single radiation is compatible with predictions made by current population ecology theory [12, 13]. It seems that self-organized similarity does not only play an important role in the maintenance of diversity, for example of plankton [30], but also in the rapid formation of organismal diversity via convergent evolution. Because resources are jointly used by several ecomorphologically similar and co-occurring cichlid species from distinct clades in LT, species numbers are maximized without increasing overall disparity. A key to the cichlid problem (i.e., why are there so many species?) might thus lie in the frequent occurrence of convergent evolution—not only between lakes but especially within a single lake and in adaptively relevant traits such as the LPJ. The question is now whether divergence via convergence is a more general pattern of diversification in species-rich communities. It would thus be

of great interest to extend the kind of integrative analysis implemented in this study to other adaptive radiations and, especially, to the cichlid adaptive radiations in Lakes Malawi and Victoria. Even more so, because a recent comparison across 46 cichlid adaptive radiations [31] suggests that the LT radiation is an outlier from an otherwise more general trend in cichlid radiations, which appear to be triggered by both ecological opportunity and sexual selection.

Experimental Procedures

Sampling

Sampling was performed under permission from the Department of Fisheries, Lake Tanganyika Research Unit, Mpulungu, Zambia. In total, we sampled more than 1,000 specimens for this study (see Supplemental Experimental Procedures and Table S1 for further details).

Phylogenetic Analyses

We analyzed one mitochondrial (ND2) and two nuclear (*ednrb1*, *phpt*) markers (see Supplemental Experimental Procedures and Table S1 for GenBank accession numbers used in this study). We relied on maximum likelihood and Bayesian methods for phylogenetic analysis using PAUP*, MRBAYES, and the BEAST package. The appropriate model of molecular evolution for the heuristic tree searches in PAUP* was determined with JMODELTEST; MRBAYES was run for ten million generations with a burn-in of 10%; data were partitioned in BEAST. We first analyzed our core data set combining the mitochondrial and nuclear DNA sequences in 71 taxa, then the core data set including *Baileychromis centropomoides*, and, finally, a mitochondrial data set including the ND2 sequences of 180 taxa (i.e., ca. 90% of all Tanganyika species). Trees derived from the latter analysis were used for lineage-through-time plots. For incongruence testing, we applied the Kishino-Hasegawa (KH) and the Shimodaira-Hasegawa (SH) test implemented in PAUP*.

Geometric Morphometric and Morphological Analyses

We assessed the body shape of 1,049 individuals using landmark-based geometric morphometrics. xy coordinates of 17 landmarks, distributed across the whole fish body (see Figure S5A), and the scale of each picture were recorded using TPSDIG [32]. Aligned Procrustes coordinates were used for a pooled-within-species regression of shape against centroid size in MORPHOJ 1.02d [33]. Species averages were then used for principal component analysis (PCA), for disparity-through-time analyses, and for the calculation of pairwise distances between species. For LPJ assessment we recorded coordinates of eight true landmarks and 20 semilandmarks describing the outline of the bone (Figure S5B). We then clustered the species according to similarity in body and LPJ shape, using agglomerative hierarchical clustering in R.

Stomach and Gut Content Analyses

Contents were removed from the intestinal tracts of 506 specimens and separated up into one or more of the following categories: sand, aufwuchs (algae), plant material, mollusks, insects (imagines and larvae), crustaceans, fish (remains), fish eggs, and fish scales. We determined volume (in %) and weight (in μg) of each category.

Stable Isotope Analysis

White muscle tissue from 727 specimens (see Table S1) was dried, pulverized, and analyzed on an elemental analyzer (Thermo Finnigan) coupled to a Finnigan Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS).

Pairwise Distance-Contrast Plots

To estimate the extent of convergence, we compared the phylogenetic distance to the morphological distance of each species pair [24]. The morphological distance was calculated as Euclidean distance from the pooled-within-species regressions of shape against centroid size using R's dist() function. In total, we had 2,485 species comparisons; therefore, we used hexagonal binning (x = 10 bins) to overcome overplotting. We also simulated neutral trait evolution on the phylogeny, using Brownian motion and Ornstein-Uhlenbeck models. Species comparisons that we derived from these simulations were then compared to our actual data by subtracting the binning counts of the simulations from those of the data. We tested for statistical significance of the difference of pointwise means between simulations and data (each 1/10 of the x axis) by bootstrapping (1,000 replications).

Disparity-through-Time Analysis

DTT analyses were performed according to Harmon et al. [34], comparing the observed data to a scenario of trait evolution estimated under a Brownian motion model. Positive deviations of the data from the simulations indicate a higher overlap in morphospace among subclades than would be expected under neutral evolution.

Evolutionary PCA

We estimated the ancestral character states for body and LPJ shape at each node in the phylogeny and calculated the extent and the direction of shape change along each branch. These branchwise estimates were then subjected to PCA to find the axes of greatest evolutionary divergence. All evolutionary PCAs were performed in MORPHOJ.

Supplemental Information

Supplemental Information includes five figures, two tables, Supplemental Experimental Procedures, and one movie and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.10.048.

Acknowledgments

We thank H. Büscher and various past and present members of the SalzburgerLab for help during fieldwork campaigns; S. Koblmüller for discussions and help with species identifications; B. Aeschbach, N. Boileau, and F. Münzel for assistance in the lab; M. Lehmann for help with stable isotope analyses; H. Bichsel, T. Bosia, M. Gschwind, I. Keller, M. Maurer, N. Rose, and F. Ronco for help with the stomach content analyses; F. Meury, D. Moser, W. Moser, and C. Mullis for assistance with transect surveys; J.P. Montoya and S. Chraiti for help with the CT scans; the Department of Fisheries, Republic of Zambia, for research permits and technical support; and H. Büscher, L. Harmon, L. Keller, C.P. Klingenberg, J.B. Losos, P. Nosil, S. Ramm, L. Schärer, M. Scheffer, D. Schluter, J.T. Streelman, M. Taylor, the SalzburgerLab, and two anonymous referees for discussion and valuable comments. This work was supported by the European Research Council (ERC; Starting Grant "INTERGENADAPT"), the Swiss National Science Foundation, the National Geographic Society, and the University of Basel.

Received: September 12, 2012
Revised: October 25, 2012
Accepted: October 29, 2012
Published: November 15, 2012

References

1. Schluter, D., and Nagel, L.M. (1995). Parallel speciation by natural selection. Am. Nat. *146*, 292–301.
2. Losos, J.B., Jackman, T.R., Larson, A., Queiroz, K., and Rodriguez-Schettino, L. (1998). Contingency and determinism in replicated adaptive radiations of island lizards. Science *279*, 2115–2118.
3. Rundle, H.D., Nagel, L., Wenrick Boughman, J., and Schluter, D. (2000). Natural selection and parallel speciation in sympatric sticklebacks. Science *287*, 306–308.
4. Schluter, D. (2000). The Ecology of Adaptive Radiation (New York: Oxford University Press).
5. Nosil, P., Crespi, B.J., and Sandoval, C.P. (2002). Host-plant adaptation drives the parallel evolution of reproductive isolation. Nature *417*, 440–443.
6. Losos, J.B., Leal, M., Glor, R.E., De Queiroz, K., Hertz, P.E., Rodríguez Schettino, L., Lara, A.C., Jackman, T.R., and Larson, A. (2003). Niche lability in the evolution of a Caribbean lizard community. Nature *424*, 542–545.
7. Blackledge, T.A., and Gillespie, R.G. (2004). Convergent evolution of behavior in an adaptive radiation of Hawaiian web-building spiders. Proc. Natl. Acad. Sci. USA *101*, 16228–16233.
8. Gillespie, R. (2004). Community assembly through adaptive radiation in Hawaiian spiders. Science *303*, 356–359.
9. Gavrilets, S., and Losos, J.B. (2009). Adaptive radiation: contrasting theory with data. Science *323*, 732–737.

Current Biology Vol 22 No 24
2368

10. Kocher, T.D., Conroy, J.A., McKaye, K.R., and Stauffer, J.R. (1993). Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. Mol. Phylogenet. Evol. *2*, 158–165.
11. Salzburger, W. (2009). The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. Mol. Ecol. *18*, 169–185.
12. Scheffer, M., and van Nes, E.H. (2006). Self-organized similarity, the evolutionary emergence of groups of similar species. Proc. Natl. Acad. Sci. USA *103*, 6230–6235.
13. terHorst, C.P., Miller, T.E., and Powell, E. (2010). When can competition for resources lead to ecological equivalence? Evol. Ecol. Res. *12*, 843–854.
14. Brakefield, P.M. (2006). Evo-devo and constraints on selection. Trends Ecol. Evol. *21*, 362–368.
15. Losos, J.B. (2011). Convergence, adaptation, and constraint. Evolution *65*, 1827–1840.
16. Peterson, A.T., Soberón, J., and Sánchez-Cordero, V. (1999). Conservatism of ecological niches in evolutionary time. Science *285*, 1265–1267.
17. Losos, J.B. (2008). Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol. Lett. *11*, 995–1003.
18. Gause, G.F. (1934). The Struggle for Existence (Baltimore: The Williams and Wilkins Company).
19. Salzburger, W., Meyer, A., Baric, S., Verheyen, E., and Sturmbauer, C. (2002). Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. Syst. Biol. *51*, 113–135.
20. Day, J.J., Cotton, J.A., and Barraclough, T.G. (2008). Tempo and mode of diversification of lake Tanganyika cichlid fishes. PLoS One *3*, e1730.
21. Salzburger, W., Mack, T., Verheyen, E., and Meyer, A. (2005). Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. BMC Evol. Biol. *5*, 17.
22. Fryer, G., and Iles, T.D. (1972). The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution (Edinburgh: Oliver & Boyd).
23. Harmon, L.J., Kolbe, J.J., Cheverud, J.M., and Losos, J.B. (2005). Convergence and the multidimensional niche. Evolution *59*, 409–421.
24. Winemiller, K.O. (1991). Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. Ecol. Monogr. *61*, 343–365.
25. Hoerner, M.E. (2011). Testing for differences in rates of speciation, extinction, and morphological evolution in four tribes of cichlids endemic to Lake Tanganyika, East Africa. Evolution *65*, 3398–3412.
26. Gillespie, R.G., and Baldwin, B.G. (2009). Island biogeography of remote archipelagoes. In The Theory of Island Biogeography Revisited, J.B. Losos and R.E. Ricklefs, eds. (Princeton, NJ: Princeton University Press), pp. 358–387.
27. Gavrilets, S., and Vose, A. (2005). Dynamic patterns of adaptive radiation. Proc. Natl. Acad. Sci. USA *102*, 18040–18045.
28. Cohen, A.S., Lezzar, K.E., Tiercelin, J.J., and Soreghan, M. (1997). New paleogeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. Basin Research *9*, 107–132.
29. Rüber, L., and Adams, D.C. (2001). Evolutionary convergence of body shape and trophic morphology in cichlids from Lake Tanganyika. J. Evol. Biol. *14*, 325–332.
30. Benincà, E., Huisman, J., Heerkloss, R., Jöhnk, K.D., Branco, P., Van Nes, E.H., Scheffer, M., and Ellner, S.P. (2008). Chaos in a long-term experiment with a plankton community. Nature *451*, 822–825.
31. Wagner, C.E., Harmon, L.J., and Seehausen, O. (2012). Ecological opportunity and sexual selection together predict adaptive radiation. Nature *487*, 366–369.
32. Rohlf, F.J. (2006). tpsDIG, Version 2.10. Department of Ecology and Evolution (Stony Brook, NY: State University of New York).
33. Klingenberg, C.P. (2011). MorphoJ: an integrated software package for geometric morphometrics. Mol. Ecol. Resour. *11*, 353–357.
34. Harmon, L.J., Schulte, J.A., 2nd, Larson, A., and Losos, J.B. (2003). Tempo and mode of evolutionary radiation in iguanian lizards. Science *301*, 961–964.

Supplementary Materials

Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

Moritz Muschick, **Adrian Indermaur** and Walter Salzburger

Current Biology, Volume *22*
Supplemental Information

Convergent Evolution within an Adaptive Radiation of Cichlid Fishes

Moritz Muschick, Adrian Indermaur, and Walter Salzburger

Supplemental Figures and Tables:

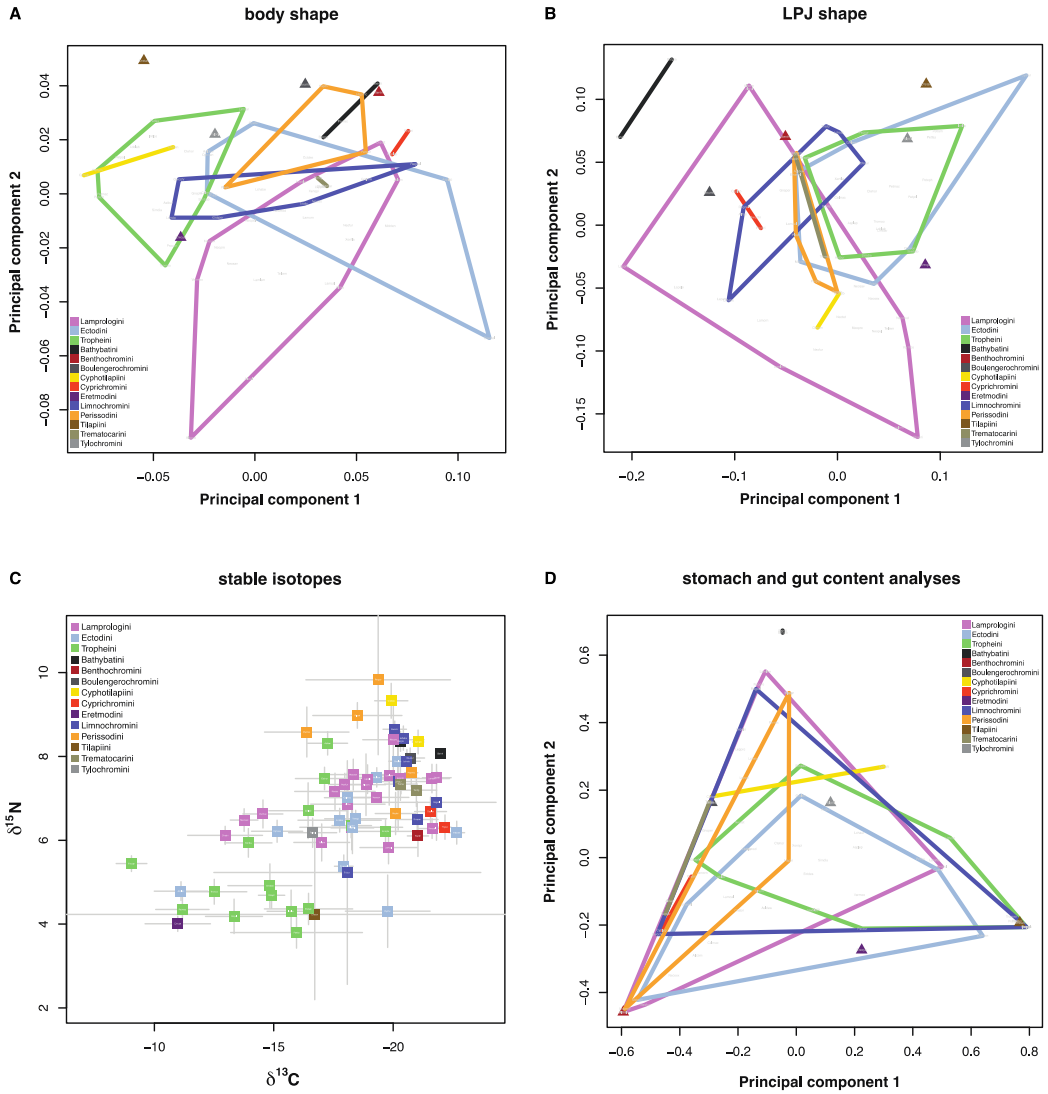


Figure S1. Morphometric Analysis of Lake Tanganyika Cichlid Fishes

Principal component analysis (PCA) of body shape (A) and LPJ shape (B) on the basis of the residuals from regression on centroid size from procrustes aligned landmarks showing a large overlap between tribes (see also [S1]). (C) Plot of stable isotope data ($\delta^{15}N$ *versus* $\delta^{13}C$) for Lake Tanganyika cichlids. (D) Principal component analysis (PCA) of stomach and gut contents showing that the tribes largely overlap in resource use.

Filled triangles in (A, B, D) represent tribes for which only one species was analyzed; grey bars in (C) indicate t-based 95% confidence intervals.

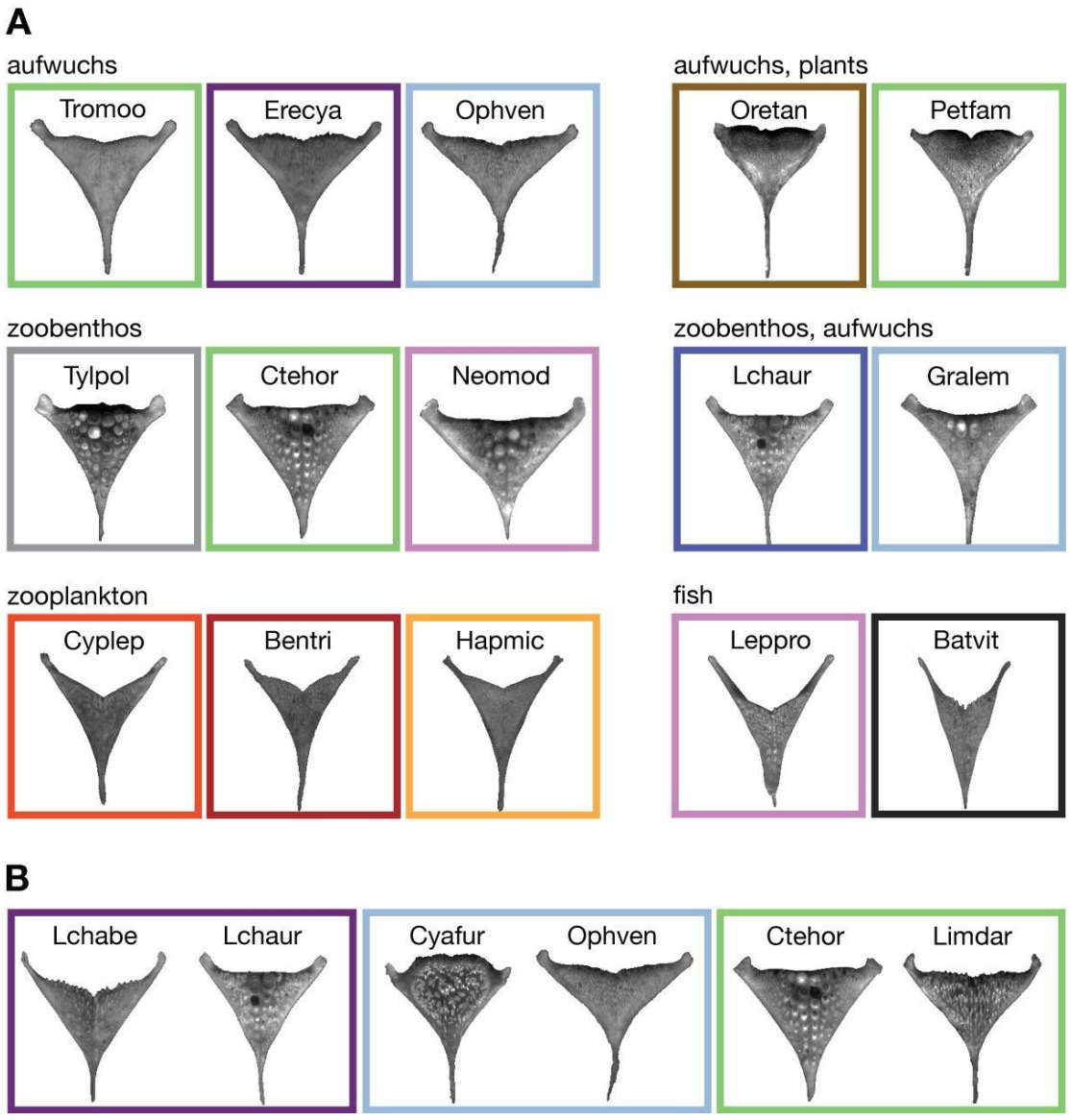


Figure S2. Convergence in Lake Tanganyika Cichlids
(A) Cichlid communities with convergent LPJs. The species in each panel belong to the same LPJ shape cluster (Figure 2C) and occur sympatrically (except for Bentri).
(B) Examples of three sister-species pairs with distinct LPJs. Colors refer to tribes (see Figure 1).

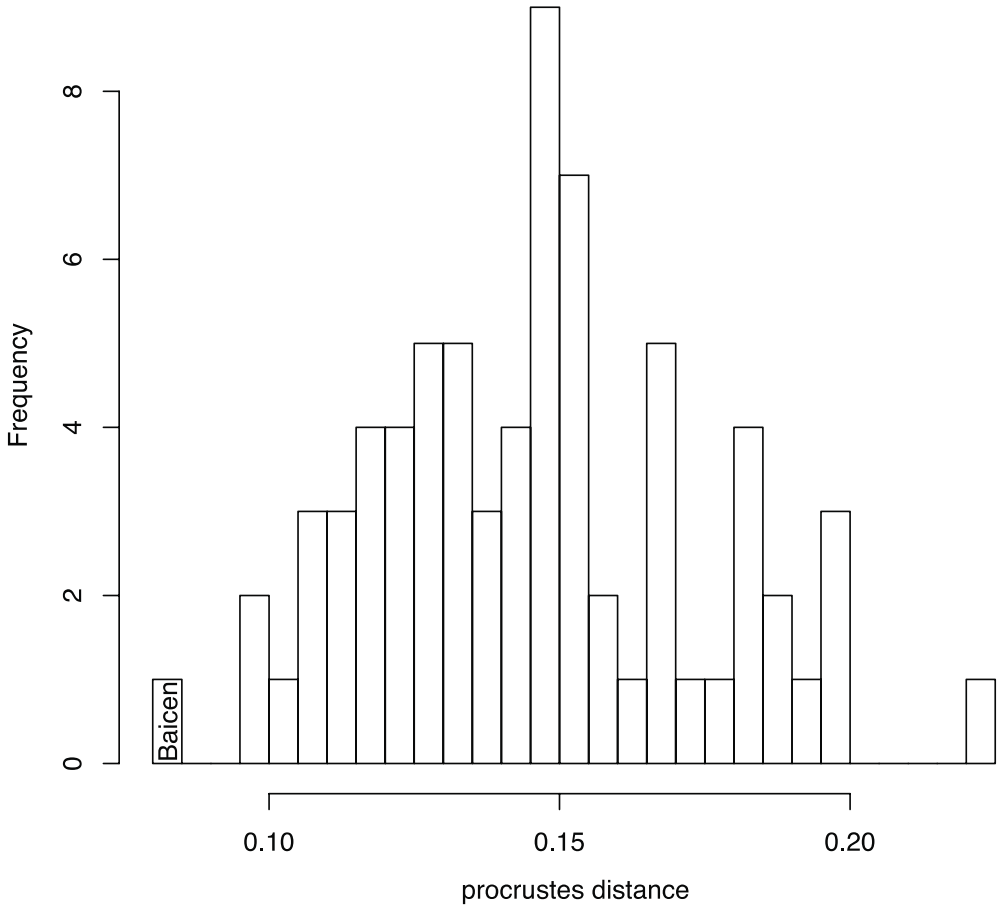


Figure S3. Similarity between a Cichlid and *Lates stappersi*
Frequency plot showing the procrustes distance based on body shape for each cichlid species in our core data set (plus *Baileychromis centropomoides*, Baicen) to *Lates stappersi*. Baicen shows the by far smallest distance of all cichlids examined.

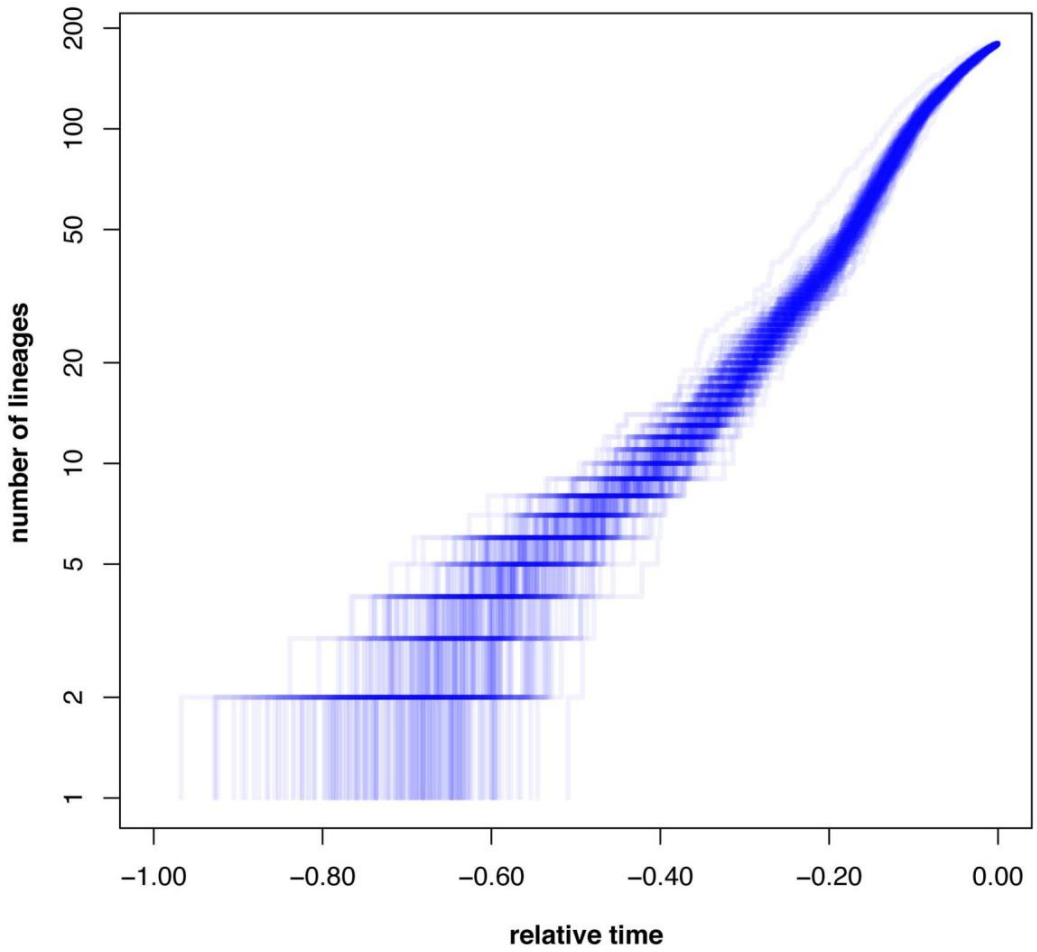


Figure S4. Lineage-through-Time Plot on the Basis of 180 Species of Lake Tanganyika Cichlids

From the posterior tree distribution, 200 trees were sampled and lineage through time (LTT) plotted individually to illustrate variance due to phylogenetic uncertainty.

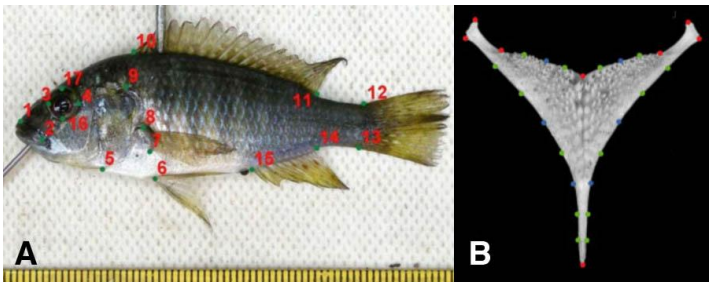


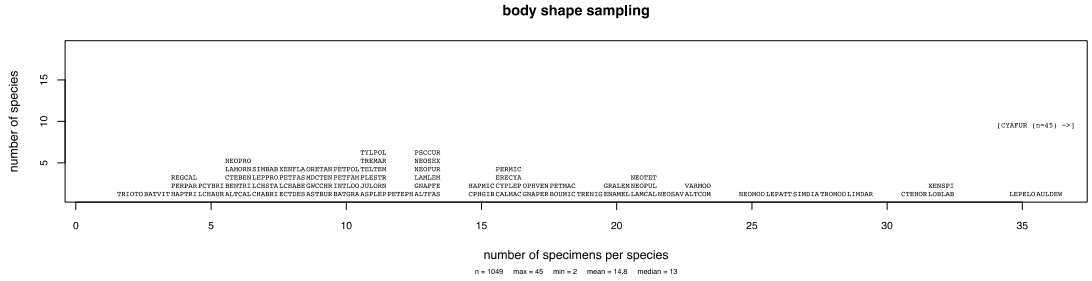
Figure S5. Distribution of Landmarks for the Morphometric Analyses of Overall Body Shape and LPJ Shape
Distribution for (A) overall body shape and (B) LPJ shape. Landmarks were treated differently in statistical analyses according to their color (see below for details).

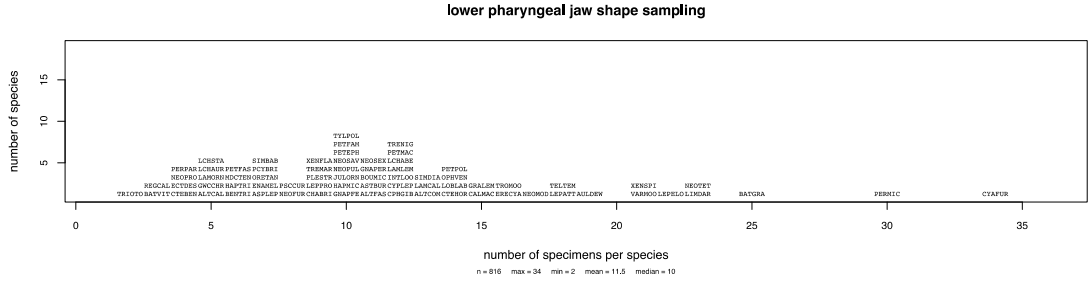
Table S1. List of Specimens Used in This Study
(A) Core dataset consisting of 71 species.

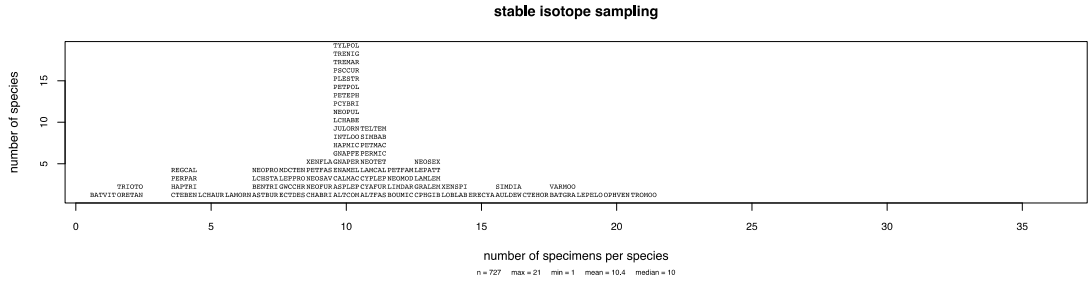
Taxonomic information					Number of specimens				GenBank accession numbers		
TID	Taxon name	tribe	fish image	LPJ	N_{body}	N_{LPJ}	N_{SIA}	N_{SGCA}	ND2	*ednrb*	*phpt*
Altcal	*Altolamprologus calvus*	Lamprologini			6	5	-	2	EF462256	JF900248	JF900177
Altcom	*Altolamprologus compressiceps*	Lamprologini			23	13	10	9	AF398229	JF900249	JF900178
Altfas	*Altolamprologus fasciatus*	Lamprologini			13	11	11	6	EF462255	JF900250	JF900179
Asplep	*Asprotilapia leptura*	Ectodini			11	7	10	9	AY337772	JF900251	JF900180
Astbur	*Astatotilapia burtoni*	Haplochromini			9	11	7	10	JF900319	JF900252	JF900181
Auldew	*Aulonocranus dewindtii*	Ectodini			36	19	16	10	AY337782	JF900253	JF900182
Batgra	*Bathybates graueri*	Bathybatini			10	25	18	2	AY663726	JF900254	JF900183
Batvit	*Bathybates vittatus*	Bathybatini			3	3	1	3	AY663728	JF900255	JF900184
Bentri	*Benthochromis tricoti*	Benthochromini			6	6	7	5	AF317264	JF900256	JF900185
Boumic	*Boulengerochromis microlepis*	Boulengerochromini			18	11	12	5	AF317229	JF900257	JF900186
Calmac	*Callochromis macrops*	Ectodini			16	15	10	10	AY337795	JF900258	JF900187
Chabri	*Chalinochromis brichardi*	Lamprologini			7	9	9	4	EF679241	JF900259	JF900188
Cphgib	*Cyphotilapia frontosa*	Cyphotilapiini			15	12	13	10	EF679242	JF900260	JF900189
Cteben	*Ctenochromis benthicola*	Cyphotilapiini			6	4	4	4	JF900320	JF900261	JF900190
Ctehor	*Ctenochromis horei*	Haplochromini			31	14	17	12	EU753935	JF900262	JF900191
Cyafur	*Cyathopharynx furcifer*	Ectodini			45	34	11	10	AY337781	JF900263	JF900192
Cyplep	*Cyprichromis leptosoma*	Cyprichromini			16	12	11	8	AF398224	JF900264	JF900193
Enamel	*Enantiopus melanogenys*	Ectodini			20	7	10	7	AY337770	JF900265	JF900194
Ectdes	*Ectodus descampsi*	Ectodini			8	4	8	2	AY337790	JF900266	JF900195
Erecya	*Eretmodus cyanostictus*	Eretmodini			16	16	15	9	AF398220	JF900267	JF900196
Gnaper	*Gnathochromis permaxillaris*	Limnochromini			17	11	10	9	JF900321	JF900268	JF900197
Gnapfe	*Gnathochromis pfefferi*	Tropheini			13	10	10	8	U07248	JF900269	JF900198
Gralem	*Grammatotria lemairii*	Ectodini			20	15	13	7	AY337787	JF900270	JF900199
Gwcchr	*Greenwoodochromis christyi*	Limnochromini			9	5	8	3	AY682528	JF900272	JF900201
Hapmic	*Haplotaxodon microlepis*	Perissodini			15	10	10	2	EF437497	JF900273	JF900202
Haptri	*Haplotaxodon trifasciatus*	Perissodini			4	6	4	11	EF437492	JF900274	JF900203
Intloo	*Interochromis loockii*	Tropheini			10	12	10	11	JF900322	JF900304	JF900232
Julorn	*Julidochromis ornatus*	Lamprologini			11	10	10	11	EF462229	JF900275	JF900204
Lamcal	*Lamprologus callipterus*	Lamprologini			21	13	11	10	AF398226	JF900276	JF900205
Lamlem	*Lamprologus lemairii*	Lamprologini			13	12	13	8	EF462271	JF900277	JF900206
Lamorn	*Lamprologus ornatipinnis*	Lamprologini			6	5	6	4	EF462260	JF900278	JF900207
Lchabe	*Limnochromis abeelei*	Limnochromini			8	12	10	3	AY682533	JF900279	JF900208
Lchaur	*Limnochromis auritus*	Limnochromini			5	5	5	3	AF398216	JF900281	JF900210
Lchsta	*Limnochromis staneri*	Limnochromini			7	5	7	5	AY682541	JF900271	JF900200
Lepatt	*Lepidiolamprologus attenuatus*	Lamprologini			26	18	13	11	EF462274	JF900282	JF900211
Lepelo	*Lepidiolamprologus elongatus*	Lamprologini			35	22	19	10	EF462268	JF900283	JF900212

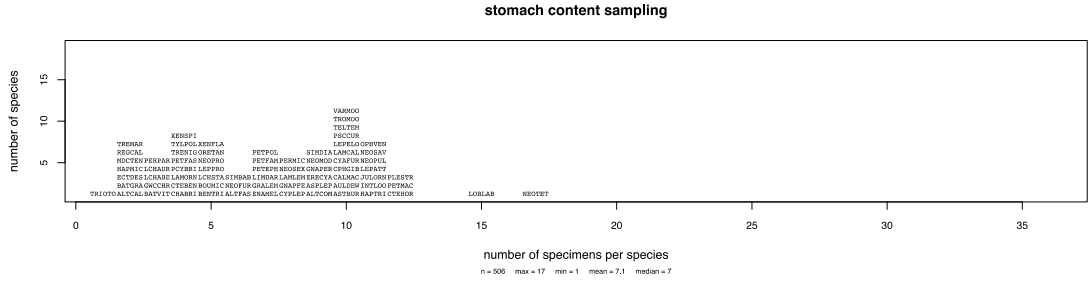
Leppro	*Lepidiolamprologus profundicola*	Lamprologini			7	9	8	5	EF462276	JF900284	JF900213
Limdar	*Limnotilapia dardennii*	Tropheini			29	23	12	7	GQ995724	JF900285	JF900214
Loblab	*Lobochilotes labiatus*	Tropheini			32	14	14	15	U07254	JF900286	JF900215
Mdcten	*Microdontochromis tenuidentatus*	Ectodini			9	6	8	2	AY337784	JF900287	JF900216
Neofur	*Neolamprologus furcifer*	Lamprologini			13	8	9	6	EF679252	JF900288	JF900217
Neomod	*Neolamprologus modestus*	Lamprologini			25	17	12	9	DQ055012	JF900289	JF900218
Neopro	*Neolamprologus prochilus*	Lamprologini			6	4	7	5	EF462248	JF900290	JF900219
Neopul	*Neolamprologus pulcher*	Lamprologini			21	10	10	11	EF462244	JF900291	JF900220
Neosav	*Neolamprologus savoryi*	Lamprologini			22	10	9	11	HM623796	JF900292	JF900221
Neosex	*Neolamprologus sexfasciatus*	Lamprologini			13	11	13	8	HM623828	JF900293	JF900222
Neotet	*Neolamprologus tetracanthus*	Lamprologini			21	23	11	17	EF462220	JF900294	JF900223
Ophven	*Ophthalmotilapia ventralis*	Ectodini			17	14	20	11	AY337774	JF900295	JF900224
Oretan	*Oreochromis tanganicae*	Tilapiini			9	7	2	5	AF317240	JF900296	JF900225
Pcybri	*Paracyprichromis brieni*	Cyprichromini			5	7	10	4	AY740378	JF900297	JF900226
Permic	*Perissodus microlepis*	Perissodini			16	30	11	8	AF398222	JF900298	JF900227
Perpar	*Perissodus paradoxus*	Perissodini			4	4	4	3	EF437500	JF900299	JF900228
Peteph	*Petrochromis ephippium*	Tropheini			12	10	10	7	JF900323	JF900300	JF900229
Petfam	*Petrochromis famula*	Tropheini			10	10	12	7	JF900324	JF900301	JF900230
Petfas	*Petrochromis fasciatus*	Tropheini			8	6	9	4	JF900325	JF900302	JF900231
Petmac	*Petrochromis macrognathus*	Tropheini			18	12	11	12	AY930068	JF900304	JF900233
Petpol	*Petrochromis polyodon*	Tropheini			10	14	10	7	JF900326	JF900305	JF900234
Plestr	*Plecodus straeleni*	Perissodini			11	9	10	12	EF437481	JF900306	JF900235
Psccur	*Pseudosimochromis curvifrons*	Tropheini			13	8	10	10	GQ995777	JF900307	JF900236
Regcal	*Reganochromis calliurus*	Limnochromini			4	3	4	2	AY682544	JF900308	JF900237
Simbab	*Simochromis babaulti*	Tropheini			7	7	11	6	GQ995782	JF900309	JF900238
Simdia	*Simochromis diagramma*	Tropheini			27	13	16	9	AY930087	JF900310	JF900239
Teltem	*Telmatochromis temporalis*	Lamprologini			11	18	11	10	EF462234	JF900311	JF900240
Tremar	*Trematocara marginatus*	Trematocarini			11	9	10	2	JF900327	JF900312	JF900241
Trenig	*Trematocara nigrifrons*	Trematocarini			19	12	10	4	JF900328	JF900313	JF900242
Trioto	*Triglachromis otostigma*	Limnochromini			2	2	2	1	AY337769	JF900280	JF900209
Tromoo	*Tropheus moorii*	Tropheini			28	16	21	10	AY930093	JF900314	JF900243
Tylpol	*Tylochromis polylepis*	Tylochromini			11	10	10	4	U07268	JF900315	JF900244
Varmoo	*Variabilichromis moorii*	Lamprologini			23	21	17	10	DQ055016	JF900316	JF900245
Xenfla	*Xenotilapia flavipinnis*	Ectodini			8	9	9	5	AY337794	JF900317	JF900246
Xenspi	*Xenotilapia spiloptera*	Ectodini			32	21	14	4	AY337788	JF900318	JF900247
total	71	14			1049	816	727	506			

(B) Frequency distribution of the specimens used for body and LPJ shape, and for stable isotope and stomach and gut content analyses.









(C) Additional ND2 sequences used in the lineage-through-time (LTT) plots.

TID	Taxon name	Tribe	GenBank accession number ND2
Altshe	*Altolamprologus sp. 'shell'*	Lamprologini	EF191107
Baicen	*Baileychromis centropomoides*	Limnochromini	AY682509
Batfas	*Bathybates fasciatus*	Bathybatini	AY663732
Batfer	*Bathybates ferox*	Bathybatini	AY663736
Bathor	*Bathybates hornii*	Bathybatini	AY663735
Batleo	*Bathybates leo*	Bathybatini	AY663729
Batmin	*Bathybates minor*	Bathybatini	AY663722
Benmel	*Benthochromis melanoides*	Benthochromini	AY682512
Calple	*Callochromis pleurospilus*	Ectodini	AY337771
Calsta	*Callochromis stappersii*	Ectodini	AY337775
Carsch	*Cardiopharynx schoutedeni*	Ectodini	AY337791
Chapop	*Chalinochromis popeleni*	Lamprologini	U07244
Cunlon	*Cunningtonia longiventralis*	Ectodini	AY337780
Cypmic	*Cyprichromis microlepidotus*	Cyprichromini	AY740346
Cyppav	*Cyprichromis pavo*	Cyprichromini	AY740382
Cypzon	*Cyprichromis zonatus*	Cyprichromini	AY740347
Hemste	*Hemibates stenosoma*	Bathybatini	AY663716
Juldic	*Julidochromis dickfeldi*	Lamprologini	EF462230
Julmar	*Julidochromis marlieri*	Lamprologini	AF398230
Julreg	*Julidochromis regani*	Lamprologini	EF462228
Jultra	*Julidochromis transcriptus*	Lamprologini	EF462231
Lamkun	*Lamprologus kungweensis*	Lamprologini	EF191084
Lamlap	*Lamprologus laparogramma*	Lamprologini	EF462278
Lammel	*Lamprologus meleagris*	Lamprologini	DQ055027
Lamoce	*Lamprologus ocellatus*	Lamprologini	EF462259
Lamsig	*Lamprologus signatus*	Lamprologini	EF191086
Lamspe	*Lamprologus speciosus*	Lamprologini	EF191102
Lamteu	*Lamprologus teugelsi*	Lamprologini	DQ055059
Lepbou	*Lepidiolamprologus boulengeri*	Lamprologini	DQ055040
Lephec	*Lepidiolamprologus hecqui*	Lamprologini	DQ055041
Lepken	*Lepidiolamprologus kendalli*	Lamprologini	EF462269
Lepnka	*Lepidiolamprologus nkambae*	Lamprologini	EF462270
Lesper	*Lestradea perspicax*	Ectodini	AY337765
Lessta	*Lestradea stappersii*	Ectodini	AY337792
Mdcrot	*Microdontochromis rotundiventralis*	Ectodini	AY337793
Neobif	*Neolamprologus bifasciatus*	Lamprologini	HM623809
Neobre	*Neolamprologus brevis*	Lamprologini	EF462264
Neobri	*Neolamprologus brichardi*	Lamprologini	AF398227
Neobue	*Neolamprologus buescheri*	Lamprologini	EF462243
Neocal	*Neolamprologus calliurus*	Lamprologini	DQ093112
Neocau	*Neolamprologus caudopunctatus*	Lamprologini	EF462272
Neochr	*Neolamprologus christyi*	Lamprologini	HM623826
Neocun	*Neolamprologus cunningtoni*	Lamprologini	DQ055054
Neocyl	*Neolamprologus cylindricus*	Lamprologini	EF462224
Neodev	*Neolamprologus devosi*	Lamprologini	EF437476
Neofal	*Neolamprologus falcicula*	Lamprologini	EF462246
Neogra	*Neolamprologus gracilis*	Lamprologini	HM623798
Neohel	*Neolamprologus helianthus*	Lamprologini	DQ055013
Neolel	*Neolamprologus leleupi*	Lamprologini	EF462251
Neolou	*Neolamprologus leloupi*	Lamprologini	EF191103
Neoloc	*Neolamprologus longicaudata*	Lamprologini	EF462250
Neolon	*Neolamprologus longior*	Lamprologini	HM623793

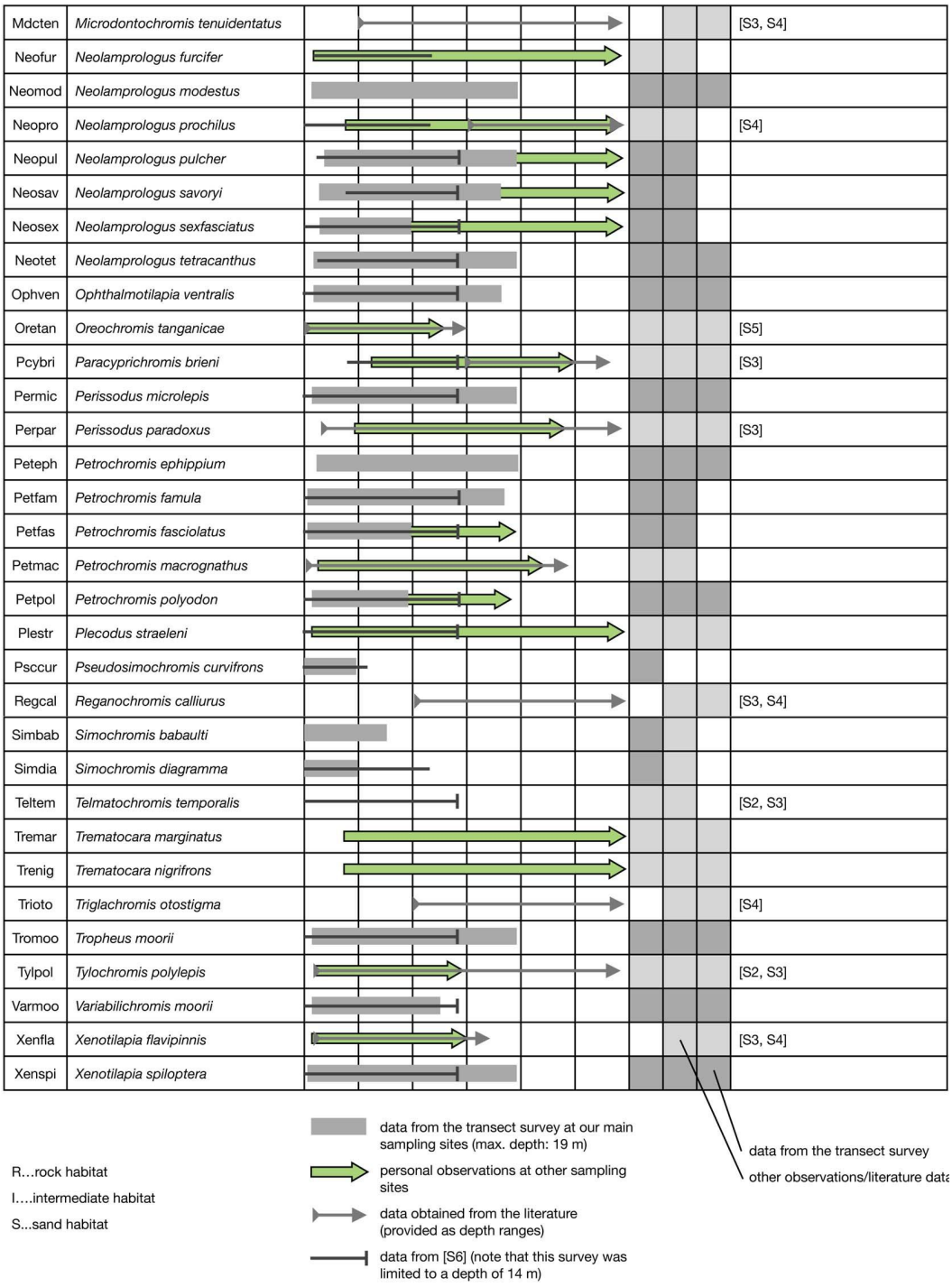
Neomar	*Neolamprologus marunguensis*	Lamprologini	AY740390
Neomee	*Neolamprologus meeli*	Lamprologini	DQ055051
Neomon	*Neolamprologus mondabu*	Lamprologini	EF462242
Neomul	*Neolamprologus multifasciatus*	Lamprologini	EF462266
Neomux	*Neolamprologus mustax*	Lamprologini	EF462223
Neonig	*Neolamprologus niger*	Lamprologini	AY740391
Neogri	*Neolamprologus nigriventris*	Lamprologini	EF462239
Neoobs	*Neolamprologus obscurus*	Lamprologini	HM623824
Neooli	*Neolamprologus olivaceous*	Lamprologini	AY740393
Neopec	*Neolamprologus pectoralis*	Lamprologini	EF462238
Neopet	*Neolamprologus petricola*	Lamprologini	HM623827
Neosim	*Neolamprologus similis*	Lamprologini	EF462261
Neosek	*Neolamprologus sp. 'eseki'*	Lamprologini	HM623794
Neokip	*Neolamprologus sp. 'Kipili'*	Lamprologini	HM623802
Neondo	*Neolamprologus sp. 'ndobnoi'*	Lamprologini	HM623802
Neospl	*Neolamprologus splendens*	Lamprologini	HM623799
Neotoa	*Neolamprologus toae*	Lamprologini	EF462222
Neotre	*Neolamprologus tretocephalus*	Lamprologini	EF462219
Neovar	*Neolamprologus variostigma*	Lamprologini	EF462253
Neoven	*Neolamprologus ventralis*	Lamprologini	EF462233
Neowal	*Neolamprologus walteri*	Lamprologini	HM623808
Neowau	*Neolamprologus wauthioni*	Lamprologini	EF191118
Ophboo	*Ophthalmotilapia boops*	Ectodini	AY337773
Ophhet	*Ophthalmotilapia heterodonta*	Ectodini	EF679254
Ophnas	*Ophthalmotilapia nasuta*	Ectodini	AY337783
Pcynig	*Paracyprichromis nigripinnis*	Cyprichromini	AY740339
Perecc	*Perissodus eccentricus*	Perissodini	EF437511
Petort	*Petrochromis orthognathus*	Tropheini	U07262
Petkat	*Petrochromis sp. 'Katete'*	Tropheini	GQ995748
Petmos	*Petrochromis sp. 'moshi'*	Tropheini	GQ995765
Pettex	*Petrochromis sp. 'Texas'*	Tropheini	GQ995766
Pettre	*Petrochromis trewavasae*	Tropheini	GQ995761
Pleela	*Plecodus elaviae*	Perissodini	EF437504
Plemul	*Plecodus multidentatus*	Perissodini	EF437505
Simmar	*Simochromis marginatus*	Tropheini	AY930088
Simple	*Simochromis pleurospilus*	Tropheini	GQ995783
Spaery	*Spathodus erythrodon*	Eretmodini	DQ055008
Spamar	*Spathodus marlieri*	Eretmodini	HM623786
Tanirs	*Tanganicodus irsacae*	Eretmodini	DQ055007
Telbif	*Telmatochromis bifrenatus*	Lamprologini	AF398228
Telbri	*Telmatochromis brichardi*	Lamprologini	EF462236
Teldho	*Telmatochromis dhonti*	Lamprologini	EF679266
Telvit	*Telmatochromis vittatus*	Lamprologini	EF462237
Ttcmac	*Telotrematocara macrostoma*	Trematocarini	AY663715
Treuni	*Trematocara unimaculatum*	Trematocarini	AF317268
Trioto	*Triglachromis otostigma*	Limnochromini	AF398217
Trobri	*Tropheus brichardi*	Tropheini	AY930086
Trodub	*Tropheus duboisi*	Tropheini	AY930085
Tropol	*Tropheus polli*	Tropheini	AY930084
Xenhec	*Xenochromis hecqui*	Ectodini	EF437513
Xenbat	*Xenotilapia bathyphila*	Ectodini	AY337789
Xenbou	*Xenotilapia boulengeri*	Ectodini	HM135111
Xencau	*Xenotilapia caudafasciata*	Ectodini	AY337777
Xenlon	*Xenotilapia longispinis*	Ectodini	AY337779

Xenoch	*Xenotilapia ochrogenys*	Ectodini	AY337767
Xensim	*Xenotilapia sima*	Ectodini	AY337785
Xenpap	*Xenotilapia sp. 'papilio sunflower'*	Ectodini	AY337776

TID Taxon identifier, which is also used in Figures 1 and 2
LPJ Lower pharyngeal jaw bone
$\mathbf{N_{body}}$ Number of specimens used for morphometric analyses of body shape
$\mathbf{N_{LPJ}}$ Number of specimens used for morphometric analyses of the lower pharyngeal jaw bone
$\mathbf{N_{SIA}}$ Number of specimens used for stable isotope analyses
$\mathbf{N_{SGCA}}$ Number of specimens used for stomach and gut content analyses

Table S2. Depth Distribution of Species Used in This Study

Taxonomic information		Depth [in m]						Habitat			Other references and notes
TID	Taxon name	0-5	5-10	10-15	15-20	20-25	25-	R	I	S	
Altcal	*Altolamprologus calvus*										[S2]
Altcom	*Altolamprologus compressiceps*										
Altfas	*Altolamprologus fasciatus*										
Asplep	*Asprotilapia leptura*										
Astbur	*Astatotilapia burtoni*										mostly riverine
Auldew	*Aulonocranus dewindtii*										
Batgra	*Bathybates graueri*										[S2, S3]
Batvit	*Bathybates vittatus*										[S4]
Baicen	*Baileychromis centropomoides*						40-100				[S3, S4]
Bentri	*Benthochromis tricoti*										[S3, S4]
Boumic	*Boulengerochromis microlepis*										[S3, S4]
Calmac	*Callochromis macrops*										
Chabri	*Chalinochromis brichardi*										
Cphgib	*Cyphotilapia gibberosa*										
Cteben	*Ctenochromis benthicola*						25+				[S2-S4]
Ctehor	*Ctenochromis horei*										
Cyafur	*Cyathopharynx furcifer*										
Cyplep	*Cyprichromis leptosoma*										
Enamel	*Enantiopus melanogenys*										[S3]
Ectdes	*Ectodus descampsi*										[S3]
Erecya	*Eretmodus cyanostictus*										
Gnaper	*Gnathochromis permaxillaris*										[S3, S4]
Gnapfe	*Gnathochromis pfefferi*										
Gralem	*Grammatotria lemairii*										[S2, S3]
Gwcchr	*Greenwoodochromis christyi*						40-150				[S2, S3]
Hapmic	*Haplotaxodon microlepis*										[S3, S4]
Haptri	*Haplotaxodon trifasciatus*										[S3]
Intloo	*Interochromis loockii*										
Julorn	*Julidochromis ornatus*										
Lamcal	*Lamprologus callipterus*										
Lamlem	*Lamprologus lemairii*										
Lamorn	*Lamprologus ornatipinnis*										[S3]
Lchabe	*Limnochromis abeelei*						50-				[S3, S4]
Lchaur	*Limnochromis auritus*										[S2, S3]
Lchsta	*Limnochromis staneri*						40-				[S3, S4]
Lepatt	*Lepidiolamprologus attenuatus*										
Lepelo	*Lepidiolamprologus elongatus*										
Leppro	*Lepidiolamprologus profundicola*										
Limdar	*Limnotilapia dardennii*										
Loblab	*Lobochilotes labiatus*										


Mdcten Microdontochromis tenuidentatus [S3, S4]
Neofur Neolamprologus furcifer
Neomod Neolamprologus modestus
Neopro Neolamprologus prochilus [S4]
Neopul Neolamprologus pulcher
Neosav Neolamprologus savoryi
Neosex Neolamprologus sexfasciatus
Neotet Neolamprologus tetracanthus
Ophven Ophthalmotilapia ventralis
Oretan Oreochromis tanganicae [S5]
Pcybri Paracyprichromis brieni [S3]
Permic Perissodus microlepis
Perpar Perissodus paradoxus [S3]
Peteph Petrochromis ephippium
Petfam Petrochromis famula
Petfas Petrochromis fasciolatus
Petmac Petrochromis macrognathus
Petpol Petrochromis polyodon
Plestr Plecodus straeleni
Psccur Pseudosimochromis curvifrons
Regcal Reganochromis calliurus [S3, S4]
Simbab Simochromis babaulti
Simdia Simochromis diagramma
Teltem Telmatochromis temporalis [S2, S3]
Tremar Trematocara marginatus
Trenig Trematocara nigrifrons
Trioto Triglachromis otostigma [S4]
Tromoo Tropheus moorii
Tylpol Tylochromis polylepis [S2, S3]
Varmoo Variabilichromis moorii
Xenfla Xenotilapia flavipinnis [S3, S4]
Xenspi Xenotilapia spiloptera
R…rock habitat
I….intermediate habitat
S...sand habitat
data from the transect survey at our main sampling sites (max. depth: 19 m)
personal observations at other sampling sites
data obtained from the literature (provided as depth ranges)
data from [S6] (note that this survey was limited to a depth of 14 m)
data from the transect survey
other observations/literature data

Supplemental Experimental Procedures

Sampling
Sampling at Lake Tanganyika, East Africa, was performed in autumn 2007, 2008, and 2011, and in spring 2010 under the permission and with guidance from the Department of Fisheries, Lake Tanganyika Research Unit, Mpulungu, Republic of Zambia. Cichlid fishes were caught with gill-nets set by snorkeling and scuba diving, by harpooning, by angling, or, in a few cases, obtained from local fishermen. For sample preparation in the field, we followed our standard operating procedure (SOP): Fishes were sized (total and standard length), weighted, sexed (whenever possible) and photographed in a standardized way using either a Nikon Coolpix P5000 or a Nikon D5000 digital camera; then, a fin-clip and a piece of white muscle tissue were taken as tissue sample (for DNA extraction and stable isotope analysis) and preserved in 96% ethanol; finally, we dissected and sun-dried the lower pharyngeal jaw apparatus and preserved the intestines in ethanol for stomach and gut content analyses. Two specimens per species were taken as voucher and preserved in ethanol. In total, we sampled more than 1000 specimens for this study (see Table S1 for details). The core dataset contains 71, thus covering more than a third of all Tanganyikan cichlid species, including all major lineages ('tribes'), and about 80% of the recognized genera. Note that we use a six letter code for the species, with the first three letters indicating the genus name and the last three letters abbreviating the species name.

Line Transect Survey
In order to obtain depth-distribution and habitat data for the most common species in our core data set, we performed transect surveys using scuba diving at our three main sampling locations in the South of Lake Tanganyika (in August and September 2011; see Table S2). Two independent rounds of fish counts were performed at each of the three locations. The sampling sites were: Toby_right_1 (8° 37' 20.97" S 31° 12' 00.37" E; transect length: 70 m), Toby_right_2 (8° 37' 19.31" S 31° 11' 59.58" E; transect length: 108 m), Toby_left_1 (8° 37' 28.79" S 31° 12' 01.75" E; transect length: 98 m), Toby_left_2 (8° 37' 30.40" S 31° 12' 01.23" E; transect length: 106 m), Mbita_1 (8° 45' 16.57" S 31° 05' 23.74" E; transect length: 60 m), and Mbita_2 (8° 45' 16.75" S 31° 05' 21.92" E; transect length: 50 m).

We used a 120 m rope with markings every 2 m, which was placed in a 90° angle to the shore. The end of the transect was determined by the beginning of sandy flats, where fish densities approximate null. Before starting with the transect dives, we determined the depth of each 2 m marking with a diving computer (Suunto Gekko) and recorded the habitat between two consecutive markings as rocks (R), sand (S) and intermediate between sand and rocks (I). Scuba dives were performed in teams of two or three divers, who recorded a predefined set of species as they were diving along the transect line and in an area of 2 m left and right of the rope. At the end of the rope, the divers rested for a period of 10 min in order to leave enough time for the fish to restore. After that, the divers returned to the shore counting the same set of species a second time (see [7]). Up to five transect dives were performed at each transect; the more shallow areas were partly covered by snorkeling.

Phylogenetic Analyses
DNA Extraction DNA was extracted from ethanol preserved tissue samples (see above) using a Qiagen Biosprint 96 DNA extraction robot and following the manufacturer's protocol.

Molecular Methods PCR amplification of the entire mitochondrial NADH Dehydrogenase Subunit 2 (ND2) gene followed the strategy described before [20] – this time, however, using Sigma RedTaq DNA polymerase (Sigma Aldrich). For the amplification of the two nuclear gene segments, *ednrb1* and *phpt*, we used the Phusion High-Fidelity DNA polymerase (New England BioLabs) in a total volume of 20µl (10µl Phusion High-Fidelity DNA master mix, 6µl water, 1µl of each primer [10µM], and 2 µl of diluted DNA extract [1:10]). For *ednrb1*, we used published primers [S8, S9]. The primers for *phpt* were 38a_F (5'-AGC AGG GTT GAC CTT CTC AA - 3') and 38a_R (5' – TGG CTA AAA TCC CCG ATG TA – 3'). PCR products were purified with the ExoSAP-IT protocol (USB) and used as template for cycle sequencing reactions in both directions with the BigDye Terminator v.3.1 kit (Applied Biosystems) in 10µl reactions. After dye removal with the BigDye XTerminator purification kit (Applied Biosystems), samples were run on an ABI3130*xl* capillary genetic analyzer (Applied Biosystems). All sequences were checked by eye and assembled with CODONCODEALIGNER v.3.5.6 (CodonCode Corporation). ND2 sequences for most of the species were already available from previous studies [20, 21, 34, S10]; all

sequences of the nuclear loci have been newly sequenced. GenBank accession numbers of all sequences used in this study are shown in Table S1.

Phylogenetic Inference No additional alignment procedure was necessary for ND2 (all sequences had the identical length of 1'047 bp); the two nuclear gene segments were aligned with MAFFT [S11] resulting in an alignment length of 542 bp for *ednrb1* and 424 bp for *phpt*. We relied on maximum likelihood and Bayesian methods for phylogenetic analysis using PAUP* [S12], MRBAYES [S13] and the BEAST package [S14]. The appropriate model of molecular evolution for the heuristic tree searches in PAUP* was determined with JMODELTEST [S15] and applying the Akaike Information Criterion. MRBAYES was run for 10'000'000 generations with a burn-in of 10% (after monitoring the level of convergence). Data were partitioned in BEAST. Three rounds of analyses were performed, first with the core data set combining the mitochondrial and nuclear DNA sequences in 71 taxa, then with the core data set including *Baileychromis centropomoides,* and third with a mitochondrial data set including the ND2 sequences of 180 taxa (i.e. ca. 90% of all Tanganyika species). The latter analysis was aimed as starting point for the lineage-through-time plots (see below).

Incongruence Testing To statistically test for incongruence between the molecular phylogeny and the grouping of taxa according to their overall and trophic morphology ('cluster analysis'; see below), we applied two classic tests implemented in PAUP*, the Kishino-Hasegawa (KH) and the Shimodaira-Hasegawa (SH) test both under a resampling-estimated log-likelihood (RELL). Note that these tests merely inform that the two topologies built from morphological characters are not supported by our molecular data and cannot *per se* be taken as evidence for convergent evolution. Valid tests for evaluating convergent evolution (pairwise distance-contrast and disparity-through-time plots) and are described below.

Lineage-Through-Time Plots In order to reconstruct diversification rates in the species flock of cichlids from Lake Tanganyika, we performed a LTT analysis with our new extensive data set including about 90% of all species. Such an analysis has been conducted before [21], albeit with a smaller data set. Still, we follow the exact same procedure as described before [21] using BEAST and the APE package [S16] in R. The main difference to the study of Day *et al.* [21] is that we refrain from inferring an absolute time scale for the Lake Tanganyika radiation, due to the lack of fossil calibrations and uncertainties with respect to the onset of the radiation (see discrepancies in previous estimates; [20, 24, 34, S10, S17]). Instead, we use a relative timing, just as with the disparity through time plots (see below), allowing for maximum compatibility between disparity and diversity plots.

Geometric Morphometric and Morphological Analyses

Body Shape We assessed the body shape of 1049 individuals using landmark-based geometric morphometric methods. The exact numbers of specimen per species are given in Table S1. *xy* coordinates of 17 landmarks, distributed across the whole fish body (see Figure. S7A), and the scale of each picture were recorded using TPSDIG [30]. Raw landmark coordinates were procrustes aligned and the resulting procrustes coordinates were used for a pooled-within-species regression of shape against centroid size in MORPHOJ 1.02d [31]. The resulting residuals were averaged for each species and used for principal component analysis (PCA), disparity through time analyses, and for the calculation of pairwise distances between species.

In a second analysis, focusing specifically on the similarity between *Baileychromis centropomoides* and *Lates sp.*, we determinded the landmark configurations of *B. centropomoides* (N=4) and all four endemic *Lates* species (*L. angustifrons*, *L. mariae*, *L. microlepis* and *L. stappersi;* based on drawings from [S18]). We first performed a canonical variates analysis (CVA) in MORPHOJ with the data from *B. centropomoides* and *Lates* and then incorporated *B. centropomoides* and *L. stappersi* (the most similar species) into the core data set and performed another CVA (Figure 3B). We also determined procrustes distances of all cichlid species to *L. stappersi*. *B. centropomoides* shows the by far smallest procrustes distances to *L. stappersi*.

Pharyngeal Jaw Shape For LPJ assessment we recorded *xy* coordinates of 28 evenly distributed landmarks describing the outline of the bone (Figure S7B). We arranged two sets of nine equidistant lines perpendicular to the posterior outline and the anterior-posterior axis respectively. That way, we could treat the intersections of these lines with the outline of the jaw as semi-landmarks. Our initial set was composed

of 8 true landmarks and 20 semi-landmarks. We subjected this data set to an iterative sliding-process in TPSRELW (10 iterations) using the minimum bending energy criterion to retain information of outline curve shape and minimize differences in landmark positions along the curve. We then pruned this data set to 14 landmarks, comprised of the 8 true landmarks (red dots in Figure S7B) and 6 slid semi-landmarks (blue dots in Figure S7B). The subsequent analyses were the same as for body shape, with the exception of accounting for the symmetry of the LPJ.

Cluster Analysis We clustered the species for their similarity in body and pharyngeal jaw shape using agglomerative hierarchical clustering in R. We used the agnes() function of the package CLUSTER [S19] and Ward's clustering method on Mahalanobis distance matrices derived from CVA in MORPHOJ.

Stomach and Gut Content Analyses
To assess the trophic specializations of the studied species, we performed stomach and gut content analyses in 506 specimens (note: this number is somewhat smaller than the number of specimens used for the other analyses, as some of the intestinal tracts were empty). For stomach and gut content analyses, the intestinal tracts were opened under a binocular (Leitz) and the entire contents were removed. Stomach and gut contents were separated up into one or more of the following categories: sand, aufwuchs (algae), plant material, mollusks, insects (imagines and larvae), crustaceans, fish (remains), fish eggs, and fish scales. We determined volume (in %) and weight (in µg; using a Kern ALS 120-4 scale) of each category. To prevent bias, roughly the same amount of time was spent on the stomach and gut content of each specimen, and the samples were blinded, i.e. the assayer was unaware of the species ID. The volumetric data, illustrated in Figure 2E, were then used to calculate Schoener's index of proportional diet overlap [S20], and to perform a PCA. We then performed a bootstrap analysis with 10.000 replicates to test whether convergent species pairs show greater similarities in Schoener's index than random pairs of species.

Stable Isotope Analyses
Stomach and gut content analyses as described above have the drawback that they only cover food uptake in the last few hours (in case of tropical fish) or days before the capture of the specimens. This problem can be overcome by determining the chemical signature of food uptake via the analysis of stable isotopes. We here apply a stable isotope analysis (SIA) on the basis of the signature of C and N stable isotopes (^{13}C and ^{15}N). To this end, we used white muscle tissue samples from 727 specimen (see Table S1), which were kept in ethanol and dried at 60°C for 24h in the laboratory. We pulverized the dried tissue using Zirconia beads and a bead-beater, and elutriated the powder in pure ethanol. The suspension was centrifuged and the supernatant decanted. The pellet was then dried at 60°C overnight and amounts of 500 µg were weighed into tin capsules and analyzed on an elemental analyzer (Thermo Finnigan) coupled to a Finnigan Delta V Advantage IRMS (Isotope Ratio Mass Spectrometer), with standard setups for N_2 and CO_2 analysis [S21]. The isotopic composition is expressed in the conventional delta notation as permil (‰) deviation *versus* atmospheric N_2 and Pee Dee Belemnite. Because of sampling at two different times of the year, in three different years and in different localities our sampling captures possible within species variation in trophic ecology.

Correlation between Morphological Clusters and Stable Isotope Signatures
We used distance based redundancy analysis as implemented in the function capscale() in the R package VEGAN [S22] and anova.cca() to test for significance of the association between morphological distances between species and their stable isotope signatures. We also estimated the phylogenetically independent correlations between data sets using phylogenetic canonical correlation analysis. We calculated principle components for each data set and used these to find the axes of largest correlation using phyl.cca() from the PHYTOOLS package [S23]. This revealed a highly significant ($p=0.0000007$) correlation ($cor=0.68$) between LPJ shape and stable isotope signatures, corroborating our findings from the disparity through time analyses.

Pairwise Distance-Contrast Plots
To estimate the extent of convergence within the Lake Tanganyika cichlid species flock we compared the phylogenetic distance between each pair of species to its morphological distance. We derived the phylogenetic distance from our molecular phylogeny using the cophenetic() function in R. The morphological distance was calculated as Euclidean distance from the pooled-within-species regressions

of shape against centroid size using R's dist() function. In total we had 2485 species comparisons, therefore we used hexagonal binning (x = 10 bins) to overcome problems with overplotting. This also allowed us a direct comparison to our modeled trait evolution scenario. To this end we calculated the variance-covariance matrix from our data considering the phylogeny by using ic.sigma() function in the R-package GEIGER [S24]. We then simulated neutral trait evolution on our phylogeny using sim.char() with Brownian motion. For a comparison to a Ornstein-Uhlenbeck model of trait evolution, we transformed the phylogeny with ouTree() using a wide range of alpha values. The species comparisons that we derived from these simulations were then compared to our actual data by subtracting the binning counts of the simulations from those of the data. This led to negative combined counts in bins with simulated comparisons being in the majority and positive ones in bins with data being in the majority. We tested for statistical significance of the difference of pointwise means between simulations and data (each $1/10^{th}$ of the x-axis) by bootstrapping (1000 bootstraps). As both simulations, Brownian motion and Ornstein-Uhlenbeck revealed highly congruent results, we only show one of them, Brownian motion, in Fig. 4.

We also estimated the number of convergent species pairs by counting those species comparisons falling below the lower 95% confidence threshold of the neutral evolution simulations. This revealed 122 and 132 species pairs that are convergent in body and LPJ shape, respectively.

Habitat and Depth Overlap

Based on our transect surveys (see above), further observations and catch-records, and available literature [S2-S6, S25], we characterized the depth distribution and the habitat for each species in our core-data set (see Table S2). These data were used to assess habitat and depth overlap between convergent forms.

We also used our transect data on 16 focal species to determine how many species co-occurred at least once within a single 2 m transect. Out of 120 comparisons, only a single species pair was never found together (Neopul-Simbab). This once more highlights the high degree of sympatry of the species in included in this study.

Disparity-through-Time Plots

Following the method of Harmon *et al*. [33], we plotted the trajectory of average subclade disparity against time for shape and stable isotope data. We compared those trajectories to ones generated from Brownian motion simulations of trait evolution using our molecular phylogeny. Positive deviations of the data from the simulations indicate a higher overlap in morphospace among subclades than would be expected under neutral evolution. As disparity measures we used average squared Euclidean distances. We averaged over 100 simulation runs to get a more reliable estimate of Brownian motion trait evolution. The plots are shown up to 80% of the time span only (from root age to present), since this analysis is prone to be affected by tip overdispersion as it approaches present due to missing terminal taxa. This analysis has been performed with the entire core data-set and with a subset of 64 taxa, in which we removed the ancestral lineages Bathybatini, Trematocarini, Tilapiini and *Tylochromis*. Figure 4 depicts the latter analysis.

A potential problem with disparity-through-time analyses is that they might be influenced by varying rates of morphological evolution between sub-clades. This is not the case in cichlids from Lake Tanganyika, as it has previously been shown that the rate of morphological evolution is relatively constant between tribes [23].

Evolutionary PCA

For body shape and LPJ shape, we estimated the ancestral character states at each node in the phylogeny from the regressions against centroid size residuals. This allowed us to calculate the extent and direction of shape change along each branch. These branch-wise estimates were then subjected to principal component analysis to find the axes of greatest evolutionary divergence within the Tanganyikan species flock. All evolutionary principal component analyses were performed in MORPHOJ. We illustrated the shape changes along the heaviest loaded axes by contrasting the reconstructed root state with the derived state along the respective axis and a scale factor of 0.1. The illustration is a warped outline drawing, with interlandmark outlines being estimated and shown for illustration purposes, but for which we have no further information on their accuracy. To counteract the distraction by largely distorted outlines, such as fins, which we never observed in nature and for which we have no direct morphometric information, we manually adjusted those outlines to be more similar in the plots. This did not influence any of our analyses or interpretations.

CT Scanning of the Pharyngeal Jaw Apparatus
To illustrate the arrangement of dentigerous bones in the pharyngeal jaw apparatus of Tanganyikan cichlids we performed a computed tomography (CT) scan. The head of an adult male *Astatotilapia burtoni* was scanned at 18µm voxel size resolution in a SkyScan 1176 in-vivo hi-res microCT scanner. Cross sections were computed from the raw images in NRECON and used to construct a virtual 3D model in OSIRIX. We removed all but the tooth-bearing pharyngeal bones from the virtual model and compiled a movie showing the phranyngeal jaw apparatus in rotation around the dorsal-ventral axis (see Movie S1).

Supplemental References

S1. Clabaut, C., Bunje, P. M., Salzburger, W., and Meyer, A. (2007) Geometric morphometric analyses provide evidence for the adaptive character of the Tanganyikan cichlid fish radiations. Evolution *61*, 560-578.

S2. Herrmann, H. J. (2002) *Buntbarsche des Tanganjikasees (*Dähne Verlag, Egestorf).

S3. Konings, A. (1998) *Tanganyikan cichlids in their natural habitat* (Cichlid Press, El Paso).

S4. Nori, M. (1997) in *Fish communities in Lake Tanganyika*, eds Kawanabe, H., Hori, M., Nagoshi, M. (Kyoto University Press) pp 277-298 .

S5. Brichard, P. (1989) *Cichlids and all other fishes of Lake Tanganyika* (THF Publications, Neptune City, NJ).

S6. Takeuchi, Y., Ochi, H., Kohda, M., Sinyinza, D., and Hori, M. (2010) A 20-year census of a rocky littoral fish community in Lake Tanganyika. Ecol. Freshw. Fish. *19,* 239-248.

S7. Dittmann, M. T., et al. (2012) Depth-dependent abundance of Midas Cichlid fish (*Amphilophus* spp.) in two Nicaraguan crater lakes. Hydrobiologia *686*, 277-285.

S8. Lang, M. et al. (2006) A BAC library of the East African haplochromine cichlid fish *Astatotilapia burtoni*. J. Exp. Zoolog. B *306B*, 35-44.

S9. Meyer, B., and Salzburger, W. (2012) A novel primer set for multilocus phylogenetic inference in East African cichlid fishes. Mol. Ecol. Res. doi: 10.1111/j.1755-0998.2012.03169

S10. Sturmbauer, C., et al. (2010) Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. Mol. Phylogenet. Evol. *57*, 266-284.

S11. Katoh, K., and Toh, H. (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinform. *9*, 286-298.

S12. Swofford, D. L. (2003) PAUP* - Phylogenetic Analyses Using Parsimony and other methods, version 4.0 v. 4.0 (Sinauer, Sunderland, MA).

S13. Ronquist, F., and Huelsenbeck, J. P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinform*. 19*, 1572-1574.

S14. Drummond. A. J., and Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. *7*, 214.

S15. Posada, D. (2008) jModelTest: phylogenetic model averaging. Mol. Biol. Evol. *25*, 1253-1256.

S16. Paradis, E., Claude, J., and Strimmer, K. (2004) APE: Analyses of Phylogenetics and Evolution in R language. Bioinform. *20,* 289-290.

S17. Genner, M. J., et al. (2007) Age of cichlids: new dates for ancient lake fish radiations. Mol. Biol. Evol. *24,* 1269-1282.

S18. Greenwood, P. H. A. (1979) Review of the family Centropomidae (Pisces, Perciformes) (*Bulletin of the British Museum of Natural History* vol. 29).

S19. Maechler, M., Rousseuw, P., Struyf, A., Hubert, M., and Hornik, K. (2011) Cluster: Cluster Analysis Basics and Extensions. R package version 1.14.1.

S20. Schoener, T. W. (1970) Nonsynchronous spatial overlap of lizards in patchy habitats. Ecology *51,* 408-418.

S21. Rutschmann, S., et al. (2011) Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol. Ecol. *20,* 4707-4721.

S22. Oksanen, J. et al. (2010) Vegan: Community Ecology Package. R package version 1.17.3.

S23. Revell, L. J. (2012) Phytools: an R package for phylogeneti comparative biology (and other things). Methods in Ecology and Evolution *3,* 217-223.

S24. Harmon, L. J., Weir, J. T., Brock, C. D., Glor, R. E., and Challenger, W. (2008) GEIGER: investigating evolutionary radiations. Bioinform. *24*, 129-131.

S25. Yamaoka, K. (1997) in *Fish communities in Lake Tanganyika*, eds Kawanabe H, Hori M, Nagoshi M (Kyoto University Press) pp 27-56.

Chapter 2

The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes

Marco Colombo*, Eveline T. Diepeveen*, Moritz Muschick, M. Emilia Santos, **Adrian Indermaur**, Nicolas Boileau, Marta Barluenga and Walter Salzburger

*These authors contributed equally to this work

Molecular Ecology (2012)

AI helped with the sampling, processing of samples, sequencing, drafting and disscusion of the manuscript

MOLECULAR ECOLOGY

Molecular Ecology (2012) doi: 10.1111/mec.12029

The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes

MARCO COLOMBO,*[1] EVELINE T. DIEPEVEEN,*[1] MORITZ MUSCHICK,*‡ M. EMILIA SANTOS,* ADRIAN INDERMAUR,* NICOLAS BOILEAU,* MARTA BARLUENGA† and WALTER SALZBURGER*

*Zoological Institute, University of Basel, Vesalgasse 1, 4051, Basel, Switzerland, †Museo Nacional de Ciencias Naturales, CSIC, José Gutierrez Abascal 2, 28006, Madrid, Spain

Abstract

The evolution of convergent phenotypes is one of the most interesting outcomes of replicate adaptive radiations. Remarkable cases of convergence involve the thick-lipped phenotype found across cichlid species flocks in the East African Great Lakes. Unlike most other convergent forms in cichlids, which are restricted to East Africa, the thick-lipped phenotype also occurs elsewhere, for example in the Central American Midas Cichlid assemblage. Here, we use an ecological genomic approach to study the function, the evolution and the genetic basis of this phenotype in two independent cichlid adaptive radiations on two continents. We applied phylogenetic, demographic, geometric morphometric and stomach content analyses to an African (*Lobochilotes labiatus*) and a Central American (*Amphilophus labiatus*) thick-lipped species. We found that similar morphological adaptations occur in both thick-lipped species and that the 'fleshy' lips are associated with hard-shelled prey in the form of molluscs and invertebrates. We then used comparative Illumina RNA sequencing of thick vs. normal lip tissue in East African cichlids and identified a set of 141 candidate genes that appear to be involved in the morphogenesis of this trait. A more detailed analysis of six of these genes led to three strong candidates: *Actb*, *Cldn7* and *Copb*. The function of these genes can be linked to the loose connective tissue constituting the fleshy lips. Similar trends in gene expression between African and Central American thick-lipped species appear to indicate that an overlapping set of genes was independently recruited to build this particular phenotype in both lineages.

Keywords: adaptive radiation, cichlid species flocks, convergent evolution, East Africa, ecological genomics, RNAseq

Received 9 March 2012; revision received 4 July 2012; accepted 15 July 2012

Introduction

Adaptive radiation is the rapid evolution of an array of species from a common ancestor as a consequence of the emerging species' adaptations to distinct ecological niches (Simpson 1953; Schluter 2000; Gavrilets & Losos 2009). It is typically triggered by ecological opportunity in form of underutilized resources—just as being provided after the colonization of a new habitat, the extinction of antagonists and/or the evolution of a novel trait, which is then termed an evolutionary 'key innovation' (Gavrilets & Vose 2005; Gavrilets & Losos 2009; Losos & Ricklefs 2009; Losos 2010; Yoder *et al.* 2010; Matschiner *et al.* 2011). Whatever the circumstances were that initiated an adaptive radiation, there is always a strong link between adaptively relevant traits and the habitat and/or foraging niche (a 'phenotype–environment correlation'; Schluter 2000). In the most illustrative examples of adaptive radiation, the Darwin's finches on the Galapagos archipelago, the *Anolis* lizards on the

Correspondence: Walter Salzburger, Fax: +41 61 267 0301; E-mail: walter.salzburger@unibas.ch
‡ Present address: Department of Animal and Plant Sciences, The University of Sheffield, Sheffield, S10 2TN, UK.
[1]These authors contributed equally to this work.

Caribbean islands and the cichlid fishes of the East African Great Lakes, this correlation exists between beak-shape and food source (finches), limb morphology and twig diameter (anoles), and the architecture of the mouth and jaw apparatus and foraging mode (cichlids) (Schluter 2000; Butler *et al.* 2007; Grant & Grant 2008; Losos 2009; Salzburger 2009).

An interesting aspect of many adaptive radiations is the frequent occurrence of convergent (or parallel) evolution (Schluter & Nagel 1995; Harmon *et al.* 2005; Arendt & Reznick 2008; Losos 2011; Wake *et al.* 2011). For example, similar ecotype morphs of anoles lizards have evolved independently on different Caribbean islands (Losos *et al.* 1998; Harmon *et al.* 2005; Losos & Ricklefs 2009), benthic–limnetic and lake–stream species pairs of threespine sticklebacks emerged repeatedly in and around postglacial lakes (Rundle *et al.* 2000; Berner *et al.* 2010; Roesti *et al.* 2012), and a whole array of convergent forms of cichlid fish emerged between the lakes of East Africa (Kocher *et al.* 1993; Salzburger 2009). Such instances of convergent evolution are generally interpreted as the result of the action of similar selection regimes in isolated settings (Schluter & Nagel 1995; Rundle *et al.* 2000; Nosil *et al.* 2002; Harmon *et al.* 2005; Losos 2011). It has further been suggested that if radiations are truly replicated (i.e. driven by adaptive processes), convergence in morphology should tightly be associated with convergence in ecology and behaviour (Johnson *et al.* 2009).

The species flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi and Tanganyika represent the most species-rich extant adaptive radiations in vertebrates (Kocher 2004; Seehausen 2006; Salzburger 2009). Several hundreds of endemic cichlid species have emerged in each lake within a period of several millions of years (as is the case for Lake Tanganyika; Salzburger *et al.* 2002; Genner *et al.* 2007) to <150 000 years (as in Lake Victoria; Verheyen *et al.* 2003). The various endemic cichlid species differ greatly in the morphology of the trophic apparatus (mouth form and shape, jaw structure and dentition) as well as in coloration and pigmentation, suggesting that both natural and sexual selection are jointly responsible for adaptive radiation and explosive speciation in cichlids (Salzburger 2009). Interestingly, convergent forms that emerged in independent cichlid adaptive radiations often show very similar coloration patterns in addition to matching body shapes and mouth morphologies (Kocher *et al.* 1993; Stiassny & Meyer 1999; Salzburger 2009). This has led to speculations whether selection alone is sufficient to explain convergence, or whether genetic or developmental constraints have contributed to the morphogenesis of these matching phenotypes (Brakefield 2006).

The present study focuses on the morphology, ecology and the genetic basis of a peculiar mouth trait in cichlid fishes, which has evolved multiple times: hypertrophied ('fleshy') lips (see Box 1 in Salzburger 2009). The exact function of the thick lips in cichlids is unknown, although this feature is generally implicated in a specific foraging mode (Fryer 1959; Fryer & Iles 1972; Arnegard *et al.* 2001). Fleshy lips are often interpreted as an adaptation for feeding on invertebrates and crustaceans hidden in crannies, with the lips being used to seal cracks and grooves to facilitate the sucking of prey (Barlow & Munsey 1976; Ribbink *et al.* 1983; Seehausen 1996; Konings 1998). Alternatively, it has been suggested that hypertrophied lips protect from mechanical shocks (Greenwood 1974; Yamaoka 1997), and that they function as taste receptors (Arnegard *et al.* 2001) or as mechanoreceptors (Fryer 1959; Fryer & Iles 1972). [Note, however, that there is no increase in sensory cells in lip tissue (Greenwood 1974).]

It is remarkable that thick-lipped species appear to be a common outcome of cichlid adaptive radiations. For example, the large cichlid assemblages in East Africa all contain at least one such taxon (Lake Victoria: *Haplochromis chilotes*; Lake Malawi: *Chilotilapia euchilus, Abactochromis labrosus*, *Otopharynx pachycheilus, Placidochromis milomo*, *Protomelas ornatus*; Lake Tanganyika: *Lobochilotes labiatus*). In addition, cichlids featuring hypertrophied lips are known from, for example, the Midas Cichlid (*Amphilophus* spp.) assemblage in the large lakes of Nicaragua, where a thick-lipped species (*A. labiatus*) is common in rocky habitats (Fig. 1). Occasionally, hypertrophied lips are also observed in other related cichlids in Nicaragua, such as in the riverine species *Tomacichla tuba* (Villa 1982) or in *Astatheros rostratus* (pers. obs.). Additional riverine representatives with hypertrophied lips are also found in South America (*Crenicichla tendybaguassu*) and Western Africa (*Thoracochromis albolabris*). Hypertrophied lips are not unique to cichlids, though. For example, the adaptive radiation of the sailfin silverside fish (Telmatherinidae) in the Malili lakes of Sulawesi (Herder *et al.* 2006) and the barbs of Lake Tana in Ethiopia (Sibbing *et al.* 1998; de Graaf *et al.* 2008) also produced thick-lipped species.

Members of the family Cichlidae are distributed in the Southern hemisphere, with a few ancestral lineages in India, Sri Lanka and Madagascar and two exceptionally species-rich clades, one in Central and South America and one in Africa (Salzburger & Meyer 2004). This biogeographical pattern is consistent with a Gondwanan origin of the Cichlidae, dating the split between American and African representatives to ~100 Ma (Salzburger & Meyer 2004; Sereno *et al.* 2004; Genner *et al.* 2007). This set-up opens the possibility to study the ecological and genetic basis of a convergent trait across one of the

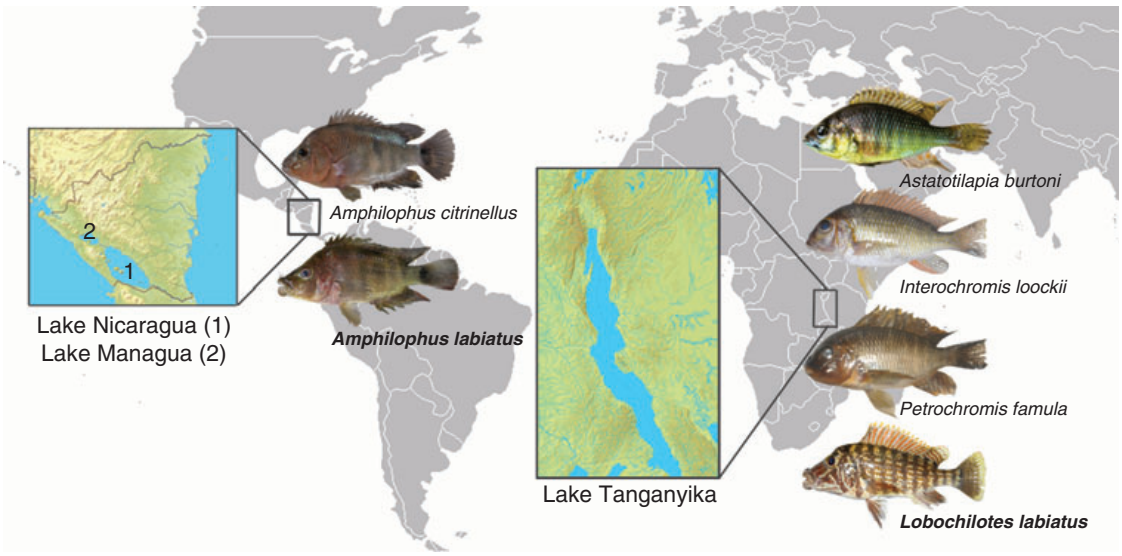


Fig. 1 Map of the Southern hemisphere showing the two study systems, the Midas Cichlid (*Amphilophus* sp.) species complex in Nicaragua, Central America, and the Tropheini in Lake Tanganyika, East Africa.

largest possible phylogenetic and geographical distances in cichlids and, hence, in the complete absence of gene flow and outside the influence of ancestral polymorphism and/or standing genetic variation.

Here, we applied an integrative approach in two cichlid fish radiations, the one of the Tropheini in East African Lake Tanganyika and the Midas Cichlid assemblage in Nicaragua, to uncover the ecological and genetic basis of the thick-lipped phenotype. More specifically, we compared the two '*labiatus*' species to one another and to their sister species by means of geometric morphometric and stomach content analyses; we placed them in their respective radiations by phylogenetic and demographic analyses; and we provide field observations on foraging strategies for one of them (*L. labiatus*). To study the genetic basis of hypertrophied lips, we first applied comparative transcriptome analyses (RNA-seq) on the basis of Illumina next-generation sequencing of juvenile and adult individuals of the African species *L. labiatus* (in comparison with a closely related species for which a genome sequence is available). In a second step, we tested candidate genes identified by RNAseq in representatives of both radiations in a quantitative real-time PCR environment.

Materials and methods

Study species

This study focuses on two thick-lipped species, *Lobochilotes labiatus* from East African Lake Tanganyika and *Amphilophus labiatus* from Nicaragua. *Lobochilotes labiatus* is a member of the rock-dwelling Tanganyikan cichlid tribe Tropheini and therefore part of the most species-rich group of cichlids, the haplochromines, which include the Tanganyikan Tropheini, many riverine species and the species flocks of Lakes Victoria and Malawi (Salzburger *et al.* 2002, 2005). The Tropheini themselves underwent a subradiation within Lake Tanganyika (see e.g. Sturmbauer *et al.* 2003). *Amphilophus labiatus* is part of the Midas Cichlid assemblage in Nicaragua and occurs in the large Central American lakes Managua and Nicaragua, where it co-occurs with the most common species in the area, *A. citrinellus* (Barlow 1976; Barluenga & Meyer 2010). For this study, we sampled a total of 84 and 74 specimens of the Central American species *Amphilophus citrinellus* and *A. labiatus*, respectively, and 143 specimens of *L. labiatus* plus 14 additional Haplochromini/Tropheini specimens from Lake Tanganyika. Exact sampling locations and dates for specimens used for the genetic analysis and GenBank accession numbers are provided in Appendix S1.

Sampling, DNA and RNA extraction

Sampling of *L. labiatus* and other Tropheini species was performed between 2007 and 2011 in the Southern part of Lake Tanganyika, East Africa; *A. labiatus* and its congeners were collected in September 2009 in the two large Nicaraguan lakes Managua and Nicaragua (see Appendix S1 for details). Fishes were processed in the field following our standard operating procedure: fishes were individually labelled, measured (total and standard length) and weighted and a photograph was taken from the left side

of each specimen using a Nikon P5000 or a Nikon D5000 digital camera (fins were spread out using clips); then, a piece of muscle tissue and a fin-clip were taken as DNA sample and preserved in ethanol; fishes were then dissected and RNA samples from lip and other tissues were preserved in RNAlater (Ambion); the whole intestinal tract was removed and stored in ethanol.

For DNA extraction, we either applied a high-salt extraction method (Bruford *et al.* 1998) or used a MagnaPure extraction robot (Roche, Switzerland) following the manufacturer's protocol. RNA was extracted according to the Trizol method with either Trizol (Invitrogen) or TRI reagent (Sigma). Lip tissue was homogenized with a PRO200 Homogenizer (PRO Scientific Inc.) or with a BeadBeater (FastPrep-24; MP Biomedicals). DNase treatment following the DNA Free protocol (Ambion) was performed to remove any genomic DNA from the samples. Subsequent reverse transcription was achieved by using the High Capacity RNA-to-cDNA kit (Applied Biosystems). For the *A. burtoni* samples, up to two individuals (adults) or up to eight individuals (juveniles) were used per sample, due to a diminutive amount of lip tissue extracted from these fishes. All other samples were taken from a single specimen.

Phylogenetic and demographic analyses

We first wanted to phylogenetically place the thick-lipped species into the respective clade of East African and Nicaraguan cichlids. We thus performed a phylogenetic analysis of the Tanganyikan cichlid tribe Tropheini (see also Sturmbauer *et al.* 2003) and used haplotype genealogies to reconstruct the evolutionary history in the much younger *Amphilophus* species assemblage in Nicaragua, where phylogenetic analyses are not expedient due to the lack of phylogenetic signal (see also Barluenga *et al.* 2006; Barluenga & Meyer 2010). We also performed mismatch analyses within *A. citrinellus*, *A. labiatus* and *L. labiatus* to compare their demographic histories.

We amplified three gene segments for each of the three focal species and additional Tropheini/Haplochromini species: the first segment of the noncoding mtDNA control region and two nuclear loci containing coding and noncoding DNA (a segment each of the *endothelin receptor 1*, *ednrb1* and the *phosphatidin phosphatase 1*, *phpt1*). We used previously published primers L-Pro-F (Meyer *et al.* 1994) and TDK-D (Lee *et al.* 1995) for the control region and ednrb1F and ednrb1R (Lang *et al.* 2006) for *ednrb1*, and so far unpublished primers 38a_F (5′-AGC AGG GTT GAC CTT CTC AA-3′) and 38a_R (5′-TGG CTA AAA TCC CCG ATG TA-3′) for *phpt1*. Polymerase chain reaction (PCR) amplification, purification and cycle sequencing were performed as described elsewhere (Diepeveen & Salzburger 2011); an ABI 3130*xl* capillary genetic analyzer (Applied Biosystems) was used for DNA sequencing.

The resulting sequences were complemented with already available sequences. In the case of the Tropheini, we also included available sequences of the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) (see Appendix S1 for GenBank accession numbers). Sequences were aligned with MAFFT (Katoh & Toh 2008) resulting in a total length of 2345 bp for the Tropheini (control region: 371 bp; ND2: 1047 bp; ednrb1: 538 bp; *phpt1:* 389 bp) and 1620 bp for *Amphilophus* (control region: 371 bp; ednrb1: 743 bp; *phpt1:* 469 bp). Maximum-likelihood and Bayesian inference phylogenetic analyses of the Tropheini were performed for each gene segment separately (not shown) and for a concatenated alignment with PAUP* (Swofford 2003) and MRBAYES (Ronquist & Huelsenbeck 2003), respectively. The appropriate model of sequence evolution was detected with JMODELTEST (Posada 2008) applying the Akaike Information Criterion (AIC). A maximum-likelihood bootstrap analysis with 100 pseudoreplicates was performed in PAUP*, and MR. BAYES was run for eight million generations with a sample frequency of 100 and a burn-in of 10%. We then used MESQUITE (www.mesquiteproject.org) to map feeding specializations on the resulting maximum-likelihood topology and to reconstruct ancestral character states with parsimony. Data on feeding mode from the Haplochromini/Tropheini species other than *L. labiatus* are based on Brichard (1989), Nori (1997), Yamaoka (1997) and Konings (1998).

Haplotype genealogies for the *Amphilophus* data set were constructed following the method described in the study by Salzburger *et al.* (2011) on the basis of a maximum-likelihood tree and sequences of the mitochondrial control region and the nuclear *ednrb1* gene (*phpt1* was not used here due to the limited number of haplotypes found). Mismatch analyses were performed on the basis of mtDNA sequences with ARLEQUIN 3.0 (Excoffier *et al.* 2005).

Geometric morphometric analyses

In order to test for similarities in overall body shape between the thick-lipped forms from Central America and East Africa, we performed geometric morphometric analyses on the basis of digital images. Body shape was quantified in a set of 58 *A. citrinellus*, 27 *A. labiatus* and 27 *L. labiatus* using 17 homologous landmarks (see Appendix S2; note that lip shape was not assessed to prevent a bias). Data acquisition was carried out using TPSDIG (Rohlf 2006), and data were analysed with MORPHOJ (Klingenberg 2011). For all shape comparisons, we used the residuals of a within-species regression of shape on centroid size to reduce allometric effects within species, in

order to retain shape differences between differently sized species. For the same reason, we only included *L. labiatus* individuals with a body size larger than 12 cm total length. We then performed a discriminant function analysis between all pairs of species and a principal component analysis (PCA). To identify morphological changes associated with the enlarged lip phenotype, we compared *A. labiatus* to its closest relative, *A. citrinellus*. In the case of *L. labiatus*, we made use of our new phylogeny of the Tropheini (Fig. 2a) and body shape data of *L. labiatus* and its nine closest relatives [*Petrochromis macrognathus*, *P. polyodon*, *P. ephippium*, *Lobochilotes labiatus*, *Simochromis diagramma*, *S. babaulti*, *Gnathochromis pfefferi*, *Pseudosimochromis curvifrons*, *Limnotilapia dardenni* and *Ctenochromis horei* (M. Muschick, A. Indermaur & W. Salzburger, unpublished data)] to reconstruct the landmark configuration of the direct ancestor to *L. labiatus*. This was carried out in MorphoJ using branch length-weighted squared-change parsimony. The changes in landmark configurations along a discriminant function (Nicaraguan species) or along the shape-change vector from the estimated ancestral shape to *L. labiatus* were increased threefold to produce Fig. 3. The shape differences between species shown in Fig. 3 accurately reflect the shape-change vectors for landmark positions. Outlines were interpolated and added to Fig. 3 to help the reader envision these shape differences in the context of fish body shape.

Stomach and gut content analyses

To assess trophic specialization of the thick-lipped cichlid species, we performed comparative stomach and gut content analyses. To this end, stomachs and guts were opened step-by-step. First, the stomach was opened and emptied under a binocular followed by the remaining parts of the intestine. All items were grouped into seven food categories: hard-shelled (crustaceans, snails, mussels), small arthropods (insects and zooplankton), fish scales, fish remains, plant seeds and plant material other than seeds. For each specimen, the wet weight of each food category was measured on a Kern ALS 120-4 scale (Kern, Germany) and was then used to calculate Schoener's index of proportional diet overlap (Schoener 1970). We analysed stomach and gut contents in a total of 159 specimens: *A. citrinellus* ($N = 58$; of which 25 had contents), *A. labiatus* ($N = 62$; 34) and *L. labiatus* ($N = 39$; 29). We note that such an analysis has the drawback that it only covers food uptake in the last few hours or days before sampling.

Field observations in Lobochilotes labiatus

The feeding behaviour of *L. labiatus* was observed at our field site near Mpulungu, Zambia, in concrete ponds ($1.5 \times 1.5 \times 1$ m). The purpose of these observations under semi-natural conditions and with wild specimens was to document if and how the lips are used in processing the main prey item identified in the stomach content analyses. The ponds were equipped with stones of ~20–30 cm diameters that covered the ground and formed caves as they occur naturally in the habitat of *L. labiatus*. Each pond was stocked with five to six freshly caught and unharmed adult individuals of *L. labiatus*. After an acclimatization period of at least 4 days, fish were offered snails of different sizes and their feeding behaviour was recorded with two underwater cameras (Canon Ixus 65 with WP-DC3 underwater case; Olympus μ tough-6000) for a period of 1 h each.

Comparative gene expression assays using RNAseq

For the identification of differentially expressed genes in thick-lipped species, we performed RNA sequencing (RNAseq) comparing lip tissue from a thick-lipped species to lip tissue from a reference species. We decided to perform these experiments in the African species *L. labiatus* and to use the closely related species *Astatotilapia burtoni* as reference taxon for several reasons such as the availability of laboratory strains and of sufficient RNA samples from adult and juvenile individuals. Most importantly, we chose this set-up because of the availability of various genomic resources for *A. burtoni*, such as a whole-genome sequence and a set of ~50 000 partly annotated expressed sequence tags (ESTs) (Salzburger *et al*. 2008; Baldo *et al*. 2011), which is crucial for the analysis and interpretation for RNAseq data. Such resources are currently not publicly available for *Amphilophus*.

In a first step, RNA was extracted from adult and juvenile individuals of *L. labiatus* and *A. burtoni* (see above for the RNA extraction protocol). RNA quality and quantity were determined on a NanoDrop 1000 spectrophotometer (Thermo Scientific) and by gel electrophoresis. RNA samples were pooled to create four samples subjected to RNA sequencing (RNAseq): (i) *A. burtoni* adult ($N = 3$); (ii) *A. burtoni* juvenile ($N = 1$); (iii) *L. labiatus* adult ($N = 2$); and (iv) *L. labiatus* juvenile ($N = 3$). Five micrograms of RNA per RNAseq sample was sent for Illumina sequencing at the Department of Biosystems Science and Engineering (D-BSSE), University of Basel and ETH Zurich. For library construction and sequencing, standard protocols were applied. Poly-A mRNA was selected using poly-T oligo-attached magnetic beads. The recovered mRNA was fragmented into smaller pieces using divalent cations under increased temperature. cDNA was produced using reverse transcriptase and random primers, followed by second-strand cDNA synthesis using DNA polymerase

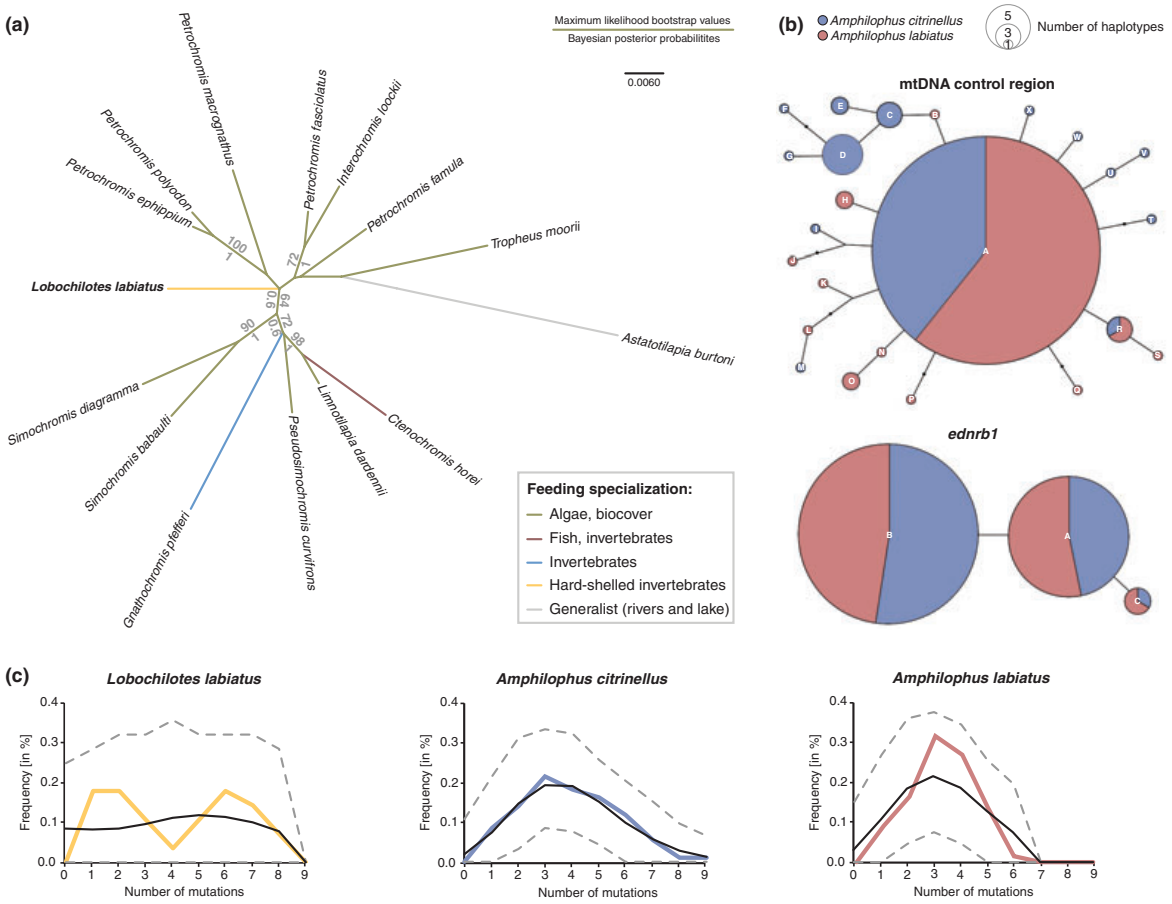


Fig. 2 Evolutionary origin of the thick-lipped species in East African Lake Tanganyika and in the Great Lakes of Nicaragua. (a) Maximum-likelihood tree of the Tropheini from Lake Tanganyika based on two mitochondrial (control region and ND2) and two nuclear (*ednrb1* and *phpt1*) gene segments (2345 bp in total) and the GTR+G+I model of molecular evolution. Numbers above the branches refer to maximum-likelihood bootstrap values, and numbers below are Bayesian posterior probabilities (note that support values are only shown for branches with bootstrap values >60). Branches are colour-coded according to feeding specializations; the trait values for internal branches have been reconstructed with MESQUITE. (b) Haplotype genealogies of the two *Amphilophus* species based on the mitochondrial control region and the nuclear *endrb1* gene. A large fraction of the haplotypes is shared between *A. citrinellus* and *A. labiatus*. (c) Results from the mismatch analysis on the basis of the mitochondrial control region showing the inferred demographic histories for *L. labiatus*, *A. citrinellus* and *A. labiatus*. Coloured lines represent the observed data, the black line indicates the best-fit model, and the dashed lines in grey indicate the upper and lower boundaries from the simulations in ARLEQUIN.

I and RNaseH. cDNA went through an end-repair process, the addition of a single 'A' base and ligation of the adapters. It was then purified and enriched with PCR to create the final cDNA library. Each library was sequenced in one lane on an Illumina Genome Analyzer IIx (read length was 76 bp). Illumina reads are available from the Sequence Read Archive (SRA) at NCBI under the accession number SRA052992.

The Illumina reads were assembled into three different data sets for further analyses: (i) a quality-filtered data set (Data set 1), where the quality of the reads was assessed with the FASTX toolkit tools implemented in GALAXY [version September/October 2011; available at http://main.g2.bx.psu.edu/ (Giardine *et al.* 2005; Blankenberg *et al.* 2010; Goecks *et al.* 2010)]; low-quality reads were discarded applying quality filter cut-off values of 22–33. (ii) a quality-filtered plus trimmed data set (Data set 2), in which all the reads were trimmed to a length of 42 bp to evaluate the effects of read length (iii) as a control for the effect of trimming and filtering, a nonquality-filtered, nontrimmed data set (Data set 3).

The reads of the three data sets were then aligned to a reference cichlid assembly (Baldo *et al.* 2011) with NOVOALIGN 2.07.06 (http://www.novocraft.com/) after indexing the reference sequences with NOVOINDEX (http://www.novocraft.com/) using default parame-

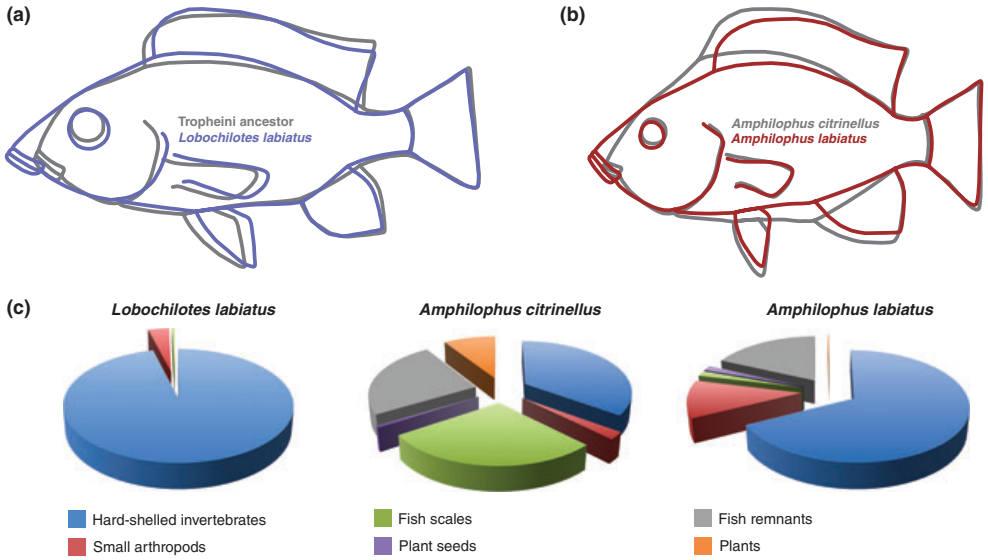


Fig. 3 Ecomorphology of the thick-lipped cichlid species in Central America and in Lake Tanganyika. (a) Body shape of *L. labiatus* in comparison with a reconstruction of the ancestor of *L. labiatus* and nine closely related Tropheini species. (b) Differences in body shape between *A. citrinellus* and *A. labiatus* along a discriminate function. In both plots, changes in landmark positions were increased threefold and interpolated outlines added for illustration purposes. Landmark locations are indicated in black on the reconstructed outlines in plot (a). (c) Analysis of stomach and gut content in the focal species. The fraction of each food category is shown.

ters. The alignment was performed using default settings with a maximum alignment score (t) of 180 and a maximum number of alignments for a single read (e) of 100; reads with multiple alignment locations were discarded. Next SAMtools version 0.1.18 (Li *et al.* 2009) was used to sort and index the files and to generate count files, which were subsequently transformed into count tables and analysed in the R package DESeq version 1.0.5 (Anders & Huber 2010). Differentially expressed genes between the four experimental groups were detected using a model based on a negative binomial distribution implemented in DESeq. Differentially expressed genes with *P*-values (adjusted for multiple testing) >0.05 and/or a quotient of variance >1.00 were discarded to reduce the number of false positives. The remaining differentially expressed genes of all pairwise comparisons were tested for multiple hits. Next the hits of the three data sets were compared with each other to create a candidate gene list, consisting of genes that were found in multiple analyses in all three data sets. Lastly, these hits were compared to the annotated *A. burtoni* ESTs of Baldo *et al.* (2011).

Comparative gene expression assays using quantitative real-time PCR

Based on their function according to gene ontology terms (GO terms; http://www.geneontology.org/) and their putative involvement in lip formation and/or hypertrophy in other organisms, six candidate genes were selected out of the list of differentially expressed genes for further characterization by means of quantitative real-time PCR (qPCR). These candidate genes are the *Bcl2 adenovirus e1b 19-kda protein-interacting protein 3* (*BNIP3*), *long-chain-fatty-acid(CoA)-ligase 4* (*ACSL4*), *histone 3.3* (*His3*), *beta actin* (*Actb*), *coatomer subunit beta* (*Copb*) and *claudin 7* (*Cldn7*; see Table 1 for primer details). qPCR experiments were performed in total of 36 cichlid specimens: *L. labiatus* (six adults, six juveniles), *A. burtoni* (six adults, six juveniles), *A. labiatus* (six adults) and *A. citrinellus* (six adults). By performing two pairwise comparisons between a thick-lipped and a normal-lipped species (a species pair each from Africa and Nicaragua), we effectively control for species-specific expression differences, as genes specific to thick-lip tissue should be upregulated in both comparisons.

The experiments were conducted on a StepOnePlus Real-Time PCR system (Applied Biosystems) as described elsewhere (Diepeveen & Salzburger 2011) using the *elongation factor 1* (*EF1*) and the *ribosomal protein SA3* (*RpSA3*) as endogenous controls. Average relative quantifications (RQ) were calculated for the six experimental groups and subsequently analysed with a two-tailed unpaired t-test using GraphPad Prism version 5.0a for Mac OS X (www.graphpad.com). We compared the expression levels between the two thick-lipped species and a closely related normally lipped species (i.e. *L. labiatus* vs. *A. burtoni* and *A. labiatus* vs. *A. citrinellus*). We also compared adults vs.

Table 1 Primers used for the quantitative real-time PCR experiments

Locus	Forward (5′–3′)	Reverse (5′–3′)
Actb	CAGGCATCAGGGTGTAATGGTT	CAGGCATCAGGGTGTAATGGTT
Copb	GAGGCTACCTTGGCTGTCAAAG	GTGCTGGATGGTTTGAGGGTAA
His3	CATCTACTGGTGGAGTGAAGAAACC	GGATCTCACGCAGAGCAACA
ACSL4	TGGTTCTGCACCGGAGATG	TCTTGCGGTCAACAATTTGTAGA
BNIP3	AACAGTCCACCAAAGGAGTTCCT	CCTGATGCTGAGAGAGGTTGTG
Cldn7	GACATCATCCGGGCCTTCT	CACCGAACTCATACTTAGTGTTGACA
EF1	GCCCCTGCAGGACGTCTA	CGGCCGACGGGTACAGT
RpSA3	AGACCAATGACCTGAAGGAAGTG	TCTCGATGTCCTTGCCAACA

juveniles in the African species, as hypertrophy in lips is much less pronounced at juvenile stages, so that this experiment also captures ontogenetic changes in lip formation. As primer efficiency was lower in the Nicaraguan samples, no direct comparisons between African and Nicaraguan tissues were possible.

Results

Phylogenetic and demographic analyses

Our phylogenetic analysis of members of the Tanganyikan cichlid tribe Tropheini based on two mitochondrial and two nuclear DNA gene segments reveals only limited phylogenetic resolution between the main lineages of the tribe (Fig. 2a). This confirms an earlier analysis based on mitochondrial DNA only, which attributed the star-like phylogeny of the Tropheini to the rapidity of lineage formation in the early stages of the adaptive radiation of this clade (Sturmbauer *et al.* 2003). Just as in the previous study, the thick-lipped species *L. labiatus* represents a separate lineage (without a closely related sister-taxon) that branches off relatively early in the phylogeny, but shows affinities to the algae-eating genera *Petrochromis* and *Simochromis*.

The haplotype genealogies of the *Amphilophus* samples based on the mitochondrial control region and the nuclear *ednrb1* gene (Fig. 2b) revealed haplotype sharing between *A. citrinellus* and *A. labiatus* (see also Barluenga & Meyer 2010). While all *Amphilophus* sequences were identical in *phpt1*, we detected three shared haplotypes in ednrb1 and 24 haplotypes in the mitochondrial control region (two shared, ten unique to *A. labiatus* and twelve unique to *A. citrinellus*).

The mismatch analyses based on the mitochondrial control region sequences revealed unimodal distributions for the two sympatrically occurring *Amphilophus* species and a bimodal distribution for *L. labiatus* (Fig. 2c). According to this analysis, the demographic expansion of the two *Amphilophus* species happened at similar times, with the one of *A. citrinellus* being slightly older than that of *A. labiatus* (mean number of differences: 3.9 vs. 3.2; τ: 3.9 vs. 3.5; see also Barluenga & Meyer 2010, who provide a relative time frame for the evolution of the Midas Cichlid species complex); the mean number of differences in *L. labiatus* was 6.4 (τ: 6.5).

Geometric morphometric analyses

The PCA of overall body shape revealed substantial overlap between the two Nicaraguan species *A. citrinellus* and *A. labiatus* (Appendix S3). The African thick-lipped species *L. labiatus* is separated from these mainly by principal component 1 (accounting for 20.2% of the variance), whereas principal component 2 (covering 16.0% of the variance) did not discriminate much between species. The discriminant function analysis, in which we compared species in a pairwise manner, revealed the main morphological differences between species. Of the two Nicaraguan species, *A. labiatus* had a more acute head, less deep body and a larger mouth than *A. citrinellus* (Fig. 3) (see also Klingenberg *et al.* 2003). These characters were even more pronounced in *L. labiatus*, when compared to either of the *Amphilophus* species. However, the distance in morphospace between the two species with fleshy lips was somewhat smaller than between *A. citrinellus* and *L. labiatus* (procrustes distance 0.08 and 0.1, respectively). We also estimated the body shape of the ancestor of *L. labiatus* and the 9 most closely related Tropheini species. A comparison of this reconstructed shape and the mean shape of our *L. labiatus* samples highlighted similar morphological differences as the comparison of the Nicaraguan species (Fig. 3), especially in the mouth region.

Stomach and gut content analyses

The fractions of food categories in guts and stomachs differed between *A. citrinellus*, *A. labiatus* and *L. labiatus* (Fig. 3c). While the diet of *A. citrinellus* did not overlap with that of *A. labiatus* (Schoener's index: 0.58) or *L. labiatus* (Schoener's index: 0.38), we found significant overlap between the two thick-lipped species *A. labiatus* and *L. labiatus* (Schoener's index: 0.71) (note that any value >0.6 is considered 'biologically significant'; see Wallace 1981). The stomach and gut contents of both

thick-lipped species consisted of a substantial fraction of hard-shelled prey (*Lobochilotes labiatus* 96%, *Amphilophus labiatus* 67.6%, *Amphilophus citrinellus* 35%).

Field observations in Lobochilotes labiatus

A careful inspection of the video material confirmed the findings from the stomach and gut content analyses that *L. labiatus* regularly feeds on snails (more than 90% of the stomach and gut content of *L. labiatus* consisted of snail shells). Small snails were engulfed using suction feeding without the lips touching the prey item or the surface (rocks) on which the items were placed. When feeding on larger snails, however, *L. labiatus* exhibited a different feeding strategy and snails were no longer taken up using suction feeding. Instead, *L. labiatus* used their lips to snatch the snails and they turned the snails a few times before they either swallowed the snails or spat them out (see Appendix S4).

Comparative gene expression assays using RNAseq

On average, ca. 42 million total reads were retrieved for each of the four RNAseq samples (*A. burtoni* adult, *A. burtoni* juvenile, *L. labiatus* adult and *L. labiatus* juvenile). Quality filtering and trimming reduced this number so that on average 21.9 (Data set 1), 24.6 (Data set 2) and 23.5 (Data set 3) million reads were aligned to the reference cichlid assembly. Five different pairwise comparisons were made to obtain genes that are differentially expressed between thick lips and normal lips (see Table 2 for the three comparisons with the highest number of genes being different). The largest number of differentially expressed genes between *L. labiatus* and *A. burtoni* was detected in adult lip tissue, with the majority of the genes being upregulated in *L. labiatus*. The total number of differentially expressed genes ranged from 9050 (Data set 3; three pairwise comparisons) to 15230 (Data set 2; five pairwise comparisons). A substantial fraction of these differentially expressed genes appeared in at least two comparisons in each data set (Data set 1: 2085 [22.1% of all hits]; Data set 2: 8078 [53.0%]; Data set 3: 1693 [18.7%]). Of these 'multiple hits', 1463 were detected in all three data sets and 560 of those could be unequivocally annotated.

A more stringent analysis, in which only loci that appeared in at least three of five comparisons were included, resulted in 231 differentially expressed genes. A functional annotation of these 231 hits with Blast2GO resulted in a total of 141 annotations (122 upregulated and 19 downregulated in *L. labiatus*; see Appendix S3). Based on their annotations, known functions and/or exceptional fold change (>1000) between *A. burtoni* and *L. labiatus*, thirteen genes were identified as good candidates for being involved in the morphogenesis of fleshy lips (Table 3).

Comparative gene expression assays using quantitative real-time PCR

The results of the comparative gene expression assays between the thick-lipped species and the normal-lipped species are depicted in Fig. 4 and Appendix S5. Overall, the qPCR experiments largely validate differential gene expression in normal and hypertrophied lip tissue as indicated by RNAseq. In the African species pair *L. labiatus* and *A. burtoni*, which were the two species used for RNAseq, differences were highly significant in four of the six genes tested: *Actb* ($P = 0.0099$), *Cldn7* ($P = 0.004$), *ACSL4* ($P = 0.0005$) and *His3* ($P = 0.0003$). However, we would like to point out one inconsistency between RNAseq and qPCR. *Actb* was actually found to be downregulated in hypertrophied lips by RNAseq, while it shows significantly higher expression levels in lip tissue in the qPCR experiments (Fig. 4).

The comparison between lip tissue in adult and juvenile *L. labiatus* and *A. burtoni* further revealed a trend towards higher expression in lip tissue of adult *L. labiatus* in *Actb*, *BNIP3*, *Cldn7* and *Copb* (Appendix S5), whereas, generally, an opposite trend is observed in *A. burtoni*, although statistical support was only found in two cases [*Cldn7* ($P = 0.0063$) and *ACSL4* ($P = 0.0328$)]. This again suggests that these genes are involved in the formation of fleshy lips. In the Nicaraguan species pair, a similar trend was observed as in the African species pair, with four of the five genes tested appearing to be upregulated in lip tissue

Table 2 Pairwise comparisons of differentially expressed genes and total number of unique differentially expressed genes in the three data sets compiled in this study

Comparison	Data set 1	Data set 2	Data set 3
AB vs. LL	7120 (4606; 2514)	7080 (4689; 2391)	7285 (4665; 2620)
AB vs. LLjuv	3611 (3395; 216)	13747 (10683; 3064)	2618 (2514; 104)
ABjuv vs. LLjuv	1116 (792; 324)	3971 (2710; 1261)	986 (687; 298)
Total	9407	15225	9050

AB, *Astatotilapia burtoni;* LL, *Lobochilotes labiatus*; juv, juvenile; numbers in brackets denote the number of upregulated and downregulated genes in *L. labiatus*.

Table 3 Thirteen candidate loci for the genetic basis of lip development in the East African cichlid *Lobochilotes labiatus*, based on RNAseq and qPCR in comparison with *Astatotilapia burtoni*, in combination with information on gene functions (in alphabetical order)

Locus	Abbreviation
ATPase mitochondrial precursor	*ATPmp*
Bcl2 adenovirus e1b 19-kda protein-interacting protein 3	*BNIP3*
Beta actin	*Actb*
Caspase-8	*Casp8*
Claudin 7	*Cldn7*
Coatomer subunit beta	*Copb*
Grainyhead-like protein 1 homolog	*Grhl1*
Heat-shock 70-kda protein 12a-like	*Hspa12al*
Histone 3.3	*His3*
Laminin subunit gamma-2	*Lamc2*
Long-chain-fatty-acid(CoA)-ligase 4	*ACSL4*
Sodium-dependent phosphate transporter 1	*Slc17a1*
Transcription factor ap-2 gamma	*Tfap2*

of *A. labiatus* as compared to *A. citrinellus* (Fig. 4; we could not amplify *BNIP3* here). We would like to note, however, that qPCR efficiency was less good in the *Amphilophus* samples, most likely because we used primers designed for the African species pair based on the available genomic resources, which also explains the limited statistical support for these comparisons. Interestingly, it seems that several loci (i.e. *Actb*, *Cldn7*, *Copb*, *His3*) are upregulated in both thick-lipped species when compared to their normally lipped relatives.

Discussion

The species flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi and Tanganyika, counting hundreds of endemic species each, are prime examples of adaptive radiation and explosive speciation (see e.g. Kocher 2004; Seehausen 2006; Salzburger 2009). Interestingly, the cichlid adaptive radiations in East Africa have independently produced ecomorphs with highly similar colour patterns and (mouth) morphologies (Kocher *et al.* 1993). Here, we explore the ecological and genetic basis of one of the particular trophic structures of cichlids, which has evolved convergently in various cichlid assemblages: fleshy lips. Instead of focusing on species with hypertrophied lips between the radiations in the East African lakes, we compare the thick-lipped phenotype between a cichlid assemblage in East African (Lake Tanganyika) and in Central American (the lake Nicaragua/Managua system), where thick-lipped species have evolved in parallel (see Fig. 1).

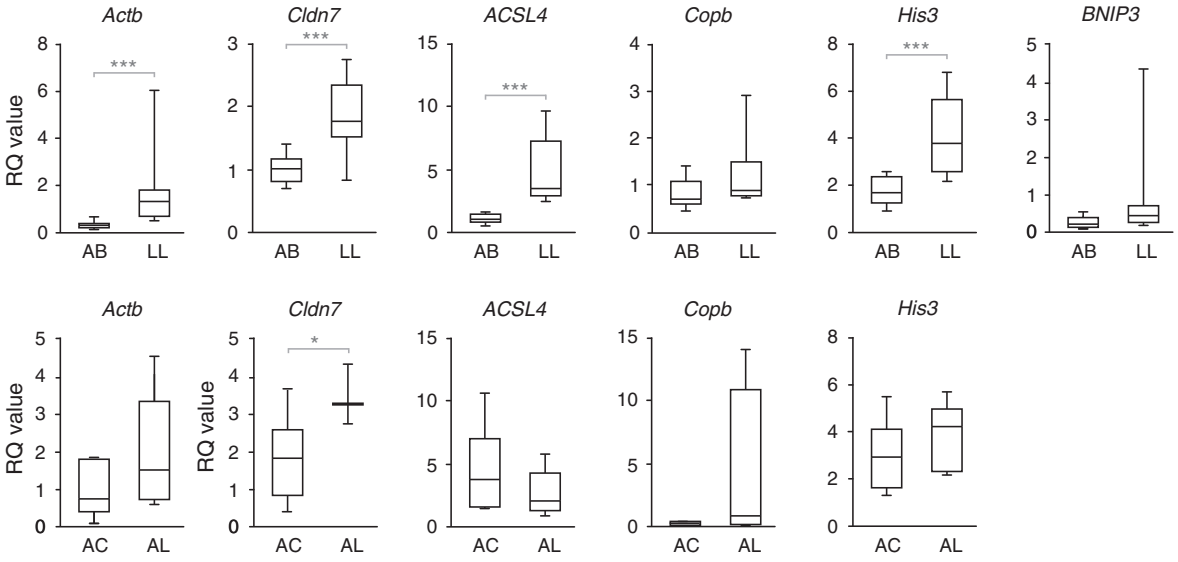


Fig. 4 Results from the comparative gene expression experiments via quantitative real-time PCR. The six genes tested in this experiment were selected on the basis of comparative RNA sequencing. All genes tested show a higher expression level in lip tissue of the Tanganyikan thick-lipped species *L. labiatus* as compared to *A. burtoni* (top panel; note that we used both juvenile and adult samples in these analyses to increase statistical power). A similar trend was found when comparing the Nicaraguan thick-lipped species *A. labiatus* to its sister species *A. citrinellus* (with the exception of *ACSL4;* lower panel). Note that *BNIP3* could not amplified in the *Amphilophus* species. *Astatotilapia burtoni* (AB); *Lobochilotes labiatus* (LL); *Amphilophus citrinellus* (AC); *Amphilophus labiatus* (AL); $*P < 0.05$; $***P < 0.01$.

The evolution of hypertrophied lips in cichlid adaptive radiations

Our phylogenetic and demographic analyses in the Tanganyikan Tropheini and the Nicaragua Midas Cichlid species complex reveal that the thick-lipped species are nested within their respective clade. The molecular phylogeny of 14 Tropheini species (Fig. 2a) shows a footprint characteristic for adaptive radiations: a 'bottom heavy' topology with only limited phylogenetic resolution at the deeper nodes due to rapid lineage formation (Gavrilets & Vose 2005). Our new analysis thus confirms previous results based on mtDNA only (Sturmbauer *et al.* 2003) or a combination of mtDNA and AFLPs (Koblmuller *et al.* 2010). In all analyses thus far, the thick-lipped species *L. labiatus* forms an independent evolutionary lineage that branches off deep in the Tropheini. Its exact position remains unclear, though. In the AFLP phylogeny of Koblmuller *et al.* (2010), *L. labiatus* appears as sister group to all Tropheini except for the genus *Tropheus*, which is sister to all other representatives of that clade (the topology has very little support, though). In our new phylogeny and the previous mtDNA trees of Sturmbauer *et al.* (2003), *L. labiatus* shows affinities to *Simochromis* and *Petrochromis* (with moderate support). In all phylogenies, however, *L. labiatus* is nested within a clade formed by various species that feed on algae and biocover (see our character state reconstruction in Fig. 2a).

In the Midas Cichlid species complex from Central America, a phylogenetic approach is not applicable with the available molecular markers. There is simply too little genetic variation, even in the rapidly evolving mitochondrial control region, as a consequence of the young age of the assemblage (see Barluenga & Meyer 2004, 2010; Barluenga *et al.* 2006). The structures of our haplotype genealogies, which now also include the analysis of a nuclear gene (Fig. 2b), confirm this scenario. In combination with the mismatch analyses (Fig. 2c), these data suggest that *A. labiatus* underwent its main demographic expansion soon after the expansion of the sympatric *A. citrinellus* populations (see Barluenga & Meyer 2010 for a large-scale analysis of the Midas Cichlid species complex).

In both species assemblages, the evolution of the thick-lipped phenotype was associated with similar modifications of overall body shape (Fig. 3a,b). Reduced body depth, a more acute head shape and a larger mouth, along with the prominently enlarged lips, can be hypothesized to be adaptations to the species' microhabitat and trophic niche. If individuals search for food in narrow rock crevices, these modifications appear advantageous. Klingenberg *et al.* (2003) already suggested that the elongation of the head, as observed in both '*labiatus*' species, increases suction power. Other morphological differences between the two thick-lipped species, such as eye size or the length of anal fin insertion, might be either due to adaptations to the specific environments or due to phylogenetic effects. Inclusion of other thick-lipped species in future studies focusing on the ecology and morphological evolution of this trait might answer this question.

The function of hypertrophied lips in cichlids

Hypertrophied lips in cichlids have been implicated in several functions. For example, it has been suggested that fleshy lips are used to seal cracks and grooves to facilitate sucking of invertebrates (Barlow & Munsey 1976; Ribbink *et al.* 1983; Seehausen 1996; Konings 1998), that they act as bumpers to protect from mechanical shock (Greenwood 1974; Yamaoka 1997) or that they function as taste (Arnegard *et al.* 2001) or mechanoreceptors (Fryer 1959; Fryer & Iles 1972). Previous food web analyses on *L. labiatus* identified this species as mollusc eater (Nori 1997).

Our ecomorphological analysis of the thick-lipped species *L. labiatus* from Lake Tanganyika and *A. labiatus* from the large lakes in Nicaragua suggests that this phenotype is indeed associated with feeding on hard-shelled prey such as snails, mussels and crustaceans in rocky habitats (Fig. 3c). We cannot, however, conclusively answer the question whether the lips are used to seal rock crevices or whether they serve as bumpers or receptors. In the underwater observations at our field site at Lake Tanganyika, small snails were usually engulfed by *L. labiatus* via suction feeding, whereas larger snails were turned around several times before being swallowed or spit out (see Appendix S4). This would classify the lips as instrument to handle hard-shelled invertebrate food (mostly molluscs). Note, however, that our observations were made in semi-natural conditions only, in the form of concrete ponds equipped with stones from the lake and filled with lake water.

Our experimental set-up could not address the possibility that phenotypic plasticity plays a role in the formation of fleshy lips, as has previously been shown in certain foraging traits in cichlid fishes (oral jaws: Meyer 1987; pharyngeal jaws: e.g. Greenwood 1965; Huysseune 1995; Muschick *et al.* 2011). Interestingly, it has been reported that thick-lipped cichlid species lose their fleshy lips under unnatural conditions in captivity (when fed with standard food; Barlow & Munsey 1976; Barlow 1976; Loiselle 1998). So far, there is no evidence for the opposite process, the plastic development of fleshy lips due to environmental or feeding properties. In the common garden experiment of Muschick *et al.*

(2011), one group of normally lipped *A. citrinellus* individuals was fed with whole snails over a period of several months, and—although not formally assessed—no increase in lip size was apparent (compared to the other two treatment groups peeled snails and crushed snails). Another study on a snail crusher (Huysseune 1995) did not report such changes either, which seems to suggest that phenotypic plasticity in the lips, if at all present, is specific to thick-lipped species only. Future common garden and feeding experiments should thus expand on this question. Such experiments, combined with molecular analyses, should focus on the plastic component of this trait and its genomic basis.

Insights into the genetic basis of hypertrophied lips in cichlids

Our comparative gene expression assays with RNA sequencing between tissue from thick and normal lips identified a set of 141 candidate genes that might be responsible for the morphogenesis or the maintenance of fleshy lips in (East African) cichlid fish (Appendix S3). Six genes were tested further by means of quantitative real-time PCR, and these experiments largely confirm the results obtained from RNAseq (Fig. 4). While there is no obvious functional connection to fleshy lips for three of these differentially expressed genes (*ACSL4*, *His3* and *BNIP3*), the observed upregulation of the remaining three (*Actb*, *Cldn7* and *Copb*) makes sense in the light of the structure of hypertrophied lips. These three genes (together with *BNIP3*) also show a higher expression in lip tissue from adult vs. juvenile *L. labiatus* (Appendix S5).

It has previously been shown that the 'fleshy' lips of the Lake Malawi cichlid *Otopharynx pachycheilus* mainly consist of loose connective tissue covered by dermis and a layer of epithelial cells (Arnegard *et al.* 2001). Interestingly, the known functions of *Actb*, *Cldn7* and *Copb* can be directly implicated in cell and/or intercell or membrane structure. The cytoplasmic *Actb* is found in high abundance in nonmuscle cells, where it promotes cell surface and cell thickness (Schevzov *et al.* 1992), which is also consistent with its upregulation in the more massive adult compared to juvenile *L. labiatus* lips (Appendix S5). The integral membrane protein *Cldn7* (among other *claudin* gene family members) constitutes the backbone of tide junctions between epithelial cells (Tsukita *et al.* 2001). The coatomer coat proteins (such as *Copb*) are involved in protein and membrane trafficking via vesicle secreting between the endoplasmic reticulum and the Golgi apparatus, plus the intra-Golgi transport (Duden 2003). In addition, they mediate lipid homoeostasis and lipid storage for energy use and membrane assembly (Soni *et al.* 2009). *Copb* might thus be involved in cellular (membrane) development but possibly also in the formation of fat cells that compose adipose tissue, a specific subtype of connective tissue. Clearly, much more work will be necessary to unravel the development and genetic basis of hypertrophied lips in cichlids, for which we herewith established a valuable starting ground.

Our results, especially the comparison of gene expression levels between the thick-lipped species in East Africa and Central America (Fig. 4), allow us to touch on ongoing discussions related to the genetic basis of convergent morphologies (reviewed in Brakefield 2006; Arendt & Reznick 2008; Elmer & Meyer 2011). Although our qPCR results in Midas Cichlid (*Amphilophus* spp.) species must be taken with caution (efficiency was lower as a consequence of using molecular tools developed for the African species leading to a lack of statistical power), we find rather similar trends in gene expression. Our results seem to indicate that a largely overlapping set of genes was recruited to develop the hypertrophied lips in Nicaraguan and African species, which are—according to most authors—separated by ~ 100 million years of evolution. This important question about the basis of convergent phenotypes should be addressed in future studies, and thick-lipped fish species, including those outside the family Cichlidae, appear as an excellent model system.

Conclusion

Our integrative evolutionary, ecological, morphological, observational and genomic analysis of thick-lipped species in East Africa and in Nicaragua reveals stunning similarities between these convergent morphs. Both thick-lipped species appear to have evolved early in the respective clade, they seem to have adapted to the same habitat (rocks) and food source (hard-shelled prey), and their evolution was associated with comparable morphological trajectories, especially in the mouth and head region. Importantly, we also show that the expression patterns of at least some genes are similar, too. We thus provide valuable resource for future studies focusing on the development of this trait and genetic basis of convergence.

Acknowledgements

We would like to thank our helpers in the field, V. Campos, C. Heule, B. Meyer, M. Roesti; E. P. van den Berghe and G. Tembo and his crew for their logistic support in Nicaragua and Africa, respectively; the Ministerio del Ambiente y los Recursos Naturales Nicaragua (MARENA) and the Lake Tanganyika Research Unit, Department of Fisheries, Republic of Zambia,

for research permits; and three anonymous referees and the Subject Editor, C. Eizaguirre, for valuable comments. This study was supported by grants from the Fundacao para Ciencia e a Tecnologia (FCT, Portugal) to M. E. S., the Swiss Academy of Sciences to A. I., the Spanish Minsiterio de Economía y Competitividad to M. B., and the European Research Council (ERC, Starting Grant 'INTERGENADAPT'), the University of Basel and the Swiss National Science Foundation (SNF, grants 3100A0_122458 and CRSII3_136293) to W. S.

References

Anders S, Huber W (2010) Differential expression analysis for sequence count data. *Genome Biology*, **11**, R106.

Arendt J, Reznick D (2008) Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? *Trends in Ecology and Evolution*, **23**, 26–32.

Arnegard ME, Snoeks J, Schaefer SA (2001) New three-spotted cichlid species with hypertrophied lips (Teleostei: Cichlidae) from the deep waters of Lake Malaŵi/Nyasa, Africa. *Copeia*, **2001**, 705–717.

Baldo L, Santos ME, Salzburger W (2011) Comparative transcriptomics of Eastern African cichlid fishes shows signs of positive selection and a large contribution of untranslated regions to genetic diversity. *Genome Biology and Evolution*, **3**, 443–455.

Barlow GW (1976) The Midas cichlid in Nicaragua. In: *Investigations of the Ichthyofauna of Nicaraguan lakes* (ed. Thorson TB), pp. 333–358. School of Life Sciences, University of Nebraska, Lincoln, Nebraska.

Barlow GW, Munsey JW (1976) The Red Devil Midas Cichlid species complex in Nicaragua. In: *Investigations of the Ichthyofauna of Nicaraguan Lakes* (ed. Thorson TB), pp. 359–370. School of Life Sciences, University of Nebraska, Lincoln, Nebraska.

Barluenga M, Meyer A (2004) The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes? *Molecular Ecology*, **13**, 2061–2076.

Barluenga M, Meyer A (2010) Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. *BMC Evolutionary Biology*, **10**, 326.

Barluenga M, Stolting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*, **439**, 719–723.

Berner D, Roesti M, Hendry AP, Salzburger W (2010) Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Molecular Ecology*, **19**, 4963–4978.

Blankenberg D, Von Kuster G, Coraor N (2010) Galaxy: a web-based genome analysis tool for experimentalists. *Current protocols in molecular biology/edited by Frederick M. Ausubel et al.* Chapter 19, Unit 19 10 11-21.

Brakefield PM (2006) Evo-devo and constraints on selection. *Trends in Ecology and Evolution*, **21**, 362–368.

Brichard P (1989) Cichlids and all Other Fishes of Lake Tanganyika, T.H.F. Publications, Neptune City, New Jersey.

Bruford MW, Hanotte O, Brookfield JFY, Burke T (1998) Multilocus and single-locus DNA fingerprinting. In: *Molecular Analysis of Populations* (ed. Hoelzel AR), pp. 283–336. Oxford University Press, New York.

Butler MA, Sawyer SA, Losos JB (2007) Sexual dimorphism and adaptive radiation in Anolis lizards. *Nature*, **447**, 202–205.

Diepeveen ET, Salzburger W (2011) Molecular characterization of two endothelin pathways in East African cichlid fishes. *Journal of Molecular Evolution*, **73**, 355–368.

Duden R (2003) ER-to-Golgi transport: COP I and COP II function (Review). *Molecular Membrane Biology*, **20**, 197–207.

Elmer KR, Meyer A (2011) Adaptation in the age of ecological genomics: insights from parallelism and convergence. *Trends in Ecology and Evolution*, **26**, 298–306.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fryer G (1959) The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with a special reference to the fishes, and a discussion on the evolution of a group of rock-frequenting Cichlidae. *Proceedings of the Zoological Society of London*, **132**, 153–281.

Fryer G, Iles TD (1972) The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution. Oliver & Boyd, Edinburgh.

Gavrilets S, Losos JB (2009) Adaptive radiation: contrasting theory with data. *Science*, **323**, 732–737.

Gavrilets S, Vose A (2005) Dynamic patterns of adaptive radiation. *Proceeding of the National Academy of Sciences U S A*, **102**, 18040–18045.

Genner MJ, Seehausen O, Lunt DH *et al.* (2007) Age of cichlids: new dates for ancient lake fish radiations. *Molecular Biology and Evolution*, **24**, 1269–1282.

Giardine B, Riemer C, Hardison RC *et al.* (2005) Galaxy: a platform for interactive large-scale genome analysis. *Genome Research*, **15**, 1451–1455.

Goecks J, Nekrutenko A, Taylor J (2010) Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biology*, **11**, R86.

de Graaf M, Dejen E, Osse JWM, Sibbing FA (2008) Adaptive radiation of Lake Tana's (Ethiopia) *Labeobarbus* species flock (Pisces, Cyprinidae). *Marine and Freshwater Research*, **59**, 391–407.

Grant PR, Grant BR (2008) How and Why Species Multiply: The Radiations of Darwin's Finches. Princeton University Press, Princeton, USA.

Greenwood PH (1965) Environmental effects on the pharyngeal mill of a cichlid fish, *Astatoreochromis alluaudi*, and their taxonomic implications. *Proceedings of the Linnean Society London*, **176**, 1–10.

Greenwood PH (1974) The cichlid fishes of Lake Victoria East Africa: the biology and evolution of a species flock. *Bulletin of the British Museum for Natural History (Zool.) Supplementary*, **6**, 1–134.

Harmon LJ, Kolbe JJ, Cheverud JM, Losos JB (2005) Convergence and the multidimensional niche. *Evolution*, **59**, 409–421.

Herder F, Schwarzer J, Pfaender J, Hadiaty RK, Schliewen UK (2006) Preliminary checklist of sailfin silversides (Teleostei: Telmatherinidae) in the Malili Lakes of Sulawesi (Indonesia), with a synopsis of systematics and threats. *Verhandlungen der Gesellschaft für Ichthyologie*, **5**, 139–163.

Huysseune A (1995) Phenotypic plasticity in the lower pharyngeal jaw dentition of *Astatoreochromis alluaudi* (Teleostei: Cichlidae). *Archives in Oral Biology*, **40**, 1005–1014.

Johnson MA, Revell LJ, Losos JB (2009) Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in Anolis lizards. *Evolution*, **64**, 1151–1159.

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics*, **9**, 286–298.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353–357.

Klingenberg CP, Barluenga M, Meyer A (2003) Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. *Biological Journal of the Linnean Society*, **80**, 397–408.

Koblmuller S, Egger B, Sturmbauer C, Sefc KM (2010) Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. *Molecular Phylogenetics and Evolution*, **55**, 318–334.

Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics*, **5**, 288–298.

Kocher TD, Conroy JA, McKaye KR, Stauffer JR (1993) Similar morphologies of cichlid fish in lakes Tanganyika and Malawi are due to convergence. *Molecular Phylogenetics and Evolution*, **2**, 158–165.

Konings A (1998) Tanganyikan Cichlids in their Natural Habitat. Cichlid Press, El Paso.

Lang M, Miyake T, Braasch I *et al.* (2006) A BAC library of the East African haplochromine cichlid fish *Astatotilapia burtoni*. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, **306B**, 35–44.

Lee WJ, Conroy J, Howell WH, Kocher TD (1995) Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution*, **41**, 54–66.

Li H, Handsaker B, Wysoker A *et al.* (2009) The sequence alignment/map format and SAMtools. *Bioinformatics*, **25**, 2078–2079.

Loiselle PV (1998) The *Amphilophus labiatus* Species Complex. The Cichlid Room Companion. Retrieved on June 05, 2012, from: http://www.cichlidae.com/article.php?id=106.

Losos JB (2009) Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles. University of California Press, Berkeley.

Losos JB (2010) Adaptive radiation, ecological opportunity, and evolutionary determinism. American Society of Naturalists E. O. Wilson award address. *American Naturalist*, **175**, 623–639.

Losos JB (2011) Convergence, adaptation and constraint. *Evolution*, **65**, 1827–1840.

Losos JB, Ricklefs RE (2009) Adaptation and diversification on islands. *Nature*, **457**, 830–836.

Losos JB, Jackmann TR, Larson A, De Queiroz K, Rodriguez-Schettino L (1998) Contingency and determinism in replicated adaptive radiations of island lizards. *Science*, **279**, 2115–2118.

Matschiner M, Hanel R, Salzburger W (2011) On the origin and trigger of the notothenioid adaptive radiation. *PLoS One*, **6**, e18911.

Meyer A (1987) Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution*, **41**, 1357–1369.

Meyer A, Morrissey JM, Schartl M (1994) Recurrent origin of a sexually selected trait in *Xiphophorus* fishes inferred from a molecular phylogeny. *Nature*, **368**, 539–542.

Muschick M, Barluenga M, Salzburger W, Meyer A (2011) Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evolutionary Biology*, **11**, 116.

Nori M (1997) Structure of littoral fish communities. In: *Fish Communities in Lake Tanganyika* (eds Kawanabe H, Hori M, Nagoshi M), pp. 277–298. Kyoto University Press, Kyoto.

Nosil P, Crespi BJ, Sandoval DP (2002) Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, **417**, 440–443.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Ribbink AJ, Marsch BA, Marsch AC, Ribbink AC, Sharp BJ (1983) A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology*, **18**, 149–310.

Roesti M, Hendry AP, Salzburger W, Berner D (2012) Genome divergence during evolutionary diversification as revealed in replicate lake–stream stickleback population pairs. *Molecular Ecology*, **21**, 2852–2862.

Rohlf FJ (2006) DIG, Version 2.10.0. Department of Ecology and Evolution. State University of New York, Stony Brook, NY.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Rundle HD, Nagel L, Wenrick Boughman J, Schluter D (2000) Natural selection and parallel speciation in sympatric sticklebacks. *Science*, **287**, 306–308.

Salzburger W (2009) The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology*, **18**, 169–185.

Salzburger W, Meyer A (2004) The species flocks of East African cichlid fishes: recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften*, **91**, 277–290.

Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C (2002) Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. *Systematic Biology*, **51**, 113–135.

Salzburger W, Mack T, Verheyen E, Meyer A (2005) Out of Tanganyika: Genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology*, **5**, 17.

Salzburger W, Renn SC, Steinke D, Braasch I, Hofmann HA, Meyer A (2008) Annotation of expressed sequence tags for the East African cichlid fish *Astatotilapia burtoni* and evolutionary analyses of cichlid ORFs. *BMC Genomics*, **9**, 96.

Salzburger W, Ewing GB, Von Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, **20**, 1952–1963.

Schevzov G, Lloyd C, Gunning P (1992) High level expression of transfected beta- and gamma-actin genes differentially impacts on myoblast cytoarchitecture. *The Journal of Cell Biology*, **117**, 775–785.

Schluter D (2000) The Ecology of Adaptive Radiation. Oxford University Press, New York.

Schluter D, Nagel LM (1995) Parallel speciation by natural selection. *American Naturalist*, **146**, 292–301.

Schoener TW (1970) Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology*, **51**, 408–418.

Seehausen O (1996) Lake Victoria Rock Cichlids, Verdujin Cichlids, Zevenhuizen, The Netherlands.

Seehausen O (2006) African cichlid fish: a model system in adaptive radiation research. *Proceeding of the Royal Society London B*, **273**, 1987–1998.

Sereno PC, Wilson JA, Conrad JL (2004) New dinosaurs link southern landmasses in the Mid-Cretaceous. *Proceeding of the Royal Society London B*, **271**, 1325–1330.

Sibbing FA, Nagelkerke LAJ, Stet RJM, Osse JWM (1998) Speciation of endemic Lake Tana barbs (*Cyprinidae*, Ethiopia) driven by trophic resource partitioning: a molecular and ecomorphological approach. *Aquatic Ecology*, **32**, 217–227.

Simpson GG (1953) The Major Features of Evolution, Columbia University Press, New York.

Soni KG, Mardones GA, Sougrat R, Smirnova E, Jackson CL, Bonifacino JS (2009) Coatomer-dependent protein delivery to lipid droplets. *Journal of Cell Science*, **122**, 1834–1841.

Stiassny MLJ, Meyer A (1999) Cichlids of the Rift Lakes. *Scientific American*, **280**, 64–69.

Sturmbauer C, Hainz U, Baric S, Verheyen E, Salzburger W (2003) Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism. *Hydrobiologia*, **500**, 51–64.

Swofford DL (2003) PAUP*—Phylogenetic Analyses Using Parsimony and Other Methods, Version 4.0, Sinauer, Sunderland, Massachusetts.

Tsukita S, Furuse M, Itoh M (2001) Multifunctional strands in tight junctions. *Nature Reviews in Molecular Cell Biology*, **2**, 285–293.

Verheyen E, Salzburger W, Snoeks J, Meyer A (2003) Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science*, **300**, 325–329.

Villa J (1982) Peces Nicaragüenses de Agua Dulce, Fondo de Promoción Cultural, Banco de América, Managua.

Wake DB, Wake MH, Specht CD (2011) Homoplasy: from detecting pattern to determining process and mechanism of evolution. *Science*, **331**, 1032–1035.

Wallace RK (1981) An assessment of diet-overlap indexes. *Transactions of the American Fisheries Society*, **110**, 72–76.

Yamaoka K (1997) Trophic ecomorphology of Tanganyikan cichlids. In: *Fish Communities in Lake Tanganyika* (eds Kawanabe H, Hori M, Nagoshi M), pp. 27–56. Kyoto University Press, Kyoto.

Yoder JB, Clancey E, Des Roches S *et al*. (2010) Ecological opportunity and the origin of adaptive radiations. *Journal of Evolutionary Biology*, **23**, 1581–1596.

M.C., E.T.D., M.E.S. and A.I. are PhD students in the group of W.S. M.C. is interested in parallel evolution events as natural replicates to test hypotheses about trait evolution and the different (or similar) genetic bases that underlie these phenotypes. E.T.D. is interested in the genetic basis of adaptive traits and the selective forces acting upon these genes. M.E.S. is interested in the ecological and developmental mechanisms underlying the emergence and diversification of novel adaptive traits. A.I. is interested in ecomorphological adaptations, phylogeography and taxonomy in cichlid fishes. M.M. recently finished his PhD in the group of W.S. and is now postdoctoral fellow with Patrik Nosil in Sheffied. His research is concerned with morphological and genomic evolution in adaptive radiations. N.B. is a technical assistant who is involved in several projects of the SalzburgerLab. M.B. is a group leader at the Natural History Museum in Madrid. Her research focuses on understanding incipient stages of speciation and the sequence of adaptations and specializations that organisms undergo after the colonization of new habitats. W.S. is Professor of Zoology and Evolutionary Biology at the University of Basel. The research of his team focuses on the genetic basis of adaptation, evolutionary innovation and animal diversification. The main model systems in the laboratory are threespine stickleback fish, Antarctic notothenioids and the exceptionally diverse assemblages of cichlid fishes. The laboratory's homepage at http://www.evolution.unibas.ch/salzburger/ provides further details on the group's (research) activities.

Data accessibility

Newly generated DNA sequences for phylogenetic and haplotype analyses have been deposited in GenBank under accession numbers JX402217–JX402407 (see Appendix S1 for details). Illumina reads from the RNA-seq experiments are available from the Sequence Read Archive (SRA) at NCBI under the accession number SRA052992. Data from the stomach and gut content analyses, the MorphoJ input files and the quantitative real-time PCR experiments have been deposited at Dryad (doi:10.5061/dryad.vf1ms).

Supporting information

Additional Supporting Information may be found in the online version of this article.

Appendix S1 List of specimens used in this study including sampling date and location and GenBank accession numbers.

Appendix S2 PCA of overall body shape of the African cichlid *Lobochilotes labiatus* and the Nicaraguan species *Amphilophus labiatus* and *A. citrinellus* (a) and distribution of landmarks for morphometric analyses (b).

Appendix S3 Blast2GO annotations of genes with differential expression between lip tissue from thick-lipped and normal-lipped cichlid species.

Appendix S4 Underwater video showing snail feeding in *Lobochilotes labiatus*.

Appendix S5 Results of the quantitative real-time PCR experiments comparing adult and juvenile lip tissue of the African cichlid species *Lobochilotes labiatus* and *Astatotilapia burtoni*.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Supplementary Materials

The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes

Marco Colombo*, Eveline T. Diepeveen*, Moritz Muschick, M. Emilia Santos, **Adrian Indermaur**, Nicolas Boileau, Marta Barluenga and Walter Salzburger

*these authors contributed equally to this work

Appendix S1 List of specimens used in this study including sampling date and location and GenBank accession numbers

Sample ID		Species	Sampling	Location	DNA/RNA sequencing/GenBank accession numbers				qPCR					
DNA	RNA		Date		mtControl	ednrb1	phpt1	RNA sequencing	BNIP3	Actb	Cldn7	Copb	His3	ACSL4
23H8		*Amphilophus citrinellus*	9/5/09	Isletas		JX402280	JX402217							
23H9		*Amphilophus citrinellus*	9/5/09	Isletas		JX402281	JX402218							
23I1		*Amphilophus citrinellus*	9/5/09	Isletas		JX402282	JX402219							
23I2		*Amphilophus citrinellus*	9/5/09	Isletas			JX402220							
24A2		*Amphilophus citrinellus*	9/5/09	Isletas		JX402283	JX402221							
24A3		*Amphilophus citrinellus*	9/5/09	Isletas		JX402284	JX402222							
24A4		*Amphilophus citrinellus*	9/5/09	Isletas		JX402285	JX402223							
24A5		*Amphilophus citrinellus*	9/5/09	Isletas		JX402286	JX402224							
25D1	31I8	*Amphilophus citrinellus*	9/9/09	Isletas		JX402288	JX402225							
25D2	30D7	*Amphilophus citrinellus*	9/9/09	Isletas		JX402289	JX402226							
25D6	30F1	*Amphilophus citrinellus*	9/9/09	Isletas			JX402227		y	y	y	y	y	y
25D7	30F3	*Amphilophus citrinellus*	9/9/09	Isletas		JX402298	JX402228							
25D8	30F9	*Amphilophus citrinellus*	9/9/09	Isletas		JX402299	JX402229							
25E1	30F6	*Amphilophus citrinellus*	9/9/09	Isletas			JX402230							
25E2	30G2	*Amphilophus citrinellus*	9/9/09	Isletas		JX402292	JX402231							
25E3	30G4	*Amphilophus citrinellus*	9/9/09	Isletas		JX402287	JX402232		y	y	y	y	y	y
25E4	30G6	*Amphilophus citrinellus*	9/9/09	Isletas		JX402293	JX402233							
25E6	30H3	*Amphilophus citrinellus*	9/9/09	Isletas			JX402234							
25E8	30H6	*Amphilophus citrinellus*	9/9/09	Isletas		JX402296	JX402235							
25E9	30H7	*Amphilophus citrinellus*	9/9/09	Isletas		JX402297	JX402236							
25D3	30E2	*Amphilophus citrinellus*	9/9/09	Isletas		JX402290								
25D4	30E7	*Amphilophus citrinellus*	9/9/09	Isletas					y	y	y	y	y	y
25E5	30G8	*Amphilophus citrinellus*	9/9/09	Isletas		JX402294			y	y	y	y	y	y
25E7	30H5	*Amphilophus citrinellus*	9/9/09	Isletas		JX402295								
25F1	30H9	*Amphilophus citrinellus*	9/9/09	Isletas		JX402298								
26I3	32A7	*Amphilophus citrinellus*	9/14/09	Managua Miraflores					y	y	y	y	y	y
26I4	32B1	*Amphilophus citrinellus*	9/14/09	Managua Miraflores					y	y	y	y	y	y
23F9		*Amphilophus labiatus*	9/5/09	Isletas	JX402360	JX402301	JX402237							
23G1		*Amphilophus labiatus*	9/5/09	Isletas	JX402354	JX402302	JX402238							
23G3		*Amphilophus labiatus*	9/5/09	Isletas			JX402239							
23G6		*Amphilophus labiatus*	9/5/09	Isletas	JX402367		JX402240							
23G9		*Amphilophus labiatus*	9/5/09	Isletas	JX402361	JX402305	JX402241							
23H3		*Amphilophus labiatus*	9/5/09	Isletas	JX402365	JX402306	JX402242							
23H6		*Amphilophus labiatus*	9/5/09	Isletas	JX402368		JX402243							
23H7		*Amphilophus labiatus*	9/5/09	Isletas	JX402375	JX402308	JX402244							
25A1	31C3	*Amphilophus labiatus*	9/9/09	Isletas	JX402371	JX402310	JX402245							
25A5	31D6	*Amphilophus labiatus*	9/9/09	Isletas	JX402364	JX402311	JX402246							
25A6	31D9	*Amphilophus labiatus*	9/9/09	Isletas	JX402369		JX402247							
25A7	31E2	*Amphilophus labiatus*	9/9/09	Isletas	JX402378	JX402312	JX402248							
25A8	31E3	*Amphilophus labiatus*	9/9/09	Isletas	JX402376	JX402313	JX402249							
25B1	31E7	*Amphilophus labiatus*	9/9/09	Isletas	JX402363	JX402314	JX402250		y	y	y	y	y	y
25B3	31F2	*Amphilophus labiatus*	9/9/09	Isletas	JX402366	JX402316	JX402251							
25B4	31F4	*Amphilophus labiatus*	9/9/09	Isletas	JX402351	JX402309	JX402252							
25B5	31F5	*Amphilophus labiatus*	9/9/09	Isletas	JX402372		JX402253							
25B6	31F7	*Amphilophus labiatus*	9/9/09	Isletas	JX402359	JX402317	JX402254							
25B7	31F9	*Amphilophus labiatus*	9/9/09	Isletas	JX402377	JX402318	JX402255							
25B9	31G3	*Amphilophus labiatus*	9/9/09	Isletas	JX402349	JX402320	JX402256							
23F8		*Amphilophus labiatus*	9/5/09	Isletas	JX402353	JX402300								
23G2		*Amphilophus labiatus*	9/5/09	Isletas	JX402374	JX402303								
23H5		*Amphilophus labiatus*	9/5/09	Isletas	JX402350	JX402307								
25B2	31E9	*Amphilophus labiatus*	9/9/09	Isletas	JX402358	JX402315								

25B8	31G2	*Amphilophus labiatus*	9/9/09	Isletas	JX402370	JX402319								
23G5		*Amphilophus labiatus*	9/5/09	Isletas	JX402373									
23G7		*Amphilophus labiatus*	9/5/09	Isletas	JX402355									
23G8		*Amphilophus labiatus*	9/5/09	Isletas	JX402356	JX402304								
23H1		*Amphilophus labiatus*	9/5/09	Isletas	JX402357									
25A3	31C9	*Amphilophus labiatus*	9/9/09	Isletas	JX402352									
25A4	31D4	*Amphilophus labiatus*	9/9/09	Isletas	JX402348									
25A9	31E5	*Amphilophus labiatus*	9/9/09	Isletas	JX402362				y	y	y	y	y	y
25C1	31G6	*Amphilophus labiatus*	9/9/09	Isletas					y	y	y	y	y	y
25C6	31H7	*Amphilophus labiatus*	9/9/09	Isletas					y	y	y	y	y	y
28A3	32G6	*Amphilophus labiatus*	9/18/09	Managua Miraflores					y	y	y	y	y	y
28C6	32H7	*Amphilophus labiatus*	9/20/09	Ometepe, San Ramon	J				y	y	y	y	y	y
35A1		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402388	JX402321	JX402257							
35A2		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402383	JX402322	JX402278							
35A3		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402398	JX402323	JX402263							
35A4		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402399	JX402324	JX402266							
35A5		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402400	JX402325	JX402268							
35A6		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402405	JX402326	JX402262							
35A7		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402391	JX402327	JX402279							
35A8		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402392	JX402328	JX402259							
35B6		*Lobochilotes labiatus*	2/21/10	Mbita Island W	JX402401	JX402329	JX402258							
36B3		*Lobochilotes labiatus*	2/22/10	Mpulungu area	JX402404	JX402330	JX402267							
36B4		*Lobochilotes labiatus*	2/22/10	Mpulungu area	JX402406	JX402331	JX402275							
36H7		*Lobochilotes labiatus*	2/23/10	Kasakalawe Lodge	JX402403	JX402333	JX402277							
43D7	81A2	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402379	JX402334	JX402261	y	y	y	y	y	y	y
43D8	81A5	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402407	JX402335	JX402260	y	y	y	y	y	y	y
43E4	81B9	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402390	JX402340	JX402264		y	y	y	y	y	y
43E5	81B4	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402381	JX402341	JX402273	y	y	y	y	y	y	y
43E6		*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402382	JX402342	JX402265							
44G8		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402395	JX402343	JX402270							
44G9		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402386	JX402344	JX402271							
44H1		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402380	JX402345	JX402269							
44H3		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402396		JX402276							
44H5		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402387	JX402346	JX402272							
44H6		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402402	JX402347	JX402274							
36H8		*Lobochilotes labiatus*	2/23/10	Kasakalawe Lodge	JX402389	JX402332								
43D9	81A7	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402384	JX402336								
43E1	81B1	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402385	JX402337								
43E2	81B3	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402393	JX402338								
43E3	81B7	*Lobochilotes labiatus*	2/28/10	Toby's Place	JX402394	JX402339								
44H4		*Lobochilotes labiatus*	3/1/10	Toby's Place	JX402397									
43D6	81A3	*Lobochilotes labiatus*	2/28/10	Toby's Place				y	y	y	y	y	y	y
55D1	81H9	*Lobochilotes labiatus*	3/11/10	Mpulungu area					y	y	y	y	y	y
55D2	81I2	*Lobochilotes labiatus*	3/11/10	Mpulungu area					y	y	y	y	y	y
55D8	79A5	*Lobochilotes labiatus*	3/11/10	Mukaka					y	y	y	y	y	y
55D9	79A9	*Lobochilotes labiatus*	3/11/10	Mukaka				y	y	y	y	y	y	y
BIB4	BMB4	*Lobochilotes labiatus*	7/20/11	Toby`s place					y	y	y	y	y	y
BIC4	BMC4	*Lobochilotes labiatus*	7/20/11	Toby`s place					y	y	y	y	y	y
BIC6	BMC6	*Lobochilotes labiatus*	7/20/11	Toby`s place					y	y	y	y	y	y
BIC7	BMC7	*Lobochilotes labiatus*	7/20/11	Toby`s place					y	y	y	y	y	y
BIC9	BMC9	*Lobochilotes labiatus*	7/20/11	Toby`s place					y	y	y	y	y	y

additional Tropheini species for phylogenetic analyses:

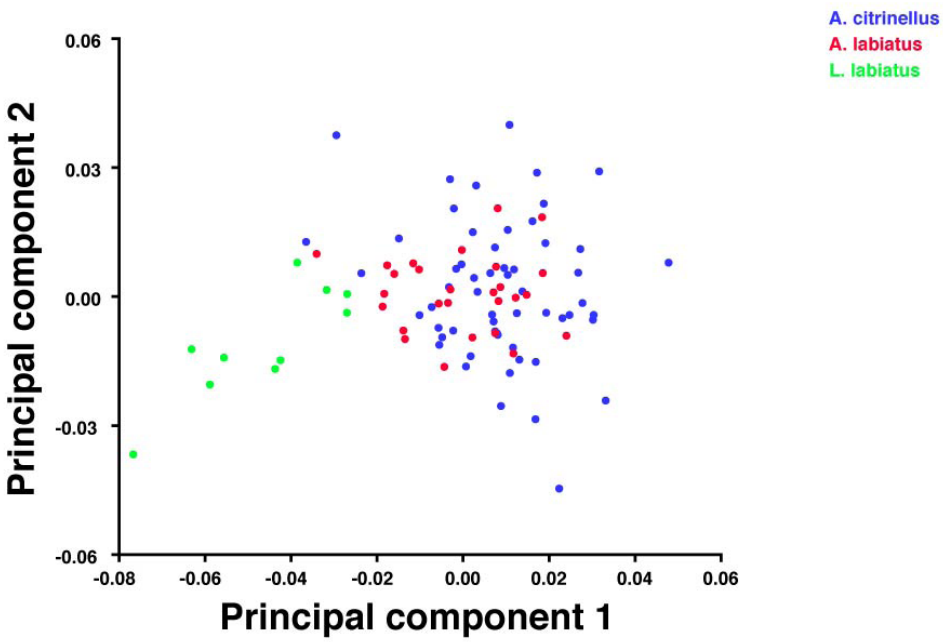
Species	**mtControl**	**ednrb1**	**phpt1**	**ND2**
Astatotilapia burtoni	AY930000	JF900252	JF900181	JF900319
Ctenochromis horei	AY301952	JF900262	JF900191	EU753935

Gnathochromis pfefferi	AY301954	JF900269	JF900198	U07248
Interochromis loockii	GQ995855	JF900304	JF900232	JF900322
Limnotilapia dardennii	AY301956	JF900285	JF900214	GQ995724
Lobochilotes labiatus	AY301958	JF900286	JF900215	U07254
Petrochromis ephippium	AY301959	JF900300	JF900229	JF900323
Petrochromis famula	AY301960	JF900301	JF900230	JF900324
Petrochromis fasciolatus	GQ995911	JF900325	JF900231	JF900325
Petrochromis macrognathus	AY929963	JF900304	JF900233	AY930068
Petrochromis polyodon	AY301967	JF900305	JF900234	JF900326
Pseudosimochromis curvifrons	AY301973	JF900307	JF900236	GQ995777
Simochromis babaulti	AY301975	JF900309	JF900238	GQ995782
Simochromis diagramma	AY301977	JF900310	JF900239	AY930087
Tropheus moorii	AY930020	JF900314	JF900243	AY930093

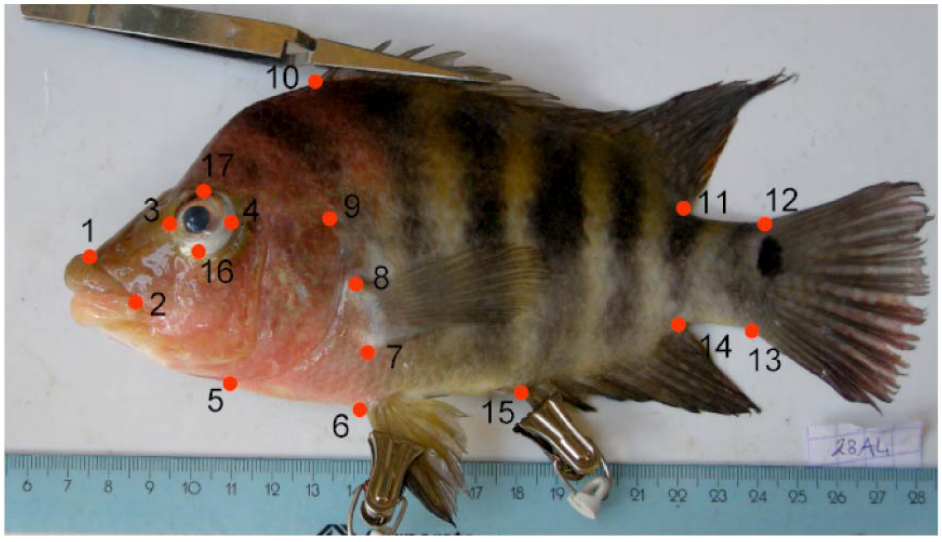
additional *Amphilophus citrniellus* mitochondrial control region sequences:

ampcit1	AY567011
ampcit2	AY567018
ampcit3	AY567012
ampcit4	AY567013
ampcit5	AY567014
ampcit6	AY567015
ampcit7	AY567016
ampcit8	AY567017
ampcit9	EF219270
ampcit10	EF219269
ampcit11	EF219268
ampcit12	EF219252
ampcit13	EF219251
ampcit14	EF219250
ampcit15	EF219249
ampcit16	EF219248
ampcit17	EF219247
ampcit18	AY567470
ampcit19	AY567469
ampcit20	AY567468
ampcit21	AY567467
ampcit22	AY567466
ampcit23	AY567465
ampcit24	AY567464
ampcit25	AY567463
ampcit26	AY567462
ampcit27	AY567461
ampcit28	AY567460
ampcit29	AY567459
ampcit30	AY567458
ampcit31	AY567457

(a)


A. citrinellus
A. labiatus
L. labiatus
Principal component 2
0.06
0.03
0.00
-0.03
-0.06
-0.08
-0.06
-0.04
-0.02
0.00
0.02
0.04
0.06
Principal component 1

(b)

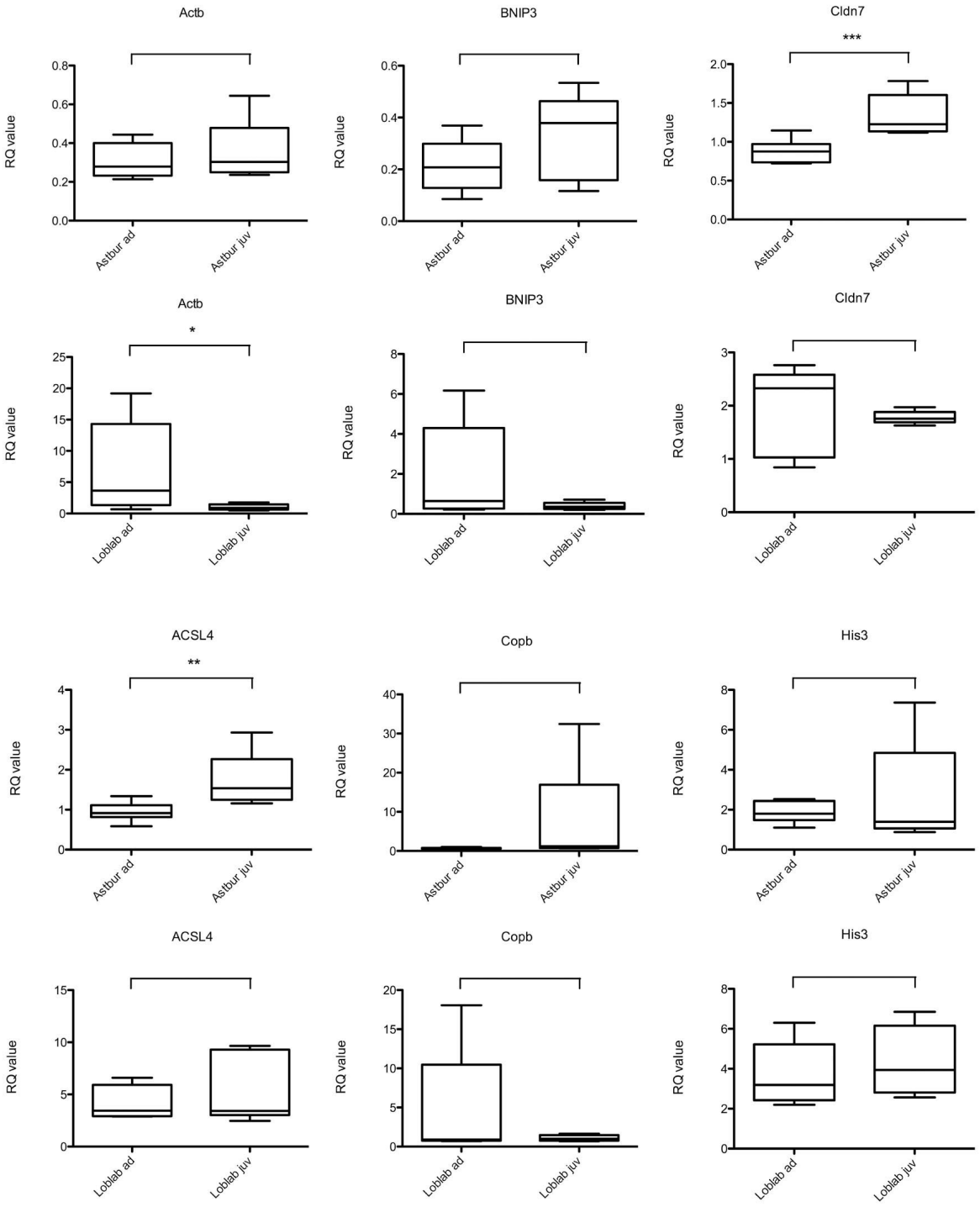

10
17
3
4
9
11
12
1
16
8
2
14
13
7
5
6
15

Blast2GO annotations of genes with differential expression between lip-tissue from thick-lipped and normal-lipped cichlid species

26s protease regulatory subunit 8
3-hydroxyanthranilate -dioxygenase
60s acidic ribosomal protein p2
actin-related protein 2-a
actin-related protein 3
activating transcription factor 4
acyl carrier mitochondrial precursor
acyl- -binding protein
adaptor-related protein complex mu 1 isoform cra_a
adaptor-related protein complex mu 1 subunit
adp-dependent glucokinase-like
adp-ribose mitochondrial-like
atp synthase subunit mitochondrial precursor
atpase mitochondrial precursor
baculoviral iap repeat-containing protein 4
bcl2 adenovirus e1b 19 kda protein-interacting protein 3
bcl2 adenovirus e1b 19 kda protein-interacting protein 3-like
beta actin
calpastatin
carboxypeptidase z-like
caspase-8
chaperonin containing subunit 6a (zeta 1)
chromobox protein homolog 3
claudin 7
cmp-n-acetylneuraminate-beta-galactosamide-alpha- -sialyltransferase 1-like
coatomer subunit beta
comm domain-containing protein 9
complement c1q tumor necrosis factor-related protein 3-like
cop9 signalosome complex subunit 8
coproporphyrinogen oxidase
cystathionine gamma-lyase
cystatin precursor
cysteine and glycine-rich protein 1
cytochrome c oxidase polypeptide viia-liver mitochondrial precursor
dcn1-like protein 1
dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase mitochondrial
dnaj homolog subfamily c member 9-like
dynactin subunit 5
ectonucleoside triphosphate diphosphohydrolase 3
estradiol 17-beta-dehydrogenase 12-b
eukaryotic translation initiation factor 3 subunit i
eukaryotic translation initiation factor 3 subunit k
eukaryotic translation initiation factor 3 subunit l
eukaryotic translation initiation factor 3 subunit m
eukaryotic translation initiation factor 4 gamma 2-like
eukaryotic translation initiation factor 4h
excitatory amino acid transporter 1 isoform 1
fk506-binding protein 2 precursor
forkhead box q1

glutamate dehydrogenase
glyoxalase domain-containing protein 4-like
grainyhead-like protein 1 homolog
granulins precursor
gtpase imap family member 4-like
gtpase imap family member 7-like
gtpase imap family member 8-like
gtpase imap family member 8-like
h1 histone
heat shock 70 kda protein 12a-like
histone
iars protein
importin-7
integrin beta-4-like
interferon-induced protein 35
isocitrate dehydrogenase
l _3
lamin b1
laminin subunit gamma-2
loc100127300 protein
long-chain-fatty-acid-- ligase 4
low quality protein: coronin-1c-like
lrr and pyd domains-containing protein 3-like
magnesium transporter 1
major vault protein
membrane magnesium transporter 1-like
methylmalonyl epimerase
microfibril-associated glycoprotein 4-like
mortality factor 4 like 1
myosin regulatory light chain smooth muscle isoform
nadh dehydrogenase
nadh dehydrogenase 1 alpha subcomplex subunit 11
nedd4 family-interacting protein 1
nedd4 family-interacting protein 1
nuclear factor erythroid 2-related factor 1-like
ornithine decarboxylase
pancreatic progenitor cell differentiation and proliferation factor
peptidylprolyl isomerase b (cyclophilin b)
phosphoglycerate kinase 1
piggybac transposable element-derived protein 4-like
pre-mrna splicing factor
PREDICTED: galectin-3-like [Oreochromis niloticus]
PREDICTED: hypothetical protein LOC100704514 [Oreochromis niloticus]
prefoldin subunit 4
probable glutathione peroxidase 8-like
programmed cell death 6-interacting protein
proteasome subunit alpha type-1
proteasome subunit alpha type-6
protein disulfide isomerase family member 4
protein fam100a-like
protein fam176b-like
protein kiaa0664-like
protein rer1

rab acceptor 1
ras-related protein rab-11b
regulator of g-protein signaling 2
renin receptor isoform 3
ribosomal l1 domain-containing protein 1-like
rilp-like protein 1
scinderin like a
scinderin like a
secretory carrier-associated membrane protein 2-like
septin 10
signal peptidase complex catalytic subunit sec11a
signal peptide peptidase-like 2a-like
small 1
sodium-dependent phosphate transporter 1
solute carrier family facilitated glucose transporter member 11-like
solute carrier family member 30
splicing factor 3b subunit 1
subfamily member 11
syntaxin 12
t-cell receptor type 1
t-complex protein 1 subunit alpha-like
t-complex protein 1 subunit theta
tbc1 domain member 15
thioredoxin domain containing 4 (endoplasmic reticulum)
threonyl-trna cytoplasmic
transaldolase
transcription factor ap-2 gamma (activating enhancer binding protein 2 gamma)
transmembrane protein 214
transmembrane protein 59 precursor
transmembrane protein 79
transposon tx1 uncharacterized 149 kda
tumor protein 63
tumor-associated calcium signal transducer 2 precursor
u6 snrna-associated sm-like protein lsm8
uap56-interacting factor-like
uncharacterized protein c22orf25-like
upf0510 protein inm02 precursor
v-type proton atpase catalytic subunit a
v-type proton atpase subunit d 1


Actb
BNIP3
Cldn7
***
Actb
*
BNIP3
Cldn7
ACSL4
**
Copb
His3
ACSL4
Copb
His3
RQ value
Astbur ad
Astbur juv
Loblab ad
Loblab juv

Chapter 3

Depth-dependent abundance of Midas Cichlid fish (*Amphilophus spp.*) in two Nicaraguan crater lakes

Marie Theres Dittmann*, Marius Roesti*, **Adrian Indermaur**, Marco Colombo, Martin Gschwind, Isabel Keller, Robin Kovac, Marta Barluenga, Moritz Muschick and Walter Salzburger

*these authors contributed equally to this work

Hydrobiologia (2012)

AI helped with fieldwork, data processing and discussion of the mauscript

Hydrobiologia (2012) 686:277–285
DOI 10.1007/s10750-012-1024-1

PRIMARY RESEARCH PAPER

Depth-dependent abundance of Midas Cichlid fish (*Amphilophus* spp.) in two Nicaraguan crater lakes

Marie Theres Dittmann · Marius Roesti · Adrian Indermaur · Marco Colombo · Martin Gschwind · Isabel Keller · Robin Kovac · Marta Barluenga · Moritz Muschick · Walter Salzburger

Received: 4 July 2011 / Revised: 2 February 2012 / Accepted: 5 February 2012 / Published online: 22 February 2012

Abstract The Midas Cichlid species complex (*Amphilophus* spp.) in Central America serves as a prominent model system to study sympatric speciation and parallel adaptive radiation, since small arrays of equivalent ecotype morphs have evolved independently in different crater lakes. While the taxonomy and evolutionary history of the different species are well resolved, little is known about basic ecological parameters of Midas Cichlid assemblages. Here, we use a line transect survey to investigate the depth-dependent abundance of *Amphilophus* spp. along the shores of two Nicaraguan crater lakes, Apoyo and Xiloá. We find a considerable higher density of Midas cichlids in Lake Xiloá as compared to Lake Apoyo, especially at the shallowest depth level. This might be due to the higher eutrophication level of Lake Xiloá and associated differences in food availability, and/or the presence of a greater diversity of niches in that lake. In any case, convergent forms evolved despite noticeable differences in size, age, eutrophication level, and carrying capacity. Further, our data provide abundance and density estimates for Midas Cichlid fish, which serve as baseline for future surveys of these ecosystems and are also relevant to past and future modeling of ecological speciation.

Keywords Sympatric speciation · Parallel adaptive radiation · Fish density estimates · Crater Lake Apoyo · Crater Lake Xiloá · Ecology

Marie Theres Dittmann and Marius Roesti contributed equally to this study.

Handling editor: Christian Sturmbauer

Electronic supplementary material The online version of this article (doi:10.1007/s10750-012-1024-1) contains supplementary material, which is available to authorized users.

M. T. Dittmann · M. Roesti · A. Indermaur · M. Colombo · M. Gschwind · I. Keller · R. Kovac · M. Muschick · W. Salzburger (✉)
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland
e-mail: walter.salzburger@unibas.ch

Present Address:
M. T. Dittmann
Group of Animal Nutrition, Institute of Agricultural Science, Swiss Federal Institute of Technology, Universitätsstrasse 2, 8092 Zurich, Switzerland

I. Keller
IFM-Geomar, Evolutionsbiologie, Düstenbrooker Weg 20, 24105 Kiel, Germany

M. Barluenga
Museo Nacional de Ciencias Naturales, CSIC, José Gutierrez Abascal 2, 28006 Madrid, Spain

Introduction

The species flocks of cichlid fishes in the East African Great Lakes Victoria, Malawi, and Tanganyika are prime model systems in evolutionary biology and, particularly, in research focusing on speciation,

adaptive radiation, and parallel evolution (reviewed in Kocher, 2004; Salzburger, 2009; Sturmbauer et al., 2011). One of the most outstanding features of the East African cichlid assemblages is their species richness, with each of the Great Lakes harboring hundreds of endemic species. The downside of this unparalleled diversity is that these species flocks are notoriously difficult to study in their entirety, which makes it attractive to study simpler cichlid communities in smaller water bodies. In the last years surveys of crater lakes cichlids proved especially fruitful, mostly due to the degree of isolation of their cichlid assemblages (Schliewen et al., 1994; Barluenga & Meyer, 2004; Barluenga et al., 2006). The probably best-studied cichlids in volcanic crater lakes belong to the Midas Cichlid species complex (*Amphilophus* spp.), which is native to Central America. Midas cichlids are abundant in the large lakes of Nicaragua (Lake Nicaragua and Lake Managua) and associated rivers in Nicaragua and northern Costa Rica. Interestingly, Midas Cichlids have also colonized various volcanic crater lakes in the area (Barlow, 1976; Barluenga & Meyer, 2004, 2010), which emerge when calderas of extinct volcanoes of the 'Pacific Ring of Fire' become filled with water.

This study focuses on the *Amphilophus* assemblages in two of these crater lakes, Apoyo and Xiloá, which contain two independent, yet ecologically and morphologically very similar sets of Midas cichlid species (Elmer et al., 2010; Geiger et al., 2010a). The lakes are similar in some aspects, such as their volcanic origin, but they do differ in others (Barlow, 1976; Sussman, 1985; Waid et al., 1999; McKaye et al., 2002; Barluenga & Meyer, 2010): With a surface area of 21.1 km^2 and a maximum depth of 142 m, Lake Apoyo is larger and deeper than Lake Xiloá, which has a surface area of 3.8 km^2 and a maximum depth of 89 m (Table 1). Also, compared to the nutrient-rich Lake Xiloá, Lake Apoyo is oligotrophic. Furthermore, they differ in the number of cichlid species. Crater Lake Apoyo is suggested to harbor six endemic species of the *Amphilophus* complex (Barlow, 1976; Stauffer et al., 2008; Geiger et al., 2010b) (Supplementary Table 1), which most likely go back to a seeding lineage from adjacent Lake Nicaragua (Barluenga et al., 2006); together with *Parachromis managuense* and the recently introduced African species *Oreochromis aureus* and *O. niloticus*, these are the only cichlids found in this lake. In Lake Xiloá three to four endemic species of the *Amphilophus* species complex are described (McKaye et al., 2002; Stauffer & McKaye, 2002) (Supplementary Table 1), which derive from the close-by Lake Managua stocks (Barluenga & Meyer, 2010). In addition to the Midas Cichlid fish, Lake Xiloá is inhabited by eight additional cichlid species, which either migrated naturally from nearby Lake Managua, or were introduced by humans, as might be the case for *Parachromis managuense* (Kullander & Hartel, 1997).

Table 1 General descriptors of size, depth, age, visibility, fish density, and population size of the crater lakes Apoyo and Xiloá

	Apoyo	Xiloá
Surface area (km^2)	21.1[a]	3.8[a]
Maximum depth (m)	142[a]	89[a]
Age (year)	<23.000[a]	ca. 10.000[a]
Secchi depth (m)	5–7	3
Cichlid density along shore (individuals per 10 m transect)	11.3	19.9
Total number of *Amphilophus* spp. along shore (estimated)	83.000	66.000

[a] Barluenga & Meyer (2010)

Here, we present a comparative study of cichlid abundance and density estimates in the two Central American calderas Lake Apoyo and Lake Xiloá. The set-up consisting of two rather similar crater lakes seeded independently by more or less the same ancestral line that subsequently radiated in parallel appears ideal to disentangle the biotic and abiotic factors influencing parallel adaptive radiation, particularly in its early stages. Many adaptive radiations appear to proceed in discrete stages starting with an initial diversification into macrohabitats (Streelman & Danley, 2003; Gavrilets & Losos, 2009), which—in fishes—is often associated with differentiation along the benthic-limnetic (pelagic) axis (Schluter & McPhail, 1992; Gíslason et al., 1999; Barluenga et al., 2006; Rutschmann et al., 2011). That independent adaptive radiations of the same group of organisms in similar ecological settings often result in similar morphologies is generally taken as strong evidence for natural selection (and the importance of ecology in speciation) (see Schluter & Nagel, 1995; Losos et al., 1998). On the other hand, the degree of similarity observed in convergent species pairs of cichlids has led some authors to question whether natural selection alone is sufficient to produce such matching morphologies, or whether genetic or developmental constraints have

contributed to the evolution of convergent forms (see, e.g., Brakefield, 2006). Even in the genomic era it is difficult to determine the relative contribution of natural selection and developmental channeling to parallel evolution. One possibility is to apply genetic and genomic experiments (reviewed in: Brakefield, 2006; Arendt & Reznick, 2008). In addition, one should inspect parallel radiations with respect to key ecological parameters. Under the assumption that ecology is the driving force behind parallel adaptive radiation, it is expected that not only the outcome of the radiations should be the same, but that the radiations should also follow the same steps and should show the same (ecological) characteristics. In the case of the parallel radiations of the Midas Cichlid in crater lakes Apoyo and Xiloá, the outcome in form of morphologically equivalent species is obviously quite similar (Fig. 1) and there is evidence that the radiations progressed in a similar fashion (Barluenga et al., 2006; Barluenga & Meyer, 2010; Elmer et al., 2010). It is not known, however, whether the communities in the seemingly similar crater lakes Apoyo and Xiloá are also similar in terms of ecological parameters such as fish densities and depth distributions.

In this study, we applied transect surveys to record the abundance of *Amphilophus* spp. in crater lakes Apoyo and Xiloá. Applying SCUBA diving and snorkeling, fish were counted at different locations and depth levels to provide data on densities of cichlids in both lakes. We hypothesized that the

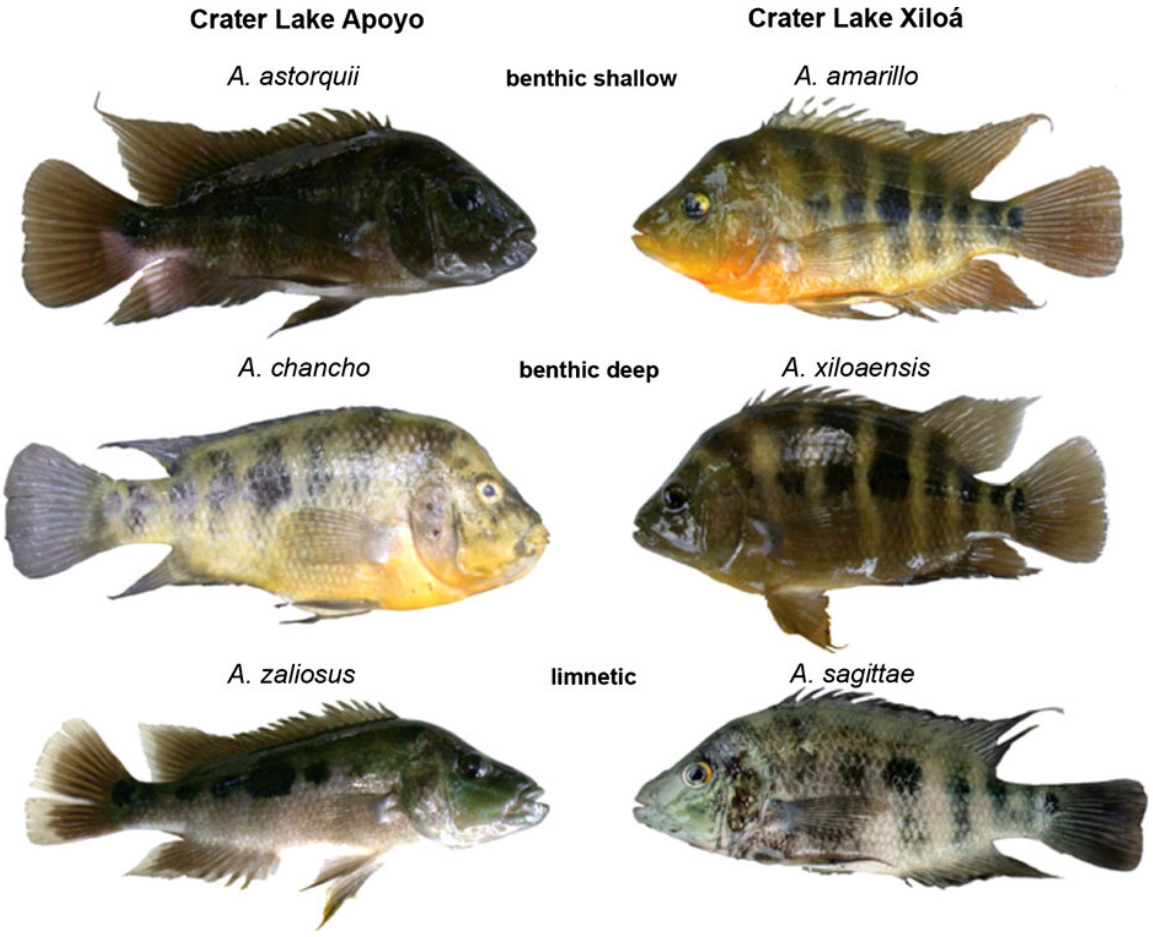


Fig. 1 Convergent phenotypes that evolved independently in the two Nicaraguan crater lakes Apoyo and Xiloá. Three species pairs are shown: benthic species using the shallow areas of the lakes; benthic species using the deeper areas of the lakes; and limnetic species inhabiting the open water column

density and distribution of Midas cichlids should be rather similar in both crater lakes due to their similar mode of origin and structure. In addition, this study aims to add ecological data in the form of abundance estimates for *Amphilophus* spp. to theoretical studies on sympatric and/or ecological speciation. Gavrilets et al. (2007), for example, investigated under which biological conditions rapid colonization of a new niche followed by sympatric or parapatric speciation in Lake Apoyo is theoretically possible. However, in their models, Gavrilets et al. (2007) were lacking empirical data on several important biological parameters (including abundance estimates). Finally, knowledge of the natural abundance of a population, species, or species group is fundamental not only to biological research but also to the management of wildlife populations. This is important in the case of crater lakes Apoyo and Xiloá, too, where cichlid fishes make up the main fraction of the ichthyofauna and provide a valuable food resource for local people (Schuster, 1957; Lin, 1961; Barlow, 1976). Importantly, through the recent introduction of African tilapiine cichlid species (*Oreochromis* spp.), the endemic cichlids of Lake Apoyo are thought to be threatened (McKaye et al., 1995; McCrary et al., 2001; Barluenga & Meyer, 2004), calling for an evaluation of the conservation status of the endemic faunas in the two crater lakes. Our data should, thus, provide important baseline references, with which upcoming impacts on the native cichlid abundance can be assessed.

Materials and methods

Study area and period

Field work was carried out in the two crater lakes Apoyo and Xiloá in Nicaragua, Central America, in September 2009. Diving was performed during the day by almost invariably good weather conditions. At the time of the study, water temperatures ranged between 29 and 31°C on all surveyed depth levels in both lakes. Transect sites were chosen randomly in both lakes, balanced, however, for different geographical locations within each lake (Supplementary Table 2). As crater lakes have a relatively homogenous habitat structure, the transects are representative of the habitat composition in each lake.

Transect surveys

We used fish counts along line transects to compare the depth-dependent abundance and density of *Amphilophus* spp. between the two lakes. Six transects were studied in the larger Lake Apoyo and four transects in the smaller Lake Xiloá. The start and end coordinates of each transect were taken with a handheld GPS from a boat (Supplementary Table 2). Depth levels at 10, 15, and 20 m were covered for each transects by a SCUBA diving buddy pair, whereas the 5 m depth level was covered by snorkelers (whenever the visibility was sufficient).

Transect length was determined by the distance covered during 10–15 min of diving (depending on the available air). Diving pace was moderate but varied between transects according to visibility and the quantity of fish that had to be counted, leading to variation in the lengths of the different transects. After having covered a transect one way, buddy pairs remained at their set depth level for 10 min to leave enough time for the fish to restore an undisturbed distribution. The end of each transect was marked with a buoy, which enabled the recording of the GPS coordinates. Buddy pairs then returned along the line transect back to the starting point. Diving was performed at 2 m above the substrate whereby dive buddies were swimming beside each other, individually counting all *Amphilophus* spp. individuals larger than ca. 5 cm within a visual field of about 4 m distance and 2 m to either side of the transect line. Snorkelers covering the 5 m depth used the same method and tried to remain at a depth of 3 m as much as possible. Owing to the difficulty to clearly identify species in sub-adult or non-breeding life stages underwater and the ongoing debate and steady changes in species classification, the overall number of *Amphilophus* spp. individuals was counted and no attempts were made to distinguish species, hybrids, or morphotypes (e.g., Barlow, 1976; McKaye et al., 2002; Bunje et al., 2007; Stauffer et al., 2008). In this visual survey a minimal bias among and within observers is expected due to individual survey differences (Thompson & Mapstone, 1997). To remove such potential confounding effects, observers alternated between different depth levels and in buddy pair partners at consecutive transects. The total number of dives over all transects was 36 (including each two persons diving back and forth), resulting in 144 single transect records.

In addition, Secchi depth measurements were taken from a boat to determine the water transparency at several random locations in both lakes.

Data analysis

To determine the average number of *Amphilophus* spp. individuals for every transect at each depth level separately, we averaged the fish counts by the two buddy team partners including the replicates from diving back and forth (Supplementary Table 3). We then calculated the average numbers of individuals per 10 m transect length for each depth level for every transect (Fig. 2), which we tested for normal distribution by applying a Shapiro–Wilk test. Using this data we tested for an overall difference in the density of *Amphilophus* spp. between lakes using Mann–Whitney U tests. We further applied a linear mixed model (LMM, LME4 package, Bates et al., 2011) to test for a difference in number and depth-distribution of individuals between the lakes by including the number of individuals counted per 10 m as the dependent variable, and lake and depth level as predictors. Assumptions of the LMM were visually checked. Since we assumed a potential difference in the depth-distribution of individuals between lakes, we included the interaction of lake and depth in the model. Furthermore, to correct for dependence in our data, we included transect as random factor. To further explore the data for effects not captured by the LMM, we applied separate Mann–Whitney U Tests for each depth level to test for depth-dependent differences in fish abundance between lakes. To roughly estimate the total number of Midas cichlids for both lakes, the numbers of fish per 10 m were extrapolated to the total circumference of the lake. This was calculated by summing up the average number of individuals at all four depth levels (Suppl. Table 3) multiplied by the circumference of the lake. All analyses was performed using R 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The average number of *Amphilophus* spp. individuals per 10 m transect length in Apoyo across all transects and depth levels was 11.3 (min = 0, max = 37, SD = 9.5), which did not differ significantly from Lake Xiloá with 19.9 fish per 10 m transect length (min = 3, max = 55, SD = 15.7) (Mann–Whitney U test, $N = 36$, $p = 0.112$). The LMM did not reveal a significant interaction between lake and depth ($t = 0.1692$, $p = 0.169$) (Fig. 2). However, testing for single depth levels between the lakes revealed a marginally significant difference at the 5 m depth level (Mann–Whitney U test, $N = 10$, $W = 18$, $p = 0.050$). The pairwise comparison of numbers of fish per 10 m transect at the other depth levels exhibited no significant difference between the lakes (Mann–Whitney U test, 10 m: $N = 10$, $p = 0.394$; 15 m: $N = 10$, $p = 0.796$; 20 m: $N = 8$, $p = 0.180$).

Extrapolating the average number of *Amphilophus* spp. individuals of all transects and depth levels to the total circumference in both lakes (Apoyo approx. 18.2 km; Xiloá approx. 8.3 km) revealed a similar

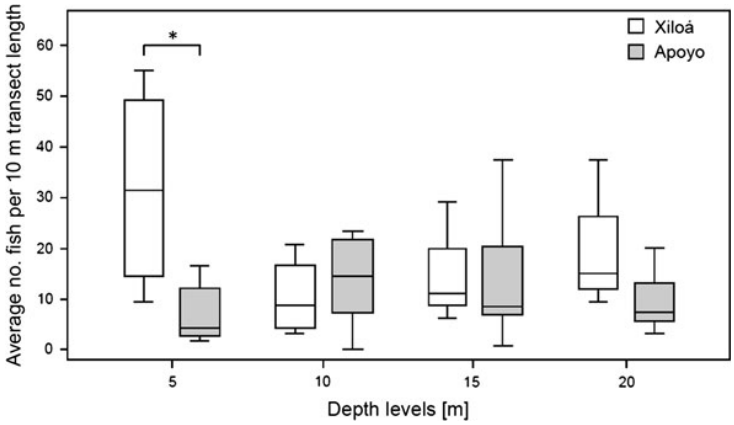


Fig. 2 Average number of *Amphilophus* spp. individuals per 10 m transect at each depth level for Lake Xiloá and Lake Apoyo. "*" denotes a marginally significant difference in cichlid fish density between the lakes (Mann–Whitney U test, $N = 10$, $p = 0.050$)

total number of fish in both lakes along the shoreline: ca. 83.000 individuals (13.000 to 150.000) in Lake Apoyo and ca. 66.000 individuals (13.000 to 120.000) in Lake Xiloá.

The Secchi depth, measured randomly several times in both lakes, ranged between 5 and 7 m in Lake Apoyo, compared to an approximately constant Secchi depth of 3 m in Lake Xiloá.

Discussion

Benefits of fish abundance estimates are diverse. The comparison of fish abundances between comparable ecosystems (e.g., between lakes) that differ in only few and well-defined ecological factors, allows to draw general conclusions on the possible impact of these factors on fish abundances and the composition and evolution of communities. This is especially the case when members of the same lineage radiated in parallel. Furthermore, in conservation biology and wildlife management, for example, changes in abundance of a fish species or population in a specific area may give an estimate for its "ecological health". This allows to define appropriate conservation strategies as well as to evaluate the (long-term) effects of habitat or species-specific conservation actions (Cheal & Thompson, 1997; Witmer, 2005). To estimate the impact of naturally induced (e.g., by a hurricane) or human-induced (e.g., by industrial fishery) changes on fish abundance, a baseline abundance needs to be established against which future levels of impact can be assessed (Jennings & Blanchard, 2004; Silvano et al., 2009). Then, abundance estimates are valuable to evaluate the relative importance and status of a fish species in an ecosystem, such as in a predator–prey relationship in the food web. Finally, mathematical modeling in fields such as evolutionary biology provides more accurate, theoretical insights into biological processes. Most often, however, theoretical approaches lack data from empirical work such as abundance estimates that would allow to make biologically reasonable assumptions and to apply mathematical models to particular case studies (see, e.g., Gavrilets et al., 2007).

The above reasons have been the motivation for this comparative study of Midas cichlid fish (*Amphilophus* spp.) abundance and density estimates in the two comparable Nicaraguan crater lakes, Apoyo and Xiloá. Despite the lack of statistical significance, our data reveal an almost twofold higher density of cichlid fish along the shoreline in Lake Xiloá as compared to Lake Apoyo. At a depth of 5 m, we found a more than fourfold higher density of Midas cichlids in Lake Xiloá (Fig. 2). Overall, however, as a consequence of the higher density of fish in the smaller lake Xiloá, the absolute numbers of *Amphilophus* spp. are relatively similar in both lakes—at least along the shore habitat covered by our survey.

Differences in food availability could explain the different densities of *Amphilophus* spp. between the two crater lakes. Indeed, the two lakes differ in their level of eutrophication: Lake Apoyo is an oligotrophic environment, whereas Lake Xiloá is relatively more eutrophic. But why would higher fish densities then only be found at shallow areas and not throughout Lake Xiloá? Eutrophication leads to a considerable reduction of ambient light at deeper waters (e.g. Koch, 2001), which can restrict photosynthesis to the shallow waters where sufficient ambient light is available for primary production (see Secchi depth in Table 1). This can directly (e.g., algae-feeders) or indirectly (e.g., through the food web) lead to higher fish densities in the shallow area. Higher fish densities in more turbid waters may also be explained by the reduced performance of predators, such as birds, which under turbid conditions have more difficulties to spot fish. It has previously been shown that reduced visibility can influence color-recognition in cichlids, and, hence, may have an impact on intraspecific (and interspecific) species recognition and communication (see, e.g., Seehausen, 1997, 2008). Whether this is also the case in Nicaraguan crater lakes remains to be tested.

An alternative explanation for the higher density of cichlids in Lake Xiloá could be the availability of ecologically more diverse niches in this lake, e.g., in the shallow area where differences in the densities of *Amphilophus* spp. are greatest. This could also explain the higher variance in fish counts at the 5 m depth level in Lake Xiloá compared to the other depth levels. Perhaps it is a combination of both factors, eutrophication and habitat complexity, that leads to higher fish densities in Lake Xiloá. A more thorough analysis of the habitat structure would be necessary to clarify this point. Furthermore, there is no knowledge on fish densities in deeper and open waters, which would allow a comprehensive comparison of both lakes. Such fish counts at deeper waters seem particularly

interesting, since we observed a distinct and clear water layer below a depth of 35 m in Lake Xiloá.

Crater lakes Apoyo and Xiloá are inhabited by a similar set of convergent *Amphilophus* ecotype morphs (Fig. 1) making the Midas Cichlid complex an ideal system to study parallel evolution (see, e.g., McKaye et al., 2002; Barluenga et al., 2006; Elmer et al., 2010). While taxonomy, morphology, and evolutionary history of the species complex is largely resolved (see Barluenga et al., 2006; Barluenga & Meyer, 2010; Elmer et al., 2010; Geiger et al., 2010a, b), little is known about basic ecological parameters such as the relative densities of the different species. Our study is the first to provide such data. We uncover a rather similar overall number of *Amphilophus* spp. individuals in both lakes, but also account differences in densities, especially in the shallow area (see above). Interestingly, the shallow areas of Lake Xiloá are not only characterized by larger densities of Midas cichlids, but also by the presence of additional cichlid species (see Supplementary Table 1). It remains unclear whether these never arrived in Lake Apoyo (e.g., because of the larger distance to a large lake), or whether these could not establish themselves there (e.g., because of the eutrophic situation). In any case, convergent phenotypes evolved in both crater lakes despite noticeable differences in size and age of the respective lake (see Table 1), in community structure (the presence/absence of other cichlid species; Supplementary Table 1), and in fish densities (Fig. 2). This corroborates the view that the initial steps of ecological speciation in fish species flocks follow similar pathways in form of a splitting into benthic and limnetic types (see, e.g., Schluter & McPhail, 1992; Salzburger, 2009), which does not seem to be dependent on phylogenetic background and parameters such as size or age of a lake or level of eutrophication. Apparently, it is enough that a benthic-limnetic axis is present in a lake (see Barluenga et al., 2006).

The Midas cichlid fauna from Lake Apoyo represents one of the most famous examples for sympatric speciation (Barluenga et al., 2006), and has attracted theoretical modeling work. Gavrilets et al. (2007), for example, investigated whether at all and under which ecological conditions sympatric speciation is likely to have occurred in lake Apoyo. One of the parameters incorporated into the model of Gavrilets et al. (2007) was the carrying capacity (K) of Lake Apoyo. Carrying capacity stands for the maximum number of individuals that can live in a particular environment given the available nutrients and without causing detrimental effects. Gavrilets et al. (2007) concluded that intermediate carrying capacities ($K = 16.000$) are propensive for sympatric speciation, whereas large carrying capacities ($K = 32.000$–51.200, depending on the model) would rather lead to the evolution of a single, generalistic species. Our estimates of K (ca. 83.000 and ca. 66.000 individuals in Lakes Apoyo and Xiloá, respectively) lie above these numbers, although these estimates refer to counts at four depth levels along the shoreline only and nothing is known about fish densities below 20 m. One also has to consider that Gavrilets et al. (2007) assumed the presence of a single age class (i.e., generation) at a given time. Our counts certainly included members from different age classes, although we lack detailed information on age distribution. Taken together, the carrying capacities assumed by Gavrilets et al. (2007) to model sympatric speciation in Lake Apoyo seem to be slightly—however not substantially—underestimated compared to our findings and it would now be interesting to evaluate what effect this has on available models.

Although a reproducing population of invasive *Oreochromis* spp. (tilapias) has been reported for Lake Apoyo in previous studies (McKaye et al., 1995; McCrary et al., 2001), we did not observe any tilapiine species during our fieldwork. These African cichlids were reported to feed on stonewort beds (*Chara* spp.) and are likely to account for the temporal elimination of these algae in Lake Apoyo (McKaye et al., 1995; McCrary et al., 2001, Canonico et al., 2005). However, we found extensive stonewort beds in Lake Apoyo. This suggests that tilapia populations might have failed to establish permanently in an oligotrophic environment such as Lake Apoyo.

Conclusions

Our study gives estimates of cichlid fish densities in two crater lakes in Nicaragua, Apoyo and Xiloá. We find that parallel ecotype morphs evolved despite noticeable differences in size, age, eutrophication level, and carrying capacity. We provide ecological data for understanding the carrying capacity of the systems in order to apply it to modeling sympatric/parapatric speciation. Furthermore, it sets baseline abundance estimates for cichlid fish in Nicaraguan

crater lakes, to which future ecological health assessments of these lakes can be compared.

Acknowledgments We are grateful to C. Heule, N. Hue and A. Theis for assisting us with diving; B. Christ and T. Suter for their helping hand at dive sites; the Ministerio del Ambiente y los Recursos Naturales Nicaragua (MARENA) for research permits; E. P. van den Berghe for logistical help and scientific expertise; and two anonymous referees, Associated Editor C. Sturmbauer and the Editor K. Martens for valuable comments on the manuscript; T. Roth for statistical support, and the "Fuerzas Armadas de Nicaragua" for boat cruises and air supply. This project was funded by grants from the European Research Council (ERC) and the Swiss NSF.

References

Arendt, J. & D. Reznick, 2008. Convergence and parallelism reconsidered: what have we learned about the genetics of adaptation? Trends in Ecology & Evolution 23: 26–32.

Barlow, G. W., 1976. The Midas cichlid in Nicaragua. In Thorson, T. B. (ed.), Investigations of the Ichthyofauna of Nicaraguan Lakes, University of Nebraska-Lincoln: 333–358.

Barluenga, M. & A. Meyer, 2004. The Midas cichlid species complex: incipient sympatric speciation in Nicaraguan cichlid fishes. Molecular Ecology 13: 2061–2076.

Barluenga, M. & A. Meyer, 2010. Phylogeography, colonization and population history of the Midas cichlid species complex (*Amphilophus* spp.) in the Nicaraguan crater lakes. BMC Evolutionary Biology 10: 326.

Barluenga, M., K. N. Stölting, W. Salzburger, M. Muschick & A. Meyer, 2006. Sympatric speciation in Nicaraguan Crater Lake cichlid fish. Nature 439: 719–723.

Bates, D., M. Mächler & B. Bolker, 2011. LME4: linear mixed-effects model using S4 classes. R package, v. 0.999375-40. http://cran.r-project.org.

Brakefield, P. M., 2006. Evo-devo and constraints on selection. Trends in Ecology & Evolution 21: 362–368.

Bunje, P. M., M. Barluenga & A. Meyer, 2007. Sampling genetic diversity in the sympatrically and allopatrically speciating Midas cichlid species complex over a 16 year time series. Bmc Evolutionary Biology 7: 25.

Canonico, G. C., A. Arthington, J. K. McCrary & M. L. Thieme, 2005. The effects of introduced tilapias on native biodiversity. Aquatic Conservation: Marine and Freshwater Ecosystems 15: 463–483.

Cheal, A. J. & A. A. Thompson, 1997. Comparing visual counts of coral reef fish: implications of transect width and species selection. Marine Ecology Progress Series 158: 241–248.

Elmer, K. R., H. Kusche, T. K. Lehtonen & A. Meyer, 2010. Local variation and parallel evolution: morphological and genetic diversity across a species complex of Neotropical Crater Lake cichlid fishes. Philosophical Transactions of the Royal Society London B 365: 1763–1782.

Gavrilets, S., A. Vose, M. Barluenga, W. Salzburger & A. Meyer, 2007. Case studies and mathematical models of ecological speciation. 1. Cichlids in a crater lake. Molecular Ecology 16: 2893–2909.

Gavrilets, S. & J. B. Losos, 2009. Adaptive radiation: contrasting theory with data. Science 323: 732–737.

Geiger, M. F., J. K. McCrary & U. K. Schliewen, 2010a. Not a simple case – A first comprehensive phylogenetic hypothesis for the Midas cichlid complex in Nicaragua (Teleostei: Cichlidae: Amphilophus). Molecular Phylogenetics and Evolution 56: 1011–1024.

Geiger, M. F., J. K. McCrary & J. R. Stauffer, 2010b. Description of two new species of the Midas cichlid complex (Teleostei: Cichlidae) from Lake Apoyo, Nicaragua. Proceedings of the Biological Society of Washington 123: 159–173.

Gíslason, D., M. M. Ferguson, S. Skúlason & S. S. Snorrason, 1999. Rapid and coupled phenotypic and genetic divergence in Icelandic Arctic charr (*Salvelinus alpinus*). Canadian Journal of Fisheries and Aquatic Sciences 56: 2229–2234.

Jennings, S. & J. Blanchard, 2004. Fish abundance with no fishing: predictions based on macroecological theory. Journal of Animal Ecology 73: 632–642.

Koch, E. W., 2001. Beyond light: physical, geological and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24: 1–17.

Kocher, T. D., 2004. Adaptive evolution and explosive speciation: the cichlid fish model. Nature Reviews Genetics 5: 288–298.

Kullander, S. & K. E. Hartel, 1997. The systematic status of cichlid genera described by Louis Agassiz in 1859: Amphilophus, Baiodon, Hypsophyrs and Parachromis (Teleostei: Cichlidae). Ichthyological Explorations of Freshwaters 7: 193–202.

Lin, S. Y., 1961. Informe al goberino de Nicaragua sobre el desarrollo de un proyecto de pesquerías continentals en dicho país. Informe F. A. O. 1347: 1–21.

Losos, J. B., T. R. Jackman, A. Larson, K. Queiroz & L. Rodriguez-Schettino, 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279: 2115–2118.

McCrary, J. K., E. P. van den Berghe, K. R. McKaye & L. J. Lopez Perez, 2001. Tilapia cultivation: a threat to native species in Nicaragua. Encuentro 58: 9–19.

McKaye, K. R., J. D. Ryan, J. R. Stauffer, L. J. Lopez Perez & E. P. van den Berghe, 1995. African tilapia in Lake Nicaragua: ecosystem in transition. BioScience 45: 406–411.

McKaye, K. R., J. R. Stauffer, E. P. van den Berghe, R. Vivas, L. J. Lopez Perez, J. K. McCrary, R. Waid, A. Konings, W. J. Lee & T. D. Kocher, 2002. Behavioral, morphological and genetic evidence of divergence of the Midas Cichlid species complex in two Nicaraguan crater lakes. Cuadernos de Investigación de la UCA 12: 19–47.

Rutschmann, S., M. Matschiner, M. Damerau, M. Muschick, M. F. Lehmann, R. Hanel & W. Salzburger, 2011. Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Molecular Ecology 20: 4707–4721.

Salzburger, W., 2009. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. Molecular Ecology 18: 169–185.

Schliewen, U. K., D. Tautz & S. Paabo, 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. Nature 368: 629–632.

Schluter, D. & J. D. McPhail, 1992. Ecological character displacement and speciation in sticklebacks. American Naturalist 140: 85–108.

Schluter, D. & L. Nagel, 1995. Parallel speciation by natural selection. American Naturalist 140: 292–301.

Schuster, W. H., 1957. Informe al goberino de Nicaragua sobre fomento de la pesca contonental. Informe F. A. O. 607: 1–13.

Seehausen, O., 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277: 1808–1811.

Seehausen, O., Y. Terai, I. S. Magalhaes, K. L. Carleton, H. D. J. Mrosso, R. Miyagi, I. van der Sluijs, et al., 2008. Speciation through sensory drive in cichlid fish. Nature 455: 620–626.

Silvano, R. A. M., M. Ramires & J. Zuanon, 2009. Effects of fisheries management on fish communities in the floodplain lakes of a Brazilian Amazonian Reserve. Ecology of Freshwater Fish 18: 156–166.

Stauffer, J. R. & K. R. McKaye, 2002. Descriptions of three new species of cichlid fishes (Teleostei: Cichlidae) from Lake Xiloá, Nicaragua. Cuadernos de Investigacíon de la Universidad Centroamericana 12: 1–18.

Stauffer, J. R., J. K. McCrary & K. E. Black, 2008. Three new species of cichlid fishes (Teleostei: Cichlidae) from Lake Apoyo, Nicaragua. Proceedings of the Biological Society Washington 121: 117–129.

Streelman, J. T. & P. D. Danley, 2003. The stages of vertebrate evolutionary radiation. Trends in Ecology & Evolution 18: 126–131.

Sturmbauer, C., M. Husemann & P. D. Danley, 2011. Explosive speciaiton and adaptive radiation of East African cichlid fishes. In Zachos, F. E. & J. C. Habel (eds.), Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas. Springer: 333–362.

Sussman, D., 1985. Apoyo caldera, Nicaragua: a major quaternary silicic eruptive center. Journal of Volcanology and Geothermal Research 24: 249–282.

Thompson, A. A. & B. D. Mapstone, 1997. Observer effects and training in underwater visual surveys of reef fishes. Marine Ecology Progress Series 154: 53–63.

Waid, R. M., R. L. Raesly, K. R. McKaye & J. K. McCrary, 1999. Zoogeografía íctica de lagunas cratéricas de Nicaragua. Encuentro 51: 65–80.

Witmer, G. W., 2005. Wildlife population monitoring: some practical considerations. Wildlife Research 32: 259.

Supplementary Materials

Depth-dependent abundance of Midas Cichlid fish (*Amphilophus spp.*) in two Nicaraguan crater lakes

Marie Theres Dittmann*, Marius Roesti*, **Adrian Indermaur**, Marco Colombo, Martin Gschwind, Isabel Keller, Robin Kovac, Marta Barluenga, Moritz Muschick, Walter Salzburger

*these authors contributed equally to this work

Supplementary Table 1: Cichlid fish diversity in lakes Apoyo and Xiloá.

Lake Apoyo – Midas cichlid species (endemic)
Amphilophus zaliosus Barlow and Munsey 1976
Amphilophus flaveolus Stauffer *et al.* 2008
Amphilophus chancho Stauffer *et al.* 2008
Amphilophus astorquii Stauffer *et al.* 2008
Amphilophus globosus Geiger *et al.* 2010
Amphilophus supercilius Geiger *et al.* 2010
Lake Apoyo – other cichlid species (introduced)
Parachromis managuense Kallander 1997
Oreochromis aureus Steindachner 1864
Oreochromis niloticus Linnaeus 1758
Lake Xiloá – Midas cichlid species (endemic)
Amphilophus xiloaensis Stauffer and McKaye 2002
Amphilophus amarillo Stauffer and McKaye 2002
Amphilophus sagittae Stauffer and McKaye 2002
Amphilophus sp. "Fat lips" (Stauffer and McKaye 2002, undescribed)
Lake Xiloá – other cichlid species (native)
Astatoheros longimanus Jordan *et al.* 1930
Archocentrus centrarchus Jordan *et al.* 1930
Amphilophus rostratus Kullander 1996
Parachromis dovii Kullander *et al.* 1997
Hypsophrys nicaraguensis Kullander *et al.* 1997
Parachromis managuense Kullander *et al.* 1997
Hypsophrys nematopus Chakrabarty *et al.* 2007
Amantitlania siquia Schmitter-Soto 2007

Supplementary Table 2: Coordinates and length of the transects in lakes Apoyo and Xiloá. Lengths were calculated by measuring start and end coordinates of each transect with a GPS device.

Lake	Transect	Start coordinate	Length [m]
Apoyo	1	11°54,554' N / 86°02,467' W	120
	2	11°54,183' N / 86°01.791' W	115
	3	11°55,626' N / 86°00,854' W	80
	4	11°56,196' N / 86°01,371' W	80
	5	11°56,002' N / 86°03,391' W	80
	6	11°92,538' N / 86°05,557' W	80
Xiloá	1	12°23,120' N / 86°31,857' W	40
	2	12°23,081' N / 86°32,259' W	40
	3	12°21.483' N / 86°32,548' W	50
	4	12°21.428' N / 86°31,510' W	50

Supplementary Table 3: Averaged numbers of cichlid fish per 10 m transect for each transect and depth level. Numbers are the averaged fish counts by the two buddy team partners including the replicates from diving back and forth.

Lake	Transect	Depth [m]				
		5	10	15	20	total
Apoyo	1	-	7.0	6.6	5.7	6.4
	2	12.3	21.7	9.9	13.2	14.3
	3	2.4	23.0	37.3	-	20.9
	4	16.2	21.8	20.4	20.1	19.6
	5	4.3	0.0	0.6	3.2	2.0
	6	1.6	6.9	6.8	7.2	5.6
	total	7.4	14.7	15.0	8.7	11.4
Xiloá	1	43.3	12.6	29.1	9.6	23.7
	2	55.0	5.5	-	37.3	32.6
	3	19.4	20.9	11.2	-	17.2
	4	9.7	3.2	6.4	15.1	8.6
	total	31.9	10.6	15.6	20.7	19.7

part two:

Phylogeography

Chapter 4

Back to Tanganyika: a case of recent trans-species-flock dispersal in East African haplochromine cichlid fishes

Adrian Indermaur*, Britta S. Meyer*, Xenia Ehrensperger, Bernd Egger, Gaspard Banyankimbona, Jos Snoeks and Walter Salzburger

* authors contributed equally to this work

Biology Letters (2014)

AI designed the study, conducted fieldwork, sequecing and helped with data analysis, disscusion and manuskript preparation

ROYAL SOCIETY OPEN SCIENCE

rsos.royalsocietypublishing.org

Brief report

Cite this article: Meyer BS, Indermaur A, Ehrensperger X, Egger B, Banyankimbona G, Snoeks J, Salzburger W. 2015 Back to Tanganyika: a case of recent trans-species-flock dispersal in East African haplochromine cichlid fishes. *R. Soc. open sci.* **2**: 140498.
http://dx.doi.org/10.1098/rsos.140498

Received: 3 December 2014
Accepted: 9 February 2015

Subject Category:
Biology (whole organism)

Subject Areas:
evolution/taxonomy and systematics

Keywords:
Haplochromis sp. 'Chipwa', adaptive radiation, superflock, Lake Victoria

Author for correspondence:
Walter Salzburger
e-mail: walter.salzburger@unibas.ch

†Present address: Evolutionary Ecology of Marine Fishes, GEOMAR Helmholtz Centre for Ocean Research, Kiel 24105, Germany.
‡These authors contributed equally to this study.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsos.140498 or via http://rsos.royalsocietypublishing.org.

Royal Society Publishing
Informing the science of the future

Back to Tanganyika: a case of recent trans-species-flock dispersal in East African haplochromine cichlid fishes

Britta S. Meyer[1,†,‡], Adrian Indermaur[1,‡], Xenia Ehrensperger[1], Bernd Egger[1], Gaspard Banyankimbona[2,3], Jos Snoeks[3,4] and Walter Salzburger[1]

[1]Zoological Institute, University of Basel, Basel 4051, Switzerland
[2]Department of Biology, University of Burundi, Bujumbura, Burundi
[3]Royal Museum for Central Africa, Tervuren 3080, Belgium
[4]Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Leuven 3000, Belgium

BSM, 0000-0002-2549-1825; WS 0000-0002-9988-1674

1. Summary

The species flocks of cichlid fishes in the East African Great Lakes are the largest vertebrate adaptive radiations in the world and illustrious textbook examples of convergent evolution between independent species assemblages. Although recent studies suggest some degrees of genetic exchange between riverine taxa and the lake faunas, not a single cichlid species is known from Lakes Tanganyika, Malawi and Victoria that is derived from the radiation associated with another of these lakes. Here, we report the discovery of a haplochromine cichlid species in Lake Tanganyika, which belongs genetically to the species flock of haplochromines of the Lake Victoria region. The new species colonized Lake Tanganyika only recently, suggesting that faunal exchange across watersheds and, hence, between isolated ichthyofaunas, is more common than previously thought.

2. Introduction

Adaptive radiation, the rapid evolution of novel species as a consequence of adaptation to distinct ecological niches, is thought to have played an important role in the origin of phenotypic diversity [1]. The species flocks of cichlid fishes in the African Great Lakes; Tanganyika, Malawi and Victoria are the most

species-rich vertebrate adaptive radiations, consisting of hundreds of endemic species each [2–4]. Lake Tanganyika, the oldest lake, harbours the genetically and phenotypically most diverse cichlid assemblage comprising 12–16 'tribes' [5]. The radiations in Lakes Malawi and Victoria involve only one of these tribes, the Haplochromini, making this the most species-rich cichlid lineage [4].

The haplochromines probably originated in the area of Lake Tanganyika, from where they colonized water bodies in large parts of Africa, including Lakes Malawi and Victoria [6–8]. This 'out of Tanganyika' scenario [6] implies that the seeding events of the haplochromine radiations in Lakes Malawi and Victoria date back to 1–5 and less than 0.25 Ma, respectively [6–9]. The latter radiation is not confined to only the basin of Lake Victoria, but includes the cichlid faunas of other lakes and rivers in the area, including Lakes Edward, George, Kivu and the Lake Rukwa drainage; it is hence referred to as the 'Lake Victoria region superflock' (LVRS) [6,7,10].

While Lake Tanganyika's cichlid assemblage has long been regarded as polyphyletic [11], the haplochromines from Lake Malawi and the LVRS were considered reciprocally monophyletic [7,12,13]. This view has recently been challenged with the analysis of large sets of nuclear DNA markers, which uncovered a polyphyletic origin of Lake Malawi's haplochromines [14,15], and high levels of shared genetic polymorphisms between the cichlid faunas of all three lakes [15,16]. These findings, together with the identification of similar or even identical genotypes across large geographical scales [17,18], suggest that the hydrologic systems in East Africa are more permeable for cichlids than previously thought. It has even been proposed that riverine species have 'transported' polymorphisms between lakes [15].

Interestingly, however, not a single case of a recent colonization of a Great Lake through a riverine lineage has been documented, and none of these lakes is known to contain a species belonging to a lineage associated with another Great Lake's radiation. Here we report the discovery of a haplochromine cichlid species in Lake Tanganyika, which belongs genetically to the LVRS.

3. Material and methods

In 2011 and 2012, we collected 12 specimens of a new haplochromine species (named *Haplochromis* sp. 'Chipwa' hereafter) in a shoreline habitat within Lake Tanganyika at Chipwa Village, between 500 and 1000 m south from the Kalambo River mouth. Five additional specimens were sampled in 2011 in the Lufubu River delta on Lake Tanganyika's western shoreline (open water distance between these locations: more than 55 km; figure 1*a*, *b*). In both localities, the new species co-occurs with the widespread haplochromine *Astatotilapia burtoni* found within Lake Tanganyika and in affluent rivers [20]. The new taxon was identified as undescribed species in the field by A.I.

For comparative reasons, we sampled additional haplochromines, including a morphologically similar species (*Haplochromis stappersii*) from rivers Malagarasi ($n = 4$) and Rusizi ($n = 1$) (electronic supplementary material, tables S1–S3). Sampling was performed using our standard operating procedure [21]; vouchers were deposited at the University of Basel or the Royal Museum of Central Africa, Tervuren.

In order to place the new taxon into a phylogenetic context, we amplified and sequenced two nuclear (*ednrb1*: 524 bp; *phpt1*: 434 bp) and two mitochondrial (mtDNA) loci (d-loop: 373 bp; ND2: 1047 bp), following the protocols described elsewhere [21,22]. These markers were chosen on the basis of the existence of large quantities of reference data on GenBank. The newly obtained sequences were inspected by eye in CodonCodeAligner, combined with available data from GenBank, aligned with MAFFT [23], and the appropriate models of molecular evolution were determined with jModelTest [24]. All specimens of the new species were identical in all four loci.

To identify the placement of the new species in the haplochromine phylogeny, we performed a stepwise approach using three different datasets: first, we wanted to confirm our *ad hoc* assumption that the new taxon does not belong to any of the Tanganyikan cichlid lineages (and genera) known to date. To this end, we combined the nuclear and ND2 sequences of the new species with a representative set including all East African cichlid lineages [21], resulting in a total of 83 taxa. The concatenated data (2001 bp) was analysed using Bayesian inference with MrBayes [25] (10 000 000 generations, four chains, two runs, 25% burn-in, three partitions: GTR + I + Γ; GTR + I + Γ; GTR + Γ) and maximum likelihood (ML) with GARLI (http://garli.nescent.org) (50 runs, 500 bootstrap replicates; three partitions: TIM3 + I + Γ; TVM + I + Γ; TPM2uf + Γ). In a second step, we focused on ND2 only, as many more reference data are available for this common marker in cichlids [6,8]. We again combined our data with available sequences from GenBank (216 taxa in total) and used MrBayes (3 000 000 generations, four chains, two runs, 25% burn-in; GTR + I + Γ) and GARLI (50 runs, 500 bootstraps; TIM2 + I + Γ). On the basis of this tree, we selected 86 taxa for an in-depth analysis focusing on the species belonging to the LVRS and its

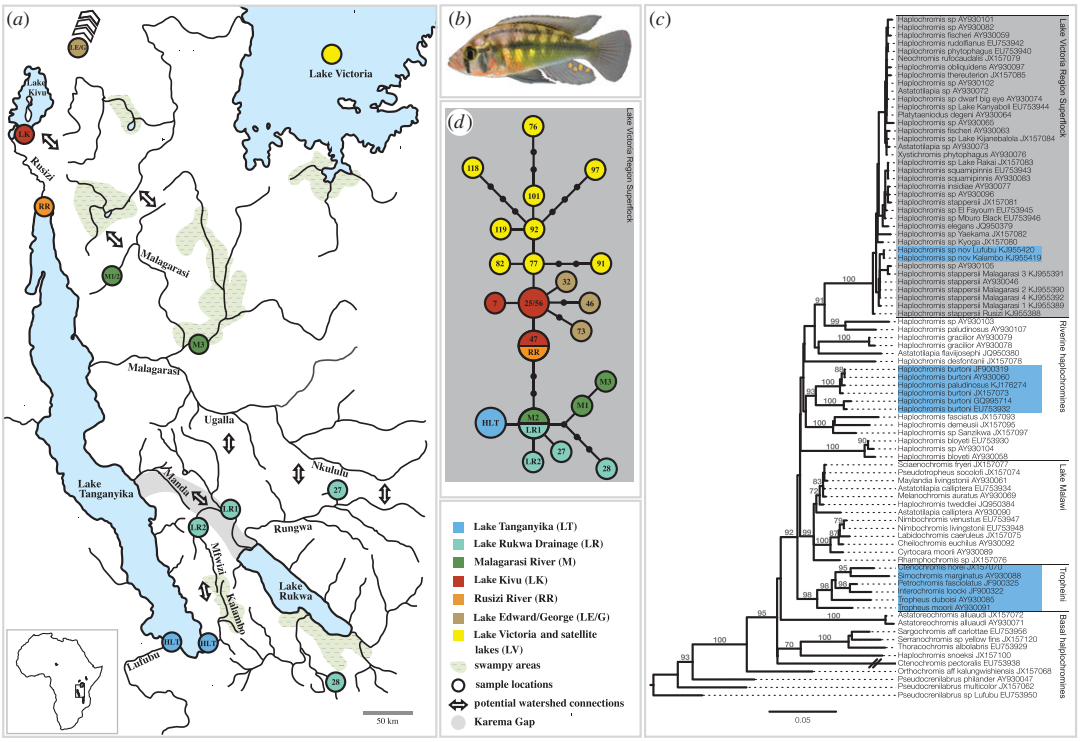


Figure 1. (*a*) Map of the study area indicating sample locations and potential watershed connections. (*b*) *Haplochromis* sp. 'Chipwa' (male) from LT. (*c*) ML phylogeny of haplochromine cichlids based on the mitochondrial ND2. *Haplochromis* sp. 'Chipwa' is firmly placed within the LVRS (grey box); the specimens from LT are depicted in blue. (*d*) Mitochondrial haplotype genealogy of representative haplotypes of the LVRS and the new species (see also the electronic supplementary material, figure S3) based on a 365 bp segment of the control region. The identification of a shared haplotype between the Malagarasi and the LR basin (M2/LR1) corroborates a recent connection between these watersheds, e.g. via 'Ugalla–Rungwa' or 'Nkululu–Rungwa' connections [19]. Colour-codes correspond to (*a*) and (*c*), haplotype numbers refer to [7].

closest sister taxa (MRBAYES: 10 000 000 generations, four chains, two runs, 25% burn-in, GTR + I + Γ; GARLI: 50 runs, 500 bootstraps, TrN + I + Γ). Finally, we integrated the mitochondrial control region sequences of *H*. sp. 'Chipwa' in the largest available dataset of members of the LVRS [7]. We performed an analysis using 178 unique mitochondrial haplotypes [7], representing about 900 specimens of the LVRS plus outgroup taxa, using GARLI (50 runs; 500 bootstraps; K81uf + I + Γ). On the basis of the resultant tree, we chose a representative subset of 27 sequences to construct a haplotype genealogy following the method described in [19] and using the first segment of the mitochondrial control region (373 bp).

4. Results

The analysis of the concatenated nuclear and mtDNA dataset resulted in highly congruent trees (electronic supplementary material, figure S1), in which *H*. sp. 'Chipwa' formed a strongly supported clade with four taxa representing the LVRS (ML bootstrap = 100, posterior probability = 1), thus confirming previous results based on a large set of nuclear DNA markers [26].

In the more inclusive ND2 phylogeny, the new species was firmly placed within the LVRS *sensu* [7] (electronic supplementary material, figure S2; ML bootstrap = 100, posterior probability = 1). Within this clade, the single ND2 haplotype of the new species from Lake Tanganyika clustered with *H. stappersii* from the Malagarasi River plus another undescribed species from Tanzania (figure 1*c*). Interestingly, two *H. stappersii* were not part of this clade: the sample from Rusizi River in Burundi and the one with unknown sampling location used by Schwartzer *et al*. [18], suggesting that specimens previously identified as *H. stappersii* are not reciprocally monophyletic and belong to at least two distinct mitochondrial lineages.

In the mtDNA haplotype genealogy, the new species was grouped into a clade of riverine taxa derived from the central haplotype of the LVRS (haplotype 25 in [7]; see the electronic supplementary material, figure S3). The reduced dataset (figure 1*d*) highlights that the single haplotype found in *H*. sp. 'Chipwa'

from Lake Tanganyika is derived from the central haplotype of this riverine clade (M2/LR1) by one mutation (nucleotide divergence: 0.29%). We refrained from performing a molecular clock analysis here, which is problematic with just one mutational difference. However, a single difference in the cichlids' mitochondrial control region is typically interpreted as recent and in the range of a maximum of tens of thousands of years [7,9].

5. Discussion

In this study, we report the discovery of a haplochromine species in Lake Tanganyika, which belongs to a clade of riverine haplochromines that is part of the LVRS (figure 1; electronic supplementary material, figures S1–S3). The phylogenetic position of the new species and the existence of identical mtDNA haplotypes on both sides of Lake Tanganyika suggest that this taxon colonized this lake recently and spread across its southern basin. Accidental translocation, e.g. with aquacultured tilapia, seems unlikely given the absence of farmed tilapia at the sampling localities. Instead, it appears likely that the new species entered Lake Tanganyika naturally.

East Africa is a geologically active area and it has been assumed that river captures mediated by tectonic movements, erosion and fluctuations in precipitation allowed for past connections between watersheds [27–30]. Since the mtDNA haplotype of the new species (HLT in figure 1) is derived from the central haplotype (M2/LR1) found in the Malagarasi and in the Lake Rukwa drainage, two alternative dispersal scenarios emerge: either via the Malagarasi River followed by southward coastal migration, or from the Lake Rukwa drainage. Given the large geographical distance between the Malagarasi River and the collection sites and that we never caught any specimen in the coastline north of the Kalambo estuary, the latter scenario appears more plausible—especially, since geological evidence suggests that Lake Rukwa was connected to Lake Tanganyika in the Early Holocene via the Karema Gap [29]. The existence of such a connection has further been corroborated with fossil molluscs and ostracods in Lake Rukwa, which resemble extant taxa from Lake Tanganyika [28]. Another recent Lake Rukwa–Lake Tanganyika connection has been hypothesized in the Kalambo-Mwimbi fault, where rivers Kalambo and Mfiwizi run, in close proximity and in opposite direction, through a swampy depression [27]. Any fish migrating downstream the Kalambo River would, however, face the challenge of a 221 m high waterfall.

With the finding of a member of the LVRS in Lake Tanganyika, we provide, to our knowledge, the first record of a cichlid species in an East African Great Lake that features genetic affinities to the fauna of another Great Lake. More precisely, we show that a haplochromine species belonging to the most recent large-scale cichlid adaptive radiation, the LVRS dated at less than 0.25 Ma [6–9], managed to migrate into the much older Lake Tanganyika, and to establish itself alongside the existing lake endemics. *Haplochromis* sp. 'Chipwa' thus represents yet another cichlid lineage that independently colonized Lake Tanganyika. Our discovery thus lends empirical support to the hypothesis that occasional migration of riverine taxa into lakes might have 'transported' genetic polymorphism between the cichlid species flocks in the East African Great Lakes [15]. Note, however, that we only demonstrated the first step required by the 'transporter hypothesis', i.e. the arrival of a distantly related haplochromine species into an established cichlid radiation. Whether this resulted in the second step, i.e. gene-flow from a divergent lineage into an established lacustrine species, remains unanswered and should be examined in the future.

Taken together, we demonstrate that recent faunal exchange occurred between the otherwise non-overlapping cichlid assemblages of the LVRS and Lake Tanganyika, thereby extending the area covered by LVRS taxa to now also include the southern part of Lake Tanganyika and affluent rivers. Our findings are in line with recent reports of shared mtDNA haplotypes across large geographical scales in haplochromines [17,18] and, particularly, with the view that faunal exchange between cichlid faunas of rivers and lakes is more common than previously thought [15]. We thus suggest that more attention should be directed towards the survey of riverine cichlid communities, which are understudied compared to the endemic faunas of Lakes Tanganyika, Malawi and Victoria.

Ethics statement. This study was performed under research permits issued by the Lake Tanganyika Research Unit, Department of Fisheries, Republic of Zambia and the cantonal veterinary office Basel (permit no. 2317).

Data accessibility. Sequence data has been deposited at GenBank under the accession numbers KJ955381-KJ955446.

Acknowledgements. We thank the Fisheries Department, Republic of Zambia and the Faculty of Sciences, University of Burundi, for permits; M. Colombo, F. Ronco, A. Rugirabirori and A. Theis for fieldwork assistance; T. C. Johnson for discussion and three anonymous referees for valuable suggestions.

Funding statement. This study was supported by the FAG-Basel (to B.S.M., A.I.), the Burchkardt-Bürgin-Stiftung (B.S.M.) and the Swiss National Science Foundation and European Research Council/ERC (W.S.).

Competing interests. We declare we have no competing interests.

References

1. Schluter D. 2000 *The ecology of adaptive radiation*. Oxford, UK: Oxford University Press.
2. Kocher TD. 2004 Adaptive evolution and explosive speciation: the cichlid fish model. *Nat. Rev. Genet.* **5**, 288–298. (doi:10.1038/nrg1316)
3. Seehausen O. 2006 African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. B* **273**, 1987–1998. (doi:10.1098/rspb.2006.3539)
4. Salzburger W. 2009 The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Mol. Ecol.* **18**, 169–185. (doi:10.1111/j.1365-294X.2008.03981.x)
5. Koblmüller S, Sefc KM, Sturmbauer C. 2008 The Lake Tanganyika cichlid species assemblage: recent advances in molecular phylogenetics. *Hydrobiologia* **615**, 5–20. (doi:10.1007/s10750-008-9552-4)
6. Salzburger W, Mack T, Verheyen E, Meyer A. 2005 Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol. Biol.* **5**, 17. (doi:10.1186/1471-2148-5-17)
7. Verheyen E, Salzburger W, Snoeks J, Meyer A. 2003 Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science* **300**, 325–329. (doi:10.1126/science.1080699)
8. Koblmüller S, Schliewen UK, Duftner N, Sefc KM, Katongo C, Sturmbauer C. 2008 Age and spread of the haplochromine cichlid fishes in Africa. *Mol. Phylogenet. Evol.* **49**, 153–169. (doi:10.1016/j.ympev.2008.05.045)
9. Genner MJ, Seehausen O, Lunt DH, Joyce DA, Shaw PW, Carvalho GR, Turner GF. 2007 Age of cichlids: new dates for ancient lake fish radiations. *Mol. Biol. Evol.* **24**, 1269–1282. (doi:10.1093/molbev/msm050)
10. Nagl S, Tichy H, Mayer WE, Takezaki N, Takahata N, Klein J. 2000 The origin and age of haplochromine fishes in Lake Victoria, East Africa. *Proc. R. Soc. Lond. B* **267**, 1049–1061. (doi:10.1098/rspb.2000.1109)
11. Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C. 2002 Phylogeny of the Lake Tanganyika cichlid species flock and its relationship to the Central and East African haplochromine cichlid fish faunas. *Syst. Biol.* **51**, 113–135. (doi:10.1080/106351502753475907)
12. Meyer A, Kocher TD, Basasibwaki P, Wilson AC. 1990 Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553. (doi:10.1038/347550a0)
13. Moran P, Kornfield I, Reinthal PN. 1994 Molecular systematics and radiation of the haplochromine cichlids (Teleostei: Perciformes) of Lake Malawi. *Copeia* **2**, 274–288. (doi:10.2307/1446977)
14. Joyce DA, Lunt DH, Genner MJ, Turner GF, Bills R, Seehausen O. 2011 Repeated colonization and hybridization in Lake Malawi cichlids. *Curr. Biol.* **21**, R108–R109. (doi:10.1016/j.cub.2010.11.029)
15. Loh YH *et al.* 2013 Origins of shared genetic variation in African cichlids. *Mol. Biol. Evol.* **30**, 906–917. (doi:10.1093/molbev/mss326)
16. Brawand D *et al.* 2014 The genomic substrate for adaptive radiation in African cichlid fish. *Nature* **513**, 375–381. (doi:10.1038/nature13726)
17. Hermann CM, Sefc KM, Koblmüller S. 2011 Ancient origin and recent divergence of a haplochromine cichlid lineage from isolated water bodies in the East African Rift System. *J. Fish Biol.* **79**, 1356–1369. (doi:10.1111/j.1095-8649.2011.03101.x)
18. Schwartzer J, Swartz ER, Vreven E, Snoeks J, Cotterill FP, Misof B, Schliewen UK. 2012 Repeated trans-watershed hybridization among haplochromine cichlids (Cichlidae) was triggered by Neogene landscape evolution. *Proc. R. Soc. B* **279**, 4389–4398. (doi:10.1098/rspb.2012.1667)
19. Salzburger W, Ewing GB, Von Haeseler A. 2011 The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Mol. Ecol.* **20**, 1952–1963. (doi:10.1111/j.1365-294X.2011.05066.x)
20. Theis A, Ronco R, Indermaur A, Salzburger W, Egger B. 2014 Adaptive divergence between lake and stream populations of an East African cichlid fish. *Mol. Ecol.* **23**, 5304–5322. (doi:10.1111/mec.12939)
21. Muschick M, Indermaur A, Salzburger W. 2012 Convergent evolution within an adaptive radiation of cichlid fishes. *Curr. Biol.* **22**, 2362–2368. (doi:10.1016/j.cub.2012.10.048)
22. Meyer BS, Salzburger W. 2012 A novel primer set for multilocus phylogenetic inference in East African cichlid fishes. *Mol. Ecol. Res.* **12**, 1097–1104. (doi:10.1111/j.1755-0998.2012.03169.x)
23. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780. (doi:10.1093/molbev/mst010)
24. Posada D. 2008 jModelTest: phylogenetic model averaging. *Mol. Biol. Evol.* **25**, 1253–1256. (doi:10.1093/molbev/msn083)
25. Ronquist F *et al.* 2012 MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **61**, 539–542. (doi:10.1093/sysbio/sys029)
26. Meyer BS, Matschiner M, Salzburger W. 2015 A tribal level phylogeny of Lake Tanganyika cichlid fishes based on a genomic multi-marker approach. *Mol. Phyl. Evol*. **83C**, 56–71. (doi:10.1016/j.ympev2014.10.009)
27. Seegers L. 1996 The fishes of the Lake Rukwa drainage. *Ann. Mus. R. Afr. Centr. Sci. Zool.* **287**, 1–407.
28. Cohen AS, Van Bocxlaer B, Todd JA, McGlue M, Michel E, Nkotagu HH, Grove AT, Delvaux D. 2013 Quanternary ostracods and molluscs from the Rukwa Basin (Tanzania) and their evolutionary and palaeobiogeographic implications. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **392**, 97–97. (doi:10.1016/j.palaeo.2013.09.007)
29. Delvaux D, Kervyn F, Vittori E, Kajara RSA, Kilembe E. 1998 Late Quaternary tectonic activity and lake level change in the Rukwa Rift Basin. *J. Afr. Earth Sci.* **26**, 397–421. (doi:10.1016/S0899-5362(98)00023-2)
30. Salzburger W, Van Bocxlaer B, Cohen AS. 2014 Ecology and evolution of the African Great Lakes and their faunas. *Annu. Rev. Ecol. Evol. Syst.* **45**, 519–545. (doi:10.1146/annurev-ecolsys-120213-091804)

Supplementary Materials

Back to Tanganyika: a case of recent trans-species-flock dispersal in East African haplochromine cichlid fishes

Adrian Indermaur*, Britta S. Meyer*, Xenia Ehrensperger, Bernd Egger, Gaspard Banyankimbona, Jos Snoeks and Walter Salzburger

* authors contributed equally to this work

Supplementary table 1: List of 83 cichlid specimens, their mitochondrial ND2 and their nuclear gene (ednrb, phpt1) accession numbers and their sample locations.

	GenBank accession numbers			Sampling information	
Species	**nd2**	**ednrb**	**phpt1**	**Locality**	**Coordinates**
Altolamprologus calvus	EF462256	JF900248	JF900177	Lake Tanganyika	-
Altolamprologus compressiceps	EF462257	JF900249	JF900178	Lake Tanganyika	-
Asprotilapia leptura	KJ955424	JF900251	JF900180	Lake Tanganyika	-
Haplochromis sp. nov. "Kalambo"	KJ955419	KJ955401	KJ955436	Kalambo River, Zambia	S08°36'06.34"; E031°11'12.73"
Haplochromis sp. nov. "Lufubu"	KJ955420	KJ955402	KJ955437	Lufubu River, Zambia	S08°33'41.25"; E030°43' 26.54"
Astatoreochromis alluaudi	KJ955410	KJ955393	KJ955429	Aquaria Stock, Lake Victoria	-
Astatotilapia burtoni	KJ955411	KJ955394	KJ955430	Aquaria Stock, Lake Tanganyika	-
Astatotilapia burtoni	JF900319	JF900252	JF900181	Lake Tanganyika	-
Astatotilapia calliptera	KJ955412	KJ955398	KJ955431	Aquaria Stock, Lake Malawi	-
Aulonocranus dewindti	AY337782	JF900253	JF900182	Lake Tanganyika	-
Baileychromis centropomoides	KJ955423	KJ955406	KJ955432	Mpulungu Market, Zambia	S8° 45' 56.737" E31° 6' 49.715"
Bathybates graueri	AY663726	JF900254	JF900183	Lake Tanganyika	-
Bathybates vittatus	AY663728	JF900255	JF900184	Lake Tanganyika	-
Benthochromis tricoti	AF317264	JF900256	JF900185	Lake Tanganyika	-
Boulengerochromis microlepis	AF317229	JF900257	JF900186	Lake Tanganyika	-
Callochromis macrops	AY337795	JF900258	JF900187	Lake Tanganyika	-
Chalinochromis brichardi	EF679241	JF900259	JF900188	Lake Tanganyika	-
Cyphotilapia gibberosa	EF679242	JF900260	JF900189	Lake Tanganyika	-
Ctenochromis horei	EU753935	JF900262	JF900191	Lake Tanganyika	-
Cyathopharynx furcifer	AY337781	JF900263	JF900192	Lake Tanganyika	-
Cyprichromis leptosoma	AY740337	JF900264	JF900193	Lake Tanganyika	-
Ectodus descampsii	AY337790	JF900265	JF900195	Lake Tanganyika	-
Enantiopus melanogenys	AY682517	JF900266	JF900194	Lake Tanganyika	-
Eretmodus cyanostictus	AF398220	JF900267	JF900196	Lake Tanganyika	-
Gnathochromis permaxillaris	JF900321	JF900268	JF900197	Lake Tanganyika	-
Gnathochromis pfefferi	U07248	JF900269	JF900198	Lake Tanganyika	-
Grammatotria lemairii	AY337787	JF900270	JF900199	Lake Tanganyika	-
Greenwoodochromis christyi	AY682528	JF900272	JF900201	Lake Tanganyika	-
Haplotaxodon microlepis	EF437497	JF900273	JF900202	Lake Tanganyika	-
Haplochromis obliquidens	KJ955416	KJ955403	KJ955433	Aquaria Stock, Lake Victoria	-
Haplochromis rockkribensis	KJ955418	KJ955404	KJ955434	Aquaria Stock, Lake Victoria	-
Haplotaxodon trifasciatus	EF437492	JF900274	JF900203	Lake Tanganyika	-
Interochromis loocki	JF900322	JF900303	JF900232	Lake Tanganyika	-
Julidochromis ornatus	EF462229	JF900275	JF900204	Lake Tanganyika	-
Lamprologus callipterus	AF398226	JF900276	JF900205	Lake Tanganyika	-
Lamprologus lemairii	EF462271	JF900277	JF900206	Lake Tanganyika	-
Lamprologus ornatipinnis	EF462260	JF900278	JF900207	Lake Tanganyika	-
Limnochromis abeelei	AY682533	JF900279	JF900208	Lake Tanganyika	-
Lepidiolamprologus attenuatus	EF462274	JF900282	JF900211	Lake Tanganyika	-
Lepidiolamprologus elongatus	EF462268	JF900283	JF900212	Lake Tanganyika	-
Lepidiolamprologus cf. profundicola	EF462276	JF900284	JF900213	Lake Tanganyika	-
Limnotilapia dardennii	GQ995724	JF900285	JF900214	Lake Tanganyika	-
Lobochilotes labiatus	U07254	JX402345	JF900215	Lake Tanganyika	-
Microdontochromis tenuidentatus	AY337784	JF900287	JF900216	Lake Tanganyika	-
Neolamprologus furcifer	EF679252	JF900288	JF900217	Lake Tanganyika	-
Neolamprologus modestus	DQ055012	JF900289	JF900218	Lake Tanganyika	-

Neolamprologus prochilus	EF462248	JF900290	JF900219	Lake Tanganyika	-
Neolamprologus pulcher	EF462244	JF900291	JF900220	Lake Tanganyika	-
Neolamprologus savoryi	HM623796	JF900292	JF900221	Lake Tanganyika	-
Neolamprologus sexfasciatus	HM623828	JF900293	JF900222	Lake Tanganyika	-
Neolamprologus tetracanthus	EF462220	JF900294	JF900223	Lake Tanganyika	-
Ophthalmotilapia ventralis	AY337774	JF900295	JF900224	Lake Tanganyika	-
Oreochromis tanganicae	AF317240	JF900296	JF900225	Lake Tanganyika	-
Paracyprichromis brieni	AY740378	JF900297	JF900226	Lake Tanganyika	-
Perissodus microlepis	AF398222	JF900298	JF900227	Lake Tanganyika	-
Plecodus paradoxus	EF437500	JF900299	JF900228	Lake Tanganyika	-
Petrochromis famula	JF900324	JF900301	JF900230	Lake Tanganyika	-
Petrochromis fasciolatus	JF900325	JF900302	JF900231	Lake Tanganyika	-
Petrochromis macrognathus	AY930068	JF900304	JF900233	Lake Tanganyika	-
Petrochromis polyodon	JF900326	JF900305	JF900234	Lake Tanganyika	-
Pharyngochromis acuticeps	KJ955421	KJ955396	KJ955438	Kafue, Zambia	-
Plecodus straeleni	EF437481	JF900306	JF900235	Lake Tanganyika	-
Pseudosimochromis curvifrons	GQ995777	JF900307	JF900236	Lake Tanganyika	-
Pseudotropheus sp. „acei“	KJ955413	KJ955399	KJ955439	Aquaria Stock, Lake Malawi	-
Pseudocrenilabrus multicolor	KJ955425	KJ955395	KJ955440	Aquaria Stock, Lake Malawi	-
Cynotilapia pulpican	KJ955414	KJ955400	KJ955442	Aquaria Stock, Lake Malawi	-
Pundamilia nyererei	KJ955417	KJ955405	KJ955441	Aquaria Stock, Lake Malawi	-
Reganochromis calliurus	AY682544	JF900308	JF900237	Lake Tanganyika	-
Rhamphochromis sp.	KJ955415	KJ955407	KJ955443	Aquaria Stock, Lake Malawi	-
Sarotherodon sp. "Barombi Mbo"	KJ955426	KJ955407	KJ955435	Barombi Mbo, Cameroon	-
Serranochromis macrocephalus	KJ955422	KJ955397	KJ955444	Kafue, Zambia	S14° 58' 25.315" E25° 55' 14.642"
Simochromis diagramma	AY930087	JF900310	JF900239	Lake Tanganyika	-
Telmatochromis dhonti/temporalis	EF679266	JF900311	JF900240	Lake Tanganyika	-
Oreochromis sp.	KJ955427	KJ955408	KJ955445	Kafue, Zambia	S14° 58' 25.315" E25° 55' 14.642"
Tilapia zillii	KJ955428	KJ955409	KJ955446	Daylan, Turkey	N36° 49' 56.349" E28° 38' 13.746"
Trematocara marginatum	JF900327	JF900312	JF900241	Lake Tanganyika	-
Trematochromis benthicola	JF900320	JF900261	JF900190	Lake Tanganyika	-
Trematocara nigrifrons	JF900328	JF900313	JF900242	Lake Tanganyika	-
Tropheus moorii	AY930093	JF900314	JF900243	Lake Tanganyika	-
Tylochromis polylepis	U07268	JF900315	JF900244	Lake Tanganyika	-
Variabilichromis moorii	DQ055016	JF900316	JF900245	Lake Tanganyika	-
Xenotilapia flavipinnis	AY337794	JF900317	JF900246	Lake Tanganyika	-
Xenotilapia spiloptera	AY337788	JF900318	JF900247	Lake Tanganyika	-

Supplementary table 2: List of 218 cichlid specimens and their mitochondrial ND2 sequence accession numbers. Specified are the original publications, their sample information and in which analysis there were used.

Species	Published in	Accession number	Locality	Coordinates	Collected by	Fig1c	FigS2
Haplochromis sp. "Chipwa"	Present study	KJ955419	Kalambo River Delta, Zambia	08°36'6.34"S; 031°11'12.73"E	*ZIUB NS_CH4*	+	+
Haplochromis sp. "Chipwa"	Present study	KJ955420	Lufubu River Delta, Zambia	8°33'41.25"S; 030°43' 26.54"E	*ZIUB NS_LU2*	+	+
Haplochromis stappersii "Malagarasi 1"	Present study	KJ955389	Malagarasi River	03°50'56.9"S; 030°18'01.3"E	*Gaspard Banyankimbona, MRAC1840*	+	+
Haplochromis stappersii "Malagarasi 2"	Present study	KJ955390	Malagarasi River	03°51'25.2"S; 030°17'53.5"E	*Gaspard Banyankimbona, MRAC1847*	+	+
Haplochromis stappersii "Malagarasi 3"	Present study	KJ955391	Muvumu-Nkobokobo	03°53'10.8"S; 030°15'16.1"E	*Gaspard Banyankimbona, MRAC12034*	+	+
Haplochromis stappersii "Malagarasi 4"	Present study	KJ955392	SOSUMO-Amont	03°59'33.8"S; 030°12'52.9"E	*Gaspard Banyankimbona, MRAC12087*	+	+
Haplochromis stappersii "Rusizi"	Present study	KJ955388	Gatumba marsh, Rusizi River	03°20'21,6"S; 029°13'56,9"E	*Gaspard Banyankimbona, MRAC6334*	+	+
Boulengerochromis microlepis	Klett & Meyer 2002	AF317229	n/a	n/a	*n/a*		+
Haplochromis burtoni	Kobelmüller et al. 2010	GQ995714	Kalambo, above falls	n/a	Kobelmüller et al. 2010, 7055	+	
Astatoreochromis alluaudi	Koblmüller et al. 2008	EU753923	Lake Kanyaboli, Kenya	n/a	n/a		+
Chetia brevicauda	Koblmüller et al. 2008	EU753924	Buzi River	n/a	n/a		+
Chetia brevis	Koblmüller et al. 2008	EU753925	Incomati River	n/a	n/a		+
Chetia flaviventris	Koblmüller et al. 2008	EU753926	Limpopo river	n/a	n/a		+
Chetia flaviventris	Koblmüller et al. 2008	EU753927	Limpopo river	n/a	n/a		+
Haplochromini sp. 'Lufubu'	Koblmüller et al. 2008	EU753928	Lufubu river, Zambia	n/a	n/a		+
Thoracochromis albolabris	Koblmüller et al. 2008	EU753929	Cunene River	n/a	n/a	+	+
Haplochromis bloyeti	Koblmüller et al. 2008	EU753930	Nyumba ya Mungu, Tanzania	n/a	n/a	+	+
Thoracochromis brauschi	Koblmüller et al. 2008	EU753931	Lake Fwa, DRC	n/a	n/a		+
Haplochromis burtoni	Koblmüller et al. 2008	EU753932	Kalambo River	n/a	n/a	+	+
Thoracochromis buysi	Koblmüller et al. 2008	EU753933	Cunene River	n/a	n/a		+
Astatotilapia calliptera	Koblmüller et al. 2008	EU753934	Lake Kisiba, Tanzania	n/a	n/a	+	+
Ctenochromis horei	Koblmüller et al. 2008	EU753935	Lake Tanganyika	n/a	n/a		+
Orthochromis machadoi	Koblmüller et al. 2008	EU753936	Cunene River	n/a	n/a		+
Haplochromis oligacanthus	Koblmüller et al. 2008	EU753937	Ngoko River, Congo	n/a	n/a		+
Ctenochromis pectoralis	Koblmüller et al. 2008	EU753938	Nyumba ya Mungu, Tanzania	n/a	n/a	+	+
Ctenochromis pectoralis	Koblmüller et al. 2008	EU753939	Nyumba ya Mungu, Tanzania	n/a	n/a		+
Haplochromis phytophagus	Koblmüller et al. 2008	EU753940	Lake Kenyaboli, Kenia	n/a	n/a	+	+
Haplochromis polli	Koblmüller et al. 2008	EU753941	Lower Congo River	n/a	n/a		+
Haplochromis rudolfianus	Koblmüller et al. 2008	EU753942	Lake Turkana	n/a	n/a	+	+
Haplochromis squamipinnis	Koblmüller et al. 2008	EU753943	Lake Edward Uganda	n/a	n/a	+	+
Haplochromis sp. 'Lake Kanyaboli'	Koblmüller et al. 2008	EU753944	Lake Kenyaboli, Kenia	n/a	n/a	+	+
Haplochromis sp. El Fayoum	Koblmüller et al. 2008	EU753945	El Fayoum Oasis, Egypt	n/a	n/a	+	+
Haplochromis sp. 'Mburo Black'	Koblmüller et al. 2008	EU753946	Lake Mburo, Uganda	n/a	n/a	+	+
Nimbochromis venustus	Koblmüller et al. 2008	EU753947	Lake Malawi	n/a	n/a	+	+
Nimbochromis livingstonii	Koblmüller et al. 2008	EU753948	Lake Malawi	n/a	n/a	+	+
Pharyngochromis acuticeps	Koblmüller et al. 2008	EU753949	Rundu, Namibia	n/a	n/a		+
Pseudocrenilabrus sp. Lufubu	Koblmüller et al. 2008	EU753950	Lufubu river, Zambia	n/a	n/a	+	+
Pseudocrenilabrus sp. Lunzua blue	Koblmüller et al. 2008	EU753951	Lunzua River, Zambia	n/a	n/a		+
Pseudocrenilabrus sp. Mweru orange	Koblmüller et al. 2008	EU753952	Lake Mweru	n/a	n/a		+
Pseudocrenilabrus sp. Olushandja	Koblmüller et al. 2008	EU753953	Cunene River, Olushandjia, Namibia	n/a	n/a		+
Sargochromis coulteri	Koblmüller et al. 2008	EU753954	Cunene River, Olushandjia, Namibia	n/a	n/a		+
Sargochromis coulteri	Koblmüller et al. 2008	EU753955	Olushandja, Namibia	n/a	n/a		+
Sargochromis aff. carlottae SK-2008	Koblmüller et al. 2008	EU753956	Kafue Flats, Zambia	n/a	n/a	+	+
Schwetzochromis neodon	Koblmüller et al. 2008	EU753957	Lake Fwa, Congo	n/a	n/a		+
Serranochromis angusticeps	Koblmüller et al. 2008	EU753958	Cunene River	n/a	n/a		+
Serranochromis angusticeps	Koblmüller et al. 2008	EU753959	Cunene River	n/a	n/a		+
Serranochromis stappersi	Koblmüller et al. 2008	EU753960	Lake Bangwuelu, Zambia	n/a	n/a		+
Serranochromis thumbergi	Koblmüller et al. 2008	EU753961	Lake Bangwuelu, Zambia	n/a	n/a		+
Benthochromis horii	Koblmüller et al. 2008	EU753962	Lake Tanganyika	n/a	n/a		+
Tylochromis polylepis	Kocher et al. 1995	U07268	Fish market, Uvira, Kongo	n/a	n/a		+
Haplochromis burtoni	Muschick et al. 2012	JF900319	Kalambo River, Zambia	n/a	ZIUB	+	+
Trematochromis benthicola	Muschick et al. 2012	JF900320	Lake Tanganyika	n/a	ZIUB		+
Gnathochromis permaxillaris	Muschick et al. 2012	JF900321	Lake Tanganyika	n/a	ZIUB		+
Interochromis loocki	Muschick et al. 2012	JF900322	Lake Tanganyika	n/a	ZIUB	+	+
Petrochromis ephippium	Muschick et al. 2012	JF900323	Lake Tanganyika	n/a	ZIUB		+
Petrochromis famula	Muschick et al. 2012	JF900324	Lake Tanganyika	n/a	ZIUB		+
Petrochromis fasciolatus	Muschick et al. 2012	JF900325	Lake Tanganyika	n/a	ZIUB	+	+
Petrochromis polyodon	Muschick et al. 2012	JF900326	Lake Tanganyika	n/a	ZIUB		+
Trematocara marginatum	Muschick et al. 2012	JF900327	Lake Tanganyika	n/a	ZIUB		+
Trematocara nigrifrons	Muschick et al. 2012	JF900328	Lake Tanganyika	n/a	ZIUB		+
Serranochromis macrocephalus "Cutato"	Musilová et al. 2013	KC146709	Cutato River, Angola	n/a	Musilová et al. C71		+
Serranochromis macrocephalus	Musilová et al. 2013	KC146710	Angola	n/a	Musilová et al. Z80_2		+
Serranochromis macrocephalus	Musilová et al. 2013	KC146711	Angola	n/a	Musilová et al. Z80_1		+
Serranochromis macrocephalus "Cuchi"	Musilová et al. 2013	KC146712	Cuchi River, Angola	n/a	Musilová et al. K03		+
Serranochromis macrocephalus "Cuchi"	Musilová et al. 2013	KC146713	Cuchi River, Angola	n/a	Musilová et al. K05		+
Serranochromis macrocephalus "Cuchi"	Musilová et al. 2013	KC146714	Cuchi River, Angola	n/a	Musilová et al. K07		+
Serranochromis macrocephalus "Cuito"	Musilová et al. 2013	KC146715	Cuito River, Angola	n/a	Musilová et al. K16		+
Serranochromis macrocephalus "Cuito"	Musilová et al. 2013	KC146716	Cuito River, Angola	n/a	Musilová et al. B51n		+
Serranochromis macrocephalus "Cuito"	Musilová et al. 2013	KC146717	Cuito River, Angola	n/a	Musilová et al. Z05		+
Serranochromis macrocephalus "Cuito"	Musilová et al. 2013	KC146718	Cuito River, Angola	n/a	Musilová et al. Z09		+
Serranochromis macrocephalus "Lomba"	Musilová et al. 2013	KC146719	Lomba, Angola	n/a	Musilová et al. C05n		+
Serranochromis macrocephalus "Lomba"	Musilová et al. 2013	KC146720	Lomba, Angola	n/a	Musilová et al. C11n		+
Serranochromis macrocephalus "Lomba"	Musilová et al. 2013	KC146721	Lomba, Angola	n/a	Musilová et al. C16n		+
Serranochromis macrocephalus "Lomba"	Musilová et al. 2013	KC146722	Lomba, Angola	n/a	Musilová et al. C17n		+
Serranochromis macrocephalus "Lomba"	Musilová et al. 2013	KC146723	Lomba, Angola	n/a	Musilová et al. C27n		+
Serranochromis macrocephalus "Cuemba"	Musilová et al. 2013	KC146724	Cuemba River, Angola	n/a	Musilová et al. V33		+
Serranochromis macrocephalus "Cuemba"	Musilová et al. 2013	KC146725	Cuemba River, Angola	n/a	Musilová et al. V35		+
Serranochromis macrocephalus "Cutato"	Musilová et al. 2013	KC146726	Cutato River, Angola	n/a	Musilová et al. C70		+
Haplochromis sp. Luando	Musilová et al. 2013	KC146727	Luando River, Angola	n/a	Musilová et al. Z38		+

Haplochromis sp. Luando	Musilovà et al. 2013	KC146728	Luando River, Angola	n/a	Musilovà et al. Z35		+
Haplochromis sp. Lomba	Musilovà et al. 2013	KC146729	Lomba, Angola	n/a	Musilovà et al. C52		+
Haplochromis sp. Lomba	Musilovà et al. 2013	KC146730	Lomba, Angola	n/a	Musilovà et al. C50n		+
Serranochromis sp.	Musilovà et al. 2013	KC146731	Angola	n/a	Musilovà et al. Z81		+
Thoracochromis sp. Huando	Musilovà et al. 2013	KC146732	Huando River, Angola	n/a	Musilovà et al. Z21		+
Tilapia sp.	Musilovà et al. 2013	unpublished			Z85		+
Haplochromis. stappersii	Salzburger et al. 2005	AY930046	Malagarasi River, Tanzania	n/a	L. De Vos (5-6/25/92)	+	+
Pseudocrenilabrus philander	Salzburger et al. 2005	AY930047	Zambezi River, Zambia	n/a	aquarium trade	+	+
Orthochromis uvinzae	Salzburger et al. 2005	AY930048	Malagarasi River, Tanzania	n/a	L. Seegers (TZ94-112b)		+
Orthochromis kasuluensis	Salzburger et al. 2005	AY930049	Tanzania	n/a	L. De Vos (T2-July 94)		+
Orthochromis rugufuensis	Salzburger et al. 2005	AY930050	Rugufu River, Tanzania	n/a	L. Seegers (TZ94-121)		+
Orthochromis rubrolabialis	Salzburger et al. 2005	AY930051	Tanzania	n/a	L. Seegers (TZ94-108)		+
Orthochromis luichensis	Salzburger et al. 2005	AY930052	Mkuti River, Luiche Basin, Tanzania	n/a	L. De Vos (T94/3)		+
Orthochromis mazimeroensis	Salzburger et al. 2005	AY930053	Nanganga, Burundi	n/a	L. De Vos (T1-5/27/93)		+
Orthochromis malagaraziensis	Salzburger et al. 2005	AY930054	Nyarungunga River, Burundi	n/a	L. De Vos (T5-5/28/93)		+
Orthochromis mosoensis	Salzburger et al. 2005	AY930055	Ruisseau Gytinya, Burundi	n/a	L. De Vos (T7-5/28/93)		+
Orthochromis malagaraziensis	Salzburger et al. 2005	AY930056	Nyarungunga River, Burundi	n/a	L. De Vos (7-2/19/93)		+
Orthochromis stormsi	Salzburger et al. 2005	AY930057	Kisangani (Lualaba River), DR Congo	n/a	L. De Vos (5/5/95)		+
Haplochromis bloyeti	Salzburger et al. 2005	AY930058	Lukaware River, Kenia	n/a	L. De Vos (F2A-12/93)	+	+
submitted as Ptyochromis sauvagei Haplochromis fischeri	Salzburger et al. 2005	AY930059	Lake Victoria (Kisumu, Kenya)	n/a	L. De Vos (F2B-12/93)	+	+
Haplochromis burtoni	Salzburger et al. 2005	AY930060	Lake Tanganyika	n/a	L. De Vos (31-02/6/92). T34	+	+
Maylandia livingstonii	Salzburger et al. 2005	AY930061	Lake Malawi	n/a	I. Kornfield	+	+
Haplochromis sp. 'Kisangani'	Salzburger et al. 2005	AY930062	Kisangani, (Lualaba River), DR Congo	n/a	L. De Vos (6/13/95)		+
submitted as Ptyochromis sauvagei Haplochromis fischeri	Salzburger et al. 2005	AY930063	Lake Victoria	n/a	A. Meyer, T44	+	+
Platytaeniodus degeni	Salzburger et al. 2005	AY930064	Lake Victoria	n/a	A. Meyer (Pd1)	+	+
Haplochromis sp. V7	Salzburger et al. 2005	AY930065	Lake Victoria	n/a	A. Meyer (V7-Feb 93)	+	+
Tropheus moorii	Salzburger et al. 2005	AY930066	Lake Tanganyika	n/a	E. Verheyen; T66		+
Tropheus moorii	Salzburger et al. 2005	AY930067	Lake Tanganyika	n/a	E. Verheyen; T67		+
Petrochromis macrognathus	Salzburger et al. 2005	AY930068	Lake Tanganyika	n/a	J. Snoeks, MRAC		+
Melanochromis auratus	Salzburger et al. 2005	AY930069	Lake Malawi	n/a	aquarium	+	+
Pseudocrenilabrus multicolor victoriae	Salzburger et al. 2005	AY930070	Lake Kanyaboli, Kenya	n/a	R. Abila (R082-2002)		+
Astatoreochromis alluaudi	Salzburger et al. 2005	AY930071	Lake Kanyaboli, Kenya	n/a	R. Abila (R101-2002)	+	+
Astatotilapia sp. R184	Salzburger et al. 2005	AY930072	Lake Kanyaboli, Kenya	n/a	R. Abila (R184-2002)	+	+
Astatotilapia sp. R185	Salzburger et al. 2005	AY930073	Lake Kanyaboli, Kenya	n/a	R. Abila (R185-2002)	+	+
Haplochromis sp. 'dwarf big eye'	Salzburger et al. 2005	AY930074	Lake Kanyaboli, Kenya	n/a	R. Abila (R280-2002)	+	+
Astatoreochromis alluaudi	Salzburger et al. 2005	AY930075	Lake Kanyaboli, Kenya	n/a	R. Abila (R281-2002)		+
Xystichromis phytophagus	Salzburger et al. 2005	AY930076	Lake Kanyaboli, Kenya	n/a	R. Abila (R670-2002)	+	+
Haplochromis insidiae	Salzburger et al. 2005	AY930077	Lake Kivu	n/a	E. Verheyen	+	+
Haplochromis gracilior	Salzburger et al. 2005	AY930078	Lake Kivu	n/a	E. Verheyen; K8	+	+
Thoracochromis brauschi	Salzburger et al. 2005	AY930080	Lake Fwa	n/a	R. Paul/E. Schraml (9792)		+
Serranochromis sp. 9793	Salzburger et al. 2005	AY930081	Lake Mweru-Wantipa, Zambia	n/a	T. Reuter / E. Schraml (9793)		+
Haplochromis sp. 9796	Salzburger et al. 2005	AY930082	Lake Mburo, Uganda	n/a	E. Schraml (9796)	+	+
Haplochromis squamipinnis	Salzburger et al. 2005	AY930083	Lake Edward	n/a	E. Schraml (9813)	+	+
Tropheus polli	Salzburger et al. 2005	AY930084	Lake Tanganyika	n/a	E. Verheyen		+
Tropheus duboisi	Salzburger et al. 2005	AY930085	Lake Tanganyika	n/a	E. Verheyen; M7	+	+
Tropheus brichardi	Salzburger et al. 2005	AY930086	Lake Tanganyika	n/a	E. Verheyen; M85		+
Simochromis diagramma	Salzburger et al. 2005	AY930087	Lake Tanganyika	n/a	E. Verheyen		+
Simochromis marginatus	Salzburger et al. 2005	AY930088	Lake Tanganyika	n/a	E. Verheyen	+	+
Cyrtocara moorii	Salzburger et al. 2005	AY930089	Lake Malawi	n/a	I. Kornfield	+	+
Astatotilapia calliptera	Salzburger et al. 2005	AY930090	Lake Malawi	n/a	I. Kornfield (A22)	+	+
Tropheus moorii	Salzburger et al. 2005	AY930091	Lake Tanganyika	n/a	E. Verheyen; 97	+	+
Cheilochromis euchilus	Salzburger et al. 2005	AY930092	Lake Malawi	n/a	I. Kornfield	+	+
Tropheus moorii	Salzburger et al. 2005	AY930093	Lake Tanganyika	n/a	E. Verheyen, 116		+
Pharyngochromis acuticeps	Salzburger et al. 2005	AY930094	Zambezi River, Zambia	n/a	C. Katongo / C. Sturmbauer		+
Thoracochromis brauschi	Salzburger et al. 2005	AY930095	Lake Fwa , Congo	n/a	Aquarium trade		+
Haplochromis sp. T13	Salzburger et al. 2005	AY930096	Upper Rusizi, Burundi	n/a	L. De Vos (T13-Aug 93)	+	+
Haplochromis obliquidens	Salzburger et al. 2005	AY930097	Lake Victoria	n/a	Aquarium trade	+	+
Sargochromis giardi	Salzburger et al. 2005	AY930098	Zambezi River, Zambia	n/a	C. Katongo / C. Sturmbauer		+
Cyclopharynx fwae	Salzburger et al. 2005	AY930099	Lake Fwa, Congo	n/a	U. Schliewen		+
Ctenochromis horei	Salzburger et al. 2005	AY930100	Lake Tanganyika	n/a	C. Sturmbauer/W. Salzburger		+
Haplochromis sp. 62	Salzburger et al. 2005	AY930101	Tanzania	n/a	L. De Vos (H62)	+	+
Haplochromis sp. 63	Salzburger et al. 2005	AY930102	Tanzania	n/a	L. De Vos (H63)	+	+
Haplochromis sp. 93/3	Salzburger et al. 2005	AY930103	Tanzania	n/a	L. Seegers (93/3)	+	+
Haplochromis sp. 93/40	Salzburger et al. 2005	AY930104	Tanzania	n/a	L. Seegers (93/40)	+	+
Haplochromis sp. 93/8	Salzburger et al. 2005	AY930105	Tanzania	n/a	L. Seegers (93/8)	+	+
Pseudocrenilabrus multicolor	Salzburger et al. 2005	AY930106	Tanzania	n/a	L. Seegers (91/137)		+
Haplochromis paludinosus	Salzburger et al. 2005	AY930107	Nanganga, Burundi	n/a	L. De Vos (T2-5/27/93)	+	+
Haplochromis gracilior	Salzburger et al. 2006	AY930079	Lake Kivu	n/a	E. Verheyen; K9	+	+
Congolapia bilineata	Schwarzer et al. 2011	JX157060	Itimbiri, DRC	n/a	ZSM		+
Lamprologus tigripictilis	Schwarzer et al. 2011	JX157061	Lower Congo, DRC	n/a	ZSM		+
Pseudocrenilabrus multicolor	Schwarzer et al. 2011	JX157062	Nile Delta, Egypt	n/a	ZSM	+	+
Orthochromis stormsi	Schwarzer et al. 2011	JX157063	Pool Malebo, DRC	n/a	ZSM		+
Orthochromis stormsi	Schwarzer et al. 2011	JX157064	Pool Malebo, DRC	n/a	ZSM		+
Orthochromis cf. stormsi 'Kisangani'	Schwarzer et al. 2011	JX157065	around Kisangani, DRC	n/a	ZSM		+
Orthochromis cf. stormsi 'Kisangani'	Schwarzer et al. 2011	JX157066	around Kisangani, DRC	n/a	ZSM		+
Orthochromis polyacanthus	Schwarzer et al. 2011	JX157067	LakeMweru, Zambia	n/a	EAWAG		+
Orthochromis aff. kalungwishiensis	Schwarzer et al. 2011	JX157068	LakeMweru, Zambia	n/a	EAWAG	+	+
Ctenochromis horei	Schwarzer et al. 2011	JX157069	Lake Tanganyika	n/a	CU		+
Ctenochromis horei	Schwarzer et al. 2011	JX157070	Lake Tanganyika	n/a	CU	+	+
Tropheus moorii	Schwarzer et al. 2011	JX157071	Lake Tanganyika	n/a	CU		+
Astatoreochromis alluaudi	Schwarzer et al. 2011	JX157072	Nile / Lake Victoria	n/a	EAWAG	+	+
Haplochromis burtoni	Schwarzer et al. 2011	JX157073	Lake Tanganyika	n/a	ZSM	+	+
Pseudotropheus socolofi	Schwarzer et al. 2011	JX157074	Lake Malawi	n/a	EAWAG	+	+

Labidochromis caeruleus	Schwarzer et al. 2011	JX157075	Lake Malawi	n/a	ZSM	+	+
Rhamphochromis sp.	Schwarzer et al. 2011	JX157076	Lake Malawi	n/a	ZSM	+	+
Sciaenochromis fryeri	Schwarzer et al. 2011	JX157077	Lake Malawi	n/a	ZSM	+	+
Astatotilapia desfontanii	Schwarzer et al. 2011	JX157078	Sahara, Tunesia	n/a	ZSM	+	+
Neochromis rufocaudalis	Schwarzer et al. 2011	JX157079	Nile / Lake Victoria	n/a	ZSM	+	+
Haplochromis sp. 'Kyoga'	Schwarzer et al. 2011	JX157080	Lake Kyoga, Uganda	n/a	ZSM	+	+
Haplochromis stappersii	Schwarzer et al. 2011	JX157081	Lake Tanganyika drainage, Burundi	n/a	ZSM	+	+
Haplochromis sp. 'Yaekama'	Schwarzer et al. 2011	JX157082	around Kisangani, DRC	n/a	ZSM	+	+
Haplochromis sp. 'Lake Rakai'	Schwarzer et al. 2011	JX157083	Nile / L. Rakai, Uganda	n/a	ZSM	+	+
Haplochromis sp. 'Lake Kijanebalola'	Schwarzer et al. 2011	JX157084	Nile / Lake Kijanebalola, Uganda	n/a	ZSM	+	+
Haplochromis thereuterion	Schwarzer et al. 2011	JX157085	Lake Victoria	n/a	ZSM	+	+
Haplochromis cf. polli 'Lefini'	Schwarzer et al. 2011	JX157086	Lefini River, ROC	n/a	MRAC		+
Haplochromis cf. polli 'Lefini'	Schwarzer et al. 2011	JX157087	Lefini River, ROC	n/a	MRAC		+
Haplochromis polli	Schwarzer et al. 2011	JX157088	Lower Congo River	n/a	ZSM		+
Haplochromis polli	Schwarzer et al. 2011	JX157089	Lower Congo River	n/a	ZSM		+
Haplochromis oligacanthus	Schwarzer et al. 2011	JX157090	Ubangi River, CAR	n/a	ZSM		+
Haplochromis oligacanthus	Schwarzer et al. 2011	JX157091	Ubangi River, CAR	n/a	ZSM		+
Haplochromis fasciatus	Schwarzer et al. 2011	JX157092	Lower Congo River	n/a	ZSM		+
Haplochromis fasciatus	Schwarzer et al. 2011	JX157093	Lower Congo River	n/a	ZSM	+	+
Haplochromis demeusii	Schwarzer et al. 2011	JX157094	Lower Congo River	n/a	ZSM		+
Haplochromis demeusii	Schwarzer et al. 2011	JX157095	Lower Congo River	n/a	ZSM	+	+
Haplochromis sp. 'Sanzikwa'	Schwarzer et al. 2011	JX157096	Sanzikwa River, DRC	n/a	ZSM		+
Haplochromis sp. 'Sanzikwa'	Schwarzer et al. 2011	JX157097	Sanzikwa River, DRC	n/a	ZSM	+	+
Haplochromis cf. bakongo	Schwarzer et al. 2011	JX157098	Kwilu River, DRC	n/a	ZSM		+
Haplochromis cf. bakongo	Schwarzer et al. 2011	JX157099	Kwilu River, DRC	n/a	ZSM		+
Haplochromis snoeksi	Schwarzer et al. 2011	JX157100	Inkisi River, DRC	n/a	MRAC	+	+
Thoracochromis callichromus	Schwarzer et al. 2011	JX157101	Lake Fwa, DRC	n/a	AMNH		+
Thoracochromis callichromus	Schwarzer et al. 2011	JX157102	Lake Fwa, DRC	n/a	AMNH		+
Cyclopharynx schwetzi	Schwarzer et al. 2011	JX157103	Lake Fwa, DRC	n/a	AMNH		+
Thoracochromis brauschi	Schwarzer et al. 2011	JX157104	Lake Fwa, DRC	n/a	AMNH		+
Schwetzochromis neodon	Schwarzer et al. 2011	JX157105	Lake Fwa, DRC	n/a	AMNH		+
Haplochromis stigmatogenys	Schwarzer et al. 2011	JX157106	Kasai River, DRC	n/a	AMNH		+
Haplochromis stigmatogenys	Schwarzer et al. 2011	JX157107	Kasai River, DRC	n/a	AMNH		+
Haplochromis sp. 'Kwango'	Schwarzer et al. 2011	JX157108	Kwango River, DRC	n/a	ZSM		+
Haplochromis sp. 'Kwango'	Schwarzer et al. 2011	JX157109	Kwango River, DRC	n/a	ZSM		+
Orthochromis torrenticola	Schwarzer et al. 2011	JX157110	Lufira, DRC	n/a	ZSM		+
Orthochromis torrenticola	Schwarzer et al. 2011	JX157111	Lufira, DRC	n/a	ZSM		+
Pharyngochromis sp. 'yellow lip'	Schwarzer et al. 2011	JX157112	Kwanza / Middel Kwanza (Angola)	n/a	SAIAB		+
Pharyngochromis sp. 'yellow lip'	Schwarzer et al. 2011	JX157113	Kwanza / Middel Kwanza, Angola	n/a	SAIAB		+
Pharyngochromis sp. 'yellow lip'	Schwarzer et al. 2011	JX157114	Kwanza / Middel Kwanza, Angola	n/a	SAIAB		+
Pharyngochromis sp. 'white tip'	Schwarzer et al. 2011	JX157115	Kwanza / Upper Lucalla, Angola	n/a	SAIAB		+
Pharyngochromis sp. 'white tip'	Schwarzer et al. 2011	JX157116	Kwanza / Upper Lucalla (Angola)	n/a	SAIAB		+
Serranochromis sp. 'red scales'	Schwarzer et al. 2011	JX157117	Kwanza / Upper Lucalla, Angola	n/a	SAIAB		+
Serranochromis sp. 'red scales'	Schwarzer et al. 2011	JX157118	Kwanza / Upper Lucalla, Angola	n/a	SAIAB		+
Pharyngochromis sp. 'yellow fins'	Schwarzer et al. 2011	JX157119	Kwanza / Upper Kwanza, Angola	n/a	SAIAB		+
Serranochromis sp. 'yellow fins'	Schwarzer et al. 2011	JX157120	Kwanza / Upper Kwanza, Angola	n/a	SAIAB	+	+
Serranochromis sp. 'black and white'	Schwarzer et al. 2011	JX157121	Kwanza / Upper Kwanza, Angola	n/a	SAIAB		+
Pharyngochromis acuticeps	Schwarzer et al. 2011	JX157122	Zambezi, Namibia	n/a	ZSM		+
Serranochromis robustus	Schwarzer et al. 2011	JX157123	Zambezi, Namibia	n/a	ZSM		+
Serranochromis macrocephalus	Schwarzer et al. 2011	JX157124	Zambezi, Namibia	n/a	ZSM		+
Serranochromis angusticeps	Schwarzer et al. 2011	JX157125	Zambezi, Namibia	n/a	ZSM		+
Serranochromis altus	Schwarzer et al. 2011	JX157126	Zambezi, Namibia	n/a	ZSM		+
Haplochromis elegans	Wagner et al. 2012	JQ950379	n/a	n/a	EAWAG, KAT_10	+	
Astatotilapia flavijosephi	Wagner et al. 2012	JQ950380	n/a	n/a	EAWAG, voucher 14	+	
Haplochromis tweddlei	Wagner et al. 2012	JQ950384	n/a	n/a	EAWAG, voucher 2_B6	+	
Haplochromis paludinosus	Weiss et al. unpublished	KJ176274	n/a	n/a	ZSM, P-AA-0595	+	

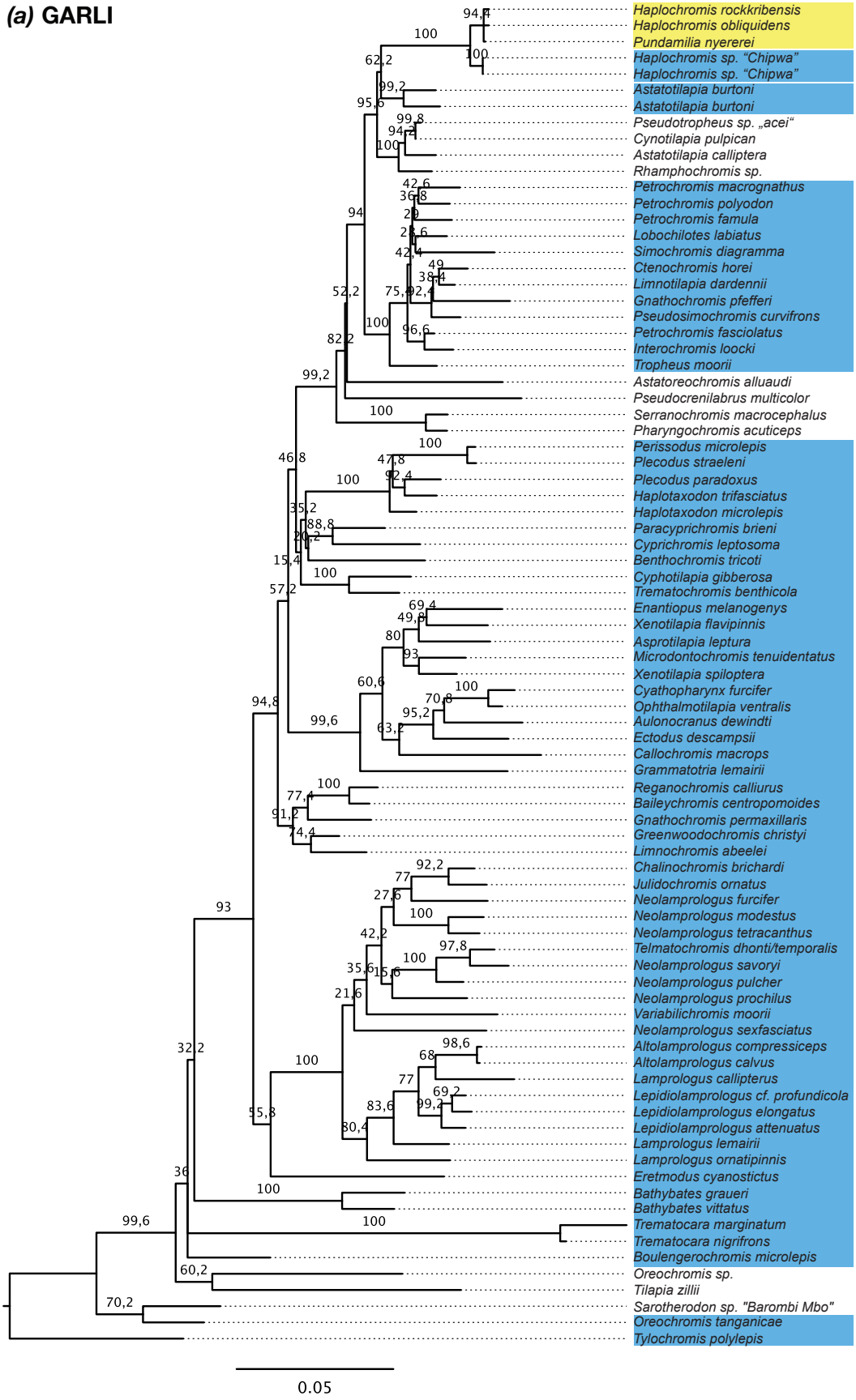
Supplementary table 3: List of the 182 haplochromine specimens and their mitochondrial control region (d-loop) accession numbers. Specified are the original publications and their sample information including haplotype number following Verheyen et al. 2003 and this study. Haplotypes used in figure 1(d) are indicated with an asterisk.

Species	Published in	Accession number	Locality	Collected by	SampleID	Haplotype in Verheyen et al. 2003 / this study
Haplochromis simpsoni	Nagl et al. 2000	AF213518	Lake Nabugabo	-	Gasi589	77
Haplochromis beadlei	Nagl et al. 2000	AF213519	Lake Nabugabo	-	Pabe593	77
Haplochromis laparogramma	Nagl et al. 2000	AF213520	Lake Victoria	-	Yila179	89
Haplochromis laparogramma	Nagl et al. 2000	AF213521	Lake Victoria	-	Yila335	80
Haplochromis laparogramma	Nagl et al. 2000	AF213522	Rusinga / Lake Victoria	-	Yila6937	25
Haplochromis lividus	Nagl et al. 2000	AF213523	Lake Victoria	-	Hali327	93
Haplochromis nubila	Nagl et al. 2000	AF213524	Lakes Nabugabo, Kayina and Kayania	-	Asnu	92*
Haplochromis chilotes	Nagl et al. 2000	AF213525	Rusinga / Lake Victoria	-	Pach	98
Haplochromis cinctus	Nagl et al. 2000	AF213526	Lake Victoria	-	Enci	77*
Haplochromis melanopterus	Nagl et al. 2000	AF213527	Lake Victoria	-	Lime	95
Neochromis nigricans	Nagl et al. 2000	AF213528	Lake Victoria	-	Neni	121
Haplochromis plagiodon	Nagl et al. 2000	AF213529	Lake Victoria	-	Papl	105
Haplochromis riponianus	Nagl et al. 2000	AF213530	Lake Victoria	-	Psri	102
Haplochromis fischeri	Nagl et al. 2000	AF213531	Lake Victoria	-	Ptsa	122
Haplochromis xenognathus	Nagl et al. 2000	AF213532	Anyanga / Lake Victoria	-	Ptxe6864	113
Haplochromis xenognathus	Nagl et al. 2000	AF213533	Anyanga / Lake Victoria	-	Ptxe6865	110
Haplochromis xenognathus	Nagl et al. 2000	AF213534	Mwanza Gulf / Lake Victoria	-	Ptxe326	109
Haplochromis xenognatus	Nagl et al. 2000	AF213535	Lake Victoria	-	Ptxe350	118*
Haplochromis nubilus	Nagl et al. 2000	AF213536	Lake Victoria	-	Asnu586	117
Prognathochromis venator	Nagl et al. 2000	AF213537	Lakes Nabugabo, Kayina and Kayania	-	Prve687	81
Prognathochromis venator	Nagl et al. 2000	AF213538	Lakes Nabugabo, Kayina and Kayania	-	Prve691	81
Haplochromis chilotes	Nagl et al. 2000	AF213539	Anyanga / Lake Victoria	-	Pach5721	79
Haplochromis chilotes	Nagl et al. 2000	AF213540	Lake Victoria	-	Pach5722	90
Haplochromis sp.'rockkribensis'	Nagl et al. 2000	AF213541	Lake Victoria	-	Haro486	108
Haplochromis sp.'rockkribensis'	Nagl et al. 2000	AF213542	Muhuru / Lake Victoria	-	Haro6745	75
Haplochromis sp.'velvetblack'	Nagl et al. 2000	AF213543	Lake Victoria	-	Havb21	115
Neochromis nigricans	Nagl et al. 2000	AF213544	Lake Victoria	-	Neni309	99
Neochromis nigricans	Nagl et al. 2000	AF213545	Lake Victoria	-	Neni817	96
Haplochromis plagiodon	Nagl et al. 2000	AF213546	Lake Victoria	-	Papl73	104
Haplochromis plagiodon	Nagl et al. 2000	AF213547	Lake Victoria	-	Papl160	91
Haplochromis plagiodon	Nagl et al. 2000	AF213548	Lake Victoria	-	Papl201	92
Haplochromis fischeri	Nagl et al. 2000	AF213549	Lake Victoria	-	Ptsa320	106
Haplochromis velifer	Nagl et al. 2000	AF213550	Lakes Nabugabo, Kayina and Kayania	-	Asve616	88
Haplochromis velifer	Nagl et al. 2000	AF213551	Lakes Nabugabo, Kayina and Kayania	-	Asve605	94
Haplochromis velifer	Nagl et al. 2000	AF213552	Lakes Nabugabo, Kayina and Kayania	-	Asve619	114
Haplochromis velifer	Nagl et al. 2000	AF213553	Lakes Nabugabo, Kayina and Kayania	-	Asve663	107
Haplochromis sp.'rockkribensis'	Nagl et al. 2000	AF213554	Lake Victoria	-	Haro6747	76*
Haplochromis sp.	Nagl et al. 2000	AF213555	WogoRiver / LakeRukwa	-	1514	27*
Haplochromis sp.	Nagl et al. 2000	AF213556	MyungaRiver / LakeRukwa	-	1605	28*
Haplochromis sp.	Nagl et al. 2000	AF213557	Kasenyi / Lake George	-	8831	73*
Haplochromis sp.	Nagl et al. 2000	AF213558	Kasenyi / Lake George	-	HT-8833	68
Haplochromis sp.	Nagl et al. 2000	AF213559	Kasenyi / Lake George	-	HT-87868786	5
Haplochromis sp.	Nagl et al. 2000	AF213560	Kasenyi / Lake George	-	HT-8801	64
Haplochromis sp.	Nagl et al. 2000	AF213561	Kasenyi / Lake George	-	HT-8837	1
Haplochromis sp.	Nagl et al. 2000	AF213562	Kasenyi / Lake George	-	HT-88348834	41
Haplochromis sp.	Nagl et al. 2000	AF213563	Kashaka / Lake George	-	HT-8924	43
Haplochromis sp.	Nagl et al. 2000	AF213564	Katwe / LakeEdward	-	HT-8880	26
Haplochromis sp.	Nagl et al. 2000	AF213566	Katwe / LakeEdward	-	HT-8879	71
Haplochromis sp.	Nagl et al. 2000	AF213567	Katwe / LakeEdward	-	HT-87688768	40
Haplochromis sp.	Nagl et al. 2000	AF213568	Katwe / LakeEdward	-	HT-8773	45
Haplochromis sp.	Nagl et al. 2000	AF213569	Katwe / LakeEdward	-	8777	46*
Haplochromis sp.	Nagl et al. 2000	AF213570	Katwe / LakeEdward	-	HT-8778	2
Haplochromis sp.	Nagl et al. 2000	AF213571	Bugoigo / LakeAlbert	-	HT-9049	66
Haplochromis sp.	Nagl et al. 2000	AF213572	Butiaba / LakeAlbert	-	HT-8990	69
Haplochromis sp.	Nagl et al. 2000	AF213573	Butiaba / LakeAlbert	-	HT-9003	44
Haplochromis sp.	Nagl et al. 2000	AF213574	Butiaba / LakeAlbert	-	HT-9019	42
Haplochromis sp.	Nagl et al. 2000	AF213575	LakeLutoto / Uganda	-	HT-8692	30
Haplochromis sp.	Nagl et al. 2000	AF213576	LakeLutoto / Uganda	-	HT-8694	31
Haplochromis sp.	Nagl et al. 2000	AF213577	LakeLutoto / Uganda	-	HT-8687	32
Haplochromis sp.	Nagl et al. 2000	AF213578	LakeChibwera / Uganda	-	HT-8947	62
Haplochromis sp.	Nagl et al. 2000	AF213579	LakeChibwera / Uganda	-	HT-8950	60
Haplochromis sp.	Nagl et al. 2000	AF213580	LakeChibwera / Uganda	-	HT-8948	61
Haplochromis sp.	Nagl et al. 2000	AF213581	LakeWamala / Lake VictoriaRegion	-	HT-8632	111

Haplochromis sp.	Nagl et al. 2000	AF213582	KatongaRiver / Lake VictoriaRegion	-	HT-8678	116
Haplochromis sp.	Nagl et al. 2000	AF213583	KatongaRiver / Lake VictoriaRegion	-	HT-8680	112
Haplochromis sp.	Nagl et al. 2000	AF213584	Kazinga Channel / L.Edward and George	-	HT-8741	70
Haplochromis sp.	Nagl et al. 2000	AF213585	Kazinga Channel / L.Edward and George	-	HT-8711	4
Haplochromis sp.	Nagl et al. 2000	AF213586	Kazinga Channel / L.Edward and George	-	HT-8718	3
Haplochromis sp.	Nagl et al. 2000	AF213587	Kazinga Channel / L.Edward and George	-	HT-87228722	39
Haplochromis sp.	Nagl et al. 2000	AF213588	MigoriRiver / Lake Victoria	-	HT-6701	87
Haplochromis sp.	Nagl et al. 2000	AF213589	Malagarazi River	-	HT-1006	na
Haplochromis sp.	Nagl et al. 2000	AF213590	Malagarazi River	-	HT-1011	na
Haplochromis sp.	Nagl et al. 2000	AF213591	Malagarazi River	-	HT-1510	na
Haplochromis sp.	Nagl et al. 2000	AF213592	Malagarazi River	-	HT-1531	na
Haplochromis sp.	Nagl et al. 2000	AF213593	Malagarazi River	-	HT-1590	na
Haplochromis sp.	Nagl et al. 2000	AF213594	Malagarazi River	-	HT-1591	na
Haplochromis sp.	Nagl et al. 2000	AF213595	Lupa River	-	HT-1597	na
Haplochromis sp.	Nagl et al. 2000	AF213596	Piti River	-	HT-1598	na
Haplochromis sp.	Nagl et al. 2000	AF213597	Piti River	-	HT-1546	na
Haplochromis sp.	Nagl et al. 2000	AF213598	Piti River	-	HT-1547	na
Haplochromis sp.	Nagl et al. 2000	AF213599	Pangani River	-	HT-1076	na
Haplochromis sp.	Nagl et al. 2000	AF213600	Pangani River	-	HT-1501	na
Haplochromis sp.	Nagl et al. 2000	AF213601	Wogo River / Lake Rukwa	-	HT-1636	na
Haplochromis sp.	Nagl et al. 2000	AF213602	Wogo River / Lake Rukwa	-	HT-1635	na
Haplochromis sp.	Nagl et al. 2000	AF213603	Wogo River / Lake Rukwa	-	HT-1515	na
Haplochromis sp.	Nagletal.2002	AF213604	Pangani River	-	HT-1530	na
Haplochromis sp.	Nagletal.2001	AF213605	Lake Chala	-	HT-1738	na
Haplochromis sp.	Nagletal.2003	AF213606	Lake Babati	-	HT-6249	na
Haplochromis sp.	Nagl et al. 2000	AF213607	Lake Manyara	-	HT-1537	na
Haplochromis sp.	Nagl et al. 2000	AF213608	Malagarazi River	-	HT-1601	na
Haplochromis sp.	Nagl et al. 2000	AF213609	Kazinga Channel / L. Edwardand George	-	HT-8746	na
Haplochromis sp.	Nagl et al. 2000	AF213610	Lake George	-	HT-8785	na
Haplochromis sp.	Nagl et al. 2000	AF213611	Lake George	-	HT-8903	na
Haplochromis sp.	Nagl et al. 2000	AF213612	Lake George	-	HT-8911	na
Haplochromis sp.	Nagl et al. 2000	AF213613	Malagarazi River	-	HT-1533	na
Haplochromis sp.	Nagl et al. 2000	AF213614	Malagarazi River	-	HT-1609	na
Astatoreochromis alluaudi	Nagl et al. 2000	AF213616	Lake Victoria	-	Asal6744	na
Astatoreochromis alluaudi	Nagl et al. 2000	AF213617	Lake Victoria	-	Asal5928	na
Pseudotropheus sp.'msobo'	Nagl et al. 2000	AF213622	Lake Malawi	-	Psms5170	na
Labeotropheus trewavasae	Nagl et al. 2000	AF213623	Lake Malawi	-	Latr5493	na
Haplochromis burtoni	Stiassny et al. 1994	AF400710	-	-	8153	na
Limnochromis auritus	Sturmbauer & Meyer 1992	AF400728	Lake Tanganyika	-	27749	na
Petrochromis orthognathus	Stiassny et al. 1994	AF400734	Lake Tanganyika	-	28818	na
Haplochromis astatodon	Verheyen et al. 2003	AY226611	Lake Kivu	E.Verheyen	K114	7
Haplochromis astatodon	Verheyen et al. 2003	AY226611	Lake Kivu	E.Verheyen	K114	7*
Haplochromis insidiae	Verheyen et al. 2003	AY226627	Lake Kivu	E.Verheyen	K080	8
Haplochromis sp.nigroides / scheffersi	Verheyen et al. 2003	AY226629	Lake Kivu	E.Verheyen	K146	9
Haplochromis astatodon	Verheyen et al. 2003	AY226631	Lake Kivu	E.Verheyen	K119	10
Haplochromis astatodon	Verheyen et al. 2003	AY226632	Lake Kivu	E.Verheyen	K131	11
Haplochromis paucidens	Verheyen et al. 2003	AY226633	Lake Kivu	E.Verheyen	K112	12
Haplochromis paucidens	Verheyen et al. 2003	AY226640	Lake Kivu	E.Verheyen	K022	13
Haplochromis paucidens	Verheyen et al. 2003	AY226641	Lake Kivu	E.Verheyen	K034	14
Haplochromis sp.crebridens / olivaceus	Verheyen et al. 2003	AY226642	Lake Kivu	E.Verheyen	K036	15
Haplochromis astatodon	Verheyen et al. 2003	AY226643	Lake Kivu	E.Verheyen	K127	16
Haplochromis sp.crebridens / olivaceus	Verheyen et al. 2003	AY226646	Lake Kivu	E.Verheyen	K060	17
Haplochromis scheffersi	Verheyen et al. 2003	AY226647	Lake Kivu	E.Verheyen	K111	18
Haplochromis graueri	Verheyen et al. 2003	AY226648	Lake Kivu	E.Verheyen	K118	19
Haplochromis graueri	Verheyen et al. 2003	AY226649	Lake Kivu	E.Verheyen	K012	20
Haplochromis astatodon	Verheyen et al. 2003	AY226650	Lake Kivu	E.Verheyen	K115	21
Haplochromis astatodon	Verheyen et al. 2003	AY226651	Lake Kivu	E.Verheyen	K124	22
Haplochromis astatodon	Verheyen et al. 2003	AY226652	Lake Kivu	E.Verheyen	K076	23
Haplochromis astatodon	Verheyen et al. 2003	AY226654	Lake Kivu	E.Verheyen	K132	24
Haplochromis astatodon	Verheyen et al. 2003	AY226655	Lake Kivu	E.Verheyen	K51	25*
Haplochromis occultidens	Verheyen et al. 2003	AY226666	Lake Kivu	E.Verheyen	K030	33
Haplochromis graueri	Verheyen et al. 2003	AY226668	Lake Kivu	E.Verheyen	K001	36
Haplochromis astatodon	Verheyen et al. 2003	AY226669	Lake Kivu	E.Verheyen	K116	37
Haplochromis sp.crebridens / olivaceus	Verheyen et al. 2003	AY226670	Lake Kivu	E.Verheyen	K057	38
Haplochromis astatodon	Verheyen et al. 2003	AY226671	Lake Kivu	E.Verheyen	K135	47*
Haplochromis paucidens	Verheyen et al. 2003	AY226687	Lake Kivu	E.Verheyen	K056	48

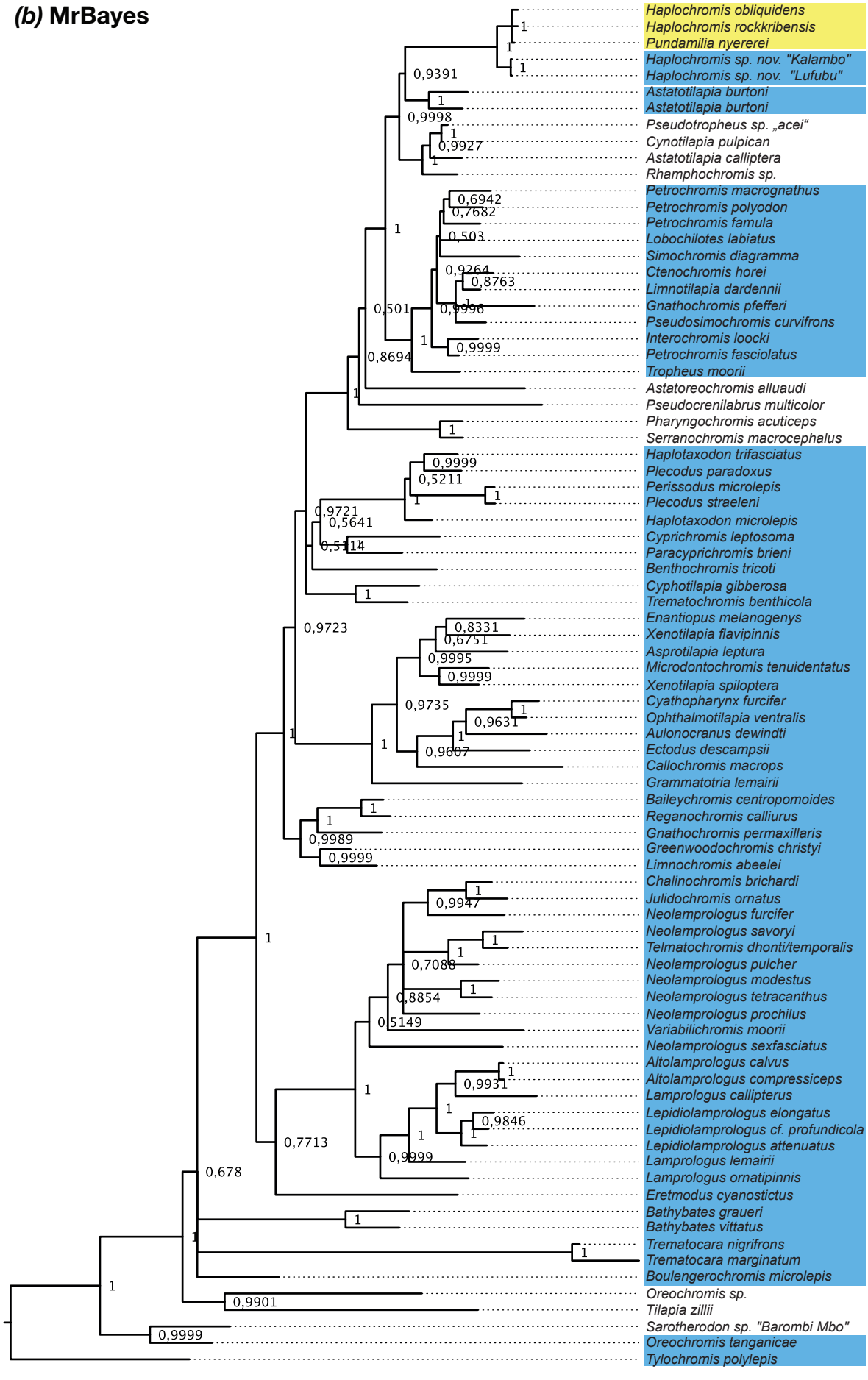
Haplochromis nigroides	Verheyen et al. 2003	AY226688	Lake Kivu	E.Verheyen	K028	49
Haplochromis astatodon	Verheyen et al. 2003	AY226691	Lake Kivu	E.Verheyen	K152	50
Haplochromis astatodon	Verheyen et al. 2003	AY226692	Lake Kivu	E.Verheyen	K138	51
Haplochromis paucidens	Verheyen et al. 2003	AY226694	Lake Kivu	E.Verheyen	K058	53
Haplochromis microchrysomelas	Verheyen et al. 2003	AY226695	Lake Kivu	E.Verheyen	K113	54
Haplochromis astatodon	Verheyen et al. 2003	AY226697	Lake Kivu	E.Verheyen	K120	55
Haplochromis microchrysomelas	Verheyen et al. 2003	AY226699	Lake Kivu	E.Verheyen	K142	56*
Haplochromis paucidens	Verheyen et al. 2003	AY226712	Lake Kivu	E.Verheyen	K174	57
Haplochromis crebridens	Verheyen et al. 2003	AY226714	Lake Kivu	E.Verheyen	K177	58
Haplochromis adolfifrederici	Verheyen et al. 2003	AY226715	Lake Kivu	E.Verheyen	K169	59
Haplochromis crebridens	Verheyen et al. 2003	AY226716	Lake Kivu	E.Verheyen	K063	74
Haplochromis sp.	Verheyen et al. 2003	AY226719	Cohoha / Bugesera Lakes	J.Snoeks	D9	82*
Haplochromis sp.	Verheyen et al. 2003	AY226720	Cohoha / Bugesera Lakes	J.Snoeks	B4	83
Haplochromis sp.	Nagl et al. 2000	AY226723	Rweru / Bugesera Lakes	-	R1	84
Haplochromis sp.	Verheyen et al. 2003	AY226726	Cohoha / Bugesera Lakes	J.Snoeks	D8	85
Haplochromis sp.	Verheyen et al. 2003	AY226727	Kachera / Uganda	E.Schraml	9803	6
Haplochromis sp.	Verheyen et al. 2003	AY226734	Victoria Nile	E.Schraml	9791	29
Haplochromis sp.	Verheyen et al. 2003	AY226735	Mugogo / Uganda	E.Schraml	9784	32
Haplochromis sp.	Verheyen et al. 2003	AY226736	aquarium trade	E.Schraml	9808	63
Haplochromis squamipinnis	Verheyen et al. 2003	AY226747	Lake Edward	E.Schraml	9813	65
Haplochromis sp.	Verheyen et al. 2003	AY226752	Nyamusingire / Uganda	E.Schraml	9765	67
Haplochromis sp.	Verheyen et al. 2003	AY226758	Nakivali / Uganda	E.Schraml	9721	72
Haplochromis sp.	Verheyen et al. 2003	AY226759	Lake Victoria	E.Schraml	9707	77
Haplochromis sp.	Verheyen et al. 2003	AY226761	Nawampasa / Lake Kyoga	E.Schraml	9788	78
Haplochromis sp.	Verheyen et al. 2003	AY226762	Lake Victoria	E.Schraml	9801	86
Haplochromis sp.	Verheyen et al. 2003	AY226763	Lake Victoria	E.Schraml	9713	91*
Haplochromis sp.	Verheyen et al. 2003	AY226764	Lake Victoria	E.Schraml	9706	92
Haplochromis sp.	Verheyen et al. 2003	AY226765	Lake Victoria	E.Schraml	9715	92
Haplochromis sp.	Verheyen et al. 2003	AY226766	Nawamapasa / Lake Kyoga	E.Schraml	9789	97*
Haplochromis sp.	Verheyen et al. 2003	AY226767	Lake Victoria	E.Schraml	9812	100
Haplochromis sp.	Verheyen et al. 2003	AY226768	Mulehe / Kabale Lakes	E.Schraml	9764	101*
Haplochromis sp.	Verheyen et al. 2003	AY226769	Lake Victoria	E.Schraml	9704	101
Haplochromis sp.	Verheyen et al. 2003	AY226779	Lake Victoria	E.Schraml	9703	103
Haplochromis sp.	Verheyen et al. 2003	AY226781	Bunyoni / Kabale Lakes	-	9727	119
Haplochromis sp.	Verheyen et al. 2003	AY226784	Bunyoni / Kabale Lakes	E.Schraml	9741	120
Haplochromis burtoni	Verheyen et al. 2003	AY226785	Cohoha / Bugesera Lakes	J.Snoeks	B6	na
Astatoreochromis alluaudi	Verheyen et al. 2003	AY226787	Cohoha / Bugesera Lakes	J.Snoeks	E9	na
Haplochromis gracilior	Verheyen et al. 2003	AY226788	Lake Kivu	-	K008	na
Haplochromis gracilior	Verheyen et al. 2003	AY226789	Lake Kivu	-	K009	na
Haplochromis gracilior	Verheyen et al. 2003	AY226790	Lake Kivu	-	K010	na
Thoracochromis brauschi	Verheyen et al. 2003	AY226791	Lac Fwa	Paul	9792	na
Serranochromis sp.WWS-2003	Verheyen et al. 2003	AY226792	Lake Mweru-Wantipa	T.Reuter	9793	na
Haplochromis stappersii	Salzburgeretal.2005	AY929941	Malagarazi River	L.DeVos	5-6 / 25 / 92	M3*
Haplochromis sp.	Salzburgeretal.2005	AY929992	Tanzania	L.Seegers	93 / 8	LR2*
Haplochromis sp.	Salzburgeretal.2005	AY930015	Tanzania	L.Seegers	92 / 12	LR1*
Cyrtocara moorii	Sturmbauer & Meyer 1992	U12554	Lake Tanganyika	-	30882	na
Haplochromis stappersii "Malagarasi1"	this study	KJ955382	Malagarazi River / Burundi	G.Banyankimbona	MRAC1840	M1*
Haplochromis stappersii "Malagarasi2"	this study	KJ955384	Malagarazi River / Burundi	G.Banyankimbona	MRAC1847	M1*
Haplochromis stappersii "Malagarasi3"	this study	KJ955385	Malagarazi River / Burundi	G.Banyankimbona	MRAC12034	M1*
Haplochromis stappersii "Malagarasi4"	this study	KJ955383	Malagarazi River / Burundi	G.Banyankimbona	MRAC12087	M2*
Haplochromis sp. "Chipwa"	this study	KJ955386	Kalambo River / Zambia	W.Salzburger	CH4	HLT*
Haplochromis sp. "Chipwa"	this study	KJ955387	Lufubu River / Zambia	W.Salzburger	LU2	HLT*
Haplochromis stappersii "Rusizi"	this study	KJ955381	Gatumbamarsh, Rusizi River	G.Banyankimbona	MRAC6334	RR*

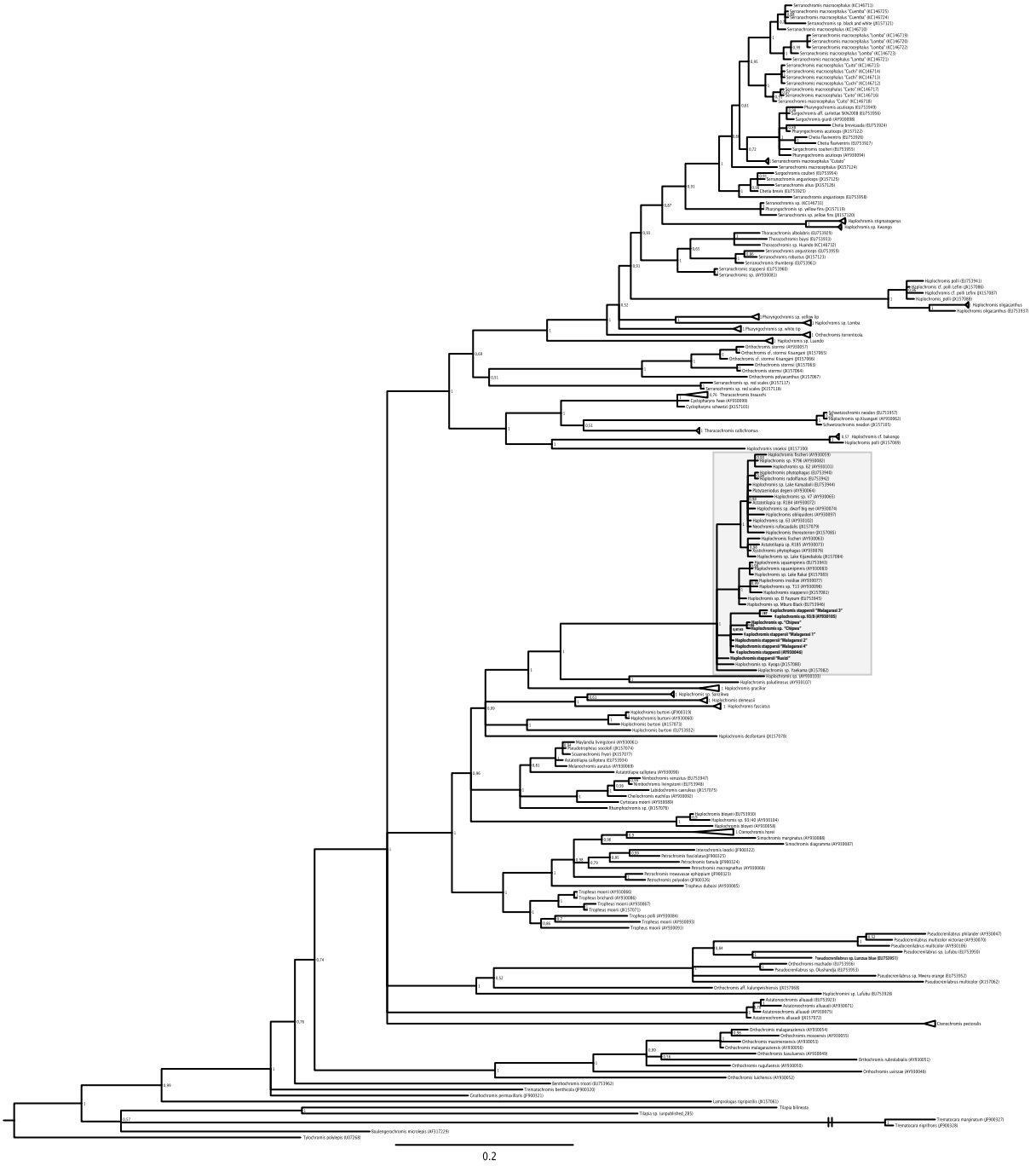
***(a)* GARLI**



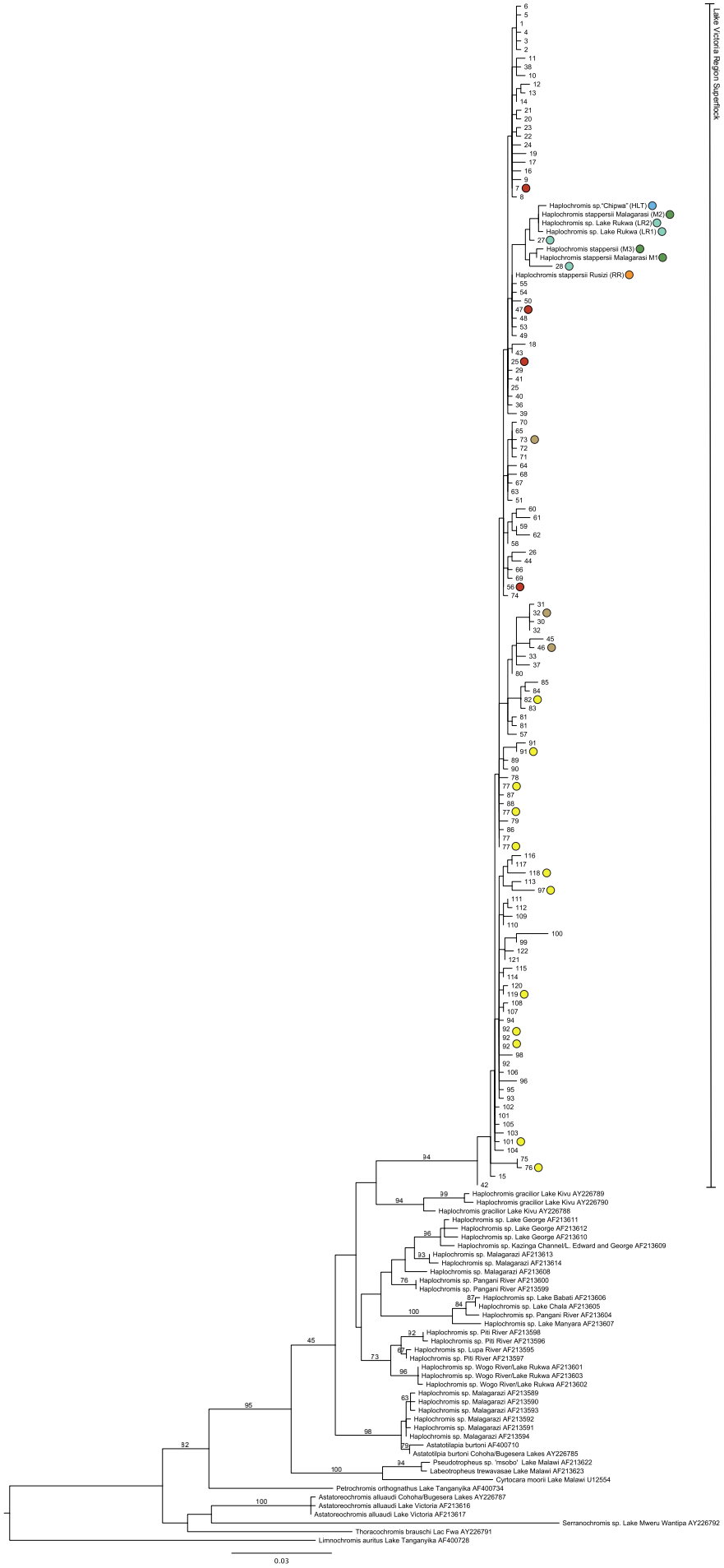
Maximum likelihood (*a*) and Bayesian (*b*) tree based on the concatenated dataset (table S1). All bootstrap support values and posterior probabilities are plotted. The geographical origin of the specimen is indicated in color (blue = Lake Tanganyika; yellow = Lake Victoria; other locations are not further indicated).

(b) MrBayes


Haplochromis obliquidens
Haplochromis rockkribensis
Pundamilia nyererei
Haplochromis sp. nov. "Kalambo"
Haplochromis sp. nov. "Lufubu"
Astatotilapia burtoni
Astatotilapia burtoni
Pseudotropheus sp. „acei“
Cynotilapia pulpican
Astatotilapia calliptera
Rhamphochromis sp.
Petrochromis macrognathus
Petrochromis polyodon
Petrochromis famula
Lobochilotes labiatus
Simochromis diagramma
Ctenochromis horei
Limnotilapia dardennii
Gnathochromis pfefferi
Pseudosimochromis curvifrons
Interochromis loocki
Petrochromis fasciolatus
Tropheus moorii
Astatoreochromis alluaudi
Pseudocrenilabrus multicolor
Pharyngochromis acuticeps
Serranochromis macrocephalus
Haplotaxodon trifasciatus
Plecodus paradoxus
Perissodus microlepis
Plecodus straeleni
Haplotaxodon microlepis
Cyprichromis leptosoma
Paracyprichromis brieni
Benthochromis tricoti
Cyphotilapia gibberosa
Trematochromis benthicola
Enantiopus melanogenys
Xenotilapia flavipinnis
Asprotilapia leptura
Microdontochromis tenuidentatus
Xenotilapia spiloptera
Cyathopharynx furcifer
Ophthalmotilapia ventralis
Aulonocranus dewindti
Ectodus descampsii
Callochromis macrops
Grammatotria lemairii
Baileychromis centropomoides
Reganochromis calliurus
Gnathochromis permaxillaris
Greenwoodochromis christyi
Limnochromis abeelei
Chalinochromis brichardi
Julidochromis ornatus
Neolamprologus furcifer
Neolamprologus savoryi
Telmatochromis dhonti/temporalis
Neolamprologus pulcher
Neolamprologus modestus
Neolamprologus tetracanthus
Neolamprologus prochilus
Variabilichromis moorii
Neolamprologus sexfasciatus
Altolamprologus calvus
Altolamprologus compressiceps
Lamprologus callipterus
Lepidiolamprologus elongatus
Lepidiolamprologus cf. profundicola
Lepidiolamprologus attenuatus
Lamprologus lemairii
Lamprologus ornatipinnis
Eretmodus cyanostictus
Bathybates graueri
Bathybates vittatus
Trematocara nigrifrons
Trematocara marginatum
Boulengerochromis microlepis
Oreochromis sp.
Tilapia zillii
Sarotherodon sp. "Barombi Mbo"
Oreochromis tanganicae
Tylochromis polylepis



Supplementary figure 2: MrBayes 50% majority rule consensus tree with branch lengths based on the ND2 data set (table S2). Posterior probabilities ≥ 0.5 are plotted. The grey box represents the Lake Victoria Region superflock.


Lake Victoria Region Superflock
Haplochromis sp."Chipwa" (HLT)
Haplochromis stappersii Malagarasi (M2)
Haplochromis sp. Lake Rukwa (LR2)
Haplochromis sp. Lake Rukwa (LR1)
Haplochromis stappersii (M3)
Haplochromis stappersii Malagarasi M1
Haplochromis stappersii Rusizi (RR)
Haplochromis gracilior Lake Kivu AY226789
Haplochromis gracilior Lake Kivu AY226790
Haplochromis gracilior Lake Kivu AY226788
Haplochromis sp. Lake George AF213611
Haplochromis sp. Lake George AF213612
Haplochromis sp. Lake George AF213610
Haplochromis sp. Kazinga Channel/L. Edward and George AF213609
Haplochromis sp. Malagarazi AF213613
Haplochromis sp. Malagarazi AF213614
Haplochromis sp. Malagarazi AF213608
Haplochromis sp. Pangani River AF213600
Haplochromis sp. Pangani River AF213599
Haplochromis sp. Lake Babati AF213606
Haplochromis sp. Lake Chala AF213605
Haplochromis sp. Pangani River AF213604
Haplochromis sp. Lake Manyara AF213607
Haplochromis sp. Piti River AF213598
Haplochromis sp. Piti River AF213596
Haplochromis sp. Lupa River AF213595
Haplochromis sp. Piti River AF213597
Haplochromis sp. Wogo River/Lake Rukwa AF213601
Haplochromis sp. Wogo River/Lake Rukwa AF213603
Haplochromis sp. Wogo River/Lake Rukwa AF213602
Haplochromis sp. Malagarazi AF213589
Haplochromis sp. Malagarazi AF213590
Haplochromis sp. Malagarazi AF213593
Haplochromis sp. Malagarazi AF213592
Haplochromis sp. Malagarazi AF213591
Haplochromis sp. Malagarazi AF213594
Astatotilapia burtoni AF400710
Astatotilpia burtoni Cohoha/Bugesera Lakes AY226785
Pseudotropheus sp. 'mscbo' Lake Malawi AF213622
Labeotropheus trewavasae Lake Malawi AF213623
Cyrtocara moorii Lake Malawi U12554
Petrochromis orthognathus Lake Tanganyika AF400734
Astatoreochromis alluaudi Cohoha/Bugesera Lakes AY226787
Astatoreochromis alluaudi Lake Victoria AF213616
Astatoreochromis alluaudi Lake Victoria AF213617
Serranochromis sp. Lake Mweru Wantipa AY226792
Thoracochromis brauschi Lac Fwa AY226791
Limnochromis auritus Lake Tanganyika AF400728
0.03

Chapter 5

Adaptive divergence between lake and stream populations of an East African cichlid fish

Anya Theis*, Fabrizia Ronco*, **Adrian Indermaur**, Walter Salzburger and Bernd Egger

*These authors contributed equally to this work.

Molecular Ecology (2014)

AI helped with sampling and discussion of the manuscript

MOLECULAR ECOLOGY

Molecular Ecology (2014) **23**, 5304–5322 doi: 10.1111/mec.12939

Adaptive divergence between lake and stream populations of an East African cichlid fish

ANYA THEIS,[1] FABRIZIA RONCO,[1] ADRIAN INDERMAUR, WALTER SALZBURGER and BERND EGGER

Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland

Abstract

Divergent natural selection acting in different habitats may build up barriers to gene flow and initiate speciation. This speciation continuum can range from weak or no divergence to strong genetic differentiation between populations. Here, we focus on the early phases of adaptive divergence in the East African cichlid fish *Astatotilapia burtoni*, which occurs in both Lake Tanganyika (LT) and inflowing rivers. We first assessed the population structure and morphological differences in *A. burtoni* from southern LT. We then focused on four lake–stream systems and quantified body shape, ecologically relevant traits (gill raker and lower pharyngeal jaw) as well as stomach contents. Our study revealed the presence of several divergent lake–stream populations that rest at different stages of the speciation continuum, but show the same morphological and ecological trajectories along the lake–stream gradient. Lake fish have higher bodies, a more superior mouth position, longer gill rakers and more slender pharyngeal jaws, and they show a plant/algae and zooplankton-biased diet, whereas stream fish feed more on snails, insects and plant seeds. A test for reproductive isolation between closely related lake and stream populations did not detect population-assortative mating. Analyses of F1 offspring reared under common garden conditions indicate that the detected differences in body shape and gill raker length do not constitute pure plastic responses to different environmental conditions, but also have a genetic basis. Taken together, the *A. burtoni* lake–stream system constitutes a new model to study the factors that enhance and constrain progress towards speciation in cichlid fishes.

Keywords: adaptive divergence, *Astatotilapia burtoni*, East African cichlid fishes, Lake Tanganyika, lake–stream system, speciation continuum

Received 29 July 2014; revision received 19 September 2014; accepted 22 September 2014

Introduction

Different environmental conditions constitute a major source of divergent natural selection between populations (reviewed in Schluter 2000; Nosil 2012). Adaptation to divergent habitats may ultimately lead to speciation, for example when reproductive isolation builds up as by-product of adaptive divergence ('ecological speciation'), or when different mutations become fixed in geographically separated populations adapting to similar environments ('mutation-order speciation') (Rundle & Nosil 2005; Schluter 2009). Both scenarios imply that speciation is a gradual process, which is evidenced by empirical data demonstrating substantial variation in the level of divergence between adjacent populations, even along environmental clines that are free of geographical barriers (Hendry *et al.* 2000; Schluter 2000; Rundle & Nosil 2005; Butlin *et al.* 2008; Mallet 2008; Berner *et al.* 2009; Nosil *et al.* 2009). This so-called speciation continuum can range from weak or no divergence between populations to strong genetic differentiation between what might then be novel pairs of sister species (Hendry *et al.* 2009; Nosil *et al.* 2009). What determines the strength of divergence between populations remains poorly understood, though.

Correspondence: Walter Salzburger and Bernd Egger, Fax: +41 61 267 0301; E-mails: walter.salzburger@unibas.ch and bernd.egger@unibas.ch

[1]These authors contributed equally to this work.

Adaptive divergence has mainly been studied in settings involving populations that differ in their degree of reproductive isolation, such as in stick insects (Nosil & Sandoval 2008), mosquitofish (Langerhans *et al.* 2007) or *Heliconius* butterflies (Mallet & Dasmahapatra 2012). Important model systems in fishes are three-spine sticklebacks and salmonids, which often occur along discrete environmental gradients such as marine–freshwater and/or lake–stream habitats (e.g. Hendry *et al.* 2000; Berner *et al.* 2008; Jones *et al.* 2012; Roesti *et al.* 2012). Stickleback lake–stream populations, for example, differ with regard to resource use and are morphologically distinct, with limnetic-foraging lake forms typically displaying shallower bodies and more and longer gill rakers than the benthic-foraging stream types (Schluter & McPhail 1992; Berner *et al.* 2008). The extent of divergence between lake and stream population pairs depends on the strength of divergent selection, on the level of gene flow and on the time since divergence (Hendry & Taylor 2004; Berner *et al.* 2010; Roesti *et al.* 2012; Hendry *et al.* 2013; Lucek *et al.* 2013). Studies in sticklebacks and salmonids also uncovered that diversification may proceed rapidly (see e.g. Hendry *et al.* 2007). In the sockeye salmon (*Oncorhynchus nerka*), for example, it took about a dozen of generations only until reproductive isolation occurred between two adjacent beach and stream populations that diverged after an introduction event (Hendry *et al.* 2000). However, ecological divergence might also fail to generate the evolution of reproductive isolation barriers (Raeymaekers *et al.* 2010).

In this study, we focus on the early phases of adaptive divergence in a prime model system for evolutionary biology, the East African cichlid fishes (see e.g. Kocher 2004; Salzburger 2009; Santos & Salzburger 2012). More specifically, we examine eco-morphological and genetic divergence in *Astatotilapia burtoni* (Günther 1894), which occurs both in East African Lake Tanganyika (LT) and inflowing rivers. Although *A. burtoni* is one of the most important cichlid model species in various fields of research including developmental biology, neurobiology, genetics and genomics, and behavioural biology (see e.g. Wickler 1962; Robison *et al.* 2001; Hofmann 2003; Lang *et al.* 2006; Salzburger *et al.* 2008; Baldo *et al.* 2011; Theis *et al.* 2012; Santos *et al.* 2014) and represents one of the five cichlid species whose genome has recently been sequenced (Brawand *et al.* 2014), surprisingly little is known about its ecology, phylogeographic distribution, population structure or genetic and phenotypic diversity in the wild.

Taxonomically, *A. burtoni* belongs to the Haplochromini, the most species-rich group of cichlids. Within the haplochromines, *A. burtoni* is nested in the derived 'modern' clade (as defined in Salzburger *et al.* 2005), the members of which are characterized by a pronounced sexual colour dimorphism with typically brightly coloured males and inconspicuous females, a polygynandrous mating system with maternal mouthbrooding, as well as egg-spots on the anal fin of males. The vast majority of haplochromines is endemic to a specific lake or river system, respectively, and specialized to certain habitat types therein. Only very few cichlid species exist that commonly occur in both truly riverine and lacustrine habitats. *Astatotilapia burtoni* is such a habitat generalist, inhabiting the shallow zones of LT as well as rivers and streams surrounding LT (Fernald & Hirata 1977; De Vos *et al.* 2001; Kullander & Roberts 2011), and thus represents an ideal species to study adaptive divergence across an environmental gradient in cichlid fishes.

So far, adaptive divergence in cichlids has mainly been investigated within lakes, for example along depth or habitat gradients (see e.g. Barluenga *et al.* 2006; Seehausen *et al.* 2008). In our study, we targeted divergence along a lake–stream environmental gradient to test whether similar mechanisms are involved in divergence along this habitat gradient as in other groups of fishes. To this end, we first established phylogeographic relationships and assessed the population structure in *A. burtoni* from the southern part of the LT drainage using mtDNA and microsatellite markers. Second, we examined morphological differences between these populations by analysing body shape, a complex quantitative trait encompassing morphological variation associated with multiple ecological factors (Webb 1984). We then focused on four lake–stream systems in detail. In addition to the body shape and population-genetic analyses, we quantified several ecologically relevant traits in these replicate lake–stream population groups, including the gill raker apparatus, which is known to respond to distinct feeding modes in fishes. The number and length of gill rakers have been identified as key elements influencing prey capture and handling in stickleback (Bentzen & McPhail 1984; Lavin & McPhail 1986; Schluter 1993, 1995; Robinson 2000). Furthermore, we examined the pharyngeal jaw apparatus, a highly diverse trait in cichlids linked to trophic diversification (Galis & Drucker 1996; Hulsey *et al.* 2006; Muschick *et al.* 2012), and used stomach content analysis as a proxy for divergent selection acting on foraging morphology. We then tested whether there were associations between shifts in resource use and trophic morphology along the lake–stream gradient that might reflect ecologically based adaptive divergence (Berner *et al.* 2009; Harrod *et al.* 2010). Finally, we conducted a mating experiment to test for reproductive isolation among a lake and stream populations. Additionally, offspring from this common garden setting was used to

evaluate levels of phenotypic plasticity in adaptive traits such as body shape and gill raker morphology.

Materials and methods

Study populations and sampling

Sampling of *A. burtoni* was carried out between February 2010 and July 2013 in the southern basin of LT and in inflowing rivers and streams, with a particular emphasis on four river systems, the Kalambo River, the Chitili Creek, the Lunzua River and the Lufubu River (Figs 1A and 2A) (see Appendix S1, Supporting information for a detailed description of these river systems). Specimens were collected using hook and line fishing, minnow traps and gill nets under the permission of the LT Research Unit, Department of Fisheries, Republic of Zambia. In total, we sampled 22 populations (several of these multiple times), resulting in a data set comprising 1425 individuals (see Tables S1 and S2A, Supporting information for details). Specimens were anaesthetized using clove oil (2–3 drops clove oil per litre water) and photographed in a standardized manner for morphometric analyses; a fin clip was taken and stored in ethanol (96%) for a DNA sample; specimens for gill raker measurements, pharyngeal jaw and stomach content analyses were preserved in ethanol (96%).

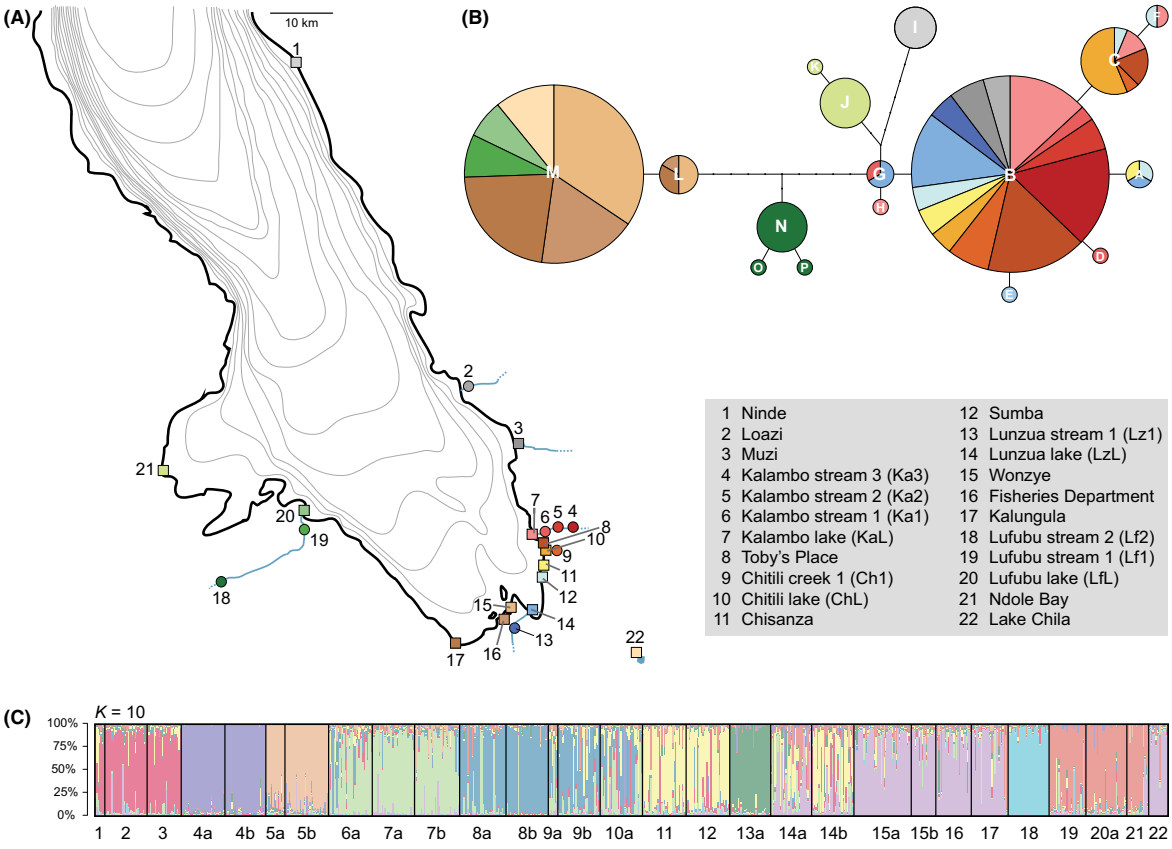


Fig. 1 Sampling locations and genetic differentiation among all populations revealed by microsatellite and mtDNA analyses. (A) The 22 sampling localities indicated by numbers on the southern part of LT (squares represent lake and circles stream populations; bathymetric lines are placed at every 100 m water depth, after Coulter 1991). Names of localities are listed in the grey box. (B) Haplotype genealogy based on mtDNA showing the 16 haplotypes (A–P) and the deep split between eastern (populations 2–14; haplotypes A–H) and western (populations 15–17, 19–20; haplotypes L and M) populations. Each colour represents a locality, which correspond to the colours on the map. (C) Structure plot based on nine microsatellite loci for all populations: the 29 population samples from 22 localities (names in the grey box; 'a' and 'b' refer to different sampling years, note that not all sampling years were analysed) group in 10 genetic clusters ($K = 10$; colours representing these clusters are decoupled from the population colours in the map). LT, Lake Tanganyika.

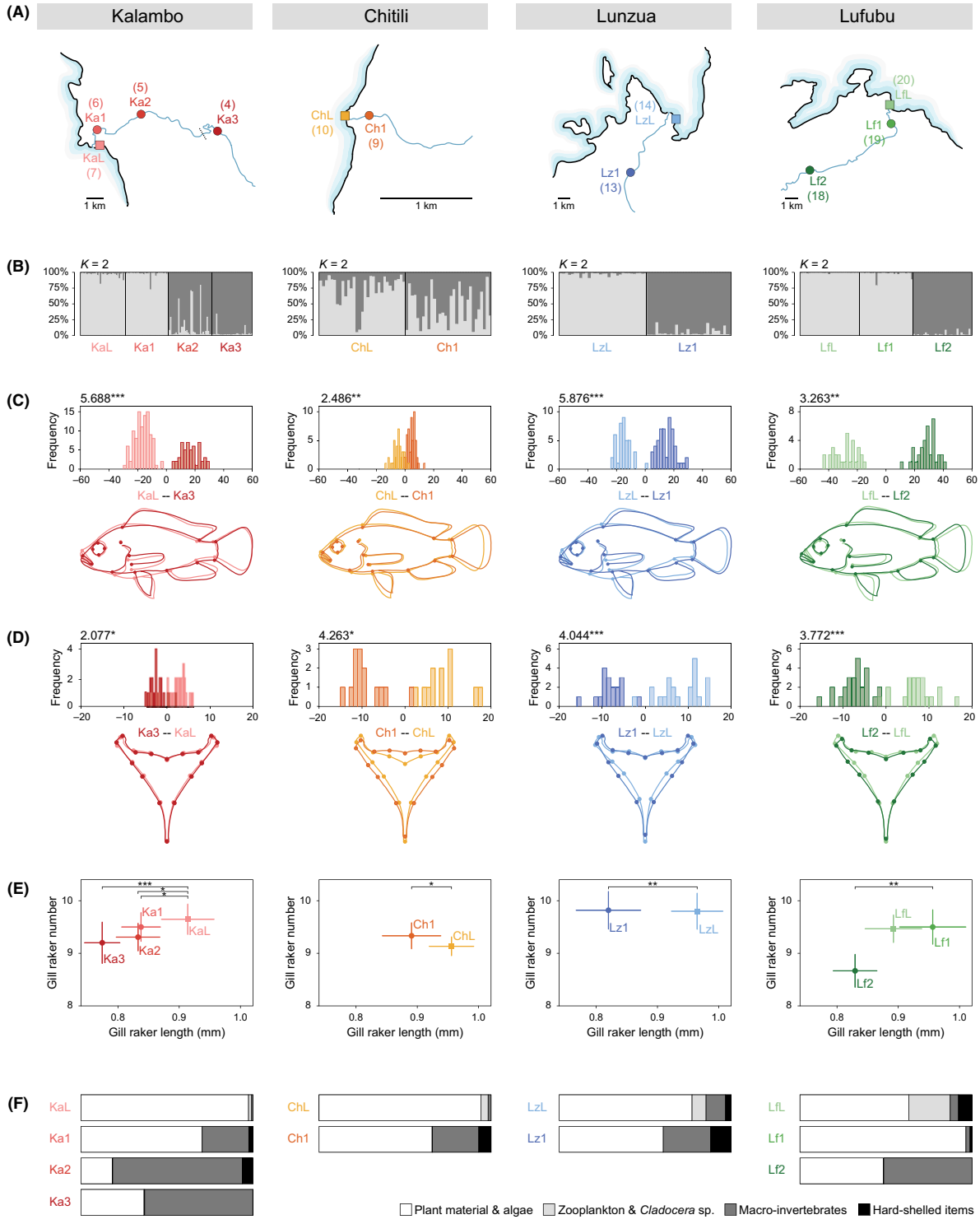


Fig. 2 Divergence between lake and stream habitats in four systems. (A) Maps showing sampling localities for each lake–stream system (see grey box in Fig. 1 for full names of localities). (B) Structure plots for each lake–stream system (shades of grey represent different genetic clusters; K = number of genetic clusters). (C) Discriminant scores of body shape comparisons and corresponding landmark shifts from the discriminant function analyses (DFA) between the lake population and the most upstream population for each lake–stream system show that lake fish generally have a deeper body and a more superior mouth position compared with stream fish. DF differences are always increased threefold in the outlines, which are drawn for illustration purposes only. DFA results are indicated with Mahalanobis distances on top of the DF score plots. (D) Discriminant scores of lower pharyngeal jaw (LPJ) shape comparisons and corresponding landmark shifts from the DFA between the lake population and the most upstream population for each lake–stream system show that lake fish generally have a slender and more elongated LPJ compared with stream fish. (E) Differences in size corrected male gill raker length and number between populations within each lake–stream system. Error bars represent 95% confidence intervals of the means. Lake fish generally have longer gill rakers compared with stream fish (Table S6, Supporting information). (F) Averaged proportions of the different stomach content categories for each population. Generally, lake fish feed more on softer and smaller food particles, whereas stream populations feed more on hard-shelled and larger food items. Significance levels: $*P < 0.05$, $**P < 0.01$ and $***P < 0.0001$.

Water current measurements

Surface water current and microhabitat current (measured directly where the fish were sighted) were determined at 10 sampling sites in July 2013. The flow regime differs between dry and wet season; however, relative differences between sampling sites are likely to be consistent. Surface current was estimated by measuring the time a float (0.5 L plastic bottle filled with 0.25 L water) travelled 10 m downstream. Measurements were taken five times at each site, and the velocity was calculated from the average of these measurements. For microhabitat current, we determined the relative level of water motion in lake and stream habitats as a proxy. To this end, we used Life Savers candies (wint-o-green flavour, individually wrapped variety; $N = 5$) to measure the relative rate of dissolution (which is directly related to water current), following the method described by Koehl & Alberte (1988). Life Savers were either tied to plants or were hand-held into the underwater habitat using a stick and line and left to dissolve for 6 min. Additionally, a baseline dissolution rate was determined by placing a candy in a bucket filled with water from the respective site (no current) for 6 min. We determined the weight of each candy before and after treatment (dried at ambient temperature for at least 2 h) to calculate the mass (g) lost relative to the baseline.

Genetics

Total DNA was extracted from fin clips preserved in ethanol applying a proteinase K digestion followed by either a high-salt (Bruford *et al.* 1998) or a MagnaPure extraction using a robotic device (MagnaPure LC; Roche Diagnostics), following the manufacturer's protocol (Roche, Switzerland). We first determined the DNA sequence of a 369-bp segment of the mitochondrial control region for 5–40 samples per location (total $N = 359$, Table S1, Supporting information) using published primers (Kocher *et al.* 1989; Salzburger *et al.* 2002). The PCR fragments of the control region were purified using ExoSAP-IT (USB), directly sequenced with the BigDye sequencing chemistry (Applied Biosystems) and analysed on an ABI 3130*xl* genetic analyzer (Applied Biosystems). Mitochondrial DNA sequences were aligned using CODONCODE ALIGNER (v.3.5; CodonCode Corporation). A maximum-likelihood analysis, using the GTR + G + I as suggested by JMODELTEST (Posada 2008), was carried out in PAUP*4.0b10 (Swofford 2002) to construct an unrooted mitochondrial haplotype genealogy following the method described in Salzburger *et al.* (2011).

A total of 786 individuals (Table S1, Supporting information) were genotyped at the following nine microsatellite loci: Ppun5, Ppun7, Ppun21 (Taylor *et al.* 2002), UNH130, UNH989 (Lee & Kocher 1996), Abur82 (Sanetra *et al.* 2009), HchiST46, HchiST68 (Maeda *et al.* 2009) and Pzeb3 (Van Oppen *et al.* 1997). Fragment size calling was carried out on an ABI 3130*xl* genetic analyzer (Applied Biosystems) in comparison with the LIZ 500(−250) internal size standard. Genotypes were determined manually using PEAK SCANNER (v.1.0; Applied Biosystems). Microsatellite scoring data were examined and rounded to valid integers using TANDEM (Matschiner & Salzburger 2009). The microsatellite data were used to calculate population pairwise F_{ST} values in ARLEQUIN (v.3.5.1.2; Schneider *et al.* 1999) and D_{EST} (Jost 2008) using the package DEMETICS (Gerlach *et al.* 2010) in R (v.3.1.0; R Development Core Team 2014). STRUCTURE (v.2.3.3; Pritchard *et al.* 2000) was then used to infer population structure. First, all 29 populations (22 localities, seven of which were sampled twice in different years) were run in a joint analysis (Markov chain Monte Carlo simulations were run for 500 000 replications, burn in = 50 000, admixture and correlated allele frequency options). Ten replicated simulations were performed for $K = 1$–16, and the most likely number of genetic clusters was inferred using the ΔK method (Evanno *et al.* 2005) implemented in the software HARVESTER (Earl & von Holdt 2012). Then, each lake–stream system

was analysed separately using the same parameters as described above and K = 1–10 for Kalambo, K = 1–6 for Lufubu, Chitili and Lunzua.

To test for isolation by distance, we conducted a simple Mantel test in R (package ecodist, Goslee & Urban 2007) using the genetic distance (pairwise F_{ST} values) and the geographic distance in metres between sites measured along the shoreline on Google Earth. For this analysis, only populations from the LT shoreline were used (N_{pop} = 13) and all riverine populations (2, 4–6, 9, 13, 18, 19; see Fig. 1) and the population from Lake Chila (22) were excluded.

Body shape

The photographs of 791 individuals (Table S1, Supporting information) were used for geometric morphometric analyses by recording the coordinates of 17 homologous landmarks (Fig. S1A, Supporting information; for details see Muschick *et al*. 2012) using TPSDIG2 (v.2.11; Rohlf 2008). The x and y coordinates were transferred to the program MORPHOJ (v.1.05f; Klingenberg 2011) and superimposed with a Procrustes generalized least squares fit (GLSF) algorithm to remove all nonshape variation (Rohlf & Slice 1990). Additionally, the data were corrected for allometric size effects using the residuals of the regression of shape on centroid size for further analyses. Canonical variate analyses (CVA; Mardia *et al*. 1979) were used to assess shape variation when several populations were compared, and discriminant function analyses (DFA) were performed for comparisons between two populations only (i.e. within some lake–stream systems). The mean shape distances of CV and DF analyses were obtained using permutation tests (10 000 permutations). Although males and females show strong body shape differences, the pooled data revealed the same results as the separate analyses for each sex (data not shown), presumably because intersexual within-population differences are smaller than intrasexual differences among populations (Fig. S2, Supporting information). Therefore, both sexes were combined in the analyses presented.

In a first step, we conducted a CVA for 20 populations and another one for the 11 shoreline populations only to test whether the clustering in morphospace shows signs of isolation by distance. Further tests for morphological isolation by distance were conducted with a simple Mantel test in the ecodist package in R using the morphological (Mahalanobis) and the geographic distance (measured in metres along the shoreline). In a second step, the lake–stream populations were tested within each system as well as in a combined data set.

Finally, we also performed a CVA focusing on the mouth position (landmarks 1, 2, 7 and 12, capturing mouth angle; Fig. S1A, Supporting information). We only used male individuals here, as this trait shows a much stronger sexual dimorphism compared with, for example, body shape.

Gill raker morphology

Following Berner *et al*. (2008), we counted gill raker number and measured the length of the 2nd, 3rd and 4th gill raker of the right first branchial arch and calculated the mean for each of 281 individuals collected from the four lake–stream systems (Table S1, Supporting information). As average gill raker length correlated positively with standard length (SL) in both sexes (males: regression, $R^2 = 0.8432$, $P < 0.0001$; females: regression, $R^2 = 0.5477$, $P < 0.0001$), mean gill raker length was regressed to SL for size correction. The individual residuals from the common within-group slope were then added to the expected gill raker length at grand mean SL (male = 0.879 mm, female = 0.783 mm) to maintain the original measurement unit. These values represent a size-independent gill raker length and were used for the comparisons between populations within each lake–stream system separately applying an ANOVA. For the Kalambo and Lufubu systems, for which we had more than two populations, a TukeyHSD was performed to adjust for multiple testing. Male (N = 155) and female (N = 126) data were analysed separately because size corrected gill raker length differed between the sexes (gill rakers are longer in females; ANOVA using size corrected values, P = 0.0095), and the sex ratios differed among populations. As we obtained similar results for males and females, we present the results of male data only. All statistical analyses were conducted in R.

Lower pharyngeal jaw morphology

Geometric morphometric analyses were applied on 224 lower pharyngeal jaw bones (LPJ) from the four lake–stream systems (Table S1, Supporting information). Pictures of the cleaned jaws were generated using an office scanner (EPSON perfection V30/V300, resolution: 4800 dpi) with a ruler on every scan to maintain size information. Following Muschick *et al*. (2012), x and y coordinates of eight homologous landmarks and 20 semilandmarks plus the image scales were acquired in TPSDIG2. After a sliding process with TPSRELW (Rohlf 2007), we reduced the initial data set to 16 landmarks consisting of eight true landmarks and eight semilandmarks (Fig. S1C, Supporting information; for details see Muschick *et al*. 2012). The symmetric components of the procrustes-aligned coordinates (GLSF algorithm) were then regressed against centroid size to correct for

allometry. The residuals of the regression were used to perform DFA for each lake–stream system by comparing each lake population with the geographically most distant stream population. Further, we conducted several CVAs comparing multiple populations within each system and over all populations of the lake–stream systems. The significance levels of the obtained mean shape distances were computed using permutation tests (10 000 permutations). As we found smaller intersexual within-population differences in LPJ shape than intrasexual differences among populations (Fig. S2, Supporting information), all analyses were conducted with pooled sexes. Statistical analyses of the morphometric data were performed in MorphoJ.

Stomach and gut content

To investigate whether the populations differ with respect to food resource use, we inspected gut and stomach contents. To this end, the intestines of 102 male individuals (Table S1, Supporting information) were opened under a binocular (LEICA, $MZ7_5$) and the content was separated into the following five categories: plant material and algae, sand, macro-invertebrates (insects and insect larvae), hard-shelled items (mollusc shells and plant seeds), and zooplankton and micro-invertebrates (mainly small shrimps of the LT endemic genus *Limnocaridina*, cladocerans and copepods). The volume (in %) of each category was determined by comparison with serial volume units. For the illustration of the proportions of food items only, the category 'sand' was excluded.

Testing for associations between genetic differentiation, morphometric traits and environment

Partial Mantel tests were applied to compare pairwise differences of morphometric traits (Mahalanobis distances for body shape, mouth position and LPJ, metric measurements for gill rakers) from lake–stream populations with the corresponding F_{ST} values, while correcting for geographic distances. In a second step, the influences of several environmental parameters (microhabitat current, proportion of hard-shelled food items and proportion of macro-invertebrates) and geographic distance on the same morphometric differences were analysed with a multiple regression on distance matrices (MRM). MRM is an extension of the partial Mantel analysis and allows multiple regression of the response matrix on any number of explanatory matrices (Lichtstein 2007). Of 10 000 permutations were performed, as recommended by Jackson & Somers (1989). All analyses were performed using the package ecodist in R. Note that we had to exclude Lf1 in these analyses due to the lack of environmental data.

Testing for reproductive isolation and trait plasticity

We evaluated reproductive isolation among lake and stream *A. burtoni* populations in triadic mating trials. The common garden setting of this pond experiment also allowed us to test for plasticity in body shape and gill raker morphology in F1 offspring.

The experiment was carried out between July 2013 and January 2014 in five concrete ponds at Kalambo Lodge, Zambia. Experimental ponds (dimensions: 3.2 × 1.4 × 0.5 m) were stocked with seven females and four males each from two stream populations (Ka3 and Lz1) and one lake population (KaL). Wild-caught adults were photographed and fin-clipped before starting the experiment. Males were selected for size to achieve a similar size distribution among the three populations within each pond. Concrete ponds were supplied with lake water; fish were fed with commercial flake food two times a day.

After a period of six months, we collected and fin-clipped all offspring plus all remaining adult fish (55 out of 165 initially introduced) from the ponds. Fish weighting more than 1 g were photographed and measured. We then genotyped all putative parental individuals and 593 offspring (i.e. all free living juveniles plus 5 individuals from each brood within a females' mouth) at five microsatellite loci (Ppun5, Ppun7, Ppun21, UNH130 and Abur82), following the methods described above. Parentage was inferred using the software CERVUS (Kalinowski *et al.* 2007), with no mismatch allowed. Offspring that were assigned to the same mother and father were combined as a single mating event, except if they belonged to different size classes (free-swimming young vs. wrigglers). In case of the detection of more than one father in broods collected from mouthbrooding females, these were treated as two mating events. Multiple paternity in *A. burtoni* has been detected previously in mate choice experiments under laboratory conditions in ~7% of genotyped broods (Theis *et al.* 2012).

We then used F1-offspring to test for a heritable component of body shape ($N = 130$) and gill raker ($N = 132$) morphology. F1 individuals were categorized as offspring resulting from the following mating combinations: KaL-KaL, Ka3-Ka3, Lz1-Lz1, Ka3-Lz1, KaL-Ka3 and KaL-Lz1 (Table S2B, Supporting information). Body shape was analysed using the same methods as described above. Due to low sample size in some of the crosses, we reduced the number of landmarks to 6 (landmarks 1, 2, 8, 12, 14 and 15; Fig. S1A, Supporting information). We first conducted CVAs for the three interpopulation crosses (KaL-Ka3, KaL-Lz1, Lz1-Ka3) and their corresponding within-population crosses (KaL-KaL, Ka3-Ka3, Lz1-Lz1) separately to test

whether (i) within-population crosses are differentiated and (ii) whether interpopulation crosses show intermediate body shape with respect to within-population crosses. Additionally, within-population F1 offspring were analysed in a CVA together with their corresponding wild-type populations to detect plastic shifts in body shape induced by the common garden setup. Moreover, we conducted a CVA to compare body shape of introduced specimens before and after the experiment, to test for plastic responses in adults. Gill raker length and number of F1 offspring were measured and analysed using the same methods as described above for wild populations. Mean gill raker length correlated positively with SL ($R^2 = 0.58$, $P < 0.0001$) and was corrected for body size. As with body shape, the three interpopulation crosses (KaL-Ka3, KaL-Lz1 and Lz1-Ka3) and their corresponding within-population crosses (KaL-KaL, Ka3-Ka3 and Lz1-Lz1) were first analysed separately. Then, within-population crosses were compared with their corresponding wild-type populations after applying a common size correction.

Results

Water current measurements

Water current was generally stronger at upstream localities, with the exception of Kalambo (water current was stronger at Ka2 than Ka3; see Table 1A for values and Appendix S1, Supporting information for habitat descriptions). As surface and microhabitat current are significantly correlated ($R^2 = 0.6155$, $P = 0.0072$), we used only microhabitat current for further analyses.

Genetics

Sequencing of the mitochondrial control region of 359 specimens revealed the presence of 16 haplotypes. The haplotype genealogy (Fig. 1B) indicates a deep split between the eastern (1–14, haplotypes A–I) and the western (15–17, 19–20, haplotypes L and M) populations. Moreover, the most upstream Lufubu population (18) comprises three haplotypes (N–P), which are clearly distinct from all other lineages. The haplotypes found at the western shoreline of LT at Ndole Bay (21, haplotypes J and K) group with the ones from the northernmost population at the eastern shoreline of LT at Ninde (1, haplotype I). The Lake Chila fish (22) contain the major mtDNA haplotype of the western haplotype lineage (haplotype M).

The analysis of nine microsatellite loci revealed moderate to strong differentiation between populations, even within lake–stream systems (Table S3A, Supporting information for population pairwise F_{ST} and D_{EST}). F_{ST} and D_{EST} values are highly congruent, and P-values (F_{ST}) and confidence intervals (D_{EST}) indicate significant differentiation between most population pairs except for some geographically adjacent populations (15 and 16 for both F_{ST} and D_{EST}, 16 and 17 for F_{ST} but not D_{EST}) and some of the populations sampled twice in two different years (4a and 4b, 7a and 7b, 15a and 15b). Based on F_{ST} and D_{EST} values, population 22 (Lake Chila) and 16 (Fisheries Department, LT) are not significantly differentiated.

Bayesian clustering with STRUCTURE of the entire data set resulted in a most likely number of $K = 10$ (Fig. 1C). The three Tanzanian populations (1–3) cluster together, despite rather large geographic distances between them.

Table 1 Microhabitat current as well as stomach and gut content information. (A) Microhabitat current (represented by dissolution rate in mg/s) at the localities from the lake–stream systems with 95% confidence intervals in brackets. (B) Average values with corresponding 95% confidence intervals in brackets for the proportions of the different stomach content categories (plant and algae, zooplankton, sand, macro-invertebrates, and hard-shelled items)

A		B					
Locality	Microhabitat current: dissolution rate (mg/s)	Population	Plants and algae	Zooplankton	Sand	Macro-invertebrates	Hard-shelled items
KaL	0.032 (±0.039)	KaL ($N = 10$)	0.954 (±0.036)	0.018 (±0.015)	0.020 (±0.037)	0.008 (±0.006)	0 (±0)
Ka1	0.280 (±0.356)	Ka1 ($N = 10$)	0.605 (±0.120)	0 (±0)	0.148 (±0.070)	0.228 (±0.095)	0.019 (±0.017)
Ka2	4.842 (±0.986)	Ka2 ($N = 10$)	0.179 (±0.090)	0.001 (±0.002)	0.009 (±0.018)	0.749 (±0.102)	0.061 (±0.031)
Ka3	2.962 (±0.888)	Ka3 ($N = 10$)	0.359 (±0.098)	0.004 (±0.005)	0.018 (±0.017)	0.618 (±0.105)	0.001 (±0.001)
ChL	1.029 (±0.223)	ChL ($N = 5$)	0.877 (±0.101)	0.039 (±0.021)	0.069 (±0.094)	0.015 (±0.010)	0 (±0)
Ch1	4.311 (±0.542)	Ch1 ($N = 10$)	0.613 (±0.148)	0.001 (±0.001)	0.064 (±0.046)	0.253 (±0.138)	0.069 (±0.053)
LzL	0.094 (±0.096)	LzL ($N = 10$)	0.565 (±0.226)	0.027 (±0.034)	0.313 (±0.227)	0.087 (±0.096)	0.008 (±0.009)
Lz1	2.749 (±0.685)	Lz1 ($N = 10$)	0.441 (±0.091)	0 (±0)	0.259 (±0.121)	0.224 (±0.099)	0.076 (±0.036)
LfL	0.693 (±0.604)	LfL ($N = 10$)	0.628 (±0.233)	0.240 (±0.257)	0.007 (±0.007)	0.047 (±0.061)	0.077 (±0.081)
Lf1	n/a	Lf1 ($N = 7$)	0.935 (±0.039)	0 (±0)	0.031 (±0.026)	0.023 (±0.031)	0.011 (±0.011)
Lf2	4.261 (±0.763)	Lf2 ($N = 10$)	0.433 (±0.164)	0.001 (±0.002)	0.117 (±0.053)	0.450 (±0.156)	0 (±0)

Along the Zambian shoreline, several 'pure lacustrine populations', that is populations not being adjacent to a river, cluster together, even when being separated by large sandy bays (16 and 17, separated by Mbete Bay; 12 and 14, separated by Chituta Bay). The population from Lake Chila (22) belongs to the same genotypic cluster as populations 15, 16 and 17 from LT. Specimens from the same population but sampled in different years always cluster together (indicated by 'a' and 'b' in Fig. 1C).

There was a strong pattern of isolation by distance for populations sampled along the shoreline (Mantel-$R = 0.5539$, $P = 0.0164$).

The separate STRUCTURE analyses for each of the four lake–stream systems are depicted in Fig. 2B. The most likely number of genetic clusters was $K = 2$ for all systems (Fig. S3, Supporting information). Note, however, that it is not possible to infer ΔK for $K = 1$.

Body shape

The CVA of body shape of the 20 sampled populations revealed a significant differentiation between all populations (Fig. S4A; Table S3B, Supporting information). The main body shape changes are described by canonical variate 1 (CV1, accounting for 32% of the variance), which shows a change in body depth, mouth position as well as in head size, and CV2 (accounting for 17% of the variance) describing additional changes in caudal peduncle and eye size.

No pattern of isolation by distance was detected regarding body shape for populations sampled along the shoreline (Mantel-$R = 0.2116$, $P = 0.1415$). The CVA plot of all shoreline populations (Fig. S4B, Supporting information) does not show closer positions in morphospace of more closely located populations, but rather indicates stronger clustering of pure lacustrine populations (of LT and Lake Chila) compared with the more scattered shoreline populations that are adjacent to streams.

When analysing each lake–stream system separately, and comparing each lake population with the most distinct corresponding stream population, it becomes apparent that lake fish generally have a deeper body and a more superior mouth position compared with stream fish. This body shape change, together with clearly partitioned discriminant scores, was found in the systems Kalambo (KaL and Ka3), Lunzua (LzL and Lz1) and Lufubu (LfL and Lf2). The lake and river populations of the Chitili system (ChL and Ch1) showed an overlap of the discriminant scores of the DFA and therefore smaller but still significant changes in body shape (Fig. 2C).

The pattern is more complex when body shape is compared within the river systems for which more than two populations have been sampled (Kalambo and Lufubu River). Three of the four Kalambo populations (KaL, Ka1 and Ka3) show a continuous shift from lake towards more upstream populations, with lake fish having a deeper body and a more superior mouth. The remaining Kalambo population (Ka2) clustered separately (Fig. S5A; Table S4A, Supporting information). The two downstream populations of the Lufubu system (LfL and Lf1) displayed a similar differentiation in body shape compared with the distinct upstream population (Lf2), again in the form of a more superior mouth position (Fig. S5A; Table S4B, Supporting information).

All populations of the lake–stream systems together show little congruence in CV1–CV2 morphospace occupation and only the populations from the two lake populations of the similar rivers Kalambo and Lunzua clustered together (KaL and LzL in Fig. 3A) and one of the Kalambo populations overlapped substantially with the first two Lufubu populations (Ka2, LfL and Lf1 in Fig. 3A). The body shape changes, however, followed similar trajectories between river and lake populations throughout all systems, as evidenced by similar unidirectional shifts in CV1 (illustrated by a bar in Fig. 3A). In all four river systems, lake fish had deeper bodies and a more superior mouth along CV1 (accounting for 45% of the variance in the CVA) (Fig. 3A and Table S5A, Supporting information).

Gill raker morphology

ANOVA detected significant differences in gill raker length between male lake and stream fish in all populations, with generally longer gill rakers in lake populations and raker length decreasing with increasing geographic distance from the lake (Fig. 2E; Table S6, Supporting information). In more detail, the lake population from the Kalambo system (KaL) showed significantly longer gill rakers compared with each of the stream populations (Ka1, Ka2 and Ka3), which did not differ significantly among each other. In the Chitili and the Lunzua system, we found a significant difference between the lake and stream populations. In Lufubu, the lake population (LfL) showed no differences in raker length compared with the first upstream population (Lf1), but gill rakers of Lf1 fish were longer compared with the most upstream population (Lf2). However, gill raker number did not differ between lake and stream fish in any of the four lake–stream systems. The results for females, which showed the same trend of longer gill rakers in lake populations compared with stream populations, are shown in Fig. S5C and Table S6 (Supporting information).

Lower pharyngeal jaw morphology

We also detected differentiation between lake and stream fish in the morphology of the LPJ (Fig. 2D). For

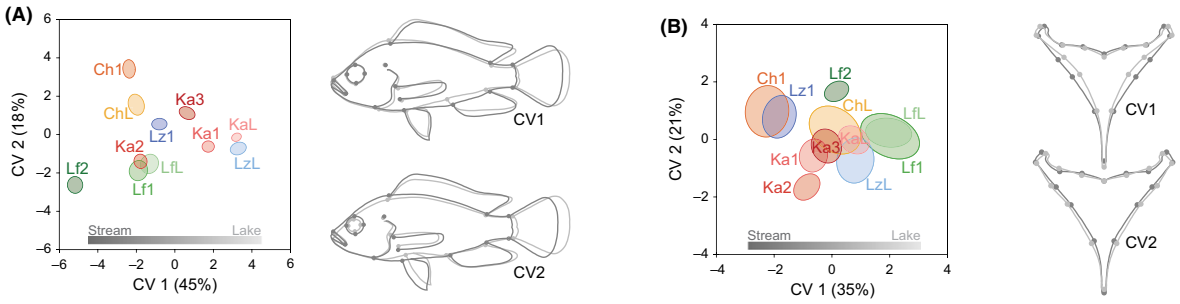


Fig. 3 Body shape and lower pharyngeal jaw (LPJ) shape differentiations of all populations from the lake–stream systems. Canonical variate analyses (CVA) plots illustrate the distribution of the populations on CV1 and CV2 (ellipses represent the 95% confidence intervals of the means) and the shifts are represented in the outline drawings (outlines are always drawn for illustration purposes only, from dark to light grey with increasing values, scaling factor 10 by default; abbreviations of locality names are defined in the grey box in Fig. 1). (A) Shifts in body shape between each lake population and their corresponding stream populations are unidirectional on the axis of CV1 (represented with the bar), indicating that lake fish have deeper bodies and a more superior mouth (Table S5A, Supporting information). (B) For LPJ morphometrics, all lake populations cluster together and show unidirectional shifts along CV1 towards their corresponding stream populations. Lake fish generally have slender and more elongated LPJ compared with stream fish (Table S5B, Supporting information).

each system, we compared the lake population to the stream population with the largest geographic distance to the lake. The Kalambo lake (KaL) and the most upstream population (Ka3) showed a minor overlap in discriminant scores and only a small but still significant difference in LPJ shape, with broader LPJ in stream fish compared with lake fish. In the Chitili, Lunzua and Lufubu systems, we found similar, yet more pronounced shifts in LPJ width. In the Chitili system, an additional shift towards a more convex posterior curve and shorter posterolateral horns in stream fish was detected. Although the underlying shape changes differed among the systems, there was a consistent shift in width of the jaws with broader LPJ in stream fish compared with lake fish.

The system specific CVA of the Kalambo River populations showed a continuous increase in LPJ width and an increasing angle of the posterolateral horns from the lake population (KaL) to the first and the second upstream populations (Ka1 and Ka2). The fourth Kalambo population (Ka3) clustered with the first upstream population (Ka1). In the Lufubu system, we found a considerable overlap in CV1 and CV2 of the lake population (LfL) and the adjacent stream population (Lf1), but a distinct LPJ shape in the furthermost upstream population (Lf2) having broader and shorter LPJ (Fig. S5B; Table S4C,D, Supporting information).

The CVA with all 11 lake–stream populations included showed a significant difference (based on Mahalanobis distances) in LPJ shape among all populations except between LfL and Lf1 (Fig. 3B; Table S5B, Supporting information). CV1 (accounting for 35% of the variance) represented mainly a change in broadness and length of the LPJ, whereas CV2 (accounting for 21% of the variance) described an additional change in angle of the posterolateral horns. In the CV1–CV2 morphospace, all lake populations clustered together, indicating similar LPJ shapes in the lake populations. All systems show a shift in LPJ shape along CV1 with broader and shorter LPJ in stream fish compared with lake fish (illustrated by a bar in Fig. 3B). Along CV2, the lake populations showed a consistent shift in angle of the posterolateral horns (except for the Kalambo system, where the shift was in the opposite direction).

Stomach and gut content

Stomach and gut content analyses revealed that *A. burtoni* is a generalist, feeding on a mixed diet composed of plant material, algae, insects, insect larvae, molluscs and planktonic components (Fig. 2F). The diet composition differed between lake and stream habitats, whereby lake fish feed more on softer and smaller food particles (plants and algae, zooplankton) and stream fish more on hard-shelled and bigger prey items (mollusc shells, plant seeds, insects and insect larvae).

In all four systems, we found a plant, algae and zooplankton-biased diet in lake fish and a parallel increase in the proportion of macro-invertebrates with increasing distance to the lake (Table 1B). In addition, the proportion of hard-shelled food items was generally higher in river populations, except for the Lufubu lake population, where a considerable proportion of hard-shelled food items has been found.

Testing for associations between genetic differentiation, morphometric traits and environment

The partial Mantel tests revealed that none of the morphometric trait differences correlated with genetic distance (F_{ST} values; Table 2A). Genetic differentiation at neutral markers therefore does not seem to be the determining factor for the observed differences among the lake and stream populations. The MRM including environmental parameters showed that the differences rather arise by the effect of environmental conditions: body shape was significantly influenced by both geographic distance and by water current. Mouth position correlated with current and was also influenced by feeding (proportion of macro-invertebrates). While gill raker length correlated with the proportion of macro-invertebrates, LPJ shape tends to be influenced by feeding on hard-shelled food items and correlated with microhabitat current (Table 2B).

Testing for reproductive isolation and trait plasticity

A total of 55 (of 165 initially introduced) wild-caught adult individuals and 593 F1 offspring were recovered from the experimental ponds. Loss of individuals was most likely due to aggressive and territorial behaviour of males. At the time the experiment was terminated, at least one female per population had survived in each pond, and in three of five ponds, at least one male per population had survived (Table S2A, Supporting information). Parentage analyses revealed that across the five ponds, all possible mating combinations occurred, but were not evenly distributed among the replicates (see Appendix S2, Supporting information for details). A qualitative inspection of the data indicated no assortative mating with respect to population but revealed that only 2–5 males reproduced per pond. Further, reproducing males were predominantly large males based on SL measurements taken at the beginning and at the end of the experiment. In *A. burtoni*, size and dominance are positively correlated (Fernö 1987), and dominant males are much more likely to reproduce. Accordingly, the observed pattern is likely a result of biased mating with respect to male size and dominance. This is also supported by comparing our observed data with a simulation assuming random mating with respect to population, but an increased mating probability of large males (see Appendix S2, Supporting information for details).

The morphometric analyses in F1 offspring revealed that while purebred (i.e. intrapopulation crosses) differed among each other in body shape in CV1 (accounting for 62–88% of the variance), between-population crosses were intermediate (Figs 4A and S6; Table S7A, Supporting information). A CVA including F1 offspring and wild populations demonstrates shifts in body shape under common garden conditions and a closer clustering of within-population crosses as compared to the corresponding wild populations (Fig. S7A; Table S8A, Supporting information). Interestingly, the body shape of introduced adult specimens also converged during the experimental period, with the stream populations (Ka3 & Lz1) becoming more like the lake population (KaL) (Fig. S7B; Table S8B, Supporting information). (Note that the experimental set-up in ponds resembles more the lake situation.)

Gill rakers were significantly longer in within-lake population offspring compared with within-stream population offspring, and intermediate in the interpopulation crosses (Fig. 4B; Table S7B, Supporting information). No difference in gill raker number was detected. Within-population offspring from the common garden experiment show a shift towards longer gill rakers compared with the corresponding wild populations (Fig. S7C; Table S8C, Supporting information).

Discussion

Phylogeography and population structure of Astatotilapia burtoni in southern LT

Overall, our study revealed an unexpectedly high degree of genetic and morphological diversity and

Table 2 Testing for associations between genetic differentiation, morphometric traits, and environment. (A) Genetic distances (F_{ST}) were correlated with morphological distances (Mahalanobis) using a partial Mantel test including geographic distance as a correction factor. (B) Combined multiple regression on distance matrices (MRM) between morphological and ecological distances

A		B				
Morphometric trait	Genetic distance (F_{ST})	Morphometric trait	Microhabitat current	Hard-shelled items	Macro-invertebrates	Geographic distance
Overall body shape	0.268 (Mantel- $R = 0.133$)	Overall body shape	0.0042**	0.2717	0.4323	0.0253*
Mouth position	0.825 (Mantel- $R = -0.226$)	Mouth position	0.0157*	0.1793	0.0175*	0.8627
Gill raker length	0.496 (Mantel- $R = -0.005$)	Gill raker length	0.4182	0.4504	0.0373*	0.2270
LPJ shape	0.762 (Mantel- $R = -0.186$)	LPJ shape	0.0219*	0.0587	0.4712	0.3425

LPJ, lower pharyngeal jaw.
Significance levels: *$P < 0.05$ and **$P < 0.01$.

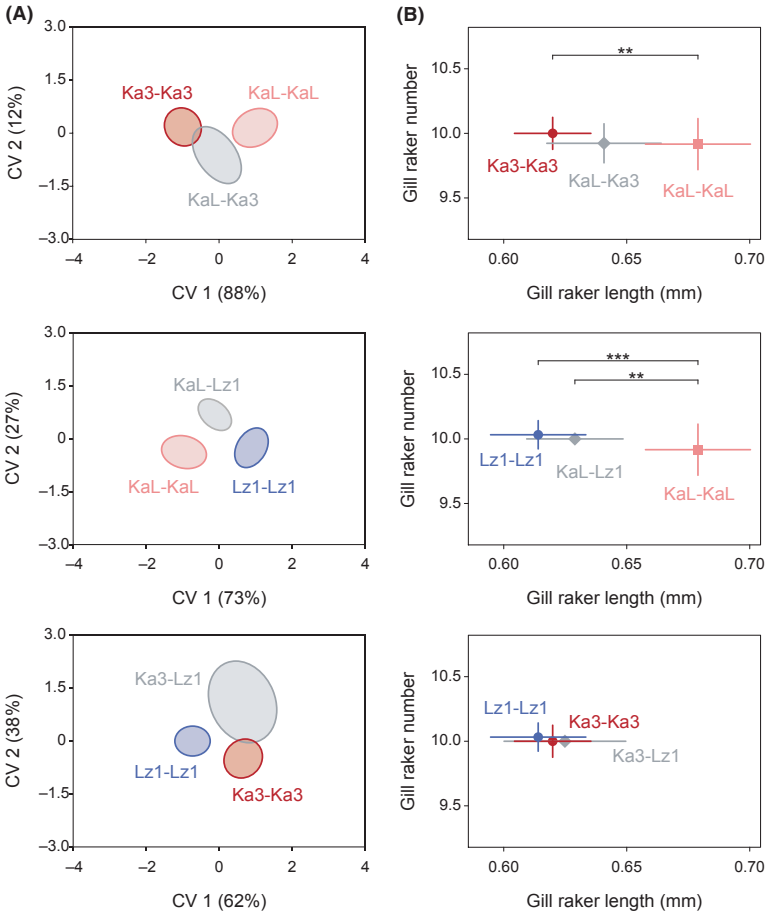


Fig. 4 Body shape (A) and gill raker comparisons (B) of each interpopulation cross with the corresponding within-population crosses from the pond experiment (Fig. S6, Supporting information for corresponding CV outlines and Table S7, Supporting information for distance and significance values).

extensive population structure in *A. burtoni* from southern LT (Figs 1, 2 and S4A, Supporting information). Notably, we identified two main mtDNA control region haplotype lineages in *A. burtoni* that are separated by 10 mutations (Fig. 1B). The genetic diversity in *A. burtoni* is thus similar to, or even exceeds the diversity observed in the same marker in the entire haplochromine cichlid assemblage of Lake Victoria (Verheyen *et al.* 2003). It has long been recognized that substantial differences exist in inter- and intraspecific genetic variation in mtDNA within different East African cichlid radiations and that the degree of differentiation reflects the respective age of a lineage rather than morphological disparity (Sturmbauer & Meyer 1992). The great diversity in mtDNA in *A. burtoni*, even across small geographic scales, thus suggests a deep coalescence time and, consequently, the presence of this species in the study area over long time periods. This is in line with a previous multispecies study that detected deep coalescence times in the only analysed *A. burtoni* population (collected in the area of our Ka3 site) based on microsatellite markers (Elmer *et al.* 2009).

The data at hand indicate that while mtDNA clearly separates the populations into an eastern (1–14) and a western clade (15–20; with the exception of population 21, see below) (Fig. 1B), such a clear-cut barrier to gene flow is not evident in the nuclear DNA markers (Fig. 1C): The population assignment tests with STRUCTURE suggest some gene exchange between populations 14 and 15, and the pairwise differences in F_{ST} and D_{EST} between populations 14 and 15 are among the smallest detected (nevertheless significant), fitting the isolation-by-distance scenario among the lacustrine populations. Similarly, while population 21 is clearly distinct in its mtDNA from the geographically nearest populations 19 and 20 (Fig. 1B), some level of gene flow between these populations is indicated based on the nuclear DNA markers (Fig. 1C). Such a pattern could be explained by male-biased dispersal along the shoreline of LT (Stiver *et al.* 2007). Male-biased dispersal and the preference

for shallow, sandy habitats would also explain why—in contrast to lake cichlids occurring in the rocky shoreline habitat of LT (e.g. Koblmüller *et al.* 2011)—long stretches of sandy shorelines do not seem to act as strong barriers to gene flow in *A. burtoni* (see e.g. 1–3, 12 and 14, 16 and 17, 20 and 21).

Recent migration along the shoreline cannot, however, explain the distribution of the main mtDNA haplotype lineages in *A. burtoni* (i.e. the clear-cut separation into an eastern and a western haplotype clade and the distinctiveness of populations 18 and 21). The bathymetry of the southern LT basin together with periodically occurring and climatically induced fluctuations in the lake level of LT (see e.g. Sturmbauer *et al.* 2001, 2005; Koblmüller *et al.* 2011) might provide one explanation for the overall structure of the mtDNA haplotype genealogy (Fig. 1B). The deep split between the eastern and the western haplotype lineages could, for example, be directly related to an underwater ridge in exactly the area between populations 14 and 15 (see fig. 1 of Koblmüller *et al.* 2011), which might have acted as migration barrier at times of low lake level stands, especially for a species associated to rivers, estuaries and shallow waters such as *A. burtoni*. Low lake level might also permit migration across what is at present two opposite shorelines of LT (see e.g. Sturmbauer *et al.* 2001; Baric *et al.* 2003), thus explaining the close relationship between population 21 from the western (Zambian/Congolese) part of LT to the eastern (Tanzanian) populations 1–3.

The close relatedness of the Lake Chila population (22) to populations sampled around Mpulungu (15–17), and especially to population 16 (Table S3A, Supporting information), is somewhat puzzling. Lake Chila is a small and shallow lake about 20 km southeast of LT, and connected to LT through a small outflow draining into LT near Sumba (population 12). However, there is no faunistic association between Lake Chila and LT, except for *A. burtoni*, and we could only detect elements of a fish fauna in Lake Chila, which is otherwise typical for the Chambeshi, Zambesi and the Zambian/Congo watersheds (*Serranochromis angusticeps*, *S. robustus*, *S. thumbergi*, *Pseudocrenilabrus* cf. *philander* and *Tilapia sparmanii*) (Skelton 1993). As Lake Chila's *A. burtoni* are genetically indistinguishable from population 16, yet distinct from population 12, and because there are reports of a recent stocking of this small lake (L. Makasa, Fisheries Department Mpulungu, personal cummunication), a human-induced translocation is the likely source of the current Lake Chila *A. burtoni* stock (despite records of the presence of *A. burtoni* in that lake more than 50 years ago as evidenced by a collection by M. Poll from 1949 deposited in the Royal Museum for Central Africa in Tervuren, Belgium).

In summary, we show that *A. burtoni* occurs along a lake–stream environmental gradient in southern LT and that several lake–stream systems have been colonized independently. One of these systems, the Lufubu, is genetically very distinct from the other three (Kalambo, Chitili and Lunzua), especially with respect to mtDNA. However, we can, at present, not infer the precise colonization history of *A. burtoni* in southern LT. In particular, we cannot assess whether any of the surveyed river populations is the source of *A. burtoni* in the area or whether all the river systems have been colonized from LT. A more thorough analysis including a denser sampling across a much larger geographic area would be necessary to fully understand the phylogeographic history and population structure of *A. burtoni*.

Adaptive divergence between lake and stream habitats in Astatotilapia burtoni

Integrative studies of fish species that occur along an environmental gradient have provided important insights into speciation (Hendry *et al.* 2000; Seehausen *et al.* 2008; Berner *et al.* 2009; Roesti *et al.* 2012). Our survey of *A. burtoni* in the southern part of LT reveals that this species occurs along a lake–stream environmental gradient and is present, in high abundance, in every suitable habitat ranging from truly lacustrine environments to river estuaries, larger rivers and small creeks draining into LT (Figs 1A and 2A). Importantly, we show that populations inhabiting the same environment tend to be morphologically similar, irrespective of their genetic background (Figs 2, 3 and S4B, Supporting information). For example, among populations sampled within LT, there is a closer morphological resemblance between the truly lacustrine populations (i.e. the populations away from any river) and between the populations near river estuaries (Fig. S4B, Supporting information). Interestingly, the only sampled lacustrine *A. burtoni* population outside from LT (from Lake Chila) clusters closely in morphospace with the truly lacustrine populations from LT (Fig. S4B, Supporting information) (note, however, that this resemblance might also be due to recent introduction; see above). In addition, while there is a strong signal of isolation by distance with respect to genetics along the shoreline of LT, this is not the case for body morphology, suggesting that similar environmental pressures, but not relatedness, mediate the emergence of similar body shapes in *A. burtoni*.

This pattern becomes even more evident when comparing the body shape between lake and stream populations from the four lake–stream systems studied in detail. Generally, we find that lake fish exhibit deeper

bodies and a more superior mouth compared with stream fish (Figs 2C and 3A) and that mouth position is correlated with feeding mode (Table 2B). In addition, we detected a significant correlation between body shape and water current (Table 2B), which is in line with adaptations to different flow rates as predicted by hydrodynamic theory (Webb 1984). However, these changes in morphology only partially agree with those found in other lake–stream systems in fishes. In sockeye salmon, for example, beach residents, too, have deeper bodies compared with their riverine counterparts (Hendry *et al.* 2000). In Canadian three-spine stickleback, on the other hand, lake fish tend to have more slender bodies compared with stream fish due to shifts in feeding modes (e.g. Schluter & McPhail 1992; Berner *et al.* 2008, 2010; Ravinet *et al.* 2013).

In addition to the body shape differences, we also detected significant shifts in trophic morphology across the lake–stream transition in *A. burtoni* (Fig. 2D,E and 3B). The morphological trajectory of the gill raker apparatus along this habitat gradient resembles that in other groups of fishes. Just as in sticklebacks (Berner *et al.* 2008; Ravinet *et al.* 2013), gill rakers are shorter in *A. burtoni* stream fish compared with lake fish. Gill rakers are an important trophic trait in fishes, and believed to function as a cross-flow filter to concentrate particles inside the oral cavity and to transport particles towards the oesophagus (Sanderson *et al.* 2001). In stickleback and other fishes, divergence in gill raker morphology is driven by differential prey resource use (e.g. Bentzen & McPhail 1984; Robinson & Wilson 1994; Skulason & Smith 1995; Berner *et al.* 2008). Likewise, in *A. burtoni*, shorter gill rakers are associated with the consumption of larger food items and longer gill rakers with smaller food particles. However, there were no significant differences in gill raker numbers between lake and stream populations. Divergence in gill raker length accompanied by stasis in gill raker number has also been found in European stickleback lake–stream population pairs, which was explained by the insufficient time for divergence and differences in the genetic architecture compared with Canadian lake–stream populations (Berner *et al.* 2010). While our population-genetic analyses based on mtDNA suggest a deep coalescence time among the major haplotype lineages in *A. burtoni*, little is known about the timing of splitting events among the studied lake–stream populations. Generally, gill raker number varies considerably among LT cichlid species (M. Rösti, personal observation), but it may be less prone to environmentally induced phenotypic variation than other morphological traits such as gill raker length and the LPJ (Lindsey 1981). We also detected sexual dimorphism in gill raker length, with females having longer gill rakers compared with males. In addition, there appears to be a sexual dimorphism in head shape, with females showing more slender yet larger heads (Fig. S1B, Supporting information). Both might be explained by functional differences due to the female mouthbrooding behaviour characteristic for haplochromines.

Trophic divergence between *A. burtoni* lake–stream populations is also evident from differences in LPJ morphology between habitats. The morphology of the oral and pharyngeal jaws is highly diverse in cichlids (Fryer & Iles 1972; Liem 1973; Salzburger 2009; Muschick *et al.* 2012) and related to functional feeding ecology (Liem 1980; Muschick *et al.* 2012, 2014). Experimentally induced, plastic changes in cichlid pharyngeal jaws have been shown to be due to the mode of feeding rather than differences in nutritional composition. For example, Nicaraguan Midas cichlids (*Amphilophus citrinellus*) fed on whole snails developed heavier and more hypertrophied LPJs compared with individuals fed on either crushed whole snails or snail bodies without shells (Muschick *et al.* 2011). Similar shifts in LPJ morphology along with different resource use are known from natural cichlid populations (Meyer 1990; Hulsey *et al.* 2008). In line with these studies, the broader and shorter LPJs of *A. burtoni* stream fish compared with lake fish may pose an adaptation to the shift in diet towards harder food items such as seeds, snails and other hard-shelled invertebrates found in stomachs of stream populations (Fig. 2F; Table 1B). In our analyses, we found that LPJ morphology tends to correlate with the proportion of hard-shelled food items, but there is also a correlation between LPJ and water current (Table 2B). This latter correlation could be due to the method used to infer LPJ shape, which might be influenced by more general shifts in head morphology across the lake–stream gradient.

Phenotypic plasticity constitutes an alternative outcome to speciation in the face of divergent selection (West-Eberhard 2005; Pfennig *et al.* 2010). The generalist species *A. burtoni* dwells in many different habitats, which could result in the evolution of highly plastic populations expressing a variety of phenotypes. On the other hand, speciation could also be initiated via plastic responses to novel environments followed by genetic assimilation (e.g. Waddington 1942; West-Eberhard 2003). Our common garden experiment demonstrated that both plastic and genetic components influence body shape and gill raker length in *A. burtoni*. The F1 offspring from the within-population matings generally show significant differentiation with respect to both body shape and gill raker length, and interpopulation crosses generally display intermediate phenotypes. This pattern, together with the conserved higher body shape and shorter gill rakers of the lake population offspring

(KaL-KaL), compared with the within-stream population crosses speaks for a genetic component underlying trait differentiation (Fig. 4). However, shifts in F1 offspring in both traits under common garden conditions compared with wild populations indicate that trait plasticity also contributes to the detected differences (Fig. S7, Supporting information). Whether these patterns also hold with regard to LPJ morphology and to what extent plasticity and heritability contribute to the detected differences in body shape and trophic traits remains to be tested in future experiments.

We did not find any evidence for assortative mating with regard to population in our mating experiment. All possible mating combinations occurred, and male dominance effects seemed to determine the observed mating patterns (Appendix S2, Supporting information). The absence of reproductive barriers in spite of strong genetic and morphological differentiation has also been reported from lake and stream stickleback (Raeymaekers *et al.* 2010). However, a transplant experiment later indicated that selection against immigrants, together with various other factors, might be contributing to reproductive isolation in this system (Räsänen & Hendry 2014). Similarly, we cannot rule out that barriers, which we did not detect in our experiment, could contribute to reproductive isolation among lake and stream populations. In *A. burtoni*, with its lek-like polygynandrous mating system, only dominant males gain access to territories as well as (several) females and are therefore able to reproduce (Fernald & Hirata 1977). Although no bias in dominance among populations was evident from our data, possible male aggression biases (and probably undetected female preferences) should be tested under more controlled conditions in the future (see Theis *et al.* 2012). As a next step, it would be interesting to test whether the genetically most distinct populations, for example Lf2 vs. KaL, are reproductively isolated.

Evidence for (ecological) speciation is often inferred via a positive correlation between the levels of (adaptive) divergence in phenotypic traits and the levels of neutral genetic differentiation between populations, when controlled for geographic distance ('isolation by adaptation', Nosil 2012). In *A. burtoni*, we did not find correlations between any morphological trait measured and F_{ST} values (Table 2A). This gene-flow approach based on neutral markers does have several caveats, though (see Nosil 2012), and a lack of signal does not necessarily exclude the possibility of (ecological) speciation. Due to the geographic isolation of some populations (e.g. populations located above waterfalls or geographically very distant populations), differentiation at neutral loci might occur without barriers to gene flow caused by divergent selection in *A. burtoni*, resulting in a failure to detect isolation by adaptation. Note that there was also no pattern of isolation by distance detectable if only lake–stream populations were included in the analysis, as opposed to the pattern detected along the shoreline (see above). However, lake and stream populations from the four lake–stream systems (and populations within systems) appear to rest at different stages of the speciation continuum. In the Chitili system, for example, the lake and stream populations are geographically close, genetically admixed and also less differentiated in body shape and gill rakers compared with the pairwise comparisons from the Kalambo, Lunzua and Lufubu systems shown in Fig. 2. Although there are several outliers in our data (e.g. relatively pronounced LPJ differentiation within the Chitili system compared with very little LPJ differences between the clearly genetically distinct populations KaL and Ka3), lake and stream populations belonging to distinct genetic clusters generally show more differentiation in morphological traits (Fig. 2).

Taken together, our study revealed the presence of multiple divergent lake–stream populations in the southern LT drainage. Phenotypic divergence between populations from the four independent lake–stream systems follows similar trajectories: Divergence in body shape is associated with different flow regimes in lake and stream habitats, whereas shifts in trophic structures are linked to differential resource use. We did not detect a signal for isolation by adaptation; however, more powerful genetic data such as genome scans may clarify the interplay between levels of gene flow and phenotypic divergence in these systems. A first test for reproductive isolation among the more closely related lake and stream populations did not reveal any population-assortative mating patterns. Importantly, analyses of F1 offspring reared under common garden conditions indicate that the detected trait differences among *A. burtoni* populations do not reflect pure plastic responses to different environmental conditions, but that these differences also have a genetic basis.

The *A. burtoni* lake–stream system constitutes a valuable model to study the factors that enhance and constrain progress towards speciation, and offers the unique possibility to contrast replicated lake–stream population pairs at different stages along the speciation continuum in cichlids. In addition, it allows evaluating parallelism across different species, that is lake–stream pairs of stickleback and cichlids. Characterizing potential reproductive barriers and the role of plasticity in phenotypic divergence in more detail, together with studies on genomic differentiation, promises to contribute to understanding the process of speciation in natural populations.

Acknowledgements

We would like to thank our helpers in the field, J. Bachmann, A. Böhne, T. Bosia, V. Campos, M. Colombo, M. Dittmann, S. Egger, Y. Klaefiger, J. De Maddalena, N. Merdas, D. Moser, M. Roesti, O. Roth, L. Schild, J. Weber, M. Zubler; H. H. Büscher and G. Tembo and his crew for their logistic support in Africa, respectively; the Lake Tanganyika Research Unit, Department of Fisheries, Republic of Zambia, for research permits. We further thank M. Muschick and M. Colombo for help in morphometric analyses, B. Meyer for help in population-genetic analyses, D. Berner and J. Raeymaekers for contributing to statistical analyses, L. Schärer for valuable suggestions, A. Bieri and S. Fischer for help with LPJ preparations and N. Rose and H. Bichsel for help with stomach content analysis. We further thank D. Berner and C. L. Peichel for valuable comments and feedback from Axios Review (axiosreview.org) on a previous version of this manuscript. This study was supported by grants from the Freiwillige Akademische Gesellschaft Basel (FAG) to AI and AT, the Swiss Academy of Sciences (SCNAT) to AI, FR and AT, the Basler Stiftung für experimentelle Zoologie to BE, FR and AT, the Swiss Zoological Society (SZS) to AT and the European Research Council (ERC, Starting Grant 'INTERGENADAPT' and Consolidator Grant 'CICHLID~X'), the University of Basel and the Swiss National Science Foundation (SNF, grant 3100A0_138224) to WS.

References

Baldo L, Santos ME, Salzburger W (2011) Comparative transcriptomics of Eastern African cichlid fishes shows signs of positive selection and a large contribution of untranslated regions to genetic diversity. *Genome Biology and Evolution*, **3**, 443–455.

Baric S, Salzburger W, Sturmbauer C (2003) Phylogeography and evolution of the *Tanganyikan cichlid* genus *Tropheus* based upon mitochondrial DNA sequences. *Journal of Molecular Evolution*, **56**, 54–68.

Barluenga M, Stoelting KN, Salzburger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature*, **436**, 719–723.

Bentzen P, McPhail JD (1984) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*) – specialization for alternative trophic niches in the Enos Lake species pair. *Canadian Journal of Zoology – Revue Canadienne De Zoologie*, **62**, 2280–2286.

Berner D, Adams DC, Grandchamp AC, Hendry AP (2008) Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. *Journal of Evolutionary Biology*, **21**, 1653–1665.

Berner D, Grandchamp AC, Hendry AP (2009) Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. *Evolution*, **63**, 1740–1753.

Berner D, Roesti M, Hendry AP, Salzburger W (2010) Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. *Molecular Ecology*, **19**, 4963–4978.

Brawand D, Wagner CE, Li YI *et al*. (2014) The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, **513**, 375–381.

Bruford MW, Hanotte O, Brookfield JFY, Burke T (1998) Multilocus and single-locus DNA fingerprinting. In: *Molecular Analysis of Populations* (ed. Hozel AR), pp. 283–336. Oxford University Press, New York, New York.

Butlin RK, Galindo J, Grahame JW (2008) Sympatric, parapatric or allopatric: the most important way to classify speciation? *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 2997–3007.

Coulter GW (1991) *Lake Tanganyika and its Life*. Oxford University Press, Oxford.

De Vos L, Seegers L, Taverne L, Thys van den Audenaerde D (2001) L'ichtyofaune du basin de la Malagarasi (Système du Lac Tanganyika): une synthèse de la connaissance actuelle. *Annales, Musée Royal de l'Afrique Centrale, Sciences Zoologiques*, **285**, 117–152.

Earl DA, von Holdt BM (2012) Structure Harvester: a website and program for visualizing Structure output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

Elmer KR, Reggio C, Wirth T, Verheyen E, Salzburger W, Meyer A (2009) Pleistocene desiccation in East Africa bottlenecked but did not extirpate the adaptive radiation of Lake Victoria haplochromine cichlid fishes. *Proceedings of the National Academy of Sciences USA*, **106**, 13404–13409.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software Structure: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Fernald RD, Hirata NR (1977) Field study of *Haplochromis burtoni* – quantitative behavioral observations. *Animal Behaviour*, **25**, 964–975.

Fernö A (1987) Aggressive behavior between territorial cichlids (*Astatotilapia burtoni*) in relation to rank and territorial stability. *Behaviour*, **103**, 241–258.

Fryer G, Iles T (1972) *The Cichlid Fishes of the Great Lakes of Africa: Their Biology and Evolution*. Oliver & Boyd, Edinburgh.

Galis F, Drucker EG (1996) Pharyngeal biting mechanics in centrarchid and cichlid fishes: insights into a key evolutionary innovation. *Journal of Evolutionary Biology*, **9**, 641–670.

Gerlach G, Jueterbock A, Kraemer P, Deppermann J, Harmand P (2010) Calculations of population differentiation based on G(ST) and D: forget G(ST) but not all of statistics!. *Molecular Ecology*, **19**, 3845–3852.

Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*, **22**, 1–19.

Günther ACLG (1894) Descriptions of the reptiles and fishes collected by Mr. E. Coode-Hore on Lake Tanganyika. *Proceedings of the Zoological Society of London, B*, **1893**, 628–632.

Harrod C, Mallela J, Kahilainen KK (2010) Phenotype-environment correlations in a putative whitefish adaptive radiation. *The Journal of Animal Ecology*, **79**, 1057–1068.

Hendry AP, Taylor EB (2004) How much of the variation in adaptive divergence can be explained by gene flow? – an evaluation using lake-stream stickleback pairs. *Evolution*, **58**, 2319–2331.

Hendry AP, Wenburg JK, Bentzen P, Volk EC, Quinn TP (2000) Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**, 516–519.

Hendry AP, Nosil P, Rieseberg LH (2007) The speed of ecological speciation. *Functional Ecology*, **21**, 455–464.

Hendry AP, Bolnick DI, Berner D, Peichel CL (2009) Along the speciation continuum in sticklebacks. *Journal of Fish Biology*, **75**, 2000–2036.

Hendry AP, Peichel CL, Matthews B, Boughman JW, Nosil P (2013) Stickleback research: the now and the next. *Evolutionary Ecology Research*, **15**, 111–141.

Hofmann HA (2003) Functional genomics of neural and behavioral plasticity. *Journal of Neurobiology*, **54**, 272–282.

Hulsey CD, García de León FJ, Rodiles-Hernández R (2006) Micro- and macroevolutionary decoupling of cichid jaws: a test of Liem's key innovation hypothesis. *Evolution*, **60**, 2096–2109.

Hulsey CD, Roberts RJ, Lin ASP, Guldberg R, Streelman JT (2008) Convergence in a mechanically complex phenotype: detecting structural adaptations for crushing in cichlid fish. *Evolution*, **62**, 1587–1599.

Jackson DA, Somers KM (1989) Are probability estimates from the permutation model of Mantel's test stable? *Canadian Journal of Zoology*, **67**, 766–769.

Jones FC, Grabherr MG, Chan YF *et al.* (2012) The genomic basis of adaptive evolution in threespine sticklebacks. *Nature*, **484**, 55–61.

Jost L (2008) G(ST) and its relatives do not measure differentiation. *Molecular Ecology*, **17**, 4015–4026.

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, **16**, 1099–1106.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353–357.

Koblmüller S, Salzburger W, Obermüller B, Eigner E, Sturmbauer C, Sefc KM. (2011) Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika. *Molecular Ecology*, **20**, 2272–2290.

Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics*, **5**, 288–298.

Kocher TD, Thomas WK, Meyer A *et al.* (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences of the USA*, **86**, 6196–6200.

Koehl MAR, Alberte RS (1988) Flow, flapping, and photosynthesis of *Nereocystis leutkeana*: a functional comparison of undulate and flat blade morphologies. *Marine Biology*, **99**, 435–444.

Kullander SO, Roberts TR (2011) Out of Lake Tanganyika: endemic lake fishes inhabit rapids of the Lukuga River. *Ichthyological Exploration of Freshwaters*, **22**, 355–376.

Lang M, Miyake T, Braasch I *et al.* (2006) A BAC library of the East African haplochromine cichlid fish *Astatotilapia burtoni*. *Journal of Experimental Zoology Part B-Molecular and Developmental Evolution*, **306**, 35–44.

Langerhans RB, Gifford ME, Joseph EO (2007) Ecological speciation in *Gambusia* fishes. *Evolution*, **61**, 2056–2074.

Lavin PA, McPhail JD (1986) Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **43**, 2455–2463.

Lee WJ, Kocher TD (1996) Microsatellite DNA markers for genetic mapping in *Oreochromis niloticus*. *Journal of Fish Biology*, **49**, 387–397.

Lichtstein JW (2007) Multiple regression on distance matrices: a multivariate spatial analysis tool. *Plant Ecology*, **188**, 117–131.

Liem KF (1973) Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. *Systematic Zoology*, **22**, 425–441.

Liem KF (1980) Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes. *American Zoologist*, **20**, 295–314.

Lindsey CC (1981) Stocks are chameleons: plasticity in gill rakers of coregonid fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1497–1506.

Lucek K, Sivasundar A, Roy D, Seehausen O (2013) Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake-stream divergence in parapatric Swiss stickleback. *Journal of Evolutionary Biology*, **26**, 2691–2709.

Maeda K, Takeda M, Kamiya K *et al.* (2009) Population structure of two closely related pelagic cichlids in Lake Victoria, *Haplochromis pyrrhocephalus* and *H. laparogramma*. *Gene*, **441**, 67–73.

Mallet J (2008) Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **363**, 2971–2986.

Mallet J, Dasmahapatra KK (2012) Hybrid zones and the speciation continuum in *Heliconius* butterflies. *Molecular Ecology*, **21**, 5643–5645.

Mardia KV, Kent J, Bibby J (1979) *Multivariate Analysis*. Academic Press, New York, New York.

Matschiner M, Salzburger W (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics*, **25**, 1982–1983.

Meyer A (1990) Ecological and evolutionary consequences of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces: Cichlidae). *Biological Journal of the Linnean Society*, **39**, 279–299.

Muschick M, Barluenga M, Salzburger W, Meyer A (2011) Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evolutionary Biology*, **11**, 116.

Muschick M, Indermaur A, Salzburger W (2012) Convergent evolution within an adaptive radiation of cichlid fishes. *Current Biology*, **22**, 2362–2368.

Muschick M, Nosil P, Roesti M, Dittmann MT, Harmon L, Salzburger W (2014) Testing the stage model in the adaptive radiation of cichlid fishes in East African Lake Tanganyika. *Proceedings of the Royal Society B*, **281**, 20140605. doi: 10.1098/rspb.2014.0605.

Nosil P (2012) *Ecological Speciation*. Oxford University Press, Oxford.

Nosil P, Sandoval CP (2008) Ecological niche dimensionality and the evolutionary diversification of stick insects. *PLoS ONE*, **3**, e1907.

Nosil P, Harmon LJ, Seehausen O (2009) Ecological explanations for (incomplete) speciation. *Trends in Ecology & Evolution*, **24**, 145–156.

Pfennig DW, Wund MA, Snell-Rood EC, Cruickshank T, Schlichting CD, Moczek AP (2010) Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, **25**, 459–467.

Posada D (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. ISBN 3-900051-07-0. Available from http://www.R-project.org/.

Raeymaekers JAM, Boisjoly M, Delaire L, Berner D, Räsänen K, Hendry AP (2010) Testing for mating isolation between ecotypes: laboratory experiments with lake, stream and hybrid stickleback. *Journal of Evolutionary Biology*, **23**, 2694–2708.

Räsänen K, Hendry AP (2014) Asymmetric reproductive barriers and mosaic reproductive isolation: insights from Misty lake-stream stickleback. *Ecology and Evolution*, **4**, 166–1175.

Ravinet M, Prodohl PA, Harrod C (2013) Parallel and nonparallel ecological, morphological and genetic divergence in lake-stream stickleback from a single catchment. *Journal of Evolutionary Biology*, **26**, 186–204.

Robinson BW (2000) Trade offs in habitat-specific foraging efficiency and the nascent adaptive divergence of sticklebacks in lakes. *Behaviour*, **137**, 865–888.

Robinson BW, Wilson DS (1994) Character release and displacement in fishes: a neglected literature. *The American Naturalist*, **144**, 596–627.

Robison RR, White RB, Illing N *et al.* (2001) Gonadotropin-releasing hormone receptor in the teleost *Haplochromis burtoni*: structure, location, and function. *Endocrinology*, **142**, 1737–1743.

Roesti M, Hendry AP, Salzburger W, Berner D (2012) Genome divergence during evolutionary diversification as revealed in replicate lake-stream stickleback population pairs. *Molecular Ecology*, **21**, 2852–2862.

Rohlf FJ (2007) *TPSRELW, Version 2.11*. Department of Ecology and Evolution, State University of New York at Stony Brook. Available from http://life.bio.sunysb.edu/morrph/.

Rohlf FJ (2008) *TPSDIG, Version 2.11*. Department of Ecology and Evolution, State University of New York at Stony Brook. Available from http://life.bio.sunysb.edu/morph/.

Rohlf FJ, Slice DE (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, **39**, 40–59.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters*, **8**, 336–352.

Salzburger W (2009) The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. *Molecular Ecology*, **18**, 169–185.

Salzburger W, Meyer A, Baric S, Verheyen E, Sturmbauer C (2002) Phylogeny of the Lake Tanganyika Cichlid species flock and its relationship to the Central and East African Haplochromine Cichlid fish faunas. *Systematic Biology*, **51**, 113–135.

Salzburger W, Mack T, Verheyen E, Meyer A (2005) Out of Tanganyika: genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evolutionary Biology*, **5**, 17.

Salzburger W, Renn SCP, Steinke D, Braasch I, Hofmann H, Meyer A (2008) Annotation of expressed sequence tags for the East African cichlid fish *Astatotilapia burtoni* and evolutionary analyses of cichlid ORFs. *BMC Genomics*, **9**, 96.

Salzburger W, Ewing GB, von Haeseler A (2011) The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology*, **20**, 1952–1963.

Sanderson SL, Cheer AY, Goodrich JS, Graziano JD, Callan WT (2001) Crossflow filtration in suspension-feeding fishes. *Nature*, **412**, 439–441.

Sanetra M, Henning F, Fukamachi S, Meyer A (2009) A microsatellite-based genetic linkage map of the cichlid fish, *Astatotilapia burtoni* (Teleostei): a comparison of genomic architectures among rapidly speciating cichlids. *Genetics*, **182**, 387–397.

Santos ME, Salzburger W (2012) Evolution. How cichlids diversify. *Science*, **338**, 619–621.

Santos ME, Braasch I, Boileau N *et al.* (2014) The evolution of cichlid fish egg-spots is linked with a *cis*-regulatory change. *Nature Communications*, **5**, 5149. doi: 10.1038/ncomms6149.

Schluter D (1993) Adaptive radiation in sticklebacks: size, shape, and habitat use efficiency. *Ecology*, **74**, 699–709.

Schluter D (1995) Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology*, **76**, 82–90.

Schluter D (2000) Ecological character displacement in adaptive radiation. *The American Naturalist*, **156**, S4–S16.

Schluter D (2009) Evidence for ecological speciation and its alternative. *Science*, **323**, 737–741.

Schluter D, McPhail JD (1992) Ecological character displacement and speciation in sticklebacks. *The American Naturalist*, **140**, 85–108.

Schneider S, Roessli D, Excoffier L (1999) *Arlequin, Version 2.0: A Software for Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Seehausen O, Terai Y, Magalhaes IS *et al.* (2008) Speciation through sensory drive in cichlid fish. *Nature*, **455**, 620–626.

Skelton P (1993) *A Complete Guide to the Freshwater Fishes of Southern Africa*. Southern Book Publishers, Halfway House.

Skulason S, Smith TB (1995) Resource polymorphisms in vertebrates. *Trends in Ecology & Evolution*, **10**, 366–370.

Stiver KA, Desjardins JK, Fitzpatrick JL, Neff JS, Quinn JS, Balshine S (2007) Evidence for size and sex-specific dispersal in a cooperatively breeding cichlid fish. *Molecular Ecology*, **16**, 2974–2984.

Sturmbauer C, Meyer A (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature*, **358**, 578–581.

Sturmbauer C, Baric S, Salzburger W, Rüber L (2001) Lake level fluctuations synchronize genetic divergences of cichlid fishes in African lakes. *Molecular Biology and Evolution*, **18**, 144–154.

Sturmbauer C, Koblmüller S, Sefc KM, Duftner N (2005) Phylogeographic history of the genus *Tropheus*, a lineage of rock-dwelling cichlid fishes endemic to Lake Tanganyika. *Hydrobiologia*, **542**, 335–366.

Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, Massachusetts.

Taylor MI, Meardon F, Turner G, Seehausen O, Mrosso HDJ, Rico C (2002) Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a *Pundamilia pundamilia* × *Pundamilia nyererei* hybrid. *Molecular Ecology Notes*, **2**, 443–445.

Theis A, Salzburger W, Egger B (2012) The function of anal fin egg-spots in the cichlid fish *Astatotilapia burtoni*. *PLoS ONE*, **7**, e29878.

Van Oppen MJH, Rico C, Deutsch JC, Turner GF, Hewitt GM (1997) Isolation and characterization of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. *Molecular Ecology*, **6**, 387–388.

Verheyen E, Salzburger W, Snoeks J, Meyer A (2003) Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science*, **300**, 325–329.

Waddington CH (1942) Canalisation of development and the inheritance of acquired characters. *Nature*, **150**, 563–565.

Webb P (1984) Body form, locomotion and foraging in aquatic vertebrates. *American Zoologist*, **24**, 107–120.

West-Eberhard MJ (2003) *Developmental Plasticity and Evolution*. Oxford University Press, New York, New York.

West-Eberhard MJ (2005) Phenotypic accommodation: adaptive innovation due to developmental plasticity. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution*, **304B**, 610–618.

Wickler W (1962) Egg-dummies as natural releasers in mouthbreeding cichlids. *Nature*, **194**, 1092–1093.

B.E., W.S., A.T. and F.R. designed the study; B.E., W.S., A.T. and F.R. wrote the manuscript. B.E. produced and analysed the population-genetic data, A.T. produced and analysed body shape data and conducted mantel test and MRM statistics, F.R. produced and analysed data on gill rakers, LPJs, stomach contents and paternity. All authors participated in sampling, were involved in the experimental design of the pond experiment and provided input on the manuscript.

Data accessibility

Mitochondrial DNA sequences: GenBank accessions KM508103–KM508461.

mtDNA sequence alignment, microsatellite genotypes, morphological data, stomach and gut content data, environmental data and common garden experiment data: Dryad doi:10.5061/dryad.pp0q1.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Landmark positions for body shape and LPJ analyses and sex differences in head shape.

Fig. S2 Comparison of intersexual within-population differences and intrasexual differences among populations in morphometric traits (body shape and LPJ).

Fig. S3 Mean likelihood (L(*K*) ± SD) over 10 STRUCTURE runs assuming *K* clusters (left); Δ*K* statistic (right).

Fig. S4 Body shape differentiation among the 20 sampled populations and among the 11 shoreline populations only.

Fig. S5 Body shape and LPJ shape differentiation within systems with more than two populations and gill raker length and number in females.

Fig. S6 Outlines to illustrate the body shape changes in F1 individuals of the pond experiment.

Fig. S7 Plasticity in body shape and gill raker length.

Table S1 Sample size details for each analysis with information about sampling year and geographic coordinates for each locality.

Table S2 Sample size details and result summary of the pond experiment.

Table S3 Pairwise genetic and morphometric (body shape) distances between populations.

Table S4 Pairwise morphometric (body shape and LPJ) distances within systems with more than two populations.

Table S5 Pairwise morphometric (body shape and LPJ) distances of all populations from the lake-stream systems.

Table S6 *P*-values for within system gill raker length comparisons for males and females.

Table S7 Pairwise morphometric (body shape and LPJ) distances between F1 crosses

Table S8 Pairwise morphometric (body shape) distances and *P*-values of gill raker comparisons among different groups of the pond experiment.

Table S9 Microsatellite diversity in populations of *Astatotilapia burtoni*.

Table S10 Genetic diversity of mtDNA sequences.

Appendix S1 Description of river systems.

Appendix S2 Pond experiment—Simulation.

Supplementary Materials

Adaptive divergence between lake and stream populations of an East African cichlid fish

Anya Theis*, Fabrizia Ronco*, **Adrian Indermaur**, Walter Salzburger and Bernd Egger

*These authors contributed equally to this work.

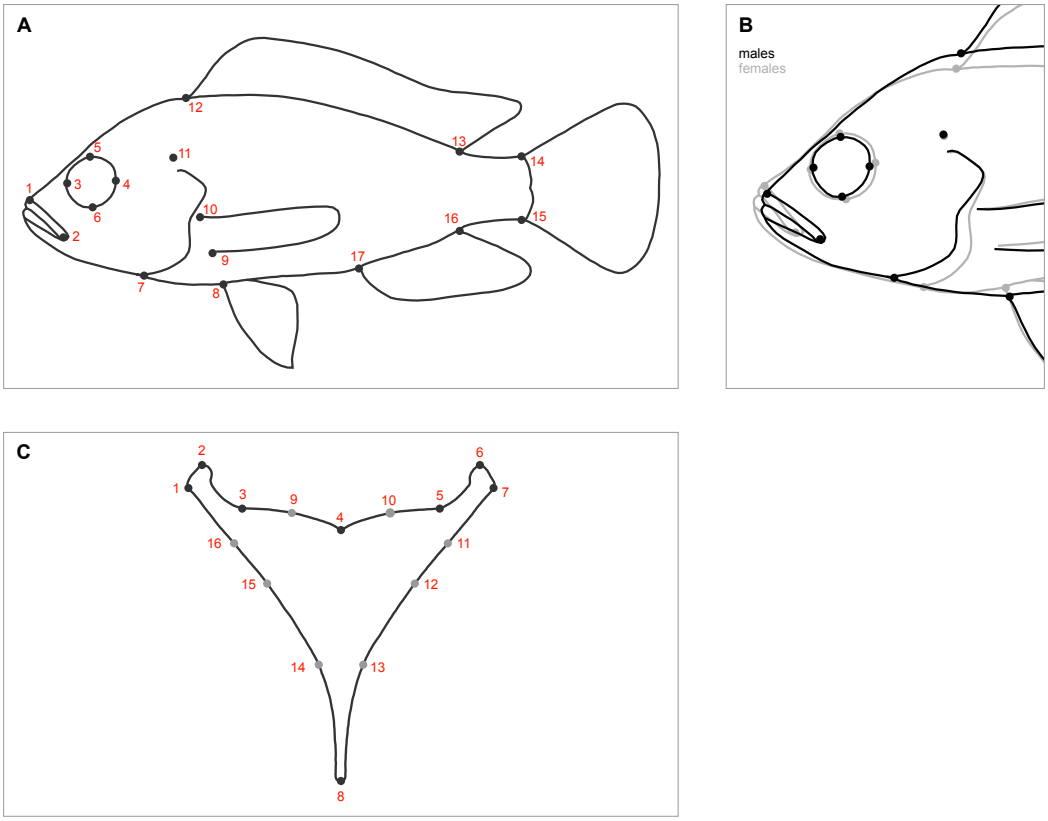


Fig. S1 Landmark positions for body shape and LPJ analyses and sex differences in head shape. (A) All 17 landmarks were used for body shape analyses comparing the wild populations, whereas only the 6 landmarks 1, 2, 8, 12, 14 and 15 were used for comparisons of the body shape of adults and F1 offspring of the pond experiment and only the four landmarks 1, 2, 7 and 12 were included in the mouth position analysis. (B) Only the landmarks describing head shape (1-8, 11 and 12) were used to compare head morphology of males (black outline) and females (grey outline). A DFA showed that females generally have more slender, but longer heads (DF differences are increased tenfold in the outlines). (C) True (black) and semi-landmarks (grey), which were included in the comparisons of the LPJ shape.

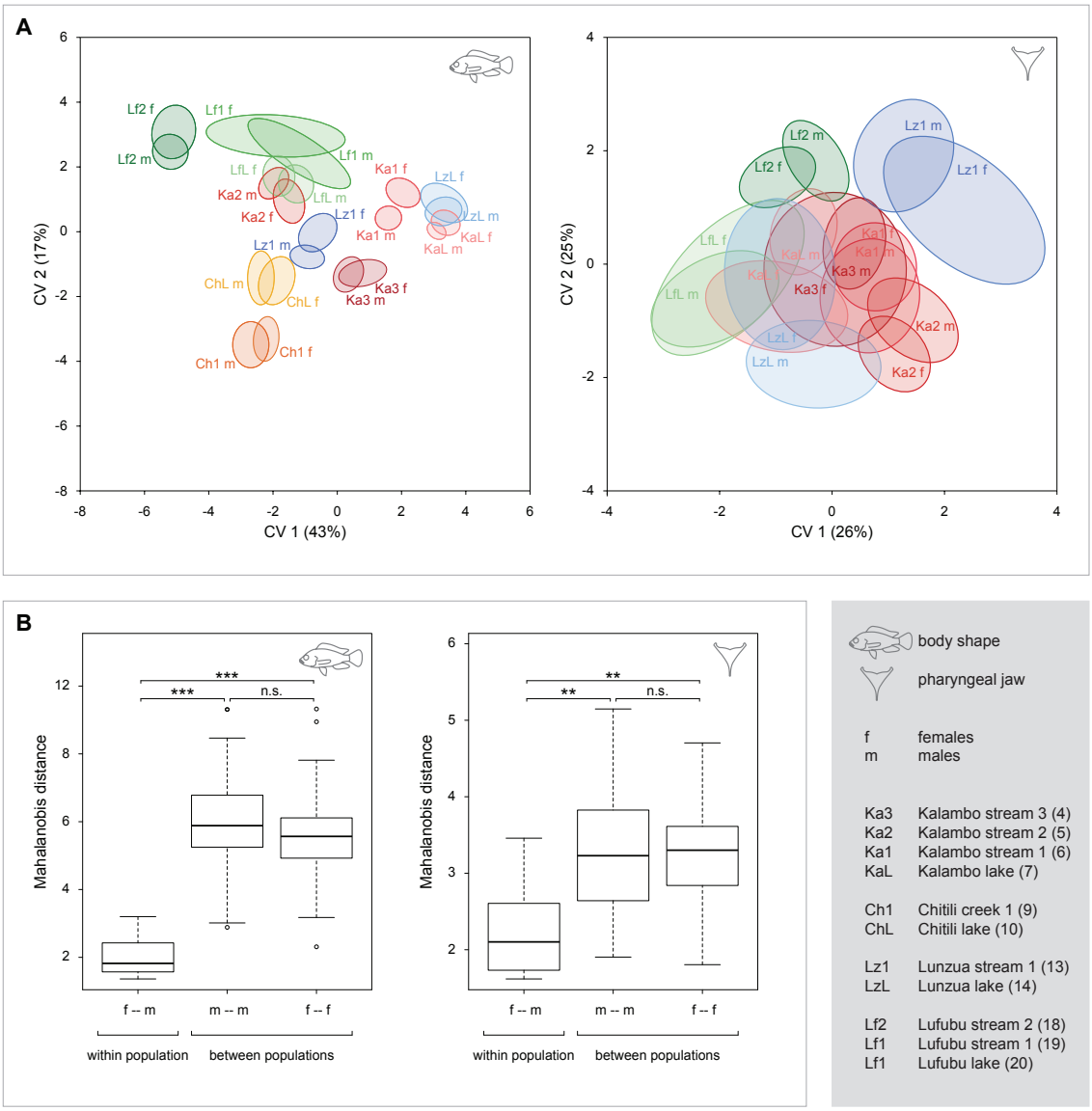


Fig. S2 Comparison of inter-sexual within population differences and intra-sexual differences among populations in morphometric traits (body shape and LPJ). (A) CVA plots show strong population specific overlap of male and female body, as well as in LPJ shape (ellipses represent the 95% confidence intervals of the means). The Chitili system was excluded for LPJ shape since sample size was low in females (Table S1). (B) ANOVAs with additional TukeyHSD show significantly smaller Mahalanobis distances in inter-sexual comparisons within populations, compared to intra-sexual comparisons among populations for body shape as well as for LPJ shape. Significance levels: $P < 0.05$*, $P < 0.01$** and $P < 0.0001$***.

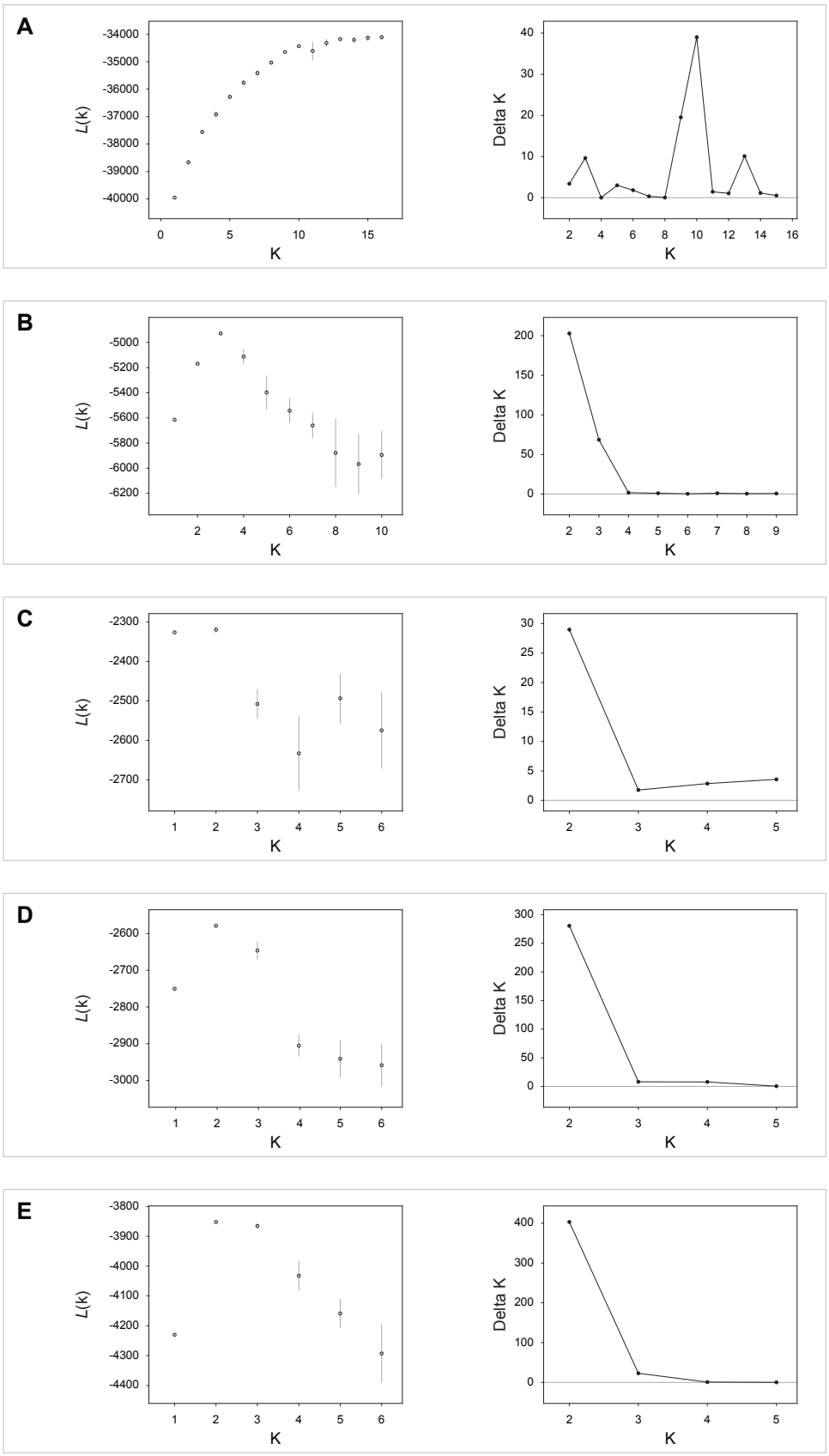
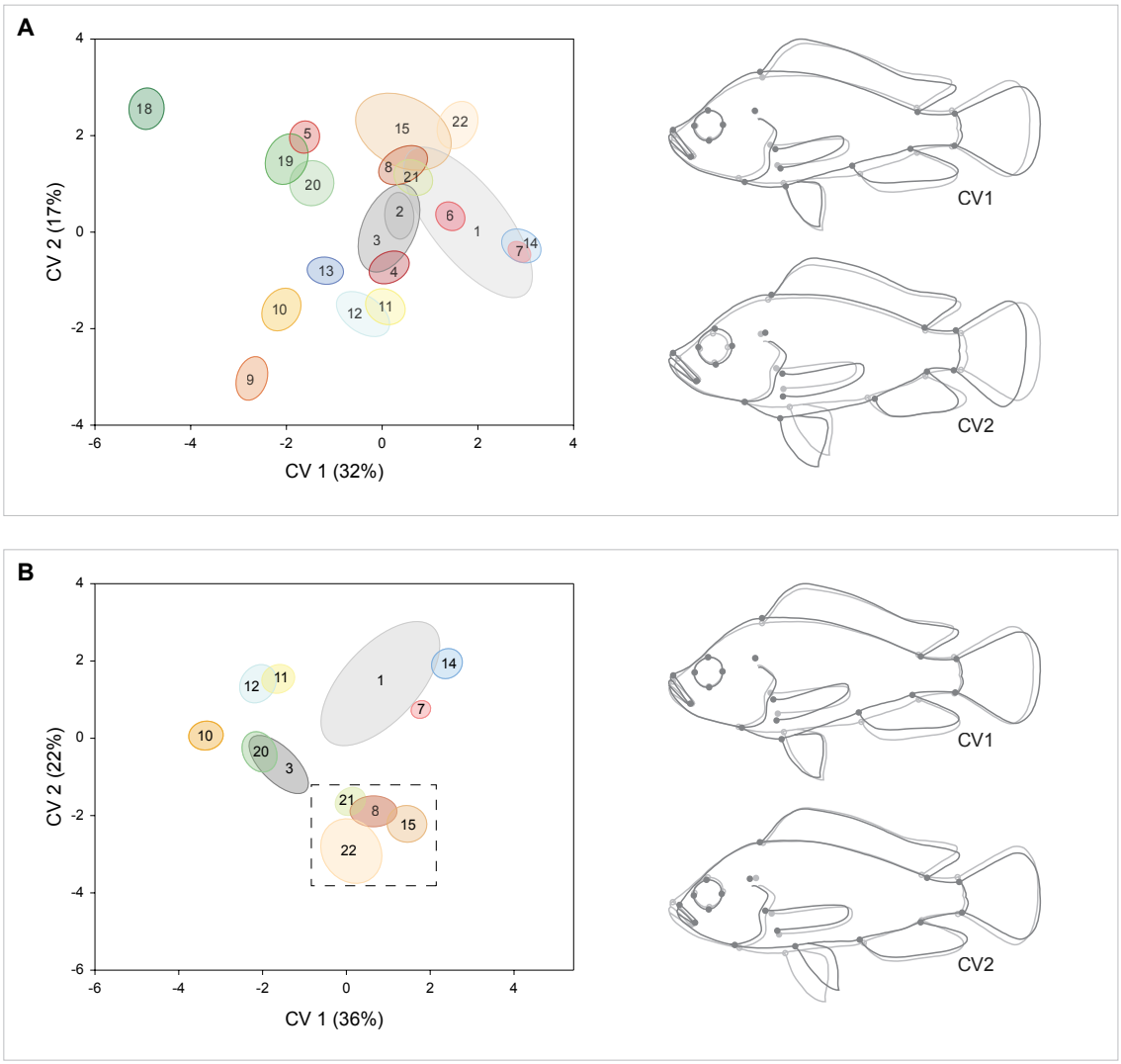


Fig. S3 Mean likelihood (L(*K*) ± SD) over 10 STRUCTURE runs assuming *K* clusters (left); Δ*K* statistic (right); (A) full data, (B) samples from the Kalambo river, (C) samples from the Chitili creek, (D) samples from the Lunzua river, (E) samples from the Lufubu river.



1 Ninde	6 Kalambo stream 1 (Ka1)	11 Chisanza	18 Lufubu stream 2 (Lf2)
2 Loazi	7 Kalambo lake (KaL)	12 Sumba	19 Lufubu stream 1 (Lf1)
3 Muzi	8 Toby's Place	13 Lunzua stream 1 (Lz1)	20 Lufubu lake (LfL)
4 Kalambo stream 3 (Ka3)	9 Chitili creek 1 (Ch1)	14 Lunzua lake (LzL)	21 Ndole Bay
5 Kalambo stream 2 (Ka2)	10 Chitili lake (ChL)	15 Wonzye	22 Lake Chila

Fig. S4 Body shape differentiation among the 20 sampled populations and among the 11 shoreline populations only (ellipses represent the 95% confidence intervals of the means). (A) Overall body shape differentiation among 20 populations (numbers and colors of the populations correspond with Fig. 1). The most extreme shape changes of the first two CVs are illustrated by landmark shifts (from grey to black with increasing values) (Table S3B). (B) CVA plot for the first two CVs and corresponding landmark shifts for the shoreline populations only. The clustering of populations in the morpho-space indicates stronger clustering of pure lacustrine populations (framed with a dashed line) compared to the other, more scattered shoreline populations, which are adjacent to streams.

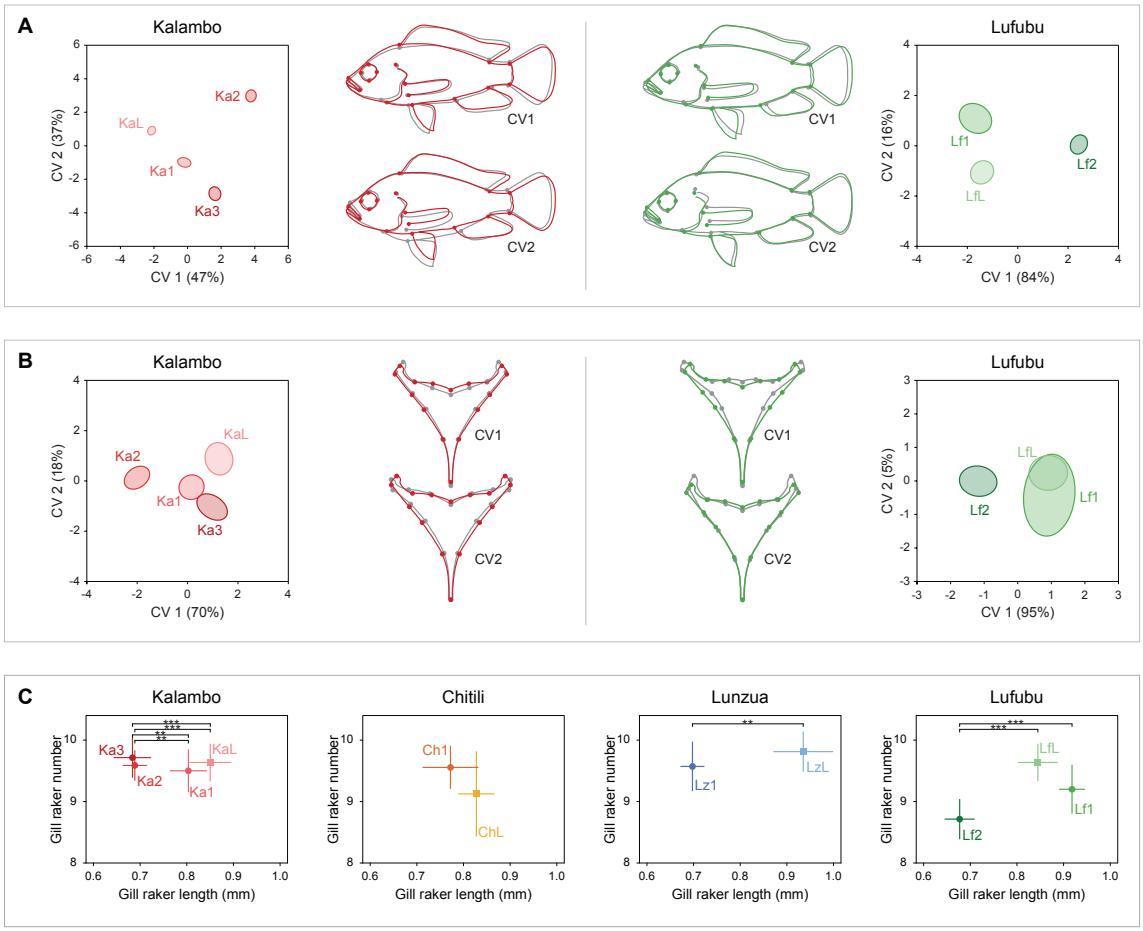


Fig. S5 Body shape and LPJ shape differentiation within systems with more than two populations and gill raker length and number in females. (A) Body shape differentiation separately for the four Kalambo populations (ellipses represent the 95% confidence intervals of the means, outlines from colored to grey with increasing CV-values, Table S4A) as well for the three Lufubu populations (Table S4B). (B) LPJ shape differentiation for the four Kalambo populations separately (Table S4C) as well for the three Lufubu populations (Table S4D). (C) Differences in size corrected female gill raker lengths and number between populations within each lake-stream system (error bars represent 95% confidence intervals of the means) (Table S6). Significance levels: $P < 0.05$*, $P < 0.01$** and $P < 0.0001$***.

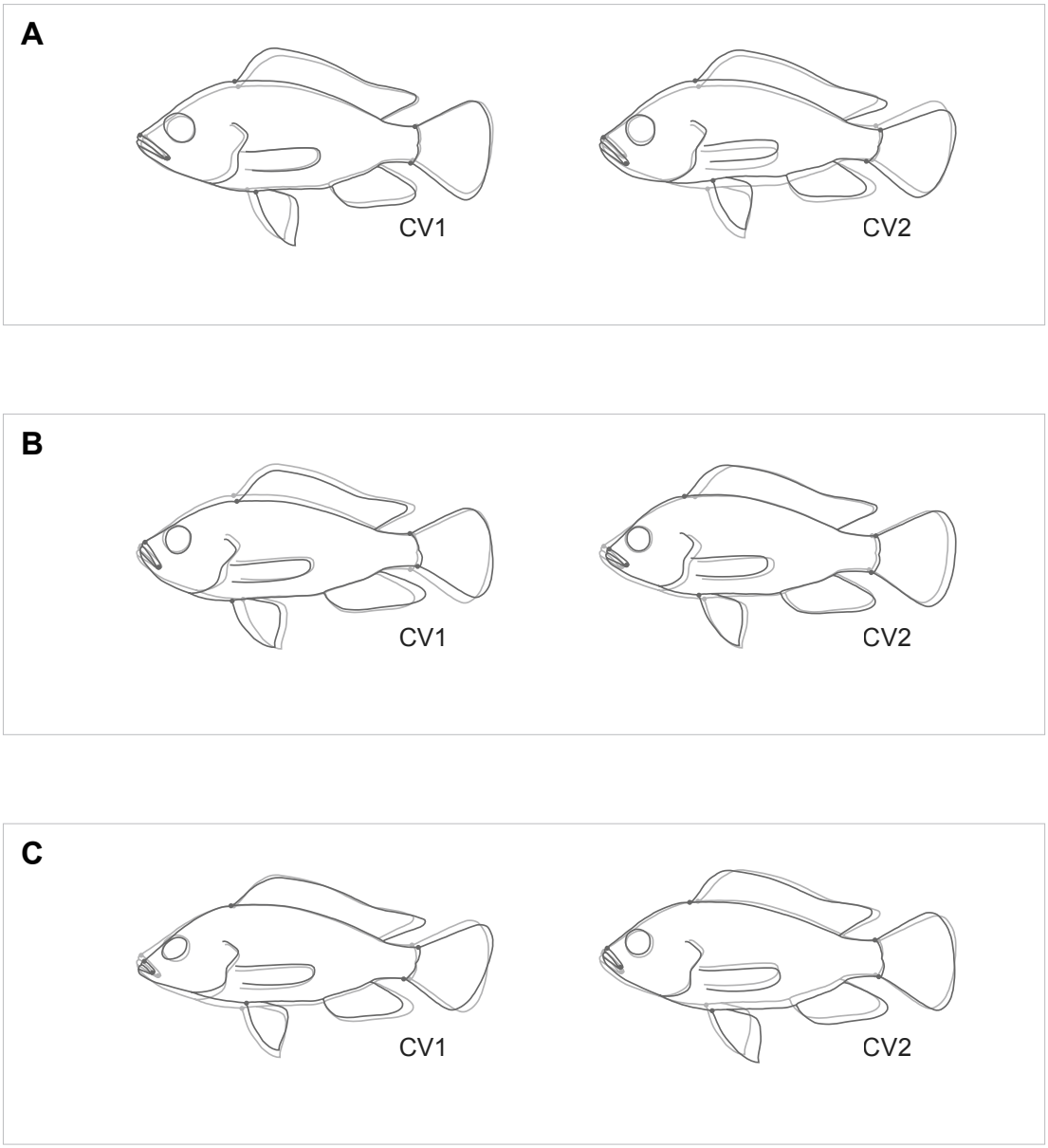
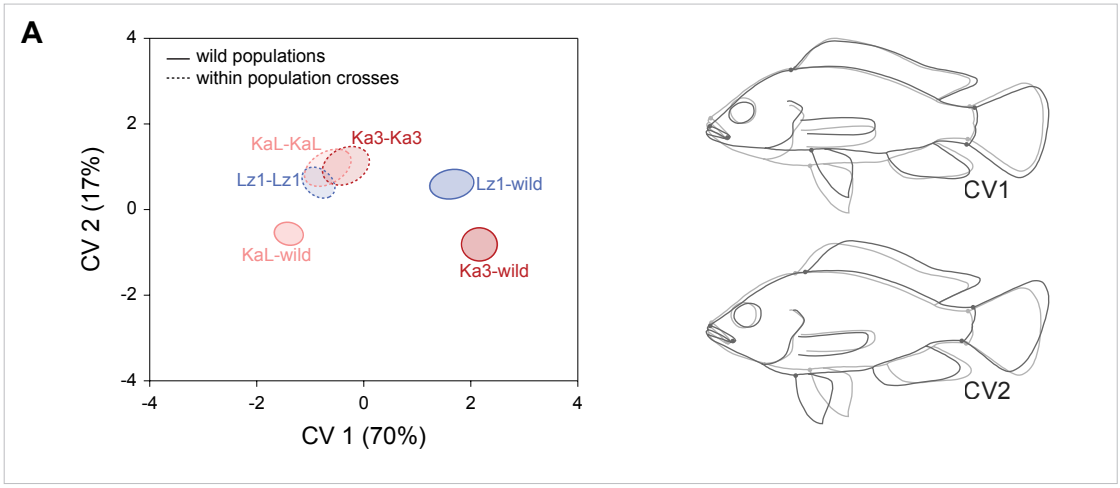
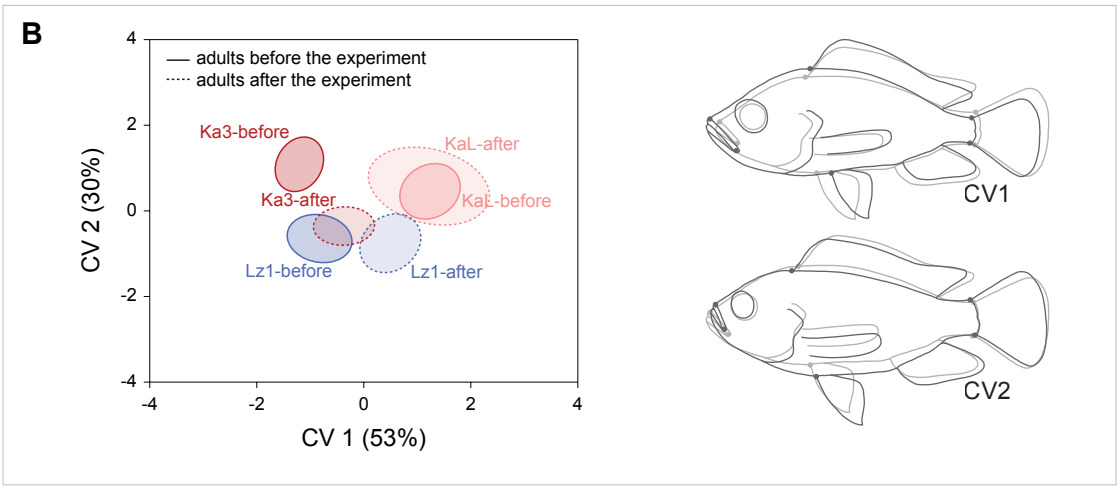


Fig. S6 Outlines to illustrate the body shape changes in F1 individuals of the pond experiment (CVA plots in Fig. 4A; distance values Table S7). From light grey to dark outlines with increasing values, scaling factor ten by default.
(A) KaL-KaL/KaL-Ka3/Ka3-Ka3, (B) KaL-KaL/KaL-Lz1/Lz1-Lz1 and (C) Ka3-Ka3/Ka3-Lz1/Lz1-Lz1.





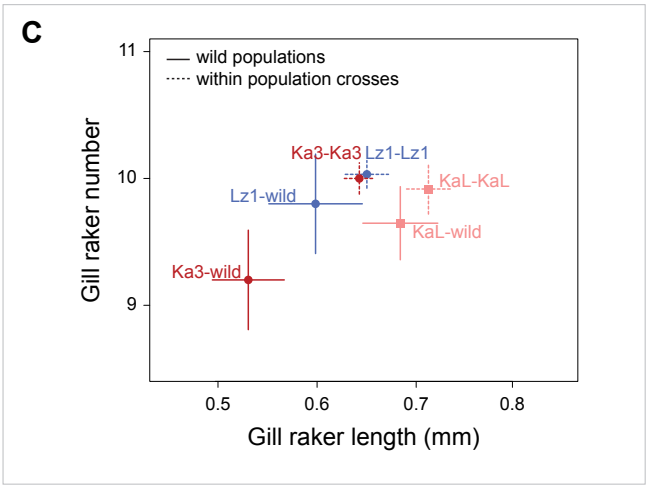


Fig. S7 Plasticity in body shape and gill raker length. (A) CVA of body shape among the within population F1 offspring and their corresponding wild populations. Outlines for illustration purposes only, from light grey to dark outlines with increasing values, scaling factor ten by default. (B) CVA comparing the body shape of surviving adults at the beginning and at the end of the experimental period. (C) Comparison of gill raker length among the within population F1 offspring and their corresponding wild populations. (Table S8)

Table S1 Sample size details for each analysis with information about sampling year and geographic coordinates for each locality (note that some individuals were used for more than one analysis).

	sampling information						body shape analysis				LPJ analysis					gill raker analysis				microsatellite analysis				mtDNA analysis				stomach and gut content analysis			
	population	locality	specification	latitude	longitude	year	total	males	females	juveniles	total	males	females	juveniles	undef. sex	total	males	females	juveniles	total	males	females	juveniles	total	males	females	juveniles	total	males	females	juveniles
Kalambo system	1	Ninde	lake population adjacent to stream	7°40'51.10"S	30°43'20.67"E	2011	7	2	5	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7	2	5	0	7	2	5	0	n/a	n/a	n/a	n/a
	2	Loazi	lake population adjacent to stream	8°18'49.55"S	31° 2'57.54"E	2011	32	17	15	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	31	16	15	0	7	7	0	0	n/a	n/a	n/a	n/a
	3	Muzi	lake population adjacent to stream	8°23'1.84"S	31° 7'47.15"E	2011	24	12	12	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	25	14	11	0	9	7	2	0	n/a	n/a	n/a	n/a
	4a Ka3	Kalambo stream 3	stream population	8°35'41.59"S	31°14'50.32"E	2010	n/a	n/a	n/a	n/a	20	10	9	1	0	n/a	n/a	n/a	n/a	32	4	10	18	27	4	9	14	n/a	n/a	n/a	n/a
	4b Ka3					2011	52	29	23	0	n/a	n/a	n/a	n/a	n/a	29	15	14	0	30	17	13	0	n/a	n/a	n/a	n/a	10	10	0	0
	5a Ka2	Kalambo stream 2	stream population	8°35'6.24"S	31°12'29.32"E	2011	n/a	n/a	n/a	n/a	14	2	12	0	0	14	2	12	0	14	1	13	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	5b Ka2					2012	38	23	15	0	14	10	4	0	0	16	11	5	0	32	19	12	1	8	6	2	0	10	10	0	0
	6a Ka1	Kalambo stream 1	stream population	8°35'35.23"S	31°11'6.18"E	2011	59	41	18	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	32	17	15	0	6	4	2	0	n/a	n/a	n/a	n/a
	6b Ka1					2012	n/a	n/a	n/a	n/a	25	13	11	0	1	28	14	14	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	10	10	0	0
	7a KaL	Kalambo lake	lake population adjacent to stream	8°36'6.27"S	31°11'13.24"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	31	27	4	0	29	25	4	0	n/a	n/a	n/a	n/a
	7b KaL					2011	102	59	43	0	23	11	8	0	4	25	17	8	0	33	19	14	0	n/a	n/a	n/a	n/a	10	10	0	0
	7c KaL					2012	n/a	n/a	n/a	n/a	3	1	2	0	0	3	0	3	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	8a	Toby's place	pure lake population	8°37'25.99"S	31°12'2.86"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	34	16	11	7	30	15	10	5	n/a	n/a	n/a	n/a
	8b					2012	33	19	14	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	31	19	12	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Chitili system	9a Ch1	Chitili creek 1	stream population	8°38'16.91"S	31°12'4.02"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	7	1	4	2	7	1	4	2	n/a	n/a	n/a	n/a
	9b Ch1					2011	53	24	29	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	31	14	17	0	10	6	4	0	n/a	n/a	n/a	n/a
	9c Ch1					2012	n/a	n/a	n/a	n/a	14	13	1	0	0	24	15	9	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	10	10	0	0
	10a ChL	Chitili lake	lake population adjacent to stream	8°38'18.42"S	31°11'55.34"E	2011	38	10	28	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	31	11	20	0	10	6	4	0	n/a	n/a	n/a	n/a
	10b ChL					2012	n/a	n/a	n/a	n/a	12	8	3	0	1	23	15	8	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	5	5	0	0
	11	Chisanza	lake population adjacent to stream	8°39'57.39"S	31°11'41.26"E	2011	41	22	19	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	32	19	12	1	9	6	3	0	n/a	n/a	n/a	n/a
	12	Sumba	lake population adjacent to stream	8°40'18.58"S	31°11'33.94"E	2011	33	22	11	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	32	21	11	0	9	6	3	0	n/a	n/a	n/a	n/a
Lunzua system	13a Lz1	Lunzua stream 1	stream population	8°47'23.51"S	31° 8'14.33"E	2011	53	36	17	0	18	11	7	0	0	n/a	n/a	n/a	n/a	30	17	12	1	7	5	2	0	10	10	0	0
	13b Lz1					2012	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	18	11	7	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	14a LzL	Lunzua lake	lake population adjacent to stream	8°44'57.13"S	31°10'21.86"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	30	21	9	0	24	20	4	0	n/a	n/a	n/a	n/a
	14b LzL					2011	40	23	17	0	11	3	8	0	0	16	5	11	0	31	16	15	0	n/a	n/a	n/a	n/a	5	5	0	0
	14c LzL					2012	n/a	n/a	n/a	n/a	11	7	4	0	0	15	10	5	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	5	5	0	0
	15a	Wonzye	pure lake population	8°45'1.76"S	31° 7'49.02"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	42	24	8	10	39	21	8	10	n/a	n/a	n/a	n/a
	15b					2012	36	17	19	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	18	10	8	0	10	6	4	0	n/a	n/a	n/a	n/a
	16	Fisheries Department	pure lake population	8°45'58.52"S	31° 6'23.99"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	26	15	5	6	24	19	5	0	n/a	n/a	n/a	n/a
	17	Kalungula	lake population adjacent to stream	8°48'33.39"S	31° 7'49.02"E	2010	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	27	17	10	0	28	17	10	1	n/a	n/a	n/a	n/a
Lufubu system	18 Lf2	Lufubu stream 2	stream population	8°41'9.37"S	30°33'51.90"E	2012	36	21	15	0	26	14	12	0	0	29	15	14	0	30	15	15	0	13	4	9	0	10	10	0	0
	19 Lf1	Lufubu stream 1	stream population	8°35'49.31"S	30°43'38.96"E	2011	27	21	6	0	12	8	4	0	0	15	10	5	0	27	21	6	0	10	8	2	0	7	7	0	0
	20a LfL	Lufubu lake	lake population adjacent to stream	8°33'36.56"S	30°43'33.79"E	2011	30	24	6	0	14	10	4	0	0	16	11	5	0	30	24	6	0	9	8	1	0	6	6	0	0
	20b LfL					2012	n/a	n/a	n/a	n/a	7	3	4	0	0	10	4	6	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	4	4	0	0
	21	Ndole bay	pure lake population	8°28'34.61"S	30°26'57.48"E	2012	45	24	21	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	16	8	8	0	13	5	8	0	n/a	n/a	n/a	n/a
	22	Lake Chila	pure lake population	8°50'8.68"S	31°22'49.44"E	2012	12	4	8	0	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	14	4	8	2	14	4	8	2	n/a	n/a	n/a	n/a
total sample size per analysis							**791**	**450**	**341**	**0**	**224**	**124**	**93**	**1**	**6**	**281**	**155**	**126**	**0**	**786**	**429**	**309**	**48**	**359**	**212**	**113**	**34**	**102**	**102**	**0**	**0**

Table S2 Sample size details and result summary of the pond experiment. (A) Number of stocked adult fish per population and information about survival and reproduction. (B) Number of F1 individuals used for body shape and gill raker analyses.

A

pond	original stock males			original stock females			surviving males			surviving females			non-surviving reproducing males			non-surviving reproducing females			reproducing males	reproducing females	genotyped offspring	mating events
	KaL	Ka3	Lz1	KaL	Ka3	Lz1	KaL	Ka3	Lz1	KaL	Ka3	Lz1	KaL	Ka3	Lz1	KaL	Ka3	Lz1				
1	4	4	4	7	7	7	1	1	2	1	3	3	0	0	1	0	0	1	3	8	148	24
2	4	4	4	7	7	7	2	1	1	1	2	5	1	2	0	0	2	1	5	11	160	26
3	4	4	4	7	7	7	1	1	2	1	3	2	0	0	1	0	0	0	2	8	95	15
4	4	4	4	7	7	7	1	0	0	1	4	4	0	1	0	0	0	0	2	9	111	18
5	4	4	4	7	7	7	2	2	0	3	3	2	0	0	0	0	0	0	2	6	79	15
total	**20**	**20**	**20**	**35**	**35**	**35**	**7**	**5**	**5**	**7**	**15**	**16**	**1**	**3**	**2**	**0**	**2**	**2**	**14**	**42**	**593**	**98**

B

F1 juveniles	body shape	gill raker
KaL-KaL	25	25
Ka3-Ka3	24	24
Lz1-Lz1	31	32
KaL-Ka3	13	13
KaL-Lz1	26	26
Ka3-Lz1	11	12
total	130	132

Table S3 Pairwise genetic and morphometric (body size) distances between populations (full names of localities are given in the grey box in Fig. 1 and Table S1). (A) Pairwise genetic differentiation: D_{EST}-values (upper triangular matrix) and F_{ST}-values (lower triangular matrix). Both indices indicate significant differentiation (P values < 0.05) between most populations (non-significant values are underlined). (B) Pairwise body shape differentiation among populations: Procrustes (upper triangular matrix) and Mahalanobis (lower triangular matrix) distances from the CVA (Fig. S4A). All comparisons showed significant body shape differences (P values < 0.05).

A

population	1	2	3	4a	4b	5a	5b	6a	7a	7b	8a	8b	9a	9b	10a	11	12	13a	14a	14b	15a	15b	16	17	18	19	20a	21	22
1		0.5450	0.6476	0.7410	0.7528	0.7166	0.7754	0.5761	0.5899	0.6407	0.6578	0.6486	0.6970	0.6573	0.6161	0.6554	0.6300	0.6773	0.6453	0.6003	0.6412	0.6333	0.6351	0.6578	0.7470	0.6216	0.6655	0.6624	0.6907
2	0.0925		0.3848	0.6392	0.6646	0.6361	0.7401	0.4525	0.4374	0.4860	0.5584	0.5472	0.5172	0.4678	0.3930	0.4036	0.4120	0.5416	0.4667	0.3968	0.4887	0.4827	0.5121	0.4972	0.6498	0.4745	0.4926	0.5976	0.5610
3	0.1118	0.0513		0.6317	0.6486	0.5918	0.6942	0.3512	0.4235	0.4443	0.5425	0.4995	0.4250	0.3380	0.3183	0.4006	0.3507	0.4906	0.3661	0.3507	0.4920	0.4609	0.5078	0.5254	0.6776	0.5332	0.5121	0.6015	0.5262
4a	0.1430	0.1134	0.1126		-0.0087	0.6502	0.5503	0.6848	0.5581	0.5785	0.5677	0.6405	0.5553	0.6398	0.6050	0.5682	0.5981	0.4527	0.4345	0.5110	0.5681	0.6076	0.5490	0.5943	0.6727	0.4306	0.4634	0.6278	0.5313
4b	0.1577	0.1256	0.1236	-0.0018		0.6382	0.5497	0.6898	0.6248	0.6422	0.6422	0.6841	0.6098	0.6267	0.6209	0.5875	0.5832	0.4352	0.4716	0.4965	0.5914	0.6429	0.5820	0.6397	0.6602	0.4656	0.5044	0.6579	0.5555
5a	0.2053	0.1379	0.1350	0.1719	0.1773		0.1128	0.3582	0.4219	0.3503	0.5523	0.5373	0.6257	0.5373	0.4357	0.5269	0.4961	0.6008	0.6377	0.5163	0.6777	0.6678	0.6311	0.5992	0.7017	0.6676	0.6408	0.5476	0.6259
5b	0.2154	0.1707	0.1659	0.1560	0.1613	0.0422		0.5291	0.4378	0.3579	0.5354	0.6415	0.5772	0.6369	0.5614	0.6299	0.6157	0.6668	0.5818	0.6264	0.6384	0.6557	0.5710	0.5932	0.7059	0.6950	0.6743	0.5443	0.5409
6a	0.1039	0.0671	0.0436	0.1309	0.1391	0.0818	0.1398		0.5291	0.1467	0.3445	0.3142	0.3983	0.3149	0.1728	0.3078	0.3022	0.5222	0.4162	0.3323	0.5202	0.5074	0.4909	0.5093	0.6767	0.5084	0.4730	0.4701	0.4930
7a	0.1052	0.0741	0.0727	0.0922	0.1110	0.1045	0.1030	0.0615		-0.0053	0.2640	0.4185	0.2659	0.4136	0.2243	0.3483	0.3616	0.5369	0.2220	0.3715	0.3418	0.3094	0.3311	0.3085	0.6529	0.4233	0.4280	0.3903	0.3756
7b	0.1240	0.0888	0.0821	0.1026	0.1220	0.0993	0.0887	0.0740	0.0006		0.2424	0.4437	0.2318	0.4171	0.2517	0.3967	0.3987	0.6047	0.2631	0.3982	0.3872	0.4222	0.3590	0.3717	0.6343	0.4850	0.4727	0.4230	0.3898
8a	0.1334	0.1011	0.1009	0.1135	0.1357	0.1437	0.1376	0.0915	0.0370	0.0346		0.1788	0.2516	0.3862	0.3416	0.5063	0.5220	0.6175	0.4398	0.5535	0.5639	0.5682	0.5506	0.5464	0.7040	0.4957	0.5201	0.4737	0.5773
8b	0.1496	0.1037	0.0865	0.1542	0.1717	0.1594	0.1958	0.0526	0.1042	0.1176	0.0789		0.4689	0.2878	0.2595	0.4627	0.4957	0.5815	0.5763	0.5227	0.6696	0.6482	0.6495	0.6972	0.7306	0.6498	0.6589	0.6168	0.6790
9a	0.1282	0.0623	0.0373	0.1267	0.1376	0.1088	0.1511	0.0227	0.0714	0.0816	0.0905	0.0450		0.2260	0.2549	0.2184	0.2473	0.4618	0.3573	0.2845	0.4853	0.4388	0.4555	0.5103	0.6709	0.5284	0.5416	0.5376	0.5607
9b	0.1372	0.0788	0.0703	0.0977	0.1154	0.1493	0.1473	0.0819	0.0333	0.0347	0.0442	0.0577	0.0789		0.0952	0.4628	0.3521	0.5596	0.3127	0.4332	0.4381	0.3847	0.3680	0.4434	0.6248	0.4096	0.4208	0.5379	0.3992
10a	0.1408	0.0874	0.0520	0.1480	0.1545	0.1422	0.1878	0.0451	0.1042	0.1151	0.0919	0.0572	0.0674	0.0142		0.3266	0.3269	0.5092	0.4391	0.3709	0.5790	0.5637	0.5762	0.5892	0.6841	0.5251	0.5744	0.6248	0.6055
11	0.1052	0.0538	0.0418	0.0929	0.1003	0.1138	0.1469	0.0379	0.0680	0.0792	0.0938	0.0752	0.0735	0.0463	0.0278		0.0892	0.4358	0.2670	0.1940	0.4398	0.4005	0.4366	0.4242	0.6550	0.4286	0.3965	0.5390	0.4644
12	0.0797	0.0474	0.0340	0.0929	0.0987	0.0987	0.1362	0.0322	0.0586	0.0703	0.0872	0.0765	0.0496	0.0470	0.0289	0.0095		0.3829	0.2465	0.1214	0.3546	0.3484	0.3775	0.3983	0.6399	0.4277	0.3935	0.5134	0.4150
13a	0.1286	0.0957	0.0786	0.1058	0.1097	0.1534	0.1869	0.0818	0.1074	0.1246	0.1375	0.1247	0.1144	0.1004	0.0810	0.0630	0.0520		0.4966	0.3472	0.5844	0.6242	0.5866	0.5359	0.6777	0.5717	0.6258	0.7185	0.5394
14a	0.1013	0.0728	0.0608	0.0618	0.0776	0.1400	0.1254	0.0868	0.0207	0.0228	0.0537	0.1200	0.0330	0.1027	0.0790	0.0513	0.0460	0.0943		0.1211	0.2074	0.1525	0.2331	0.3151	0.6680	0.3023	0.2637	0.4265	0.3091
14b	0.0800	0.0455	0.0313	0.0796	0.0845	0.1014	0.1384	0.0330	0.0630	0.0730	0.0920	0.0751	0.0587	0.0469	0.0289	0.0119	0.0069	0.0453	0.0410		0.3132	0.2815	0.3157	0.3570	0.6836	0.3839	0.3665	0.5661	0.3695
15a	0.1004	0.0633	0.0654	0.0823	0.0913	0.1293	0.1302	0.0857	0.0362	0.0444	0.0764	0.1247	0.0480	0.1050	0.0791	0.0560	0.0474	0.0959	0.0198	0.0432		-0.0202	0.0808	0.1652	0.6550	0.4034	0.3584	0.4657	0.1277
15b	0.0960	0.0566	0.0603	0.0878	0.0998	0.1330	0.1390	0.0822	0.0350	0.0477	0.0769	0.1247	0.0425	0.1022	0.0740	0.0538	0.0426	0.0983	0.0215	0.0396	-0.0021		0.0648	0.2240	0.6696	0.3461	0.3325	0.4295	0.2123
16	0.0943	0.0607	0.0644	0.0843	0.0938	0.1262	0.1218	0.0812	0.0379	0.0480	0.0784	0.1235	0.0398	0.1034	0.0761	0.0576	0.0454	0.0960	0.0250	0.0420	0.0065	0.0020		0.0748	0.6558	0.3653	0.3424	0.4662	0.0575
17	0.1135	0.0689	0.0703	0.0950	0.1074	0.1238	0.1287	0.0876	0.0378	0.0487	0.0771	0.1384	0.0510	0.1095	0.0833	0.0605	0.0503	0.0473	0.0987	0.0318	0.0143	0.0178	0.0054		0.6973	0.3978	0.3745	0.4929	0.1040
18	0.1978	0.1483	0.1541	0.1644	0.1690	0.2100	0.2161	0.1686	0.1471	0.1575	0.1709	0.2147	0.1493	0.1894	0.1664	0.1491	0.1383	0.1394	0.1771	0.1413	0.1270	0.1286	0.1272	0.1364		0.5617	0.6571	0.6803	0.6186
19	0.0853	0.0528	0.0581	0.0713	0.0799	0.1208	0.1345	0.0735	0.0500	0.0606	0.0691	0.1175	0.0415	0.0965	0.0748	0.0491	0.0422	0.0419	0.0966	0.0345	0.0360	0.0292	0.0289	0.0348	0.1021		0.0676	0.3984	0.4382
20a	0.0838	0.0661	0.0766	0.0693	0.0795	0.1396	0.1526	0.0900	0.0583	0.0716	0.0767	0.1278	0.0548	0.1045	0.0910	0.0652	0.0583	0.0556	0.1043	0.0405	0.0527	0.0449	0.0452	0.0508	0.1318	0.0115		0.4544	0.3790
21	0.1505	0.0956	0.0956	0.1143	0.1255	0.1459	0.1379	0.0772	0.0418	0.0603	0.0828	0.1481	0.0809	0.1343	0.1040	0.0822	0.0616	0.0739	0.1364	0.0555	0.0463	0.0504	0.0509	0.0611	0.1485	0.0404	0.0550		0.5562
22	0.1214	0.0745	0.0720	0.0898	0.0982	0.1330	0.1253	0.0862	0.0471	0.0573	0.0894	0.1371	0.0528	0.1137	0.0884	0.0633	0.0506	0.0994	0.0395	0.0502	0.0170	0.0168	0.0047	0.0183	0.1344	0.0620	0.0392	0.0751	

B

population	1	2	3	4b	5b	6a	7b	8b	9b	10a	11	12	13a	14b	15b	18	19	20a	21	22
1		0.0295	0.0404	0.0484	0.0233	0.0511	0.0327	0.0228	0.0420	0.0379	0.0369	0.0457	0.0438	0.0276	0.0316	0.0395	0.0401	0.0319	0.0324	0.0337
2	4.9489		0.0220	0.0320	0.0279	0.0346	0.0225	0.0190	0.0273	0.0188	0.0194	0.0258	0.0269	0.0236	0.0205	0.0290	0.0231	0.0219	0.0223	0.0235
3	5.5377	3.5329		0.0309	0.0398	0.0303	0.0224	0.0337	0.0370	0.0269	0.0275	0.0268	0.0380	0.0331	0.0257	0.0399	0.0332	0.0340	0.0164	0.0252
4b	6.8230	5.1413	5.0948		0.0458	0.0220	0.0252	0.0382	0.0314	0.0300	0.0290	0.0198	0.0238	0.0330	0.0430	0.0473	0.0338	0.0381	0.0326	0.0364
5b	6.0672	4.7985	5.2939	5.3491		0.0537	0.0371	0.0169	0.0350	0.0307	0.0322	0.0426	0.0397	0.0297	0.0304	0.0289	0.0313	0.0258	0.0309	0.0322
6a	5.8194	4.2485	4.5856	3.9140	5.8216		0.0250	0.0433	0.0456	0.0399	0.0379	0.0276	0.0342	0.0373	0.0418	0.0518	0.0402	0.0435	0.0339	0.0349
7b	4.6544	3.9309	4.7515	4.4821	5.9213	3.6119		0.0271	0.0362	0.0290	0.0259	0.0266	0.0319	0.0202	0.0262	0.0423	0.0322	0.0300	0.0178	0.0219
8b	5.0837	3.6133	4.9001	4.8953	3.2746	4.5877	4.1048		0.0323	0.0262	0.0267	0.0343	0.0311	0.0225	0.0214	0.0292	0.0260	0.0210	0.0259	0.0227
9b	7.4268	5.4561	5.8027	5.2976	5.6321	6.4922	6.4594	5.7587		0.0157	0.0207	0.0267	0.0232	0.0344	0.0426	0.0369	0.0295	0.0302	0.0383	0.0405
10a	6.4120	4.3150	4.5920	4.9249	4.8566	5.5860	5.4444	4.8670	2.7773		0.0122	0.0208	0.0246	0.0301	0.0313	0.0275	0.0191	0.0199	0.0275	0.0309
11	5.3302	3.9713	4.4396	5.4390	5.5530	5.2339	4.0371	4.7344	4.6413	3.2506		0.0175	0.0226	0.0237	0.0307	0.0297	0.0185	0.0201	0.0266	0.0330
12	5.3235	4.1567	3.9157	5.0405	5.4377	4.7458	4.4480	4.7718	4.3730	3.1550	2.6681		0.0195	0.0302	0.0370	0.0382	0.0253	0.0309	0.0307	0.0355
13a	5.5699	3.9608	4.9743	4.2981	4.7427	4.7551	5.0274	4.2964	4.2820	4.1919	4.4350	3.7757		0.0279	0.0411	0.0370	0.0266	0.0302	0.0390	0.0384
14b	4.7761	4.7438	5.9381	6.0316	6.3602	5.3310	3.1124	4.8900	7.2722	6.4282	4.6146	5.0731	5.0915		0.0280	0.0366	0.0313	0.0276	0.0266	0.0304
15b	5.9430	4.0590	4.9601	5.8978	4.9754	5.3011	4.3202	3.1856	7.2228	5.9421	5.1375	5.6335	5.6291	5.0148		0.0324	0.0308	0.0271	0.0189	0.0211
18	8.6069	6.8003	7.1742	7.4456	5.6354	7.6059	8.6780	6.9380	7.0098	6.1314	7.5099	7.0728	5.8962	8.8590	7.8515		0.0236	0.0221	0.0355	0.0380
19	7.2622	5.0887	5.7369	5.7504	5.0762	5.5812	6.1696	5.2621	6.2073	4.7727	4.8053	5.2103	5.2406	6.9723	5.7678	5.6799		0.0119	0.0298	0.0312
20a	6.1171	4.7340	5.5192	5.5934	4.9598	5.3450	5.4051	4.8552	5.8256	4.1782	4.1792	4.7464	4.7929	6.1352	5.5719	5.7718	1.9520		0.0277	0.0276
21	5.4945	3.6410	3.4655	4.2158	3.8495	4.1331	3.5529	3.3760	5.7653	4.4868	4.2807	4.3444	4.9261	5.0558	3.5793	6.8204	5.0064	4.6630		0.0200
22	6.1168	5.0319	5.0086	5.6808	4.4431	5.0999	5.0892	3.7617	6.9245	5.5506	5.8239	5.5646	5.7433	6.4322	4.3525	7.5547	5.6727	5.1649	3.7673	

Table S4 Pairwise morphometric (body shape and LPJ) distances within systems with more than two populations. Procrustes (upper triangular matrix) and Mahalanobis (lower triangular matrix) distances from the CVA (Fig. S5A & B) (non-significant values are underlined). (A) Pairwise body shape differentiation among the four Kalambo populations. (B) Pairwise body shape differentiation among the three Lufubu populations. (C) Pairwise LPJ shape differentiation among the four Kalambo populations. (D) Pairwise LPJ shape differentiation among the three Lufubu populations.

A

population	KaL	Ka1	Ka2	Ka3
KaL		0.0251	0.0368	0.0253
Ka1	3.8781		0.0535	0.0220
Ka2	6.3056	6.0287		0.0456
Ka3	5.3659	4.2863	6.3437	

B

population	LfL	Lf1	Lf2
LfL		0.0122	0.0182
Lf1	2.1637		0.0208
Lf2	4.0045	4.2414	

C

population	KaL	Ka1	Ka2	Ka3
KaL		0.0175	0.0217	0.0158
Ka1	1.9260		0.0122	0.0192
Ka2	3.3438	2.4847		0.0257
Ka3	1.9681	1.8445	3.2216	

D

population	LfL	Lf1	Lf2
LfL		0.0064	0.0266
Lf1	0.6663		0.0258
Lf2	2.1046	2.1603	

Table S5 Pairwise morphometric (body shape and LPJ) distances of all populations from the lake-stream systems. Procrustes (upper triangular matrix) and Mahalanobis (lower triangular matrix) distances from the CVA (Fig. 3) (non-significant values are underlined). (A) Pairwise body shape differentiation. (B) Pairwise LPJ shape differentiation.

A

population	Ka3	Ka2	Ka1	KaL	Ch1	ChL	Lz1	LzL	Lf2	Lf1	LfL
Ka3		0.0457	0.0220	0.0253	0.0314	0.0295	0.0238	0.0331	0.0474	0.0341	0.0386
Ka2	5.6679		0.0535	0.0369	0.0349	0.0312	0.0396	0.0296	0.0288	0.0301	0.0243
Ka1	3.9344	5.9029		0.0251	0.0455	0.0393	0.0341	0.0373	0.0518	0.0406	0.0442
KaL	4.5737	6.1411	3.5549		0.0361	0.0288	0.0320	0.0202	0.0423	0.0321	0.0301
Ch1	5.4110	5.8736	6.5003	6.6979		0.0158	0.0231	0.0344	0.0370	0.0291	0.0300
ChL	5.0602	4.9994	5.5704	5.7198	2.7821		0.0242	0.0302	0.0278	0.0182	0.0196
Lz1	4.3098	5.0077	4.6585	5.0996	4.3658	4.2179		0.0279	0.0371	0.0268	0.0307
LzL	6.0366	6.6764	5.2110	3.0927	7.4857	6.6939	5.1698		0.0366	0.0309	0.0273
Lf2	7.7497	5.8296	7.8774	9.0269	7.0925	6.1512	6.0970	9.2435		0.0229	0.0214
Lf1	5.6788	4.9360	5.4026	6.1579	6.0796	4.5449	5.0511	6.9778	5.6664		0.0121
LfL	5.4917	4.7787	5.1340	5.3243	5.6660	3.8408	4.5813	6.0876	5.7561	2.0123	

B

population	Ka3	Ka2	Ka1	KaL	Ch1	ChL	Lz1	LzL	Lf2	Lf1	LfL
Ka3		0.0273	0.0214	0.0160	0.0414	0.0086	0.0277	0.0257	0.0317	0.0287	0.0247
Ka2	2.7659		0.0122	0.0226	0.0260	0.0310	0.0254	0.0202	0.0193	0.0188	0.0212
Ka1	1.8301	2.1889		0.0188	0.0269	0.0256	0.0191	0.0171	0.0193	0.0234	0.0232
KaL	2.0079	2.9577	2.0749		0.0421	0.0162	0.0278	0.0158	0.0255	0.0238	0.0216
Ch1	3.5801	3.4244	3.1368	4.1603		0.0445	0.0280	0.0419	0.0291	0.0377	0.0392
ChL	1.7643	3.2636	2.5246	1.9657	3.6404		0.0296	0.0291	0.0347	0.0318	0.0270
Lz1	2.5159	3.3232	2.2841	2.9991	2.8786	2.8934		0.0313	0.0200	0.0392	0.0388
LzL	2.8324	3.2146	1.9479	2.1601	4.4232	3.1740	3.7602		0.0252	0.0233	0.0239
Lf2	3.0152	3.6780	2.7341	2.4114	3.3427	2.9693	2.9560	3.0189		0.0310	0.0339
Lf1	3.1319	3.5420	3.3800	2.6641	4.5602	3.1829	4.4215	3.2175	3.0839		0.0074
LfL	2.8893	3.6144	3.2140	2.3889	4.4794	2.8310	4.2185	3.0297	3.0012	0.6559	

Table S6 P values for within system gill raker length comparisons for males and females. P values were obtained with an ANOVA and adjusted with a TukeyHSD in systems with more than two populations to correct for multiple testing (Fig. 2E, Fig. S5C).

sex	Kalambo						Chitili	Lunzua	Lufubu		
	KaL-Ka1	KaL-Ka2	KaL-Ka3	Ka1-Ka2	Ka1-Ka3	Ka2-Ka3	ChL-Ch1	LzL-Lz1	LfL-Lf1	LfL-Lf2	Lf1-Lf2
males	0.0211*	0.0149*	< 0.0001***	0.9979	0.0864	0.1407	0.0419*	0.0003**	0.1544	0.1107	0.0017**
females	0.3340	< 0.0001***	< 0.0001***	0.0001**	0.0001**	0.9967	0.1531	0.0001**	0.0840	< 0.0001***	< 0.0001***

Table S7 Pairwise morphometric (body shape and LPJ) distances between F1 crosses. (A) Pairwise morphometric distances described by Procrustes (upper triangular matrix) and Mahalanobis (lower triangular matrix) distances from the CVAs comparing each inter-population cross with the corresponding within population crosses (non-significant values are underlined, for CVA plots see Fig. 4A). (B) P values for pairwise comparisons of gill raker length among all within and inter-population crosses (Fig. 4B).

A

F1 juveniles	KaL-KaL	KaL-Ka3	Ka3-Ka3
KaL-KaL		0.0086	0.0097
KaL-Ka3	1.2961		0.0048
Ka3-Ka3	1.9713	1.2240	

F1 juveniles	KaL-KaL	KaL-Lz1	Lz1-Lz1
KaL-KaL		0.0081	0.0110
KaL-Lz1	1.3536		0.0078
Lz1-Lz1	1.8514	1.3714	

F1 juveniles	Ka3-Ka3	Ka3-Lz1	Lz2-Lz1
Ka3-Ka3		0.0081	0.0079
Ka3-Lz1	1.6021		0.0090
Lz1-Lz1	1.4724	1.7618	

B

F1 juveniles	Ka3-Ka3	Lz1-Lz1	KaL-Ka3	KaL-Lz1	Ka3-Lz1
KaL-KaL	0.00078	0.00004	0.22130	0.00588	0.02763
Ka3-Ka3		0.99788	0.82486	0.98741	0.99975
Lz1-Lz1			0.57282	0.86382	0.98707
KaL-Ka3				0.98122	0.96682
KaL-Lz1					0.99990

Table S8 Pairwise morphometric (body shape) distances and P values of gill raker comparisons among different groups of the pond experiment. Procrustes (upper triangular matrix) and Mahalanobis (lower triangular matrix) distances of the CVA comparing body shape among the within population F1 offspring and their corresponding wild populations (A) and among population of surviving adults at the beginning and at the end of the experimental period (B). (C) Comparison of gill raker length among the within population F1 offspring and their corresponding wild populations. (Fig. S7)

A

F1 and wild populations	KaL-KaL	Ka3-Ka3	Lz1-Lz1	KaL-wild	Ka3-wild	Lz1-wild
KaL-KaL		0.0094	0.0108	0.0119	0.0266	0.0241
Ka3-Ka3	1.5840		0.0080	0.0145	0.0261	0.0225
Lz1-Lz1	1.3126	1.3175		0.0154	0.0309	0.0284
KaL-wild	2.1099	2.0466	1.7501		0.0235	0.0242
Ka3-wild	3.4504	3.2127	3.3877	3.6574		0.0103
Lz1-wild	2.8738	2.2854	2.9527	3.2975	1.9800	

B

parental populations	KaL-before	Ka3-before	Lz1-before	KaL-after	Ka3-after	Lz1-after
KaL-before		0.0215	0.0218	0.0079	0.0134	0.0106
Ka3-before	2.6663		0.0131	0.0196	0.0132	0.0225
Lz1-before	2.4212	1.9504		0.0211	0.0109	0.0184
KaL-after	1.1066	2.5476	2.4792		0.0127	0.0138
Ka3-after	1.9615	1.7624	1.0353	2.0022		0.0119
Lz1-after	1.8311	2.5275	1.7273	1.7073	1.2464	

C

F1 and wild populations	Ka3-Ka3	Lz1-Lz1	KaL-wild	Ka3-wild	Lz1-wild
KaL-KaL	0.00214	0.00401	0.69902	< 0.00001	0.00005
Ka3-Ka3		0.99760	0.30149	< 0.00001	0.42067
Lz1-Lz1			0.47098	< 0.00001	0.20750
KaL-wild				< 0.00001	0.01044
Ka3-wild					0.09142

Table S9 Microsatellite diversity in populations of *Astatotilapia burtoni*. N_G, number of genotypes per locus; N_A, number of alleles per locus; H_O, obsevered heterozygosity; H_E, expected heterozygosity. Deviations from Hardy-Weinberg expectations at a 0.05 significance level after sequential Bonferroni correction are indicated in bold print.

sampling information			locus									
population	year		Ppun7	Ppun21	UNH130	Abur82	Ppun5	HchiST46	HchiST68	UNH989	Pzeb3	average
Ninde	2011	N_G	7	7	6	3	7	1	7	6	7	5.67
		N_A	6	4	6	3	8	1	3	7	4	4.67
		H_O	0.85714	0.42857	0.83333	0.33333	0.85714	na	0.00000	0.66667	0.42857	0.55
		H_E	0.8022	0.73626	0.87879	0.60000	0.91209	na	0.48352	0.83333	0.57143	0.73
Loazi	2011	N_G	31	31	30	31	27	31	28	28	31	29.78
		N_A	27	20	21	15	24	2	18	22	6	17.22
		H_O	0.93548	0.87097	**0.70000**	0.74194	0.88889	0.12903	0.71429	0.92857	0.70968	0.74
		H_E	0.9413	0.92491	0.92147	0.86409	0.94689	0.12269	0.85390	0.92208	0.71232	0.80
Muzi	2011	N_G	25	25	24	25	25	24	24	24	24	24.44
		N_A	15	14	15	15	15	2	12	18	6	12.44
		H_O	0.96000	0.84000	0.79167	0.76000	0.72000	0.04167	0.79167	**0.66667**	0.58333	0.68
		H_E	0.90531	0.90531	0.91135	0.90776	0.90367	0.04167	0.86968	0.94681	0.64539	0.78
Kalambo stream 3	2010	N_G	32	31	32	32	32	32	32	32	32	31.89
		N_A	9	11	15	7	16	2	6	8	7	9.00
		H_O	0.90625	0.87097	0.87500	0.84375	0.84375	0.46875	0.65625	0.71875	0.75000	0.77
		H_E	0.82192	0.83131	0.88790	0.76290	0.91915	0.44792	0.75694	0.84226	0.80655	0.79
	2011	N_G	30	30	30	30	30	30	30	30	30	30.00
		N_A	11	10	10	7	15	2	4	10	6	8.33
		H_O	0.93333	0.76667	0.76667	0.73333	0.63333	0.43333	0.63333	**0.80000**	0.60000	0.70
		H_E	0.87627	0.79887	0.87175	0.78588	0.89492	0.48079	0.75763	0.82316	0.73446	0.78
Kalambo stream 2	2011	N_G	14	14	13	14	14	1	13	14	14	12.33
		N_A	7	12	10	6	13	1	6	6	6	7.44
		H_O	0.64286	0.71429	0.69231	0.42857	0.71429	na	0.69231	0.64286	0.78571	0.66
		H_E	0.69312	0.79630	0.84308	0.43915	0.92593	na	0.71385	0.72751	0.76720	0.74
	2012	N_G	32	32	32	32	31	32	32	32	32	31.89
		N_A	9	13	9	10	14	2	9	12	6	9.33
		H_O	0.50000	0.56250	0.84375	0.59375	0.74194	0.09375	0.81250	0.78125	0.71875	0.63
		H_E	0.57391	0.62351	0.74504	0.68006	0.84294	0.09077	0.82440	0.76885	0.75198	0.66
Kalambo stream 1	2011	N_G	32	32	32	32	31	32	32	31	32	31.78
		N_A	21	23	23	20	19	3	12	20	6	16.33
		H_O	0.96875	0.90625	0.93750	0.93750	0.83871	0.15625	**0.37500**	0.93548	0.40625	0.72
		H_E	0.91419	0.94643	0.93800	0.94444	0.94289	0.17708	0.76935	0.91962	0.49603	0.78
Kalambo lake	2010	N_G	31	31	30	31	30	30	31	31	31	30.67
		N_A	18	25	22	19	20	3	14	19	5	16.11
		H_O	0.96774	0.93548	0.86667	0.96774	**0.76667**	0.26667	0.54839	1.00000	0.58065	0.77
		H_E	0.91645	0.94342	0.94407	0.93971	0.94068	0.24350	0.80539	0.93178	0.59598	0.81
	2011	N_G	33	33	32	33	33	33	33	33	33	32.89
		N_A	18	21	20	18	22	3	17	20	5	16.00
		H_O	0.87879	0.93939	0.84375	0.87879	0.81818	0.24242	0.81818	0.9697	0.42424	0.76
		H_E	0.87832	0.93706	0.93204	0.91422	0.95058	0.29324	0.86993	0.93986	0.43357	0.79
Toby's place	2010	N_G	34	34	31	33	34	34	34	34	33	33.44
		N_A	13	14	20	14	15	2	11	16	7	12.44
		H_O	0.91176	0.85294	0.93548	0.93939	0.88235	0.05882	0.73529	0.91176	0.54545	0.75
		H_E	0.80114	0.84372	0.88525	0.87925	0.90386	0.11238	0.83055	0.89245	0.52214	0.74
	2012	N_G	31	31	28	31	31	31	31	27	31	30.22
		N_A	11	10	12	13	14	2	4	9	5	8.89
		H_O	0.96774	0.83871	0.82143	0.90323	0.96774	0.03226	0.61290	0.74074	0.48387	0.71
		H_E	0.85405	0.77737	0.80779	0.83765	0.85616	0.03226	0.68324	0.83718	0.45267	0.68
Chitili creek 1	2010	N_G	7	7	7	7	7	1	7	7	6	6.22
		N_A	9	7	9	7	9	1	7	5	4	6.44
		H_O	1.00000	0.71429	0.85714	**0.42857**	1.00000	na	0.85714	1.00000	1.00000	0.86
		H_E	0.94505	0.89011	0.91209	0.85714	0.93407	na	0.85714	0.83516	0.72727	0.87
	2011	N_G	31	31	31	31	31	31	30	29	31	30.67
		N_A	15	17	14	13	16	2	12	12	5	11.78
		H_O	0.90323	0.80645	0.64516	0.80645	0.87097	0.06452	0.70000	0.89655	0.32258	0.67
		H_E	0.87996	0.87943	0.86039	0.85087	0.90164	0.06346	0.85424	0.87719	0.34320	0.72
Chitili lake	2011	N_G	31	31	29	31	31	1	28	30	31	27.00
		N_A	16	13	18	19	19	1	14	14	6	13.33
		H_O	0.87097	0.90323	0.96552	0.83333	0.86667	na	**0.57143**	0.73333	0.41935	0.77
		H_E	0.90375	0.91698	0.92257	0.91808	0.91751	na	0.88636	0.91751	0.46007	0.86
Chisanza	2011	N_G	32	30	30	32	31	32	31	32	32	31.33
		N_A	25	24	19	23	20	2	13	21	10	17.44
		H_O	0.96875	1.00000	**0.63333**	0.81250	0.83871	0.37500	**0.61290**	**0.75000**	0.59375	0.73
		H_E	0.94792	0.95593	0.92712	0.94990	0.91539	0.30952	0.85669	0.92808	0.67560	0.83
Sumba	2011	N_G	32	31	32	32	32	32	32	32	31	31.78
		N_A	27	25	22	23	20	2	14	20	9	18.00
		H_O	0.93750	0.96774	**0.75000**	**0.81250**	0.78125	0.21875	**0.53125**	**0.62500**	0.70968	0.70
		H_E	0.96081	0.95346	0.93204	0.94891	0.93056	0.19792	0.90228	0.92808	0.72343	0.83
		p	0.10437	0.41513	**0.00142**	**0.00379**	**0.01741**	1.00000	**0.00000**	**0.00000**	0.37605	0.21
Lunzua stream 1	2011	N_G	30	30	30	30	30	30	30	30	30	30.00
		N_A	11	13	12	12	13	2	10	9	6	9.78
		H_O	0.83333	0.90000	0.66667	0.66667	**0.60714**	0.23333	**0.60000**	0.70000	0.60000	0.65
		H_E	0.87458	0.85480	0.83446	0.75819	0.86104	0.20980	0.85706	0.82542	0.60791	0.74
Lunzua lake	2010	N_G	30	30	29	30	28	28	30	29	30	29.33
		N_A	24	29	31	26	20	3	18	22	8	20.11
		H_O	0.93333	0.96667	0.89655	0.93333	0.89286	0.42857	**0.63333**	0.93103	0.66667	0.81
		H_E	0.95876	0.96667	0.97217	0.96271	0.94675	0.38247	0.92825	0.95523	0.5887	0.85
	2011	N_G	31	31	31	31	31	31	31	31	31	31.00
		N_A	25	30	27	25	20	3	23	23	8	20.44
		H_O	0.87097	1.00000	**0.74194**	0.80645	0.80645	0.32258	**0.70968**	0.87097	0.67742	0.76
		H_E	0.94553	0.96616	0.96140	0.95928	0.94236	0.32311	0.94342	0.94553	0.68852	0.85
Wonzye	2010	N_G	41	42	42	42	37	42	42	42	42	41.33
		N_A	29	33	30	24	23	3	15	24	10	21.22
		H_O	**0.95122**	**0.95238**	**0.88095**	**0.95238**	**0.86486**	0.38095	**0.61905**	**0.90476**	0.73810	0.80
		H_E	0.95574	0.96944	0.95668	0.95726	0.95002	0.32014	0.82760	0.94894	0.79891	0.85
	2012	N_G	18	18	17	18	15	18	18	18	18	17.56
		N_A	23	23	22	20	12	2	13	19	9	15.89
		H_O	0.94444	0.83333	0.88235	1.00000	0.66667	0.16667	0.83333	0.94444	0.88889	0.80
		H_E	0.96349	0.96508	0.96791	0.96825	0.91264	0.15714	0.82698	0.95714	0.85556	0.84
Fisheries Department	2010	N_G	25	26	24	25.00000	26	26	26	26	26	25.56
		N_A	27	20	24	23	17	3	15	23	11	18.11
		H_O	1.00000	1.00000	0.95833	0.80000	0.80769	0.15385	**0.61538**	**0.76923**	0.84615	0.77
		H_E	0.94694	0.93288	0.96188	0.95020	0.93363	0.14706	0.88612	0.94872	0.84238	0.84
Kalungula	2010	N_G	26	27	26	26	20	27	27	27	27	25.89
		N_A	23	20	18	19	15	3	15	17	10	15.56
		H_O	0.88462	0.92593	0.92308	0.84615	**0.70000**	0.11111	0.62963	**0.70370**	0.70370	0.71
		H_E	0.94344	0.94340	0.92809	0.94118	0.92821	0.10832	0.84696	0.90566	0.82250	0.82
Lufubu stream 2	2012	N_G	30	30	29	30	30	30	30	29	30	29.78
		N_A	9	20	14	17	15	2	6	10	5	10.89
		H_O	0.80000	0.96667	0.79310	0.93333	0.75862	0.03333	0.60000	**0.96552**	**0.30000**	0.68
		H_E	0.71469	0.94407	0.76830	0.90904	0.91712	0.03333	0.60904	0.69752	0.58136	0.69
Lufubu stream 1	2011	N_G	27	27	27	27	24	27	27	27	27	26.67
		N_A	19	28	21	25	21	3	16	21	12	18.44
		H_O	0.88889	0.88889	**0.66667**	0.85185	**0.62500**	0.25926	**0.74074**	0.92593	0.85185	0.74
		H_E	0.95318	0.96995	0.95038	0.94200	0.94592	0.23410	0.92872	0.94829	0.85325	0.86
Lufubu lake	2011	N_G	30	30	30	30	30	30	29	30	30	29.89
		N_A	18	28	22	22	24	4	14	19	13	18.22
		H_O	0.9	0.86667	**0.46667**	0.93333	**0.73333**	0.46667	**0.55172**	**0.83333**	0.86667	0.74
		H_E	0.93616	0.9661	0.86723	0.93164	0.95028	0.45989	0.79492	0.94746	0.83051	0.85
		p	0.08827	0.12133	**0.00008**	0.77188	**0.00048**	0.61206	**0.00000**	**0.00672**	0.33487	0.22
Ndole bay	2012	N_G	16	16	8	16	13	16	16	15	16	14.67
		N_A	15	9	12	7	12	2	7	10	7	9.00
		H_O	1.00000	0.93750	0.87500	0.68750	**0.61538**	0.18750	0.66750	0.53333	0.68750	0.69
		H_E	0.93347	0.88105	0.96667	0.74194	0.93846	0.17540	0.71371	0.85057	0.74395	0.77
Lake Chila	2012	N_G	13	14	13	14	12	1	14	14	14	12.11
		N_A	13	19	15	16	14	1	12	12	8	12.22
		H_O	1.00000	1.00000	0.76923	1.00000	0.75000	na	0.78571	0.71429	0.85714	0.86
		H_E	0.92000	0.96825	0.94154	0.94974	0.94203	na	0.84656	0.88624	0.84656	0.91

Table S10 Genetic diversity of mtDNA sequences. N, number of sequences per population; H, number of haplotypes; He, gene diversity; π, nucleotide diversity.

population	*N*	*H*	*He*	*π*
Ninde	7	1	0.00000	0.00000
Loazi	7	1	0.00000	0.00000
Muzi	9	1	0.00000	0.00000
Kalambo stream 3	27	1	0.00000	0.00000
Kalambo stream 2	8	1	0.00000	0.00000
Kalambo stream 1	6	3	0.60000	0.00182
Kalambo lake	29	3	0.25400	0.00071
Toby's place	30	2	0.18600	0.00051
Chitili creek 1	17	2	0.44100	0.00120
Chitili lake	10	2	0.55600	0.00151
Chisanza	9	3	0.41700	0.00182
Sumba	9	3	0.58300	0.00227
Lunzua stream 1	7	1	0.00000	0.00000
Lunzua lake	24	4	0.30800	0.00098
Wonzye	49	2	0.08000	0.00022
Fisheries Department	24	1	0.00000	0.00000
Kalungula	28	1	0.00000	0.00000
Lufubu stream 2	13	3	0.41000	0.00119
Lufubu stream 1	10	1	0.00000	0.00000
Lufubu lake	9	1	0.00000	0.00000
Ndole	13	2	0.15400	0.00042
Lake Chila	14	1	0.00000	0.00000

Appendix S1: Description of river systems

Kalambo

The catchment of the Kalambo River is located mainly in Tanzania, with a small portion in Zambia. The lake population of the Kalambo system (KaL) was collected at Chipwa village, close to the Kalambo River mouth at the border between Zambia and Tanzania (Fig. A1A, Fig. 1A and Fig. 2A). The habitat at Chipwa is characterized by mainly sandy bottom with bulrush (*Typha* spp.) vegetation and a maximum depth of 1.5 m. The first riverine population (Ka1) was sampled 1500 m upstream from KaL, within a slowly flowing, maximally 3 m deep water and vegetation comprising mainly hippo grass (*Vossia cuspidata*). The second upstream population (Ka2) originates from predominantly rocky habitat with a maximum depth of 1 m. The third upstream population (Ka3) is separated from downstream populations by the Kalambo Falls – with a drop of more than 200 m the second-tallest waterfall in Africa. Compared to Ka2 there is less water current at Ka3, fewer rocks but more vegetation (predominantly reeds and hippo grass).

Chitili

The Chitili Creek is a very small yet permanent stream flowing through Chitili village, and is therefore greatly affected by human activities including agriculture (Fig. A1B). The corresponding lake population (ChL) dwells in a heterogeneous shallow (max. 0.6 m) habitat with rock and sand bottom covered with aquatic plants and hippo grass belts. At the relatively close upstream sampling site, the creek is narrow, shallow (max. 0.3 m deep) and densely vegetated.

Lunzua

Although the Lunzua catchment is almost three times smaller in area than that of the Kalambo, both catchments are comparable with regard to slope angles, water discharge rates and drainage densities (Sichingabula 1999; Kakogonzo *et al.* 2000). The habitat of the Lunzua lake population (LzL) is similar to KaL, with mostly sandy bottom, bulrush vegetation and relatively shallow waters (max. 0.6 m depth) (Fig. A1C). A 3 m tall waterfall close to the river mouth and several rapids separate the lake population from the upstream riverine population (Lz1). The habitat at Lz1 consists mainly of sand and mud bottom, the water depth was around 0.5 m.

Lufubu

The Lufubu River is the largest tributary of southern LT (Langenberg *et al*. 2003). The sampling site at the river mouth (LfL) is shallow (0.3 – 2 m), densely vegetated with papyrus (*Cyperus papyrus*), hippo grass and balsa wood trees (*Aeshynomene elaphroxylon*) (Fig. A1D). The first upstream population (Lf1) was sampled at a location with very similar habitat conditions to LfL with very slowly flowing water. The upstream population (Lf2) was collected more than 30 km upstream the estuary, with habitat comprising pebbles and submerged vegetation and fast flowing waters (max. depth 0.5 m).

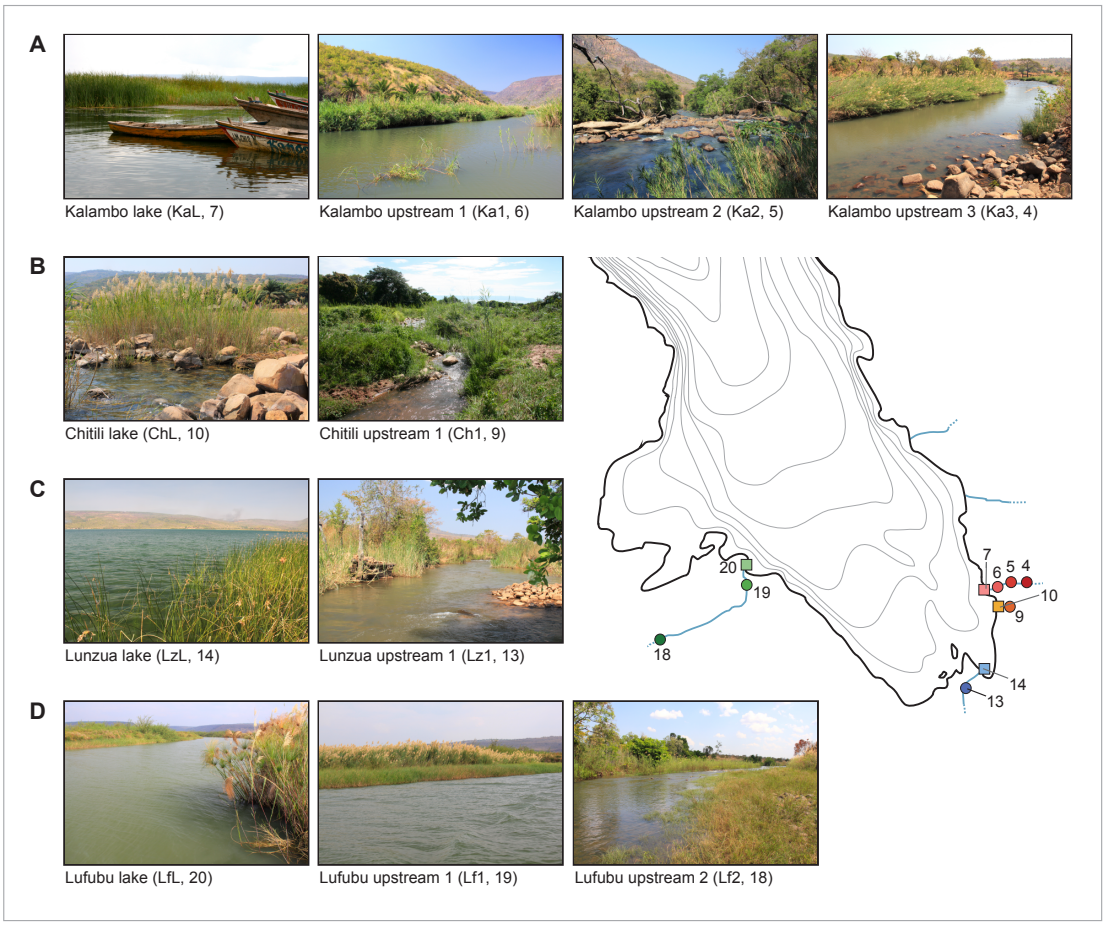


Fig. A1 Map of the southern part of LT (altered from Fig. 1A) showing the populations of the four lake-stream systems with corresponding habitat photographs. (A) The four Kalambo populations, (B) the two populations from the Chitili Creek, (C) the two Lunzua populations and (D) the three populations from the large Lufubu River.

References

Kakogozo B, Kahindo N, Mwenyemaile B, Drieu O (2000) Etude Hydrologique du Bassin Nord-Ouest du Lac Tanganyika (R. D. Congo). *Pollution Control and Other Measures to Protect Biodiversity in Lake Tanganyika*, UNDP/GEF/RAF/92/G32. 44p.

Langenberg V, Nyamushahu S, Roijackers R, Koelmans A (2003) External nutrient sources for Lake Tanganyika. *Journal of Great Lakes Research*, **29**, 169–180.

Sichingabula H (1999) Analysis and results of discharge and sediment monitoring activities in the Southern Lake Tanganyika basin, Zambia. *Pollution Control and Other Measures to Protect Biodiversity in Lake Tanganyika*, UNDP/GEF, RAF/92/G32, Technical Report No: 4.

Appendix S2: Pond experiment– Simulation

To test whether biased mating with respect to body size might explain the observed pattern, we simulated the experiment under the conditions of random mating with an increased mating probability, however, for large males. The simulations were conducted for each pond separately, with the observed number of male and female survivors per pond and reproductively active individuals (based on the paternity analyses, Table S2A). The frequencies for all 9 possible mating combinations were simulated for the observed number of mating events per pond (Table S2A) with 1'000 iterations. We tested 43'910 models with different mating probabilities for four dominant males per pond: for the two largest males at the starting point of the experiment (accounting for dominance in the early phase) and at the end point of the experiment (accounting for dominance in the late phase). We assigned dominance for two males per phase (early and late) to include possible dynamics in dominance ranks. The models covered a range from 1- to 20-fold mating probabilities for the four dominant males. Females were sampled randomly with equal probabilities in each model. To find the best fitted model we calculated the absolute deviation of the observed data form each of the iterations per model (Δ_{SIM}). Then the sum of the mean Δ_{SIM} (SUM_{Δ}) over all ponds was calculated. Therefore the model with the smallest SUM_{Δ} represents the model, which fits the observed data best. The macro for the simulations was written in R.

Comparing the SUM_{Δ} of the 43'910 models revealed that the model assuming random mating (without dominance) shows the highest SUM_{Δ} whereas several models accounting for biased mating with respect to size fit the observed data very well (Fig. A2). Generally, the model improves with increasing probability for the largest male to mate at the end point of the experiment. Further, SUM_{Δ} decreases with increasing mating probability for the largest male at the starting point of the experiment, achieving an optimum when the probability to mate is 10- to 12-fold higher for the largest, i.e. dominant male(s). If the mating probabilities for the two largest males (starting point and end point) increase, SUM_{Δ} decreases asymptotically resulting in several well fitting models. Thereby an increasing mating probability for the second largest male in the late phase does not substantially contribute to an improvement of the model. However the model improves with 4- to 6-fold higher mating probability for the second largest male in the early phase.

Comparing the best-fitting models with the observed data revealed that the observed frequencies of all mating combinations overlap with the 95% confidence limits of the simulated model (1'000 iterations) in all 5 ponds (Fig. A3). This suggests that the model assumptions of an increased mating probability for the largest males (10- to 12-fold higher for males in the early phase and 15- to 20-fold higher in the late phase of the experiment), plus a 4- to 6-fold higher probability for the second largest males in the early phase, explain best the observed frequencies of mating combinations. The lower mating probability for the dominant male in the early phase in combination with an increased probability for the second largest males might reflect an unstable dominance status and relatively early changes in dominance ranks. The observed aggressive territorial fights within the first two weeks (which led to high mortality in the early phase of the experiment) also support this.

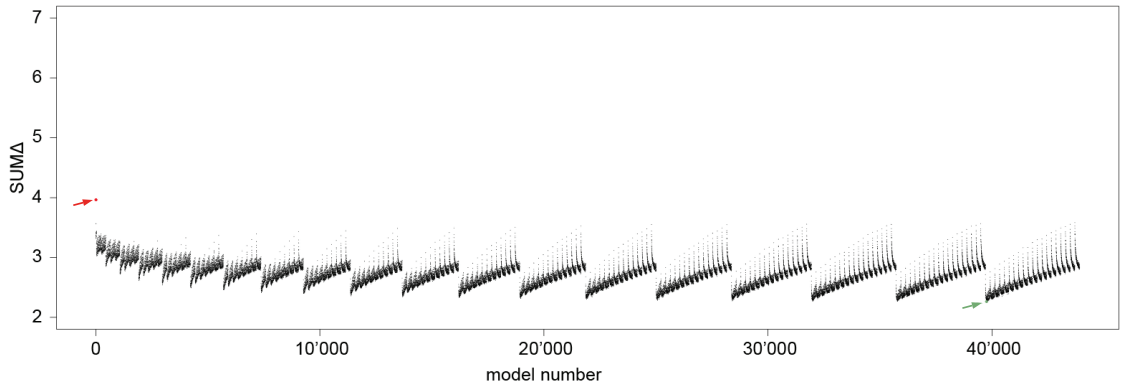


Fig. A2 SUM_{Δ} of the 43'910 models tested. The different combinations of mating probabilities (from 1- to 20-fold) for the four dominant males sorted by increasing mating probabilities for (*i*) the largest male at the end point of the experiment, (*ii*) the largest male at the starting point of the experiment, (iii) the second largest males at the end point and (*iiii*) the second largest males at the starting point of the experiment. The model without assigning any dominance to the males is marked in red and the best fitting model (lowest SUM_{Δ}) in green.

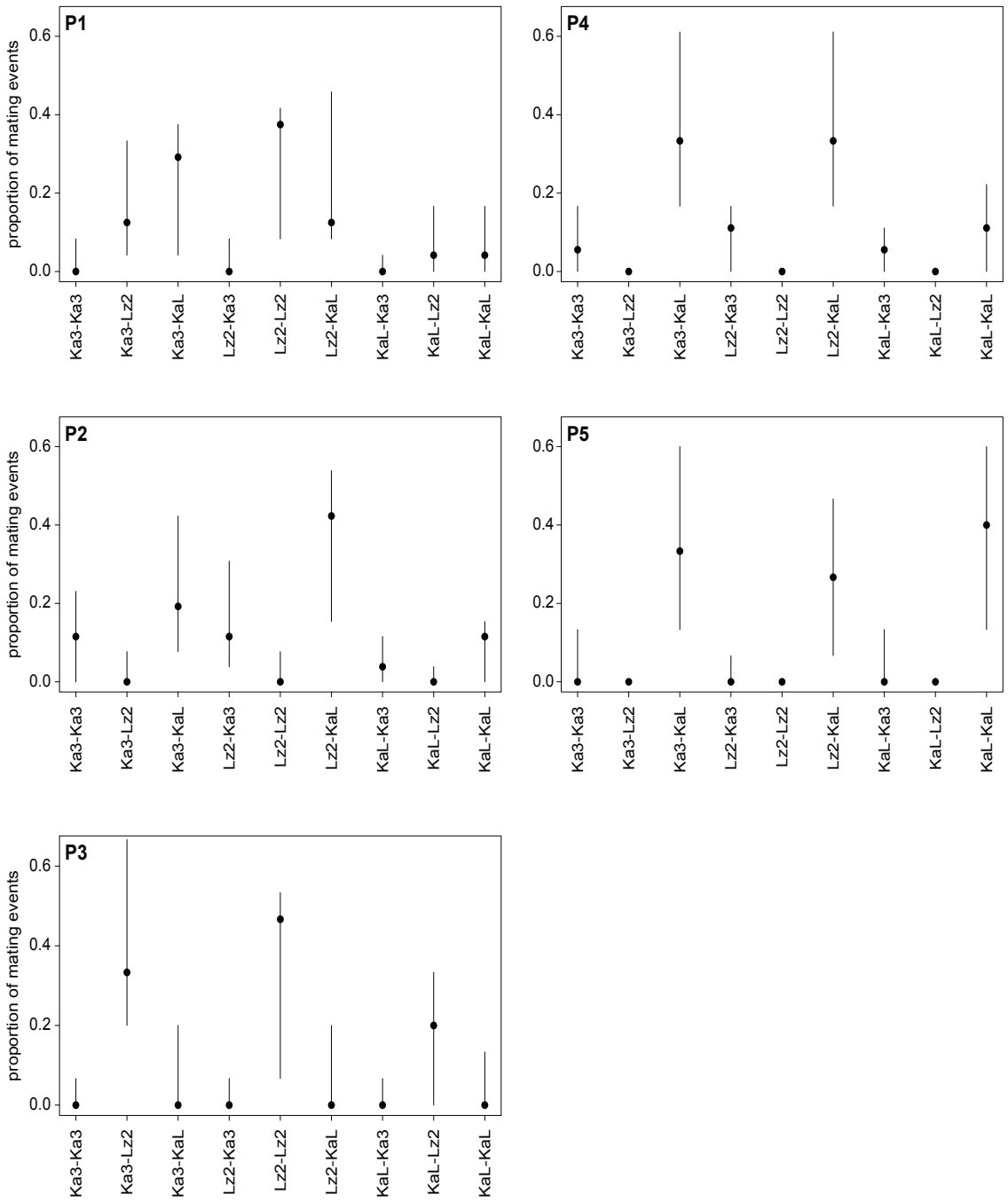


Fig. A3 Observed frequencies of mating combinations per replicate (filled circles) and simulated mating combinations with 1‘000 iterations (bars show the 95% confidence limits) using the best fitting model (green arrow in Fig. A2) with following mating probabilities: 10-folded and 5-folded mating probabilities for the largest and the second largest males at the starting point of the experiment and 20- and 1-folded probabilities for the largest and the second largest males at the end point of the experiment.

Chapter 6

Phylogeographic and phenotypic assessment of a basal haplochromine cichlid fish from Lake Chila, Zambia

Bernd Egger, Yuri Klaefiger, **Adrian Indermaur**, Stephan Koblmüller, Anya Theis, Simon Egger, Thomas Näf, Maarten Van Steenberge,Christian Sturmbauer, Cyprian Katongo and Walter Salzburger

Hydrobiologia (2015)

AI participated in designing the study, conducted sampling and sequencing and helped in discussion and drafting the manuscript

Hydrobiologia (2015) 748:171–184
DOI 10.1007/s10750-014-1919-0

ADVANCES IN CICHLID RESEARCH

Phylogeographic and phenotypic assessment of a basal haplochromine cichlid fish from Lake Chila, Zambia

B. Egger · Y. Klaefiger · A. Indermaur · S. Koblmüller · A. Theis · S. Egger · T. Näf · M. Van Steenberge · C. Sturmbauer · C. Katongo · W. Salzburger

Received: 24 February 2014 / Accepted: 19 May 2014 / Published online: 1 July 2014

Abstract The basal haplochromine genus *Pseudocrenilabrus* comprises three valid species, although the current taxonomy most probably underestimates species richness. Previous phylogeographic studies on the *P. philander* species complex revealed a clear structuring of populations, shaped by river capture events. Here we report the discovery of *P.* cf. *philander* in Lake Chila, a small lake south of Lake Tanganyika. We were interested whether discrete morphs, similar to what has been found in Lake Mweru and the Lunzua River, were present in Lake Chila. We evaluated the phenotypic variability of the population in relation to other lacustrine and riverine populations by quantifying colouration and body shape. To place the specimens in a phylogeographic framework, we inferred a phylogeny based on the most variable part of the mitochondrial control region. We found two divergent mtDNA lineages in Lake Chila and tested for population structure and admixture between the lineages using microsatellite data. Our study reveals a complex phylogeographic pattern and demonstrates admixture of distant mtDNA lineages in Lake Chila, producing a hybrid swarm with substantial phenotypic variability. Unlike in Lake Mweru, *Pseudocrenilabrus* has not diversified further into discrete morphs in Lake Chila, probably because

Guest editors: S. Koblmüller, R. C. Albertson, M. J. Genner, K. M. Sefc & T. Takahashi / Advances in Cichlid Research: Behaviour, Ecology and Evolutionary Biology

Electronic supplementary material The online version of this article (doi:10.1007/s10750-014-1919-0) contains supplementary material, which is available to authorized users.

B. Egger (✉) · Y. Klaefiger · A. Indermaur · A. Theis · S. Egger · T. Näf · W. Salzburger (✉)
Zoological Institute, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland
e-mail: bernd.egger@unibas.ch

W. Salzburger
e-mail: walter.salzburger@unibas.ch

S. Koblmüller · C. Sturmbauer
Department of Zoology, Karl-Franzens University of Graz, Universitatsplatz 2, 8010 Graz, Austria

M. Van Steenberge
Zoology Department, Ichthyology, Royal Museum for Central Africa, Leuvensesteenweg 13, 3080 Tervuren, Belgium

M. Van Steenberge
Laboratory of Biodiversity and Evolutionary Genomics, Department of Biology, Katholieke Universiteit Leuven, Charles Deberiotstraat 32, 3000 Louvain, Belgium

C. Katongo
Department of Biological Sciences, University of Zambia, P.O. Box 32379, Lusaka, Zambia

of the long-term instability of the lake and the presumed recency of the admixture event.

Keywords Phylogeography · Nuptial colouration · *Pseudocrenilabrus* · Hybridization

Introduction

Cichlid fishes from the East African Great Lakes, Tanganyika (LT), Malawi (LM) and Victoria (LV), are well-known model systems for studying the mechanisms underlying adaptive radiation and explosive speciation (see e.g. Kocher, 2004; Salzburger, 2009; Santos & Salzburger, 2012). Within African cichlids, the Haplochromini stand out as the most species-rich lineage, comprising the species flocks of LM and LV, the Tropheini from LT, as well as riverine and lacustrine species from northern, eastern, southern and central Africa and the levant (Turner et al., 2001; Verheyen et al., 2003; Joyce et al., 2005; Salzburger et al., 2005; Koblmüller et al., 2008a). The majority of haplochromine cichlids belongs to the derived 'modern' clade (as defined in Salzburger et al., 2005), the members of which are mostly lacustrine, characterized by a pronounced sexual colour dimorphism with typically brightly coloured males and inconspicuous females, a polygynandrous mating system with maternal mouthbrooding, as well as egg-spots on the anal fin of males. The cichlid fauna of many rivers and smaller lakes, especially in central and southern Africa, is typically dominated by more basal haplochromine lineages. These lineages are considered comparably species poor, which has been explained by the lack of ecological opportunity in temporally unstable riverine ecosystems (Joyce et al., 2005). One of these basal riverine haplochromine lineages is represented by the genus *Pseudocrenilabrus,* which is distributed across many river systems and ichthyogeographic regions in northern, eastern, central and southern Africa (Skelton, 1991). The genus currently comprises three valid species, *P. multicolour* (two subspecies: *P. m. multicolour* and *P. m. victoriae*), *P. nicholsi* and *P. philander* (three subspecies: *P. p. dispersus*, *P. p. luebberti* and *P. p. philander*), although the current taxonomy likely underestimates species richness (Twentyman-Jones et al., 1997; Katongo et al., 2005; Stelkens & Seehausen, 2009). *Pseudocrenilabrus* are all considered generalist species, typically inhabiting calm parts of rivers, swamps and flooded areas (Greenwood, 1989). Males of the genus *Pseudocrenilabrus* generally show less elaborate nuptial colouration compared to 'modern' haplochromines and lack egg-spots, but most populations feature a red to orange blotch on the posterior margin of their anal fin.

The phylogeographic relationships within the genus *Pseudocrenilabrus* have so far mainly addressed the *P. philander* species complex in southern Zambian rivers. Two previous studies revealed a clear structuring of populations, possibly shaped by tectonic movements that allowed for past temporal connections between watersheds (Katongo et al., 2005; Koblmüller et al., 2012). Based on sequences of the most variable part of the mitochondrial control region (d-loop), Katongo et al. (2005) identified four distinct clades: the Chambeshi-Bangweulu clade, the Lake Mweru clade, the Lunzua clade and the Kafue–Zambezi clade. In more recent studies, Koblmüller et al. (2008a, 2012) included a previously undescribed haplochromine species from the Lufubu River (*P.* sp. 'Lufubu A'), which turned out as the most basal lineage in the genus. *P.* sp. 'Lufubu A' is found in sympatry with another *Pseudocrenilabrus* that represents a fifth lineage within the *P. philander* species complex (*P.* sp. 'Lufubu B'; Koblmüller et al., 2012). Despite the existence of several subspecies and many geographically separated, often morphologically distinct populations (Greenwood, 1989; Katongo et al., 2005), the genus was considered species poor in comparison to other riverine taxa (Skelton, 1994). However, Koblmüller et al. (2008b) described a population from the upper Lunzua River that contains two (blue and yellow) colour morphs sharing a single mitochondrial haplotype, but showing weak differentiation at nuclear markers suggesting that they might be undergoing incipient speciation. In addition, Stelkens and Seehausen (2009) reported the occurrence of at least 13 distinct morphs of *Pseudocrenilabrus* cf. *philander* in Lake Mweru. The morphs were assigned to two divergent mitochondrial lineages, of which the more frequent one diversified with respect to eco-morphology and nuptial colouration. In mate choice experiments, it was shown that the degree of divergence between morphological traits, but not genetic distance, was associated with the level of reproductive isolation between morphs (Stelkens & Seehausen, 2009). The existence of a small adaptive radiation in Lake Mweru

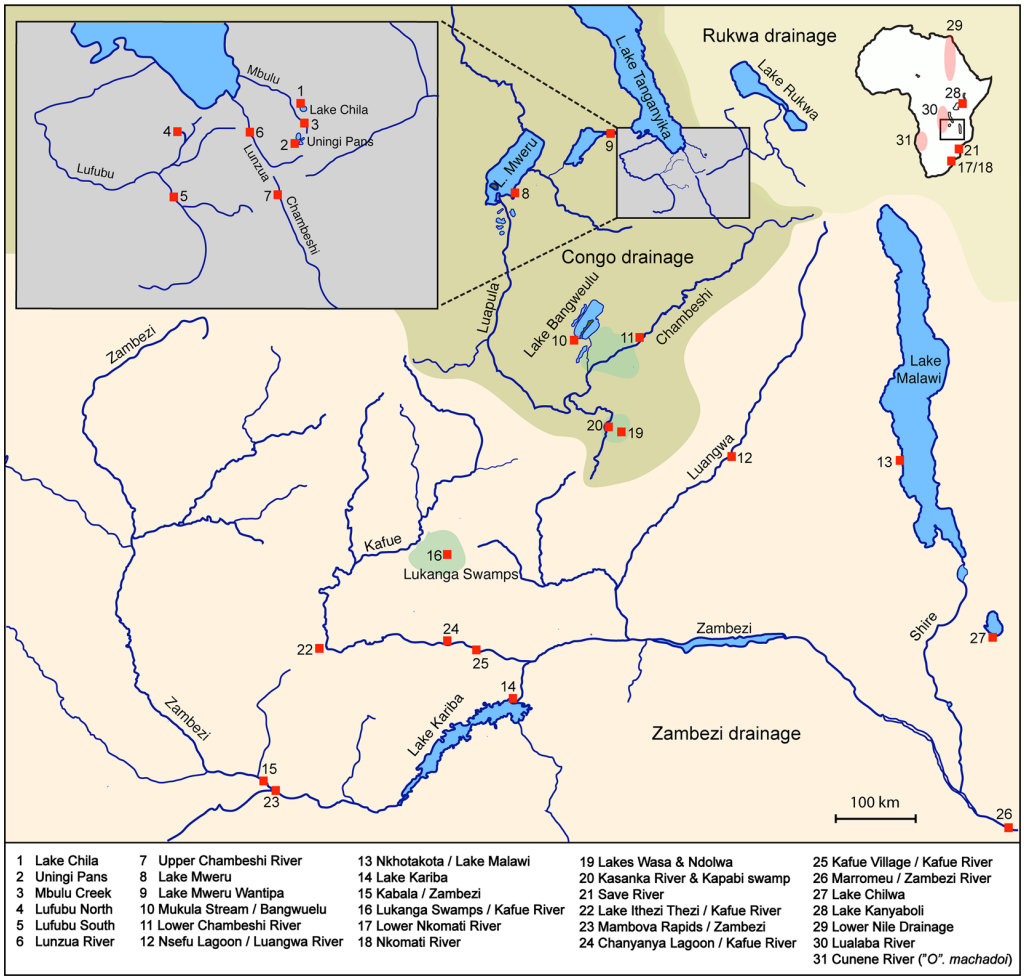


Fig. 1 Simplified map of the major water bodies in our study area showing the 28 sampling sites (*red squares*). Locations 29–31 roughly indicate the natural range of specimens acquired from the aquaria trade or where the exact location was unknown (translucent *red areas*). *Dark green* patches indicate swampy areas. *Different background colours* designate the major drainages indicated in the figure, namely Zambezi, Congo and Rukwa (including eastward draining rivers). Sampling site 19 and 20 each designate two sites that are very close together and belong to the same system

suggests that *Pseudocrenilabrus* are more likely to diversify in a stable heterogeneous (lake) environment, providing more ecological opportunity as compared to rivers (see e.g. Schluter, 2000; Wagner et al., 2012).

During a field trip in February 2012, we discovered a population of *P.* cf. *philander* in Lake Chila, a small (approximately 1,200 m long and 900 m wide) and shallow (maximum depth = 4 m) but permanent lake 20 km south of Lake Tanganyika (Fig. 1). Apart from *P.* cf. *philander*, the lake harbours a cichlid fauna typical for the Chambeshi, Zambezi and the Zambian/Congo watersheds (*Serranochromis angusticeps*, *S. robustus*, *S. thumbergi*, *Tilapia sparmanii*, *Oreochromis macrochir*) and *Astatotilapia burtoni* from the LT basin (see also Skelton, 1993). *Pseudocrenilabrus* from this population showed phenotypes distinct from other populations belonging to the *P. philander* species complex, with deeper bodies compared to nearby riverine populations and very elaborate colour patterns in males. We evaluated the phenotypic variability of the Lake Chila population in relation to other lacustrine (Mweru-Wantipa) and riverine (Lunzua and Chambeshi) populations by quantifying male nuptial colouration and body shape based on

standardized photographs. To place the Lake Chila specimens in a phylogeographic context, we reconstructed a phylogeny based on the most variable part of the mitochondrial control region using available *Pseudocrenilabrus* sequences from GenBank and additional samples recently collected from the area (Fig. 1). Interestingly, we found that two divergent mtDNA lineages were present in the small lake and further tested for population structure (in relation to neighbouring riverine populations) and admixture between the two mtDNA lineages using microsatellite data.

Materials and methods

Sampling

Sampling of *Pseudocrenilabrus* spp. was carried out during several field trips to Zambia between September 2003 and February 2012 (see Fig. 1; Tables S1, S2 and S3 for details on sample size and locations). Specimens were collected using gill nets and hook and line fishing under the permission of the Lake Tanganyika Research Unit, Department of Fisheries, Ministry of Agriculture and Livestock, Republic of Zambia. Fish were anaesthetized using clove oil (2–3 drops clove oil per litre water) and photographed in a standardized manner for later colour pattern and geometric morphometric analyses. Fin clips were taken from the specimens directly in the field and subsequently preserved in 96 % ethanol for further whole genomic DNA extraction. From each sampling location, at least one whole specimen was preserved in 96 % ethanol.

Male body colouration

To evaluate differences in nuptial colouration within and between populations, we used standardized photographs of males from Lake Chila ($n = 49$), Lunzua River ($n = 7$), Mbulu Creek ($n = 2$), Lufubu River ($n = 3$), Chambeshi River ($n = 2$), Lake Mweru-Wantipa ($n = 15$) and the Uningi Pans ($n = 3$) (Table S3) to extract nine features related to colouration (see Salzburger et al., 2006): anal fin colour (red/yellow/red–yellow/none); anal fin blotch colour and presence (orange/red/none); dorsal fin colouration (black–red/red–grey/none); pelvic fin colouration (intensity of black stripe); caudal fin pattern (spotted/half spotted); dorsal body colouration (bluish/yellowish/blue–yellowish/none); central body colouration (bluish/yellowish/blue-yellowish/none); ventral body colouration (bluish/yellowish/blue-yellowish/none) and eye bar presence. Characters were translated into a categorical data matrix and analysed in a Multiple Correspondence Analysis (MCA) in R (v.3.0.3, R Development Core Team, 2014; package FactoMineR, Husson et al., 2014).

Body shape

The photographs of males from Lake Chila ($n = 49$), Lunzua River ($n = 18$), Lufubu River ($n = 5$), Chambeshi River ($n = 2$) and Lake Mweru-Wantipa ($n = 14$) (Table S3) were used to obtain data for the geometric morphometric analyses by recording the coordinates of 17 homologous landmarks (for details see Muschick et al., 2012) using TPSDIG2 (v.2.11; Rohlf, 2008). The *x* and *y* coordinates were transferred to the program MORPHOJ (v.1.05f; Klingenberg, 2011) and superimposed with a Procrustes generalized least squares fit (GLSF) algorithm to remove all non-shape variation (Rohlf & Slice, 1990). Additionally, the data were corrected for allometric size effects by using the residuals of the regression of shape on centroid size for further analyses. A canonical variate analysis (CVA; Mardia et al., 1979) was used to assess shape variation among the populations. The mean shape distances of the CV analysis were obtained using permutation tests (10,000 replications). Additionally, a PCA was conducted to assess within-population variance in body shape for Lake Chila only.

Molecular methods

Total DNA was extracted from fin clips preserved in ethanol applying a proteinase *K* digestion followed by either a high-salt (Bruford et al., 1998) or a Magna Pure extraction using a robotic device (Magna Pure LC, Roche Diagnostics) and following the manufacturer's protocol (Roche, Switzerland).

We genotyped a total of 249 *Pseudocrenilabrus* specimens from the Lunzua River ($n = 167$; 73 specimens sampled in 2004 partly used in Koblmüller et al., 2008b; 94 specimens sampled in 2010), Mbulu Creek ($n = 13$, sampled in 2010) and Lake Chila ($n = 69$, sampled in 2012) (see Table S2 for details) at

5 microsatellite loci (HchiST46, HchiST94 (Maeda et al., 2008), UNH002 (Kellogg et al., 1995), Pmv3 and Pmv4 (Crispo et al., 2007)).

Fragment size calling was carried out on an ABI 3130*xl* genetic analyser (Applied Biosystems) in comparison to the LIZ 500(−250) (Applied Biosystems) size standard. Genotypes were determined manually using Peak Scanner (v.1.0; Applied Biosystems), controlled and rounded to integers with the software TANDEM (v.1.09; Matschiner & Salzburger, 2009). STRUCTURE (v.2.3.3; Pritchard et al., 2000) was then used to infer population structure (Markov chain Monte Carlo simulations were run for 500,000 replications, burn-in = 50,000, admixture and correlated allele frequency options). Ten replicated simulations were performed for $K = 1$–8 and the most likely number of genetic clusters was inferred using the ΔK method (Evanno et al., 2005) implemented in the software STRUCTURE HARVESTER (Earl & von Holdt, 2012). Initially, we intended to genotype all 249 *Pseudocrenilabrus* spp. specimens with a larger set of microsatellite loci, but only 5 loci (see above) could be amplified in both the Lake Chila and the Lunzua River/Mbulu Creek samples. We, therefore, tested additional loci and selected, based on amplification success and the level of polymorphism, 7 loci for the Lake Chila subset (HchiST46, HchiST94 (Maeda et al., 2008), UNH002 (Kellogg et al., 1995), Pmv3, Pmv4 (Crispo et al., 2007), Ppun21 (Taylor et al., 2002), Pzeb3 (Van Oppen et al., 1997) and 6 loci for the Lunzua River/Mbulu Creek subset: (Pmv1, Pmv3, Pmv4, Pmv15 (Crispo et al., 2007), UNH989 and UNH002 (Kellogg et al. 1995)). We then performed STRUCTURE analyses for the Lake Chila set and the Lunzua River/Mbulu Creek set separately to test for substructure within the two datasets. Conditions were the same as for the combined dataset, except the ten replicated simulations were performed for $K = 1$–5 for Lake Chila and $K = 1$–10 for Lunzua River/Mbulu Creek. Genetic differentiation among all populations and between morphs within the Lunzua River samples, as well as between yellow morphs sampled in 2004 and 2010 (the low sample size of blue males from the same location did not allow for a contrast between different sample years) was estimated as θ_{ST} (Weir & Cockerham, 1984) in ARLEQUIN (v.3.5; Excoffier & Lischer, 2010) for both the dataset containing 5 loci and the Lunzua River/Mbulu Creek dataset with 6 loci.

We also determined the DNA sequence of the most variable part of the mitochondrial control region (359 bp in total) for 82 samples (see Table S1 for details) using published primers (L-ProF or L-Pro-F_Tropheus and TDK-D; Meyer et al., 1994; Lee et al., 1995; Koblmüller et al., 2011). Amplification and sequencing were performed as described elsewhere (Duftner et al., 2005; Koblmüller et al., 2011). The PCR fragments of the control region were purified using ExoSAP-IT (USB), directly sequenced with the BigDye sequencing chemistry (Applied Biosystems) and analysed on an ABI 3130*xl* genetic analyser (Applied Biosystems). Additionally, sequences of the most variable part of the mitochondrial control region for *Pseudocrenilabrus* spp. were obtained from GenBank (from Joyce et al., 2005; Katongo et al., 2005; Koblmüller et al., 2008a, 2012; Wagner et al., 2012; see Table S1 for details). Note that we also included '*Orthochromis' machadoi* (Poll, 1967), since previous studies demonstrated the placement of this species within the genus *Pseudocrenilabrus* (see e.g. Koblmüller et al., 2008a). Together with the sequences from GenBank (total $n = 155$), the mitochondrial DNA sequences were aligned in MAFFT v.6 (Katoh et al., 2002) under the FFT-NS-i option, i.e. with fast construction of an initial alignment followed by iterative refinement until convergence, with default gap penalties. Identical sequences were collapsed into haplotypes using DNA collapser implemented in the online tool FaBox (Villesen, 2007). Bayesian inference (BI) was carried out in MrBayes v.3.2.2 (Ronquist et al., 2012). Posterior probabilities were obtained from MCMC simulations in two independent runs (10 chains with 10 million generations each, chain temperature: 0.25, trees sampled every 1,000 generations) using the best-fit model of molecular evolution as suggested by JMODELTEST (Posada, 2008). A 50 % majority-rule consensus tree was constructed after a one million generation burn-in (chain stationarity and run parameter convergence were checked with Tracer v.1.6 (Rambaut et al., 2013), using posterior probability as a measure of clade support).

Results

Nuptial colouration

Results from the MCA on the colour matrix including all populations are shown in Fig. 2A. Dimension 1

Table 1 Pairwise population differentiation between *Pseudocrenilabrus* populations and morphs

Dataset 1 (5 loci)	Lunzua loc. 1 (blue '04)	Lunzua loc. 1 (yellow '04)	Lunzua loc. 1 (yellow '10)	Lunzua loc. 2 (blue '10)	Lunzua loc. 2 (yellow '10)	Mbulu yellow ('10)
Lunzua loc. 1 (yellow '04)	−0.01474					
Lunzua loc. 1 (yellow '10)	0.16427***	0.17607***				
Lunzua loc. 2 (blue '10)	0.41273***	0.37886***	0.16337**			
Lunzua loc. 2 (yellow '10)	0.18593***	0.19913***	0.03094*	0.05452		
Mbulu (yellow '10)	0.16224**	0.17008***	0.08917**	0.19097**	0.11138**	
Chila ('12)	0.32118***	0.38302***	0.29676***	0.30133***	0.35078***	0.19948***
Dataset 2 (6 loci)	Lunzua loc. 1 (blue '04)	Lunzua loc. 1 (yellow '04)	Lunzua loc. 1 (yellow '10)	Lunzua loc. 2 (blue '10)	Lunzua loc. 2 (yellow '10)	
Lunzua loc. 1 (yellow '04)	−0.01156					
Lunzua loc. 1 (yellow '10)	0.08357***	0.08048***				
Lunzua loc. 2 (blue '10)	0.24296***	0.24822***	0.15457***			
Lunzua loc. 2 (yellow '10)	0.14404***	0.14151***	0.04734***	0.02517		
Mbulu (yellow '10)	0.24440***	0.28047***	0.24497***	0.28296***	0.27547***	

Significance levels: * <0.05; ** <0.01; *** <0.001

Hydrobiologia (2015) 748:171–184

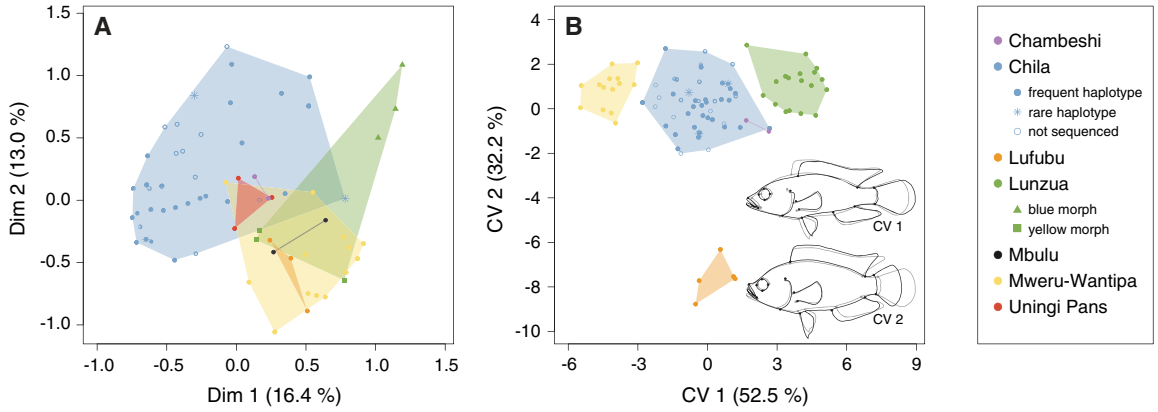


Fig. 2 **A** MCA based on nine male nuptial colouration traits. **B** CVA on male body shape based on 17 landmarks. *Green triangles* represent blue morphs and *green squares* yellow morphs from the Lunzua River (see Koblmüller et al. 2008b). *Filled blue circles* represent specimens assigned to the more frequent mitochondrial haplotype lineage; *blue stars* represent specimens assigned to the less frequent mtDNA lineage (*empty blue circles* represent individuals for which no mitochondrial sequence data was available)

explained 16 % and Dimension 2 explained 13 % of the variation. The traits explaining most of the variation were related to anal fin, pelvic fin and central body colouration (data not shown). The samples from Lake Chila show the widest distribution in trait-space; however, there were no distinct phenotypic clusters detectable within the population, e.g. with respect to mitochondrial lineage assignment (Fig. 2A). Specimens from Lake Mweru-Wantipa and the Lunzua River partly overlapped with Lake Chila phenotypes. Within the males from Lunzua River, blue and yellow morphs were separated along the axis of Dimension 2. Yellow morphs from Mbulu Creek clustered with yellow morphs from the Lunzua River. Specimens from the Lufubu, Chambeshi and Uningi Pans fell within the distribution range of samples from Lake Mweru-Wantipa and values did not overlap with the majority of the Lake Chila specimens (Fig. 2A). While the separation of colour morphs within the Lunzua River population is mainly due to blue and yellow central body colouration and the presence/absence of an anal fin blotch, phenotypic variation in the Lake Chila population is due to a more complex interplay of several traits (e.g. colour of anal fin blotch; colour of anal, dorsal, pelvic and caudal fin; ventral, dorsal and central body colouration). The MCA restricted to specimens from Lake Chila did not detect any clustering that would indicate the presence of distinct morphs (Fig. S1A).

Body shape

The CVA of the overall body shape of the sampled populations revealed a significant differentiation between all populations (Fig. 2B; all pairwise population comparisons $P < 0.05$). The main body shape changes are described by canonical variate 1 (CV1, accounting for 53 % of the variance), which shows mainly a prolongation of the head shape (with riverine Lunzua fish having longer heads and a more slender body shape), and CV2 (accounting for 32 % of the variance) describing additional changes in body shape and mouth position (with fish from the Lufubu River having longer caudal peduncles, more slender bodies and a more inferior position of the mouth). The PCA on body shape for the Lake Chila population only did not detect any clustering that would indicate the presence of distinct morphs (Fig. S1B).

Population structure

Bayesian clustering with Structure of the combined dataset (including population samples from the Lunzua River, Mbulu Creek and Lake Chila) based on five microsatellites revealed a clear geographic pattern. The most likely number of $K = 2$ separated one genotypic cluster comprising the two riverine populations from the cluster representing the Lake Chila stock (Fig. 3A). The separate Structure analysis for

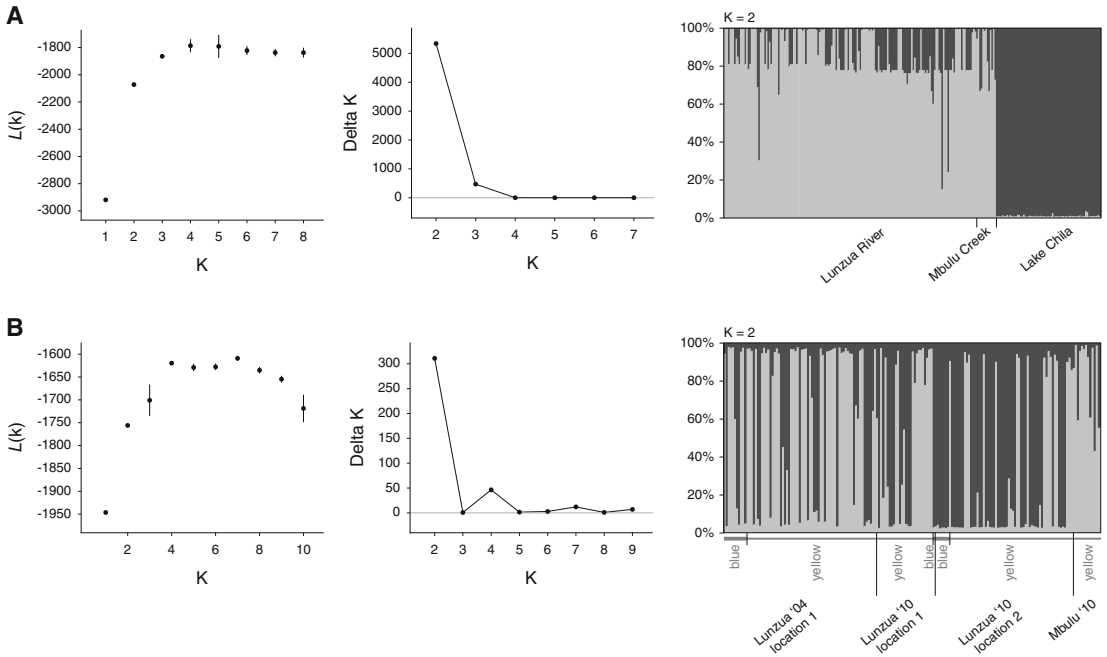


Fig. 3 Bayesian clustering analysis of *Pseudocrenilabrus* populations. **A** Dataset 1 (5 microsatellite loci) including samples from the Lunzua River, Mbulu Creek and Lake Chila. **B** Dataset 2 (6 microsatellite loci) including samples from the Lunzua River, Mbulu Creek and Lake Chila. *Left* mean likelihood ($L(K) \pm$ SD) over 10 runs assuming K clusters. *Middle* ΔK statistic (see Evanno et al. 2005). *Right* STRUCTURE plots for the most likely number of genetic clusters (K) as inferred from the ΔK statistic

the Lake Chila fish with seven microsatellites did not detect additional substructure within the population ($K = 1$, data not shown). The analysis of the dataset comprising only the Lunzua River and Mbulu Creek specimens based on six microsatellites, resulted in the most likely number of $K = 2$ (Fig. 3B). There was no clear genetic clustering detectable with regard to population or morph (Fig. 3B).

For the dataset including five microsatellite loci (Dataset 1, Table 1), pairwise comparisons revealed significant differentiation between morphs sampled in the years 2004 and 2010 (e.g. between yellow morphs from 2004 to 2010 from location 1) and between different sample locations (i.e. between Lunzua River locations 1 and 2; between Lake Chila and all other populations/morphs; between Mbulu Creek and all other populations/morphs), but not between blue and yellow morphs sampled within the same year.

Results from the Lunzua River/Mbulu Creek dataset (Dataset 2, Table 1) comprising 6 microsatellite loci (without the population from Lake Chila) are in line with those from the reduced dataset, with significant differentiation in all contrasts except between blue and yellow morphs sampled in the same year.

Phylogeography

Collapsing of sequences of the mitochondrial control region resulted in a total of 55 haplotypes (see Fig. 4; Table S1 for details). Our new BI phylogenetic reconstruction was largely in agreement with results from previous studies (Katongo et al., 2005; Koblmüller et al., 2012). The BI tree was rooted with *P.* sp. 'Lufubu A', which was identified as basal to all other *Pseudocrenilabrus* in previous phylogenetic studies (see Koblmüller et al., 2008a, 2012). Our new Lufubu River samples grouped with those downloaded from GenBank (Ht20 & Ht34; Fig. 4). The remaining haplotypes clustered into two major mitochondrial lineages: One comprised the Kafue–Zambezi clade and specimens from the Upper Luapula area: Lake

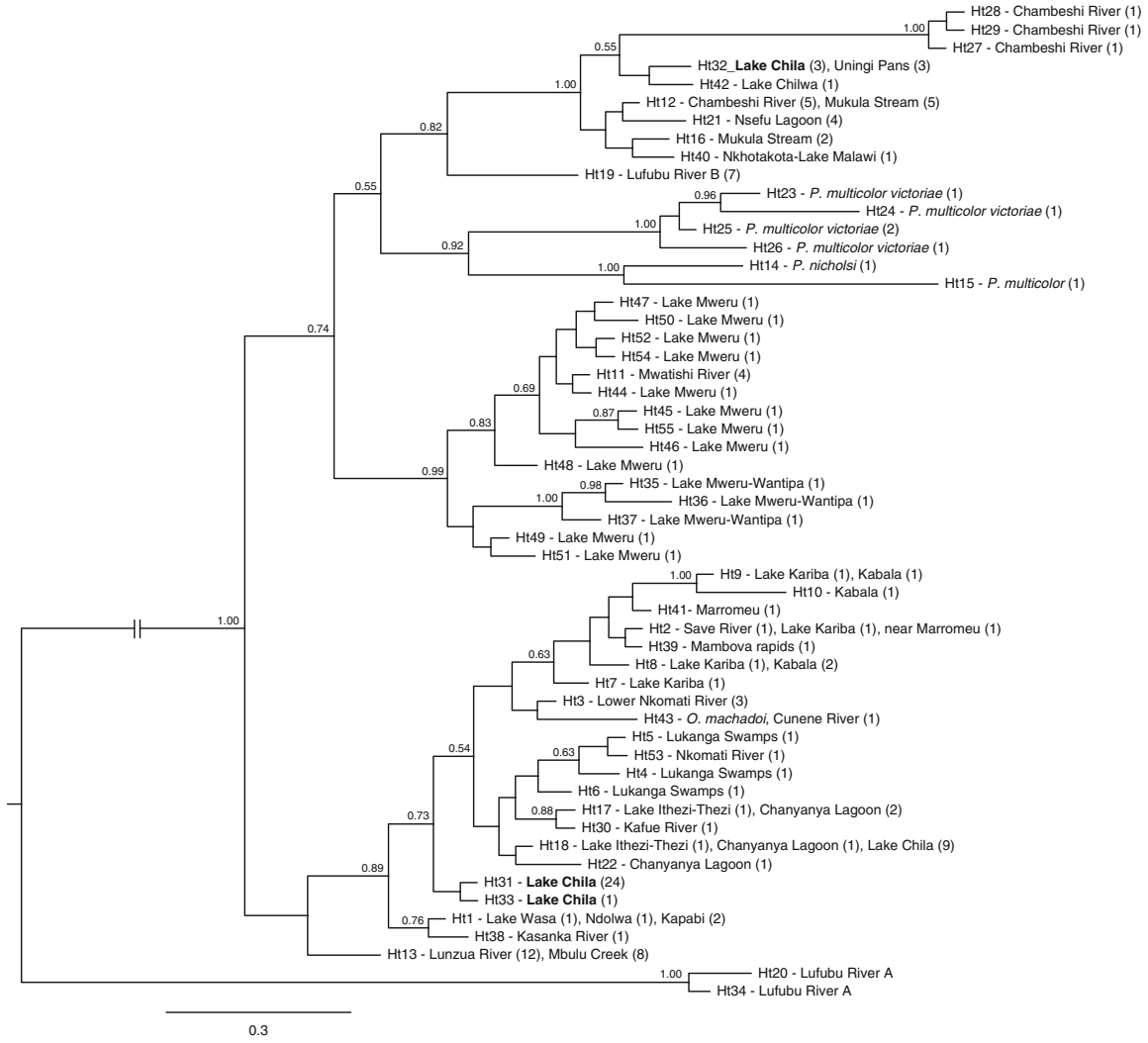


Fig. 4 Bayesian inference haplotype tree, rooted with *Pseudocrenilabrus* sp. 'Lufubu A'. Only posterior probabilities ≥0.50 are shown

Wasa, Kasanka River, Ndolwa and Kapabi in Kasanka NP (Ht1 & Ht38), as well as samples from further south (Cunene, Save and Nkomati basins). Our specimens from the Lunzua River and Mbulu Creek shared the haplotype with the previously published samples from the Lunzua River (Ht13) and formed the sister group to this lineage, although with very low posterior probabilities. '*Orthochromis' machadoi* and, interestingly, two *P.* cf. *philander* haplotypes from Lake Chila (Ht31 & Ht33) were resolved within the Kafue–Zambezi clade. In the other major mitochondrial lineage, *P.* sp. 'Lufubu B' (Ht19) was placed as sister group to the Chambeshi–Bangweulu clade. The newly sampled specimens from the Chambeshi River (Ht27, Ht28 & Ht29) grouped in this clade, as well as new individuals from Lake Chila and the Uningi Pans (Ht32) plus the two specimens from the geographically distant Malawi drainage and nearby basins (Lake Chilwa, Ht42 & Nkhotakota, Ht1). The samples from Lake Mweru-Wantipa (Ht35, Ht36 & Ht37) grouped within the Lake Mweru clade. Specimens from the Lake Victoria region, the remainder of the Congo drainage and the Nile, comprising the species *P. nicholsi* and *P. multicolour* (including the subspecies

P. m. victoriae), were placed as sister group to the Chambeshi–Bangweulu lineage, although with very low posterior probabilities.

Discussion

In this study, we reassessed the phylogeography of *Pseudocrenilabrus* in the watersheds of Zambia with a particular focus on a newly discovered lacustrine population of *Pseudocrenilabrus* cf. *philander* from Lake Chila, a small and shallow lake about 20 km south of LT. Males from this population displayed deeper bodies and more elaborate colour patterns compared to other known populations from the *P. philander* species complex. Interestingly, sequencing of the mitochondrial control region revealed the presence of two divergent mtDNA haplotype lineages in Lake Chila, with the more frequently sampled lineage (Ht31 & Ht33, ~90 % of Lake Chila mtDNA sequences) being associated with the Kafue–Zambezi clade, whereas the less frequent lineage (Ht32, ~10 % of Lake Chila mtDNA sequences) was placed within the Chambeshi clade (Fig. 4; Table S1). The exact origin of the two lineages remains unclear, and we cannot exclude the possibility that *Pseudocrenilabrus*, especially from the Zambezi–Kafue lineage, have been accidentally translocated in the course of a stocking event with *Oreochromis macrochir* (Thys van den Audenaerde, 1994; Lawrence Makasa, Fisheries Department Mpulungu, personal communication). However, this would not affect our conclusions about the maintenance of genetic and phenotypic diversity within Lake Chila.

We conducted a MCA based on nuptial colour traits of males to compare phenotypic diversity between different *Pseudocrenilabrus* populations. This analysis (and the MCA on the Lake Chila population only) did not result in the clustering of males with respect to mtDNA lineage assignment or any pattern that would indicate the presence of distinct morphs, but suggested a rather extensive colour pattern variation within the Lake Chila population, distinct from the other populations included in the analysis (Fig. 2A). Note, however, that males from Lake Chila that share the less frequent mtDNA haplotype with fish from the Uningi Pans (Ht32) showed a distinct phenotype (Fig. 2A), further rejecting an association between mtDNA lineage and nuptial colour pattern. The MCA separated blue and yellow morphs from the Lunzua River and revealed differences in nuptial colouration, although with overlapping distributions, among some of the included populations (e.g. Lake Mweru-Wantipa, Lake Chila and Lunzua River, see Fig. 2A).

The CVA on body shape detected significant population differentiation for all analysed populations, with the lacustrine populations from Lakes Mweru-Wantipa and Chila having shorter heads and deeper bodies compared to the riverine populations (Fig. 2B), indicating adaptation to different flow regimes in lake and riverine habitat (Webb, 1984). The PCA on the Lake Chila population did not reveal clustering of distinct phenotypes, rejecting the idea of eco-morphological divergence within the small lake. However, due to the bias in sample sizes of lake and stream populations, we cannot exclude that phenotypic variability of some of the included riverine populations may be underestimated.

In addition to a lack of discrete colour morphs within Lake Chila, the population assignment test with STRUCTURE (based on both datasets with 5 and 6 microsatellite loci) indicated no genetic substructure within the lake (Fig. 3), suggesting complete admixture between the two divergent mtDNA haplotype clades (the STRUCTURE analysis did infer distinct genetic clusters for Lake Chila and the populations from Lunzua River and Mbulu Creek; see Fig. 3).

Introgressive hybridisation between lineages has been proposed to facilitate the colonisation of new environments by increasing genetic variation and generating unique phenotypes via transgressive segregation (Kolbe et al., 2004; Seehausen, 2004). Such a genetically admixed 'hybrid swarm' often exceeds morphospace occupation when compared to parental populations (Lucek et al., 2010; Tobler & Carson, 2010). Thus, selection can act on a broadened working surface and new, adaptive trait combinations may enable the exploitation of previously not utilized niches (Seehausen, 2004).

Stelkens & Seehausen (2009) discovered two divergent mtDNA lineages in Lake Mweru, a rather large lake 150 km west of the southern end of LT (see Fig. 1). In Lake Mweru, one of the mitochondrial lineages was present in several distinct morphs (the study reports 'at least 13 distinct phenotypes'), whereas the other mtDNA lineage was represented by a single generalist phenotype only, and appeared to be generally very rare. The level of reproductive isolation between these morphs has been shown to

correlate positively with divergence in nuptial colour pattern and eco-morphological divergence, but not with genetic differentiation (Stelkens & Seehausen, 2009). Lake Mweru is much larger (131 km long and 56 km wide) and deeper (max. 27 m deep) than Lake Chila, and diversification in Lake Chila might be impeded due to the comparative long-term instability of the lake and the presumed recency (assuming that the Kafue–Zambezi haplotypes in Lake Chila result from unintentional stocking) of the admixture between the two distinct genetic lineages.

Given the small radiation in Lake Mweru, and the phenotypic and genetic variability in Lake Chila, it is puzzling why *Pseudocrenilabrus* did not diversify in any of the other lakes of the region despite its presence in most of the basins (Seehausen, 2006; Stelkens & Seehausen, 2009).

During several sampling trips to rivers draining into southern LT (Kalambo, Lunzua and Lufubu), we observed that *Pseudocrenilabrus* were present in the more upstream regions of these rivers, whereas the dominant cichlid species in the downstream areas was *Astatotilapia burtoni*. We never found the two species in sympatry in any of the rivers (see also Seegers, 1996; Theis et al., unpublished). In Lake Chila, however, the two species co-occur, although *Pseudocrenilabrus* are much more abundant and we only caught *A. burtoni* in very low numbers and in a restricted area. Further, *A. burtoni* were smaller in body size and less intensively coloured compared to populations from LT or inflowing rivers (Theis et al., unpublished). Lake Chila is located 1,600 m above sea level and *Pseudocrenilabrus* cf. *philander* is known to be tolerant to temperatures as low as 16 °C (Loiselle, 1982). It seems that under these conditions, *P.* cf. *philander* is able to compete against the apparently less temperature-tolerant *A. burtoni*. Competitive exclusion of the two generalist species in combination with differing temperature tolerance might also explain the mutually exclusive distribution ranges of *A. burtoni* and *P. philander* in Zambian rivers. Lake Mweru, to our knowledge, does not harbour any 'modern' haplochromine species, which could partly explain why *Pseudocrenilabrus* successfully utilized the provided ecological opportunities in this lake (Stelkens & Seehausen, 2009).

Our extended dataset on the *P. philander* species complex also provides new insights into the phylogeographic relationships of the genus. Overall, our mitochondrial phylogenetic reconstruction is largely in line with previous phylogenies from Katongo et al. (2005) and Koblmüller et al. (2012). However, an even more complex phylogeographic pattern emerges with the inclusion of additional samples. Our samples from the Lufubu River, which were assigned to *P.* sp. 'Lufubu A', grouped together with sequences from the most basal *Pseudocrenilabrus* lineage (Koblmüller et al., 2012; Fig. 4). The remaining taxa formed two major mitochondrial clades, one representing the Zambezi–Kafue drainage, and the other representing a lineage of mainly Congolese origin (see Figs. 1 and 4). The new samples from the Upper Luapula area (locations 19 and 20) were placed within the Zambezi–Kafue clade, indicating past connections of the Kafue/Zambezi and Chambeshi watersheds—in line with the presumed Zambezian influences of the ecoregion's ichthyofauna (Jackson, 1961, 1986; Balon, 1977; Scott, 2005). However, other specimens from locations 7, 10 and 11, which are part of the Chambeshi drainage, clustered with samples from Lake Mweru and Lake Mweru-Wantipa, which are part of the Congo drainage. The Bangweulu-Chambeshi subregion is known to harbour ichthyofaunal elements from both the Zambezi and Congo (Van Steenberge et al., 2014), and our phylogenetic inference demonstrates the occurrence of two mitochondrial lineages in the subregion, one belonging to the Zambezian and the other to the Congo drainage *Pseudocrenilabrus* clades. These phylogeographic patterns are in line with previous studies on other cichlid species (Joyce et al., 2005; Katongo et al., 2007) and African tigerfish (Goodier et al., 2011), all of which imply repeated and fairly recent faunal exchange between the Zambezi and Zambian Congo system by capture of entire river systems as well as small headwater creeks, despite the longstanding separation of the main courses (Stankiewizc & de Wit, 2006; Cotterill & de Wit, 2011).

The second *Pseudocrenilabrus* lineage found in the Lufubu, *P.* sp. 'Lufubu B', was placed as sister group to the Chambeshi clade, indicating a second wave of colonisation of the Lufubu river via the upper Congo system by a derived haplotype lineage (see also Koblmüller et al., 2012). Moreover, sequences from fish collected in Lake Chilwa and Nkhotakota/LM were resolved in this clade, which suggests a past connection between the upper Malawi and Chambeshi drainages, possibly via the Luangwa (note that

specimens from the Luangwa, Nsefu Lagoon also grouped in the same clade, Figs. 1, 4; see also Tweddle & Skelton, 2008).

Our specimens from the Lunzua River and Mbulu Creek all shared a single mitochondrial haplotype with previously published sequences (Koblmüller et al., 2008b, 2012) and were resolved, although weakly supported, as sister to the Zambezi clade. The dispersal route of this haplotype between the Lunzua River and Mbulu Creek is puzzling, given that the Uningi Pans, which contain a different haplotype, are located in between both river's headwaters (see Figs. 1, 4). The Lunzua and Mbulu, however, might have been connected downstream during a severe low surface level in LT (the two rivers enter LT in the Chituta Bay; McGlue et al., 2008)—or alternatively, gene flow between the two streams might have been enabled via past river capture of small headwaters. The two populations did show genetic differentiation at nuclear markers, as evidenced by significant pairwise θ_{ST} values (Table 1). We also detected genetic differentiation between the two sampling locations in Lunzua from 2010 and interestingly, also between specimens sampled from the same location in the years 2004 and 2010, corroborating the idea that genetic bottlenecks induced by strong seasonal variation of flood plains and small river confluences have a strong impact on the population dynamics of cichlid fish in general and on *Pseudocrenilabrus* in particular (Koblmüller et al., 2008b; Crispo & Chapman, 2010; Hermann et al., 2011). In contrast to Koblmüller et al. (2008b), blue and yellow morphs (both in 2004 and 2010) were not genetically differentiated (Table 1), which might be explained by the use of a different set of microsatellite markers.

Taken together, our study reveals a rather complex phylogeographic pattern and demonstrates introgression between distant mitochondrial lineages in a basal haplochromine cichlid, providing additional evidence for the role of hybridisation in the evolution of haplochromines (Joyce et al., 2011; Schwarzer et al., 2012). The occurrence of divergent mtDNA haplotypes and extensive morphological variation in Lake Chila, together with the small radiation in Lake Mweru, which contrast the low genetic and phenotypic diversity found in rivers, suggest that *Pseudocrenilabrus* are more prone to diversify in a lake habitat providing more ecological opportunity, especially when more derived 'modern' haplochromines are absent. That *Pseudocrenilabrus* did not (yet) diversify further in Lake Chila might be related to the small size and hence comparative long-term instability of Lake Chila and the presumed recency of the admixture between the two distinct genetic lineages.

Acknowledgments We would like to thank our helpers in the field, J. Bachmann, T. Bosia and L. Schild, the Kasanka Trust, the Lake Tanganyika Research Unit, Department of Fisheries, Republic of Zambia, for research permits and Radim Blazek and Martin Reichard for providing samples. This study was supported by grants from the European Research Council (ERC, Starting Grant 'INTERGENADAPT'), the University of Basel, and the Swiss National Science Foundation (SNF, Grant 3100A0_138224) to W. S.

References

Balon, E. K., 1977. The golden mudsuckers (*Labeo* spp.) from above and below the Victoria Falls: Sibling species along different invasion routes. Hydrobiologia 53: 3–12.

Bruford, M. W., O. Hanotte, J. F. Y. Brookfield & T. Burke, 1998. Multilocus and single-locus DNA fingerprinting; molecular genetic analysis of populations, a practical approach. In Hoelzel A. R. (eds), Oxford University Press, Oxford: 287–336.

Cotterill, F. P. D. & M. J. de Wit, 2011. Geoecodynamics and the Kalahari epeirogeny: linking its genomic record, tree of life and palimpsest into a unified narrative of landscape evolution. South African Journal of Geology 114: 489–514.

Crispo, E., C. Hagen, T. Glenn, G. Geneau & L. J. Chapman, 2007. Isolation and characterization of tetranucleotide microsatellite markers in a mouth-brooding haplochromine cichlid fish (*Pseudocrenilabrus multicolor victoriae*) from Uganda. Molecular Ecology Notes 7: 1293–1295.

Crispo, E. & L. J. Chapman, 2010. Temporal variation in population genetic structure of a riverine African cichlid fish. Journal of Heredity 101: 97–106.

Duftner, N., S. Koblmüller & C. Sturmbauer, 2005. Evolutionary relationships of the Limnochromini, a tribe of benthic deep water cichlid fishes endemic to Lake Tanganyika. Journal of Molecular Evolution 60: 277–289.

Earl, D. A. & B. M. von Holdt, 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359–361.

Evanno, G., S. Regnaut & J. Goudet, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611–2620.

Excoffier, L. & H. E. L. Lischer, 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567.

Goodier, S. A. M., F. P. D. Cotterill, C. O'Ryan, P. H. Skelton & M. J. de Wit, 2011. Cryptic diversity of African tigerfish (genus Hydrocynus) reveals palaeogeographic signatures

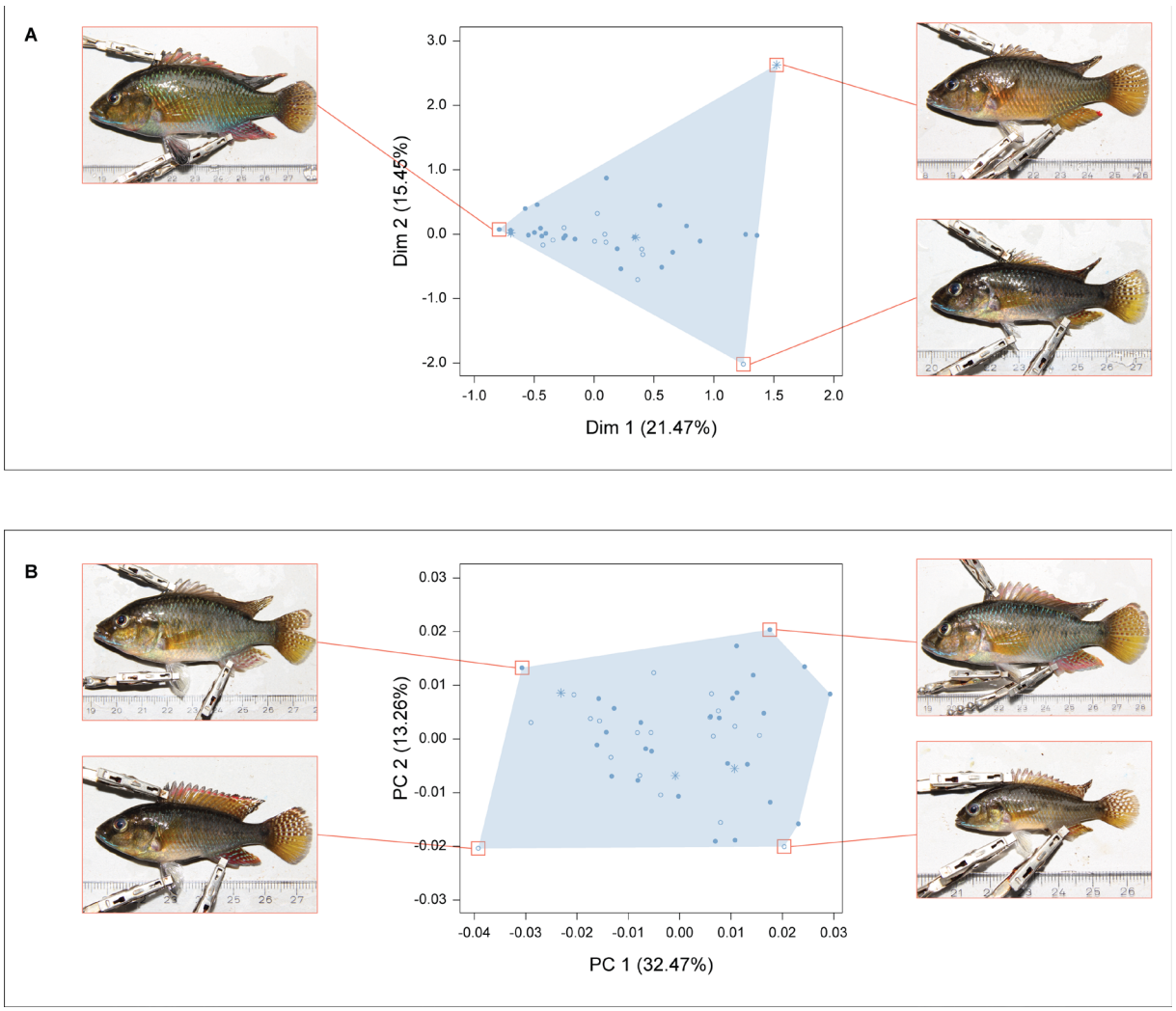
of linked neogene geotectonic events. PLOS One 6: e28775.
Greenwood, P. H., 1989. The taxonomic status and phylogenetic relationships of Pseudocrenilabrus Fowler (*Teleostei*, *Cichlidae*). Ichthyological Bulletins of the J. L. B. Smith Institute of Ichthyology 54: 1–16.
Hermann, C. M., K. M. Sefc & S. Koblmüller, 2011. Ancient origin and recent divergence of a haplochromine cichlid lineage from isolated water bodies in the East African Rift system. Journal of Fish Biology 79: 1356–1369.
Husson, F., J. Josse, S. Le & J. Mazet, 2014. FactoMineR: multivariate exploratory data analysis and data mining with R. R package version 1: 26.
Jackson, P. B. N., 1961. *The fishes of Northern Rhodesia*. The government printer, Lusaka: 140.
Jackson, P. B. N., 1986. Fish of the Zambezi system. In Davies, B. R. & K. F. Walker (eds), The ecology of river systems. W. Junk, Dordrecht: 269–288.
Joyce, D. A., D. H. Lunt, R. Bills, G. F. Turner, C. Katongo, N. Duftner, C. Sturmbauer & O. Seehausen, 2005. An extant cichlid fish radiation emerged in an extinct Pleistocene lake. Nature 435: 90–95.
Joyce, D. A., D. H. Lunt, M. J. Genner, G. F. Turner, R. Bills & O. Seehausen, 2011. Repeated colonization and hybridization in Lake Malawi cichlids. Current Biology 21: R108–R109.
Katoh, K., K. Misawa, K. Kuma & T. Miyata, 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059–3066.
Katongo, C., S. Koblmüller, N. Duftner, L. Makasa & C. Sturmbauer, 2005. Phylogeography and speciation in the Pseudocrenilabrus philander species complex in Zambian rivers. Hydrobiologia 542: 221–233.
Katongo, C., S. Koblmüller, N. Duftner, L. Mumba & C. Sturmbauer, 2007. Evolutionary history and biogeographic affinities of the serranochromine cichlids in Zambian rivers. Molecular Phylogenetics and Evolution 45: 326–338.
Kellogg, K. A., J. A. Markert, J. R. Stauffer & T. D. Kocher, 1995. Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. Proceedings of the Royal Society B 260: 79–84.
Klingenberg, C. P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353–357.
Koblmüller, S., U. K. Schliewen, N. Duftner, K. M. Sefc, C. Katongo & C. Sturmbauer, 2008a. Age and spread of the haplochromine cichlid fishes in Africa. Molecular Phylogenetics and Evolution 49: 153–169.
Koblmüller, S., K. M. Sefc, N. Duftner, C. Katongo, T. Tomljanovic & C. Sturmbauer, 2008b. A single mitochondrial haplotype and nuclear genetic differentiation in sympatric colour morphs of a riverine cichlid fish. Journal of Evolutionary Biology 21: 362–367.
Koblmüller, S., W. Salzburger, B. Obermüller, E. Eigner, C. Sturmbauer & K. M. Sefc, 2011. Separated by sand, fused by dropping water: habitat barriers and fluctuating water levels steer the evolution of rock-dwelling cichlid populations in Lake Tanganyika. Molecular Ecology 20: 2272–2290.
Koblmüller, S., C. Katongo, H. Phiri & C. Sturmbauer, 2012. Past connection of the upper reaches of a Lake Tanganyika tributary with the upper Congo drainage suggested by genetic data of riverine cichlid fishes. African Zoology 47: 182–186.
Kocher, T. D., 2004. Adaptive evolution and explosive speciation: The cichlid fish model. Nature Reviews Genetics 5: 288–298.
Kolbe, J. J., R. E. Glor, L. R. G. Schettino, A. C. Lara, A. Larson & J. B. Losos, 2004. Genetic variation increases during biological invasion by a Cuban lizard. Nature 431: 177–181.
Lee, W. J., J. Conroy, W. H. Howel & T. D. Kocher, 1995. Structure and evolution of teleost mitochondrial control regions. Journal of Molecular Evolution 41: 54–66.
Loiselle, P. V., 1982. Pseudocrenilabrus; the dwarf African mouthbrooders, part one: the Pseudocrenilabrus multicolor complex. Freshwater and Marine Aquarium 51(30–35): 59–63.
Lucek, K., D. Roy, E. Bezault, A. Sivasundar & O. Seehausen, 2010. Hybridization between distant lineages increases adaptive variation during a biological invasion: stickleback in Switzerland. Molecular Ecology 19: 3995–4011.
Mardia, K. V., J. Kent & J. Bibby, 1979. Multivariate analysis. Academic Press, New York.
Maeda, K., H. Takeshima, S. Mizoiri, N. Okada, M. Nishida & H. Tachida, 2008. Isolation and characterization of microsatellite loci in the cichlid fish in Lake Victoria, Haplochromis chilotes. Molecular Ecology Resources 8: 428–430.
Matschiner, M. & W. Salzburger, 2009. TANDEM: integrating automated allele binning into genetics and genomics workflows. Bioinformatics 25: 1982–1983.
Meyer, A., J. M. Morrissey & M. Schartl, 1994. Recurrent origin of a sexually selected trait in Xiphophorus fishes inferred from a molecular phylogeny. Nature 368: 539–541.
McGlue, M. M., K. E. Lezzar, A. S. Cohen, J. M. Russell, J. J. Tiercelin, A. A. Felton, E. Mbede & H. H. Nkotagu, 2008. Seismic records of late Pleistocene aridity in Lake Tanganyika, tropical East Africa. Journal of Paleolimnology 40: 635–653.
Muschick, M., A. Indermaur & W. Salzburger, 2012. Convergent Evolution within an Adaptive Radiation of Cichlid Fishes. Current biology 22: 2362–2368.
Poll, M., 1967. Contribution à la faune ichthyologique de l'Angola. Publicações. Culturais da Companhia de Diamantes Angola 75: 1–381.
Posada, D., 2008. jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25: 1253–1256.
Pritchard, J. K., M. Stephens & P. Donnelly, 2000. Inference of population structure using multi-locus genotype data. Genetics 155: 945–959.
R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.
Rambaut A., M. A. Suchard, D. Xie & A. J. Drummond, 2013. Tracer v1.6. http://beast.bio.ed.ac.uk/Tracer.
Rohlf, F. J., 2008. TPSDIG, Version 2.11. Department of Ecology and Evolution, State University of New York at

Stony Brook, Stony Brook. http://life.bio.sunysb.edu/morph/.
Rohlf, F. J. & D. E. Slice, 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology 39: 40–59.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., Larget, B., Liu, L., M. A. Suchard & J. P. Huelsenbeck, 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.
Salzburger, W., 2009. The interaction of sexually and naturally selected traits in the adaptive radiations of cichlid fishes. Molecular Ecology 18: 169–185.
Salzburger, W., T. Mack, E. Verheyen & A. Meyer, 2005. Out of Tanganyika: Genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. BMC Evolutionary Biology 5: 17.
Salzburger, W., H. Niederstätter, A. Brandstätter, B. Berger, W. Parson, J. Snoeks & C. Sturmbauer, 2006. Color-assortative mating in the cichlid species *Tropheus moorii* from Lake Tanganyika, East Africa. Proceedings of the Royal Society London B-Biological Sciences 273: 257–266.
Santos, M. E. & W. Salzburger, 2012. How cichlids diversify. Science 338: 619–621.
Schwarzer, J., E. R. Swartz, E. Vreven, J. Snoeks, F. P. D. Cotterill, B. Misof & U. K. Schliewen, 2012. Repeated trans-watershed hybridization among haplochromine cichlids (Cichlidae) was triggered by Neogene landscape evolution. Proceedings of the Royal Society B 279: 4389–4398.
Schluter, D., 2000. The ecology of adaptive radiation. Oxford University Press, New York.
Scott, L., 2005. Bangweulu-Mweru. In M. L. Thieme, R. Abell, M. L. Stiassny, P. Skelton, B. Lehner, E. Dinerstein, G. G. Teugels, N. Burgess, A. K. Toham & D. Olson (eds), Freshwater ecoregions of Africa and Madagascar: a conservation assessment. Island Press, New York: 185–186.
Seegers, L., 1996. The fishes of the Lake Rukwa drainage. Annales du Musée royal de l'Afrique Centrale. Sciences zoologiques, Tervuren.
Seehausen, O., 2004. Hybridization and adaptive radiation. Trends in Ecology and Evolution 19: 198–207.
Seehausen, O., 2006. African cichlid fish: a model system in adaptive radiation research. Proceedings of the Royal Society B 273: 1987–1998.
Skelton, P. H., 1991. Pseudocrenilabrus. In Daget, J., J. P. Gosse, G. G. Teugels & D. F. E. T. van den Audenaerde (eds), Check-list of freshwater fishes of Africa (CLOFFA). ISNB, Brussels; MRAC, Tervuren; and ORSTOM, Paris, 4: 394–398.
Skelton, P. H., 1993. *Scientific and common names of southern African freshwater fishes*. J. L. B. Smith Institute of Ichthyology, Grahamstown:1–34.
Skelton, P. H., 1994. Diversity and distribution of freshwater fishes in East and Southern Africa. Annales du Musée de la République de l'Afrique Centrale, Zoologique 275: 95–131.
Stankiewics, J. & M. de Wit, 2006. A proposed drainage evolution model for Central Africa—did the Congo flow east? Journal of African Earth Sciences 44: 75–84.
Stelkens, R. B. & O. Seehausen, 2009. Phenotypic divergence but not genetic distance predicts assortative mating among species of a cichlid fish radiation. Journal of Evolutionary Biology 22: 1679–1694.
Taylor, M., F. Meardon, G. F. Turner, O. Seehausen, H. Mrosso & C. Rico, 2002. Characterization of tetranucleotide microsatellite loci in a Lake Victorian, haplochromine cichlid fish: a Pundamilia × Pundamilia nyererei hybrid. Molecular Ecology Notes 2: 443–445.
Thys van den Audenaerde, D. F. E., 1994. Introduction of aquatic species into Zambian waters, and their importance for aquaculture and fisheries. Aquaculture for Local Community Development Programme, field document no. 24.
Tobler, M. & E. W. Carson, 2010. Environmental variation, hybridization, and phenotypic diversification in Cuatro Ciénegas pupfishes. Journal of Evolutionary Biology 23(7): 1475–1489.
Turner, G. F., O. Seehausen, M. E. Knight, C. J. Allender & R. L. Robinson, 2001. How many species of cichlid fishes are there in African lakes? Molecular Ecology 10: 793–806.
Tweddle, D. & P. H. Skelton, 2008. New species of 'Barbus' and Labeobarbus (Teleostei: Cyprinidae) from the South Rukuru River, Malawi, Africa. Smithiana Bulletin 8: 25–39.
Twentyman-Jones, V., A. J. Ribbink & D. Voorvelt, 1997. Colour clues to incipient speciation of Pseudocrenilabrus philander (*Teleostei*, *Cichlidae*). South African Journal of Science 93: 529–537.
Van Oppen, M. J. H., Rico C., Deutsch, J. C., G. F. Turner & G. M. Hewitt, 1997. Isolation and characterization of microsatellite loci in the cichlid fish Pseudotropheus zebra. Molecular Ecology 6: 387–388.
Van Steenberge, M., E. Vreven & J. Snoeks, 2014. The fishes of the Upper Luapula area (Congo basin): a fauna of mixed origin. Ichthyological Exploration of Freshwaters 24: 289–384.
Verheyen, E., W. Salzburger, J. Snoeks & A. Meyer, 2003. On the origin of the superflock of cichlid fishes from Lake Victoria, East Africa. Science 300: 325–329.
Villesen, P., 2007. FaBox: an online toolbox for fasta sequences. Molecular Ecology Notes 7: 965–968.
Wagner, C. E., L. J. Harmon & O. Seehausen, 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature 487: U124–U366.
Webb, P., 1984. Body form, locomotion and foraging in aquatic vertebrates. American Zoologist 24: 107–120.
Weir, B. & C. C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Supplementary Materials

Phylogeographic and phenotypic assessment of a basal haplochromine cichlid fish from Lake Chila, Zambia

Bernd Egger, Yuri Klaefiger, **Adrian Indermaur**, Stephan Koblmüller, Anya Theis, Simon Egger, Thomas Näf, Maarten Van Steenberge,Christian Sturmbauer, Cyprian Katongo and Walter Salzburger



Supplementary Figure 1:

Supplementary Table 1:

Sample ID	Nr.	Species	Colour morph	Collection year	Locality	Location number (Fig.1)	Drainage	Geographical coordinates	Reference (first publ.)	Collector
Lunzua_T03-1-D20	1	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1D19	2	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F20	3	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H11	4	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A2	5	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A4	6	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A5	7	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H12	8	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A8	9	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A9	10	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B10	11	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-D17	12	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E1	13	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E4	14	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E5	15	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E7	16	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E8	17	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E9	18	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E10	19	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E11	20	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E12	21	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E13	22	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E14	23	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E15	24	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E16	25	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1E18	26	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F5	27	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G12	28	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1E19	29	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F2	30	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F3	31	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F6	32	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F12	33	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F15	34	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F17	35	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G2	36	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G3	37	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G4	38	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G5	39	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G7	40	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G13	41	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G14	42	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3G20	43	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H1	44	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H2	45	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H3	46	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H4	47	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H5	48	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H7	49	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H8	50	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H9	51	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H10	52	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H14	53	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T04-6C19	54	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A10	55	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B3	56	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B4	57	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B5	58	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B6	59	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B7	60	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B9	61	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F10	62	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F7	63	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F8	64	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F11	65	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F13	66	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G1	67	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G8	68	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3G19	69	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H6	70	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H13	71	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T04-6C20	72	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A7	73	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_37B4	74	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37B8	75	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C6	76	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C9	77	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D2	78	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D4	79	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D5	80	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D6	81	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D7	82	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A1	83	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A2	84	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A3	85	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A4	86	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D1	87	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A5	88	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54E5	89	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37A1	90	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37A3	91	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A6	92	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A7	93	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A8	94	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B1	95	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B2	96	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B4	97	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B5	98	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54E4	99	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54F3	100	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C7	101	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54F5	102	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54H1A	103	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F1	104	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F2	105	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F3	106	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54I2	107	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F1	108	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54C8	109	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54D3	110	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54D8	111	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F2	112	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F4	113	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F6	114	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F7	115	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F8	116	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F9	117	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54G1	118	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B6	119	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B7	120	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B8	121	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C3	122	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C4	123	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C5	124	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C6	125	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54G4	126	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab

Supplementary Table 2:

Sample ID	Nr.	Species	Colour morph	Collection year	Locality	Location number (Fig.1)	Drainage	Geographical coordinates	Reference (first publ.)	Collector
Lunzua_T03-1-D20	1	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1D19	2	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F20	3	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H11	4	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A2	5	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A4	6	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A5	7	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H12	8	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A8	9	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A9	10	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B10	11	*Pseudocrenilabrus cf. philander*	blue	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-D17	12	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E1	13	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E4	14	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E5	15	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E7	16	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E8	17	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E9	18	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E10	19	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E11	20	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E12	21	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E13	22	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E14	23	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E15	24	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1-E16	25	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1E18	26	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F5	27	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G12	28	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1E19	29	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F2	30	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F3	31	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F6	32	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F12	33	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F15	34	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F17	35	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G2	36	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G3	37	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G4	38	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G5	39	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G7	40	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G13	41	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G14	42	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3G20	43	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H1	44	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H2	45	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H3	46	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H4	47	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H5	48	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H7	49	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H8	50	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H9	51	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H10	52	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H14	53	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T04-6C19	54	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A10	55	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B3	56	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B4	57	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B5	58	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B6	59	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B7	60	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2B9	61	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F10	62	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F7	63	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F8	64	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F11	65	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1F13	66	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G1	67	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-1G8	68	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3G19	69	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H6	70	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T03-3H13	71	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T04-6C20	72	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_T05-2A7	73	*Pseudocrenilabrus cf. philander*	yellow	2004	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	Koblmüller *et al.* 2008b	Koblmüller *et al.*
Lunzua_37B4	74	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37B8	75	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C6	76	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C9	77	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D2	78	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D4	79	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D5	80	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D6	81	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D7	82	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A1	83	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A2	84	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A3	85	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A4	86	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54D1	87	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A5	88	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54E5	89	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37A1	90	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_37A3	91	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A6	92	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A7	93	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86A8	94	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B1	95	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B2	96	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B4	97	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_86B5	98	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54E4	99	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54F3	100	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54C7	101	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 1	6	LT	8°57'38''S, 31°10'38''E	This study	Salzburger Lab
Lunzua_54F5	102	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54H1A	103	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F1	104	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F2	105	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86F3	106	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54I2	107	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F1	108	*Pseudocrenilabrus cf. philander*	blue	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54C8	109	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54D3	110	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54D8	111	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F2	112	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F4	113	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F6	114	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F7	115	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F8	116	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54F9	117	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54G1	118	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B6	119	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B7	120	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86B8	121	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C3	122	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C4	123	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C5	124	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_86C6	125	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab
Lunzua_54G4	126	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57'17.57''S, 31°9'42.70''E	This study	Salzburger Lab

Lunzua_86C7	127	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86C8	128	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86C9	129	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D1	130	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D2	131	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54G6	132	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D3	133	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D4	134	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D5	135	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D6	136	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D7	137	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54G8A	138	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D8	139	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86D9	140	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E1	141	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E2	142	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E3	143	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54H2	144	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54H3	145	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E4	146	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E5	147	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E6	148	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54H5	149	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E7	150	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E8	151	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86E9	152	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54H7A	153	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F4	154	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F5	155	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F6	156	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F7	157	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F8	158	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86F9	159	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86G1	160	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86G2	161	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54I3	162	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54E6	163	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_54E7	164	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86B9	165	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86C1	166	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
Lunzua_86C2	167	*Pseudocrenilabrus cf. philander*	yellow	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
37C7b_Mbulu	168	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37C9_Mbulu	169	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D1_Mbulu	170	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D2_Mbulu	171	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D3_Mbulu	172	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D4_Mbulu	173	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D5_Mbulu	174	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D6_Mbulu	175	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D7_Mbulu	176	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D8_Mbulu	177	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D9_Mbulu	178	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37E3_Mbulu	179	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37E4_Mbulu	180	*Pseudocrenilabrus cf. philander*	yellow	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
Lake_Chila_DRA5	181	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRA6	182	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRA7	183	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB1	184	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB2	185	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB3	186	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB4	187	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB5	188	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB6	189	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB7	190	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRB9	191	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRC2	192	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRC3	193	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRC5	194	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_DRC6	195	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA1	196	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA2	197	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA3	198	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA4	199	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA5	200	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA6	201	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA7	202	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA8	203	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFA9	204	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB1	205	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB2	206	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB3	207	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB4	208	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB5	209	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB6	210	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB7	211	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB8	212	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFB9	213	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC1	214	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC2	215	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC3	216	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC4	217	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC5	218	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC6	219	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC7	220	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC8	221	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFC9	222	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD1	223	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD2	224	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD3	225	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD4	226	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD5	227	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD6	228	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD7	229	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD8	230	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFD9	231	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE1	232	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE2	233	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE3	234	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE4	235	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE5	236	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE6	237	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE7	238	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE8	239	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFE9	240	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFF1	241	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFF2	242	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFF9	243	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI2	244	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI3	245	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI4	246	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI5	247	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI7	248	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
Lake_Chila_EFI8	249	*Pseudocrenilabrus cf. philander*	-	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab

Supplementary Table 3:

Sample ID	Nr.	Colour matrix	Body shape	Species	Collection year	Locality	Location number	Drainage	Geographical coordinates	Reference (first pub.)	Collector
DRA5	1	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRA6	2	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRA7	3	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB1	4	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB2	5	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB5	6	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB6	7	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB7	8	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRB9	9	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRC1	10		X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRC2	11	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRC4	12	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRC5	13	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA1	14	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA2	15	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA3	16	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA4	17	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA5	18	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA6	19	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA7	20	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA8	21	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFA9	22	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB1	23	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB2	24	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB3	25	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB4	26	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB5	27	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB6	28	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB7	29	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB8	30	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFB9	31	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFC1	32	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFC2	33	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFC3	34	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFC4	35	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFC5	36	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFD3	37	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFD5	38	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFD7	39	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFD9	40	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFE2	41	X		*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFE6	42	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFF9	43	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI2	44	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI3	45	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI4	46	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI7	47	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI8	48	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFI9	49	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
EFE9	50	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Chila	1	LT	8°50′5.02″S, 31°22′53.286″E	This study	Salzburger Lab
DRA1	51	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Upper Chambeshi River	7	CO	9°08′22.634″S, 31°22′31.097″E	This study	Salzburger Lab
DRA2	52	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Upper Chambeshi River	7	CO	9°08′22.634″S, 31°22′31.097″E	This study	Salzburger Lab
DRD6	53	X	X	*Pseudocrenilabrus* sp.'Lufubu A'	2012	Lufubu River	4	LT	9°03′40.111″S, 30°55′35.694″E	This study	Salzburger Lab
DRD7	54	X	X	*Pseudocrenilabrus* sp.'Lufubu A'	2012	Lufubu River	4	LT	9°03′40.111″S, 30°55′35.694″E	This study	Salzburger Lab
DRD8	55	X	X	*Pseudocrenilabrus* sp.'Lufubu A'	2012	Lufubu River	4	LT	9°03′40.111″S, 30°55′35.694″E	This study	Salzburger Lab
DRD9	56		X	*Pseudocrenilabrus* sp.'Lufubu A'	2012	Lufubu River	4	LT	9°03′40.111″S, 30°55′35.694″E	This study	Salzburger Lab
DRE1	57		X	*Pseudocrenilabrus* sp.'Lufubu A'	2012	Lufubu River	4	LT	9°03′40.111″S, 30°55′35.694″E	This study	Salzburger Lab
FJA8	58		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJA9	59		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB1	60		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB2	61		X	*Pseudocrenilabrus* cf. *philander* 'blue'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB3	62		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB4	63		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB5	64		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB6	65		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB7	66		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJB8	67		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC1	68		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC2	69		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC3	70		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC4	71		X	*Pseudocrenilabrus* cf. *philander* 'blue'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC5	72		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC6	73		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC8	74		X	*Pseudocrenilabrus* cf. *philander* 'blue'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
FJC9	75		X	*Pseudocrenilabrus* cf. *philander* 'yellow'	2013	Lunzua River	6	LT	8°57′32.598″S, 31°10′15.608″E	This study	Salzburger Lab
54C7	76	X		*Pseudocrenilabrus* cf. *philander* 'blue'	2010	Lunzua River, location 1	6	LT	8°57′38″S, 31°10′38″E	This study	Salzburger Lab
54F1	77	X		*Pseudocrenilabrus* cf. *philander* 'blue'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
54F4	78	X		*Pseudocrenilabrus* cf. *philander* 'yellow'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
54F5	79	X		*Pseudocrenilabrus* cf. *philander* 'blue'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
54F7	80	X		*Pseudocrenilabrus* cf. *philander* 'yellow'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
54I2	81	X		*Pseudocrenilabrus* cf. *philander* 'blue'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
54I3	82	X		*Pseudocrenilabrus* cf. *philander* 'yellow'	2010	Lunzua River, location 2	6	LT	8°57′17.57″S, 31°9′42.70″E	This study	Salzburger Lab
37D2	83	X		*Pseudocrenilabrus* cf. *philander*	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
37D9	84	X		*Pseudocrenilabrus* cf. *philander*	2010	Mbulu Creek	3	LT	8°51′27.83″S, 31°21′50.04″E	This study	Salzburger Lab
FBA1	85	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA2	86	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA3	87	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA4	88	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA5	89	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA7	90	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA8	91	X		*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBA9	92	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBB7	93	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBB8	94	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBB9	95	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBC1	96	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBC3	97	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBC4	98	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
FBC5	99	X	X	*Pseudocrenilabrus* cf. *philander*	2012	Lake Mweru Wantipa	9	CO	8°31′30.708″S, 30°07′39.99″E	This study	Salzburger Lab
IMG_6984	100	X		*Pseudocrenilabrus* cf. *philander*	2012	Uningi Pans	2	LT	8°55′54.392″S, 31°21′45.984″E	This study	Salzburger Lab
IMG_7006	101	X		*Pseudocrenilabrus* cf. *philander*	2012	Uningi Pans	2	LT	8°55′54.392″S, 31°21′45.984″E	This study	Salzburger Lab
IMG_7009	102	X		*Pseudocrenilabrus* cf. *philander*	2012	Uningi Pans	2	LT	8°55′54.392″S, 31°21′45.984″E	This study	Salzburger Lab

part three:

Asymmetrical Polymorphism

Chapter 7

A field based assessment of attack strategy and feeding success in the scale eating cichlid fish *Perissodus microlepis* (Perciformes, Cichlidae)

Adrian Indermaur, Anya Theis, Bernd Egger and Walter Salzburger

in preparation

AI helped design the study, conducted field work and conducted sample processing and drafted the manuscript.

Chapter 7

A field based assessment of attack strategy and feeding success in the scale eating cichlid fish *Perissodus microlepis* (Perciformes, Cichlidae)

Adrian Indermaur[x], Anya Theis[x] , Bernd Egger[x] and Walter Salzburger [x]
[x]: Zoological Institute, University of Basel; Switzerland

Abstract
Asymmetries are a special case of natural morphological variation and are relatively common in fishes.
Particularly interesting cases are the scale-eating cichlid fish, Perissodini, from Lake Tanganyika. These display a handed foraging behavior where an asymmetric 'left' mouth morph feeds on the scales of the right side of its prey fish and a 'right' morph bites the scales of the left side. This morphological polymorphism previously was hypothesized to be maintained via negative frequency dependent selection and its developmental and morphological base has been studied extensivly in the last years. Despite these efforts it is not known how such a polymorphism can arise in the first place. We conducted a field-based experiment reassessing attack strategies under semi-natural conditions and evaluated feeding success of mixed morph populations as opposed to single morph populations of the scale eater *Perissodus microlepis.* We could confirm laboratory findings of previous studies that *P. microlepis* feed preferentially on the side of its prey to which its mouth is bent under semi-natural conditions. Further we could show that mixed morph populations of *P. microlepis* have a higher feeding success than non-mixed ones thus demonstrating the selective advantage necessary for such an asymmetrical polymorphism to arise.

Introduction

Morphological variation plays a crucial role for the ecological and adaptive evolution of natural populations (Nosil 2012). This variation is most often manifested in a symmetrical continuous trait variance among individuals within populations, but there are also cases where the natural symmetry is broken and morphological asymmetries are present (Palmer 1994, 2010). Particularly in fishes, mouth asymmetries seem to be a rather common phenomenon (Nakajima *et al.* 2004). Often these are random asymmetries, meaning there are right and left sided individuals in a population at a certain frequency as opposed to dextral- and sinistral asymmetries, where only the right respectively left sided individuals are present (Van Valen 1962; Palmer 2009, 2010).
One of the most fascinating examples of a random morphological mouth asymmetry is found in several species of the cichlid fish tribe Perissodini, which show an extensive mouth asymmetry and have become a model organism and textbook example for the study of behavioural and morphological laterality (Fryer & Iles 1972; Futuyama 2009) as well as frequency dependent selection (Hori 1993; Takeuchi *et al.* 2012). The Perissodini, a group of cichlid fish (Cichlidae) endemic to Lake Tanganyika in East Africa, are a relatively species poor lineage with nine

described species (Liem & Stewart 1976; Koblmüller *et al.* 2007). They exhibit a particularly specialized feeding mode living on fish scales and epidermis to various degrees - they range from a mainly zooplankton based feeding regime (*Haplotaxodon spp.* and *Xenochromis heqcui)* to almost exclusively feeding on scales and epidermis as seen in one of the most specialized species, *Perissodus microlepis* (Takahashi et al. 2007a; 2007b).

Perissodus microlepis hunts and feeds by ambushing its prey fish from the rear, attack the flanks of the victim and bites out a single or a bunch of scales and epidermis. To this end, lurking in shady spaces e.g. structured habitats could allow hunting individuals' to improve efficiency. Possessing a mouth morphology bent to either side also enables *Perissodus spp*. to attack from steeper rear angles and hence increases overall feeding success as prey species have a lower probability of detecting and avoiding the attacker (Takeuchi *et al.* 2012). Such an angled feeding morphology could go along with a lateralized feeding behaviour as Hori already suggested in his famous study from 1993. He observed in the field, that individuals with a mouth opening to the right and hence a longer left lower jaw (termed 'lefties') seem to attack their prey on its left side while individuals with a mouth opening to the left and hence a longer right lower jaw (termed 'righties') attack on the right side accordingly. This finding was later verified with a one-predator-one-prey setting in laboratory experiments (Lee *et al.* 2012; Takeuchi *et al.* 2012).

Hori further showed that natural populations of *P. microlepis* fluctuate around a 50:50 lefties-to-righties-ratio with an amplitude of 0.15 and a wavelength of about 5 years. As the mechanism maintaining this natural polymorphism, he postulated negative frequency dependent selection where the rare morph would have a selective advantage over the common one. Prey fish would accustom to being attacked from one side more often, driving them to be more alert on this side creating a benefit and a higher feeding success for the obverse oriented individuals (Hori 1993).

Despite extensive research on the morphological laterality of *P. microlepis*, the trait is still not fully understood and it is not clear what the selective and developmental pathways are that lead to its formation and persistence. In order to tackle these questions, a number of recent studies focused more on the developmental and quantitative aspects of this asymmetry (Lee et al. 2012; Kusche *et al.* 2012; Hata *et al.* 2013; Takeuchi et al. 2012), many of these though reported non-concordant results. Concerning the mouth morphology Hori (1993) had described it as a bimodal anti-symmetry with no intermediate morphs present for the system, while a later study empirically stated that natural populations of *P. microlepis* exhibit a continuously unimodal anti-symmetry with no discrete dimorphism (Kusche et al. 2012). However, this study measured the bending angle solely via external measurements and Takeuchi et al. (2012) could show by measuring the anti-symmetry using external as well internal skeletal structures that the trait indeed seems to have a discretely bimodal distribution, which is not detectable in external examination. Further discussions were raised with regard to heritability and genetic maintenance of the trait. Examining wild caught broods and their parents, it had been stated that a Mendelian one locus two allele mechanism controls the polymorphism, where the lefty allele is dominant, and the dominant locus is homozygous lethal (Hori *et al.* 2007). The same study alternatively suggested the possibility of cross-incompatibility with predominance in lefty homozygotes. As main mechanism for maintaining this morphological and genetic

polymorphism, disassortative mating had been put forward (Takahashi & Hori 2008). Lee et al. (2010) later though refuted this theory, as the authors found purely random mating in natural populations. These mechanisms were also contradicted by Van Dooren (2010), who showed that not Mendelian inheritance, but rather both, polygenetic as well as environmentally plastic effects seem to contribute to the formation of the asymmetrical trait. It had been shown that morphological asymmetry is very weakly expressed in juvenile fish and only fully forms during development. Opposed to that, a handedness (e.g. preference for one attack side) is present very early in live preceding any morphological laterality and does not seem to change throughout live and hence might facilitate or even induce the plastic formation of the asymmetry (Lee *et al.* 2012). Further experiments showed a control group of test fish to increase their asymmetry during the course of the experiment while a treatment group, which was inhibited from feeding on their preferred side, did not significantly increase their asymmetry. This also strongly points towards a unidirectional plasticity being involved in the trait (Van Dooren *et al.* 2010).
In summary, the exact mechanisms of this laterality are not yet fully understood, but the trait is supposed to be the outcome of developmentally plastic processes and environmental factors mediated by an intrinsic handedness e.g. the preference of the fish for one side of the prey. Considering evolutionary timescales, frequency dependent selection could very well explain the maintenance of the 50:50 ratios in populations but cannot fully explain how such a polymorphic trait can emerge.
In this study we conducted a field based enclosure experiment with the scale eating cichlid *P. microlepis* under a semi natural setting with interacting communities in Lake Tanganyika in order to (i) confirm laboratory one-predator-one-prey findings about asymmetrical attack strategies and (ii) test the hypothesis whether a polymorphic population would have an overall higher feeding success and hence a selective advantage over a monomorphic population. We additionally assessed the potential influence of different habitat structures (rocky vs. sandy) on the attack strategies as well as on the overall feeding success of *P. microlepis.* All together we aimed to disentangle causalities in the evolution of this system, and to demonstrate the selective advantage of such a dimorphic trait, which would be necessary to help to explain how such asymmetries can arise in natural populations.

Materials and Methods

The experiment was carried out at Rift Valley Tropicals (S 8° 37’ 25.99”; E 31° 12’ 2.86”) on the southern shore of Lake Tanganyika in northern Zambia during two field seasons in September 2012 and September 2013. We used the scale eating cichlid fish *P. microlepis* as predator and as prey species the algae grazing cichlid species *Interochromis loocki* and *Tropheus moori* (Tropheini). They were caught by the authors and local fishermen using monofilament gillnet of 6 mm mesh size by chasing the individuals and carefully, with least damage to the scale cover possible removed from the net using snorkelling and SCUBA.
Prior to the experimental setup fishes were kept species-wise in concrete ponds (1 x 1 x 1 m) to settle and cope with the stress induced by catching and general fish handling. *Perissodus microlepis* individuals were scored by eye for mouth orientation since quantitative degree of mouth asymmetry could not be taken into account. Three examiners (AI, AT and WS) carried

out visual scoring independently in the two discrete categories 'lefties' and 'righties'. *Perissodus microlepis* were only used for the experiment if all three examiners agreed on the mouth morph and an easily visible laterality was present.

Experimental Setup

The experimental setup (Fig. 1) consisted of 6 equally sized underwater cages (2 x 2 x 2 m) which are made of a hollow steel frame covered by a sturdy net with 6 mm mesh size. The cages are open to the bottom allowing for a natural interaction of the fishes with the substrate. The cages are distributed between 6 and 9 m depth around 30 m off shore. Half of the cages had a homogeneous sandy ground while the other half was equipped with a rocky substrate similar to natural rocky areas providing lots of potential hiding places for prey and predator species (Fig. 1A).
In an initial phase 2 experimental trial runs were carried out to get familiar with the experimental procedure. During these trials the state of the fish as well as the cages were checked regularly to assess feeding rates and account for the speed of predation not to risk an effect of oversaturation. From this data, most suitable prey type and experiment duration were defined. For the experiment itself a total of 3 rounds were performed, with each round consisting of 3 cages in rocky and 3 in sandy habitat. In every round each of the 6 cages was stocked with 20 prey specimens (10 *I. loocki* and 10 *T. moorii*) and 14 predator specimens (*P. microlepis*). In each habitat triplet, one cage was stocked with exclusively right skewed *P. microlepis* (R), one with solely left skewed individuals (L) and a third one with a mixed population (7 lefties and 7 righties; M) (Fig. 1A). Therefore we had produced two types of population mixtures: monomorphic experimental populations (L and R) and polymorphic experimental populations (M). Assignment of these groups to the cages was altered in rotation within habitat type in each trial to avoid cage position effects. Prey fish as well as predators were randomly size distributed among the 6 cages within each round. While doing so we secured a homogenous size distribution in every cage.
Cages were sealed and the experiment was run for 3 days. Afterwards all fishes were re-caught using SCUBA and 6 mm mesh sized gillnets, euthanized and immediately stored in 96% Ethanol for transportation and processing.

Data assessment

In a first step our aim was to examine whether attack strategy correlates with mouth asymmetry (lefties feed more on left prey side and righties feed more on right prey side). Using binoculars (Leica S6E with LeicaL2 light source) prey fish were examined for the amount of missing scales on each body side. Missing scales were assessed and counted by two different examiners (AI and AT). The average of the two counts was then used as value for further transformation in order to minimize count errors. Where large parts of the scale cover were missing we excluded the data of this area on both sides of the fish to avoid introducing any possible bias due to handling.
In a second step, the feeding success of *P. microlepis* from mono- and polymorphic experimental

populations was compared. Feeding success was determined as a combination of feeding event (if predators were or were not able to feed) and intestinal scale count e.g. the amount of scales found, if they were able to feed. To this end preserved fish were dissected to examine the content of the intestinal tract and scales were counted if present. Since very little is known about the mode as well as the rate of digestion of scales in *P. microlepis* and digested scales form a homogenous mass, we only counted intact or slightly digested scales, which were still recognizable as discrete entity being easy to quantify. We hence refrained from including digested material in the study. Counts of intestinal scales could be done only once (AI), since the specimen and its intestine were damaged during dissection. This did not allow for a second identical procedure, but we are confident to rule out counting bias because the scales are a discrete entity and were very easy visible due to high contrast on black background.

Statistical analyses

To test for a putative correlation between attack strategy and mouth morph (lefties/righties) of the two monomorphic populations (L/R) we categorized the absolute number of missing scales into the attack strategies 0 and 1. If more scales were missing on the left than on the right side of a preys' flank it was coded as 1 (predators strategy to attack more often on the right side of the prey), and if less scales were missing on the left than on the right flank it was coded as 0 (predator strategy to attack more often on the left side of the prey). These attack strategy categories as response variable, together with the fixed effects mouth morph (righties/lefties) and habitat (rocky/sandy) were used to apply a generalized linear mixed model (GLMM) with a logistic link function with the package lme4 (Bates *et al.* 2014)(See Supplementary Table 2A). This model as all further statistical analyses were done using the statistical software R (v.3.0.2; R Core Team 2013). The factor cage was included as a random effect to account for within cage dependence of the data. Afterwards the modelled proportion of prey with more scales missing on the left side of the body kept in the cages with either only righties or only lefties was calculated with the probability-logit-inverse function plogis.

To analyse the feeding success of *P. microlepis* we applied a hurdle model with the package glmmadmb (Fournier *et al*. 2012; Skaug *et al.* 2013) (See Supplementary Table 2B), which separates the data into two sets to disentangle on one hand if the experimental populations had different proportions of feeding events in general and on the other hand if the intestinal scale count differed among the ones that were able to feed. For the first part of the hurdle procedure to describe the probability for feeding events we fit a model to the binary part of the data, which means that all zeroes (no scales in stomach) were coded as 0 and all non-zeroes (scales in stomach) were coded as 1. In a GLMM with logistic link function we tested if feeding events correlate with experimental populations mixture (mono-/polymorphic) as a fixed effect. Cage was again included as a random effect. Due to the fact that neither standard length (SL) nor habitat (rocky/sandy) improved the model significantly (ANOVA model comparison; $p_{\text{with SL}} = 0.6766$, $p_{\text{with habitat}} = 0.9128$), these parameters were not included as further fixed effects.

In the second part of the hurdle procedure, to compare the intestinal scale count of *P. microlepis* among the experimental populations mixtures (mono-/polymorphic) a truncated

negative binomial distribution (NB1) was fitted to the non-zero outcomes of the counted intestinal scales. Additionally to the experimental populations, SL and habitat were included as fixed effects. The factor cage was again included as a random effect. This model was also repeated with the logarithmic prey-predator-ratio as an offset, after checking for a correlation of prey-predator-ratio with experimental population. This correlation was performed with a GLMM with a logistic link function with the package lme4 (Bates *et al.* 2014), the additional fixed effect habitat and the random effect cage. (See Supplementary Table 2C)

Results

The sample size of experimental individuals underwent a considerable reduction due to loss of prey as well as predator individuals. Of the formerly stocked 252 *P. microlepis* 162 could be recaptured at the end of the trials and of the 360 stocked prey individuals 260 survived (*T. moori:* 118 of 180; *I. loocki:* 142 of 180; for cage specific sample sizes see Supplementary Table 1). In addition 6 non-stocked individuals were found, which were also included in further analysis since they served as prey as well. Despite the reduction in sample size, the size distribution was kept stable throughout the cages (mean SL ± sd; *P. microlepis* = 78.9 ± 9.0; prey = 74.9 ± 12.2; for cage specific SL distribution see Supplementary Table 1).

In a first step to examine whether attack strategy correlates with mouth asymmetry (lefties feed more on left prey side and righties feed more on right prey side), prey fish from experimental population L and R were scored for the amount of missing scales on each body side. All 207 recaptured prey individuals showed missing scales. Scale counts revealed a high variability from 1 to 109 scales per individual (mean missing scales ± sd = 16.58937 ± 15.09406; for cage and experimental population specific scale information see Supplementary Table 1). Missing scales were most often not exclusively found on one side of the preys' body: Only 8 individuals showed missing scales exclusively on one body side while the majority of the 207 individuals had missing scales on both body sides. The proportion of prey with more scales missing on the left than on the right body side and vice versa were significantly influenced by mouth morph of the predator (GLMM; $n = 207$, $z = 6.309$, $p < 0.0001$; Fig. 2A) and therefore seems to correlate with the attack strategies of *P. microlepis* - with lefties attacking from the left side whereas righties attacked from the right side in the majority of cases. Contrarily to mouth morph, no effect of habitat on attack strategy was found (GLMM; $n = 207$, $z = 1.513$, $p = 0.13$).

In the second part we tested whether *P. microlepis* of polymorphic were more successful than monomorphic experimental populations with regard to feeding events and number of ingested scales. The dissection of the 162 *P. microlepis* intestines revealed that only 106 individuals were able to succeed at a recent feeding event and therefore contained intestinal scales (monomorphic experimental populations: 66 of 111 individuals; polymorphic experimental populations: 40 of 51 individuals). *Perissodus microlepis* therefore had a higher probability for feeding events if they were kept in cages with polymorphic experimental populations than the ones in the cages with only monomorphic experimental populations (GLMM; $n = 162$, $z = -2.32$, $p = 0.0204$; Fig. 2B).

The intestinal scale count of the 162 successful *P. microlepis* individuals shows a range of 1 to 44 scales (mean intestinal scales ± sd, range; mixed experimental populations = 7.0 ±

6.8, 1-31; single experimental populations = 7.5 ± 8.1, 1-44; for details on intestinal scale count information per cage see Supplementary Table 1). Even though polymorphic morph experimental populations did have a higher feeding success due to a higher chance of feeding events, it seems that they could not individually feed on more scales than the monomorphic morph experimental populations. The intestinal scale count of the successfully feeding *P. microlepis* was only found to be significantly influenced by SL, but neither by population mixture nor by habitat (GLMM; n = 106, $z_{\text{experimental population mixture}} = 0.32$, $p_{\text{experimental population mixture}} = 0.7470$; $z_{SL} = -2.13$, $p_{SL} = 0.033$; $z_{habitat} = -1.36$, $p_{habitat} = 0.1730$).

These results must be treated with caution since number of intestinal scales is the response variable, which could be influenced by the variable predator-prey-ratio (Supplementary Table 1). Including predator-prey-ratio as a correction we find that polymorphic experimental populations do have a higher feeding rate than monomorphic experimental populations (GLMM; n = 106, z = -3.17, p = 0.0015). Again, the feeding rate was significantly influenced by SL, but not by habitat (GLMM; n = 106; $z_{SL} = -2.81$, $p_{SL} = 0.0049$; $z_{habitat} = -1.75$, $p_{habitat} = 0.0801$). However, this correction for predator-prey-ratio could have created these results artificially by acting as a confounding factor: Due to the limited replicate number the average predator-prey-ratio is coincidentally much lower in polymorphic experimental populations than in monomorphic experimental populations, which was not explainable by habitat (GLMM; n= 106, $z_{\text{experimental population mixture}} = 3.131$, $p_{\text{experimental population mixture}} = 0.0017$; $z_{habitat} = 0.059$, $p_{habitat} = 0.9530$) and therefore the correlations of predator-prey-ratio with the response variable intestinal scale count and the fixed effect experimental population mixture can not be disentangled. The models with and without offset were represented by nearly identical AIC values and should both be taken into account (see Supplementary Table 2).

Integrating the two steps of the hurdle model, we demonstrate that the polymorphic experimental populations of *P. microlepis* have a higher feeding success than monomorphic experimental populations. This is attributable to a higher probability on feeding events but possibly also to a potential increase in intestinal scale count.

Discussion

In this study we conducted a field based enclosure experiment in a semi-natural environment to reassess attack strategies and feeding success of *P. microlepis* populations.

By firstly examining the missing scale data on the prey fishes, we clearly confirm laboratory findings on attack strategy of previous studies (Takeuchi *et al.* 2012; Lee *et al.* 2012) that also under semi-natural circumstances and with community interactions, the two ecological mouth morphs of *P. microlepis* show a preference of feeding on their respective suitable side (e.g. lefties feeding preferably on the left flank while righties feed more on the right side of the prey species) (Fig. 2A). Seeing scales also missing from the not preferred flank of both prey fish species is in relative contrast to other studies, which have shown this preference to be quite strong and in some cases rather exclusive. With relatively few (20% in Takeuchi et al. 2012), or no attacks directed to the 'wrong' flank (Lee *et al.* 2012). This difference might arise through the one-predator-one-prey set up of other studies conducted so far. It seems easily imaginable that the predator has to refrain from its optimal hunting strategy under semi-natural or natural

conditions, where fishes encounter each other in differing orientations on multiple occasions. Contrarily (or additionally) it might be an indication that our setup provided *P. microlepis* with more opportunity for strike due to the slightly elevated prey density compared to natural communities as well as the lack of dilution by other species (Sturmbauer *et al.* 2008).

In a second step by counting the scales from the intestines of *P. microlepis* we estimated overall relative feeding success of the mono- and polymorphic populations while feeding success is made up of two factors, which we analysed separately: (i) the opportunity to feed e.g. the actual feeding events and (ii) the amount of intestinal scales per fed predator.

The opportunity to feed was higher in *P. microlepis* living in a polymorphic population than in individuals from monomorphic populations and therefore it seems that they have an increased chance of striking an attack (Fig. 2B). This seems to be attributable to the fact that in a polymorphic populations of *P. microlepis* prey species have much less chance to adapt to the attack strategies of the scale eater. This would support the hypothesis of negative frequency dependent selection acting on polymorphic populations of *P. microlepis* (Hori 1993). Another explanation could be that the polymorphic populations theoretically have access to a proportionally larger area of prey surface (scales) as opposed to a purely monomorphic population where only half of the prey surface is available for optimal hunting strategy and hence this would be a density dependent effect.

In the case of intestinal scale count it was less clear if *P. microlepis* from polymorphic populations have an advantage over individuals from monomorphic populations. The fact that smaller sized *P. microlepis* had a higher feeding rate than larger ones, which could be due to the lower intimidation effect of smaller individuals, was consistent but the increase in the amount of fed scales from individuals of polymorphic populations was only visible if the different prey-predator-ratios were incorporated as a correction in the analyses. These differences in the ratio of prey to predator arose through varying sample sizes per cage due to loss of experimental individuals, this had several reasons which were difficult to avoid in semi-natural setups: (i) experimental cages are set up at 6 to 9 meters depth. Even though this is well within the depth distribution of both *P. microlepis* and our prey species *I. loocki* and *T. moorii* (Muschick *et al.* 2012) we had to recompress the fish in order to stock the cages. *Perissodus microlepis* proved to be rather sensitive to this procedure and some individuals might have died from compressed swim bladder. (ii) Confined space might have promoted territorial disputes with diminished opportunity of escape and caused the fish to fight and injure each other. Because prey-predator-ratio was found to be a confounding factor and correlated both with feeding success but as well with experimental population mixtures, it was unsecure to correct for it in the model. Without this correction we could not confirm the previously found increase of the intestinal scale count of polymorphic populations. Nevertheless, even without an elevated intestinal scale count e.g. without the individual specimens being able to feed more, the increased possibility for a feeding event alone would be enough to provide a significant selective advantage for a polymorphic population over a monomorphic one since this corresponds to a increased feeding rate over time. Such a selective advantage would act as promoter in natural populations and drive divergent selection between the two morphs of *P. microlepis* in an initial phase of trait divergence. Negative frequency dependent selection as demonstrated by Hori (1993) seems to be the stabilizing force, which keeps populations at an

equal morph ratio. In a sense *P. microlepis* populations can be seen as divergent populations that persist in one interbreeding species since the selective advantage of the trait arises though the intrinsic bimodality.

Habitat did not seem to have an effect on neither the attack strategy nor the overall feeding success of *P. microlepis*. This is somewhat surprising since we had expected to see an effect of habitat, either decreasing feeding success of *P. microlepis* because prey fish have the opportunity to hide from the predator or contrarily, increasing feeding success due to the possibility of *P. microlepis* to attack its prey from an ambush. Not seeing any effect might be due to the above-mentioned effects offsetting each other on the one hand or could be an artefact of limited sample size with respect to habitat on the other (3 replicates). It could also be that the feeding behaviour of *P. microlepis* in natural settings does not depend on or even benefit from richly structured habitats and that the individuals rely much more on the evaluation of the preys behaviour.

Irrespectively, the effect of habitat with regard to feeding strategy should be re-evaluated with a larger number of replicates in this setting. Further investigations in this study system should focus on the issue of the degree of disassortative mating within the species since previous studies have yielded inconclusive results on this (Takahashi & Hori 2008; Kusche *et al.* 2012). Along with this the genetic basis of the trait should be investigated in order to make predictions about population dynamics with respect to the mouth morphology.

Overall we could show that *P. microlepis* do, also in semi-natural settings, preferably but not exclusively attack prey species on the flank corresponding to their mouth laterality. This confirms experimental studies of the past and confirms their validity for natural settings. Secondly we were able to empirically demonstrate that polymorphic populations of *P. microlepis* seem to have a higher probability for feeding success than monomorphic populations, probably mainly attributable to an increased probability of feeding events. The selective force resulting from such a strong ecological advantage might be the driving force in the formation of asymmetrical mouth polymorphism of *P. microlepis* and potentially also other asymmetrical scale eaters from Lake Tanganyika.

Aknowledgements

We are grateful to the fisheries department of Zambia, Mpulungu (especially to Dr. Lawrence Makasa and Dr. Dani Syninza) for permission and support in the field. We thank Gilbert Tembo with his family, crew and the local fishermen at Rift Valley Tropicals for their ever welcoming and hospitable nature and their indispensable help. We are deeply endepted to Yuri Kläfiger, Fabrizia Ronco, Marie Dittmann and Hugo Gante for their help with SCUBA in the field. We owe gratitude to Carolin Göppert and Lukas Widmer for valuable help and discussions in developing the protocol for data assessment. AI was funded by European Research Council (ERC) Starting Grant 'INTERGENADAPT' and Freiwillige Akademische Gesellschaft Basel (FAG). AT was funded by the University of Basel, the Freiwillige Akademische Gesellschaft Basel (FAG) and the Swiss Zoological Society (SZS). WS was funded by the European Research Council (ERC) Starting Grant 'INTERGENADAPT', the University of Basel and the Swiss National Found (SNF).

References

1. Bates, D., Maechler, M., Bolker, B. and Walker, S. 2014 lme4: Linear mixed-effects models using Eigen and S4. R package version 1.0-6. http://CRAN.R-project.org/package=lme4

2. Fournier, DA., Skaug, HJ., Ancheta, J., Ianelli J., Magnusson, A., Maunder, M., Nielsen, A. and Sibert, J. 2012 "AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models._Optim. Methods Softw._,*27*, pp. 233-249.

3. Fryer, G. & Iles, T. 1972 The cichlid fishes of the Great Lakes of Africa: their biology and evolution. Edinburgh, UK: Oliver & Boyd.

4. Futuyama, D.J. 2009 Evolution Sinauer Associates, Sunderland, MA 633 p.

5. Hata, H., Yasugi, M., Takeuchi, Y., Takahashi, S., Hori, M. 2013 Measuring and evaluating morphological asymmetry in fish: distinct lateral dimorphism in the jaws of scale-eating cichlids Ecology and Evolution 3 14 2045-7758 http://dx.doi.org/10.1002/ece3.849

6. Hori, M. 1993 Frequency-dependent natural-selection in the handedness of scale-eating cichlid fish. Science 260, 216 – 219 (doi:10.1126/science.260.5105.216)

7. Hori, M., Ochi, H., & Kohda, M. 2007 Inheritance Pattern of Lateral Dimorphism in Two Cichlids (a Scale Eater, Perissodus microlepis, and an Herbivore, Neolamprologus moorii) in Lake Tanganyika. *Zoological Science*, *24*(5), 486–492. doi:10.2108/zsj.24.486

8. Koblmüller, S., Egger, B., Sturmbauer, C. & Sefe, K. 2007 Evolutionary history of Lake Tanganyika's scale-eating cichlid fishes. Mol. Phylogenet. Evol. 44, 1295 – 1305. (doi:10.1016/j.ympev.2007.02.010)

9. Kusche H., Lee, H. J., & Meyer, A. 2012 Mouth asymmetry in the textbook example of scale-eating cichlid fish is not a discrete dimorphism after all. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1748), 4715–4723. doi:10.1111/j.1420-9101.2008.01539.x

10. Lee, H. J., Kusche, H., and Meyer, A. 2012 Foraging behavior in scale-eating cichlid fish: its potential role in shaping morphological asymmetry. PLoS ONE 7:e44670.

11. Lee, H. J., Pittlik, S., Jones, J. C., Salzburger, W., Barluenga, M. & Meyer, A. 2010 Genetic support for random mating between left and right-mouth morphs in the dimorphic scale-eating cichlid fish Perissodus micro- lepis from Lake Tanganyika. J Fish Biol. 76, 1940 – 1957. (doi:10.1111/j.1095-8649.2010.02620.x)

12. Liem, K., and D. Stewart. 1976 Evolution of the scale-eating cichlid fishes of Lake Tanganyika: a generic revision with a description of a new species. Bull. Mus. Comp. Zool. 147:313 – 350.

13. Muschick, M., Indermaur, A., & Salzburger, W. 2012 Convergent Evolution within an Adaptive Radiation of Cichlid Fishes. *Current Biology*, *22*(24), 2362–2368. doi:10.1016/j.cub.2012.10.048

14. Nakajima, M., Matsuda, H., and Hori, M. 2004 Persistence and Fluctuation of Lateral Dimorphism in Fishes. *The American Naturalist* , Vol. 163, No. 5 (May) , pp. 692-698

15. Nosil, P. 2012 Oxford University Press, 08.03.2012 - 304 Seiten

16. Palmer, A. R. 2005 Antisymmetry. *Variation*, 359-397.

17. Palmer, A. R. 2004 Symmetry breaking and the evolution of development. Science 306, 828–833. (doi:10.1126/ science.1103707)

18. Palmer, A. R. 2009 Animal asymmetry. Curr. Biol. 19, R473–R477 (doi:10.1016/j.cub.2009.04.006

19. Palmer, AR 2010 Scale-eating cichlids: from hand(ed) to mouth. Journal of Biology 9: 11.

20. Palmer, A. R. 1994 Fluctuating asymmetry: a primer. In Developmental instability: its origins and evolutionary implications (ed. T. A. Markow), pp. 335 – 364. Dordrecht, The Netherlands: Kluwer.

21. R Core Team 2013 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

22. Sturmbauer, C., Fuchs, C., Harb, G., Damm, E., Duftner, N., Maderbacher, M., et al. 2008 Abundance, distribution, and territory areas of rock-dwelling Lake Tanganyika cichlid fish species. *Hydrobiologia*, *615*(1), 57–68. doi:10.1007/s10750-008-9557-z

23. Skaugk H., Fournier, D., Nielsen, A., Magnusson, A. and Bolker, B. ($Date: 2013-07-1613:52:38-0400 (Tue, 16 Jul 2013)$). Generalized Linear Mixed Models using AD Model Builder R package version 0.7.7.

24. Takahashi, R., Moriwaki, T., and Hori, M. 2007 Foraging behaviour and functional morphology of two scale-eating cichlids from Lake Tanganyika. J. Fish Biol. 70:1458 – 1469.

25. Takahashi, R., Watanabe, K., Nishida, M., & Hori, M. 2007 Evolution of feeding specialization in Tanganyikan scale-eating cichlids: a molecular phylogenetic approach. *BMC Evolutionary Biology*, *7*(1), 195. doi:10.1186/1471-2148-7-195

26. Takahashi, T., & Hori, M. 2008 Evidence of disassortative mating in a Tanganyikan cichlid fish and its role in the maintenance of intrapopulation dimorphism. *Biology Letters*, *4*(5), 497–499. doi:10.1046/j.1365-294x.2000.00964.x

27. Takeuchi, Y., Hori, M. and Oda, Y. 2012 Lateralized kinematics of predation behavior in a Lake Tanganyika scale-eating cichlid fish. PLoS ONE 7:e29272.

28. Van Dooren, T. J. M., Van Goor, H. A., and Van Putten, M. 2010 Handedness and asymmetry in scale-eating cichlids: antisymmetries of different strength. Evolution 64:2159 – 2165.

29. Van Valen, L. 1962 A study of fluctuating asymmetry. Evolution 16:125 – 142

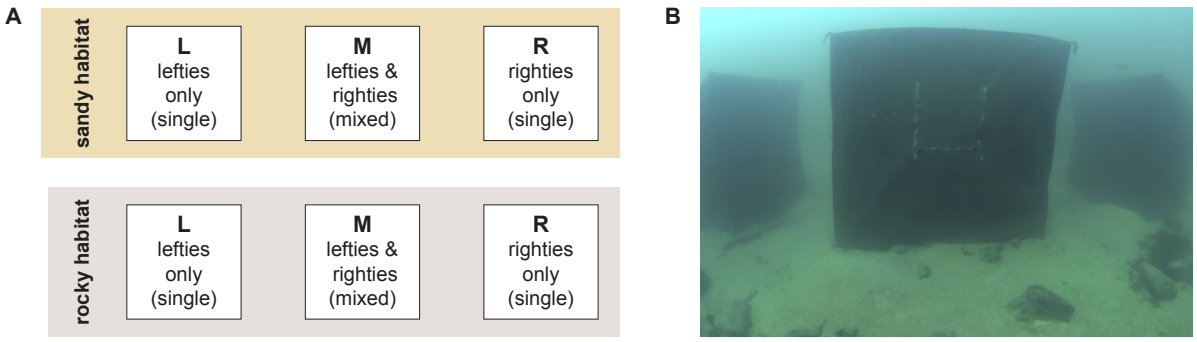


Figure 1 Schematic view of the experimental set up (A) and underwater photograph of one of the cages (B).

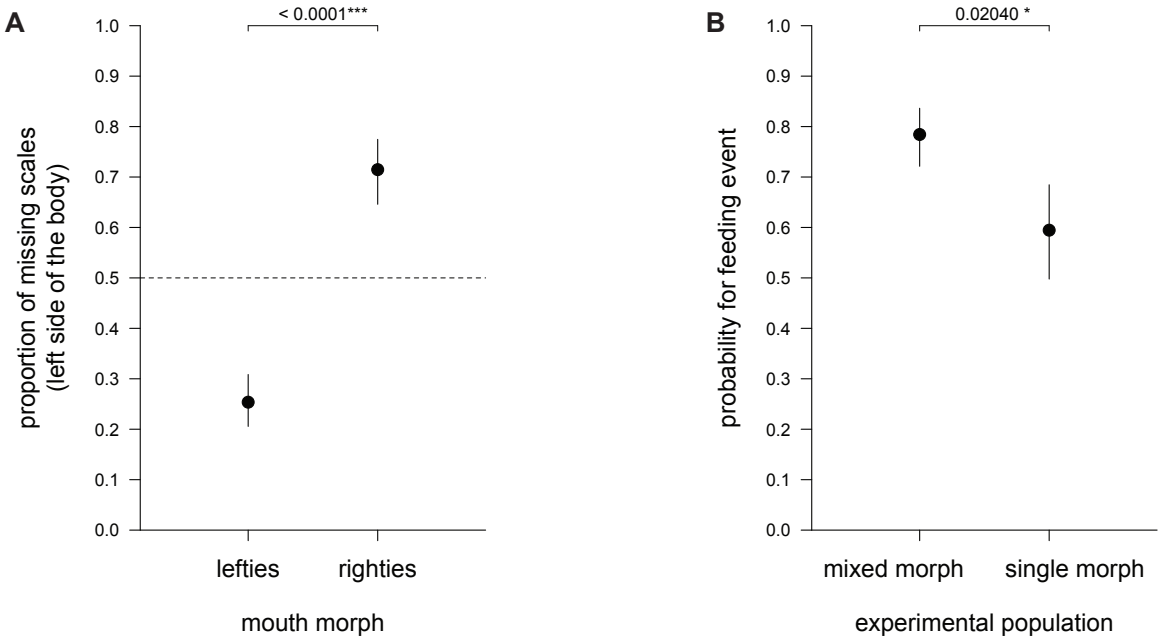


Figure 2 (A) Proportions of missing scale on the left body side of the prey species for each mouth morph. (B)Probability for a feeding event for mixed and single morph populations.

Supplementary Materials

A field based assessment of attack strategy and feeding success in the scale eating cichlid fish *Perissodus microlepis* (Perciformes, Cichlidae)

Adrian Indermaur, Anya Theis, Bernd Egger and Walter Salzburger

Supplementary Table 1:

cage information				sample size *P. microlepis*			sample size prey				prey-predator-ratio	lefties-to-righties-ratio		standard length (mm) average (mean ± sd) range (min - max)		scale informations average (mean ± sd) range (min - max)					
number	experimental population	experimental population mixture	habitat	survivals (successful feeders) / original stock			stocked prey survivals / original stock		non-stocked prey	total				*P. microlepis*	prey	missing scales on the prey's body side				intestinal scale counts of *P. microlepis*	
				lefties	righties	total	*I. loocki*	*T. moori*				wanted	achieved			left	right	difference (left - right)	total (left + right)	all individuals	successful feeders only
1	L	monomorphic	rocky	8 (6) / 14	-	8 (6) / 14	10 / 10	7 / 10	2	19	2.3750	100 : 0	100 : 0	82.500 ± 9.636 72.0 - 98.0	76.500 ± 11.897 61.5 - 96.0	9.553 ± 7.774 1.0 - 28.5	7.342 ± 12.147 0.5 - 55.5	2.211 ± 9.631 -27.0 - 17.0	16.895 ± 17.979 2.5 - 84.0	7.000 ± 10.650 0.0 - 32.0	9.333 ± 11.518 2.0 - 32.0
2	L	monomorphic	rocky	9 (6) / 14	-	9 (6) / 14	10 / 10	7 / 10	0	17	1.8889	100 : 0	100 : 0	82.444 ± 8.218 72.0 - 101.0	75.912 ± 10.263 60.5 - 95.0	9.029 ± 3.233 5.0 - 16.0	4.765 ± 2.768 0.0 - 10.0	4.265 ± 3.981 -3.0 - 10.5	13.794 ± 4.514 7.0 - 24.5	5.556 ± 7.970 0.0 - 24.0	8.333 ± 8.595 2.0 - 24.0
3	L	monomorphic	rocky	9 (5) / 14	-	9 (5) / 14	10 / 10	9 / 10	0	19	2.1111	100 : 0	100 : 0	83.444 ± 7.502 74.0 - 95.0	71.632 ± 12.533 51.5 - 93.5	6.368 ± 3.872 1.0 - 16.0	6.868 ± 9.853 0.0 - 41.5	-0.500 ± 8.288 -27.5 - 12.5	13.237 ± 12.468 3.5 - 57.5	1.556 ± 1.944 0.0 - 5.0	2.800 ± 1.789 1.0 - 5.0
4	L	monomorphic	sandy	7 (4) / 14	-	7 (4) / 14	10 / 10	10 / 10	1	21	3.0000	100 : 0	100 : 0	81.286 ± 8.995 69.0 - 93.0	75.429 ± 12.225 57.0 - 104.0	9.238 ± 3.750 3.0 - 19.5	4.310 ± 3.520 0.0 - 17.0	4.929 ± 4.597 -3.5 - 17.0	13.548 ± 5.637 5.0 - 30.5	4.714 ± 5.090 0.0 - 11.0	8.250 ± 3.594 3.0 - 11.0
5	L	monomorphic	sandy	6 (3) / 14	-	6 (3) / 14	8 / 10	5 / 10	0	13	2.1667	100 : 0	100 : 0	77.167 ± 3.251 74.0 - 82.0	71.716 ± 9.671 60.5 - 90.0	11.231 ± 7.568 4.5 - 30.5	6.077 ± 3.605 2.0 - 12.0	5.154 ± 7.072 -5.0 - 18.5	17.308 ± 9.514 7.5 - 42.5	4.667 ± 5.645 0.0 - 12.0	9.333 ± 3.786 5.0 - 12.0
6	L	monomorphic	sandy	8 (4) / 14	-	8 (4) / 14	8 / 10	10 / 10	0	18	2.2500	100 : 0	100 : 0	79.625 ± 6.567 68.0 - 86.0	66.417 ± 9.345 50.5 - 83.5	8.750 ± 5.735 1.0 - 24.5	7.250 ± 5.048 0.0 - 19.0	1.500 ± 4.159 -6.5 - 8.5	16.000 ± 9.972 1.0 - 43.5	2.000 ± 2.828 0.0 - 8.0	4.000 ± 2.828 2.0 - 8.0
	total L			**47 (28) / 84**	**-**	**47 (28) / 84**	**56 / 60**	**48 / 60**	**3**	**107**	**2.2766**	**100 : 0**	**100 : 0**	**81.319 ± 7.619 68.0 - 101.0**	**73.054 ± 11.490 50.5 - 104.0**	**8.911 ± 5.543 1.0 - 30.5**	**6.084 ± 7.219 0.0 - 55.5**	**2.827 ± 6.816 -27.5 - 18.5**	**14.995 ± 10.919 1.0 - 84.0**	**4.191 ± 6.405 0.0 - 32.0**	**7.036 ± 7.010 1.0 - 32.0**
7	R	monomorphic	rocky	-	8 (5) / 14	8 (5) / 14	10 / 10	4 / 10	2	16	2.0000	0 : 100	0 : 100	78.875 ± 9.672 67.0 - 92.0	79.250 ± 10.415 61.5 - 98.5	3.719 ± 2.966 0.0 - 11.0	6.219 ± 4.091 0.0 - 14.0	-2.500 ± 4.037 -11.0 - 5.0	9.938 ± 5.896 2.5 - 25.0	3.625 ± 4.207 0.0 - 12.0	5.800 ± 3.899 2.0 - 12.0
8	R	monomorphic	rocky	-	11 (8) / 14	11 (8) / 14	9 / 10	9 / 10	0	18	1.6364	0 : 100	0 : 100	73.909 ± 10.358 61.0 - 97.0	76.722 ± 11.030 60.0 - 98.0	11.972 ± 16.607 2.5 - 70.5	16.111 ± 15.590 0.0 - 64.0	-4.139 ± 12.678 -31.5 - 32.0	28.083 ± 29.613 3.5 - 109.0	8.000 ± 9.154 0.0 - 25.0	11.000 ± 9.055 2.0 - 25.0
9	R	monomorphic	rocky	-	11 (6) / 14	11 (6) / 14	9 / 10	10 / 10	1	20	1.8182	0 : 100	0 : 100	79.818 ± 11.898 63.0 - 103.0	71.650 ± 11.930 50.5 - 96.0	4.925 ± 3.711 0.5 - 14.0	9.325 ± 7.836 0.5 - 29.0	-4.400 ± 6.504 -22.5 - 4.5	14.250 ± 10.394 3.5 - 42.0	7.091 ± 13.308 0.0 - 44.0	13.000 ± 16.186 1.0 - 44.0
10	R	monomorphic	sandy	-	11 (7) / 14	11 (7) / 14	9 / 10	8 / 10	0	17	1.5455	0 : 100	0 : 100	76.273 ± 6.166 64.0 - 85.0	77.382 ± 11.608 62.0 - 102.5	4.118 ± 2.281 1.0 - 8.5	5.500 ± 4.287 1.5 - 19.5	-1.382 ± 3.706 -11.0 - 3.5	9.618 ± 5.781 3.5 - 28.0	4.455 ± 7.062 0.0 - 22.0	7.000 ± 7.895 1.0 - 22.0
11	R	monomorphic	sandy	-	12 (9) / 14	12 (9) / 14	7 / 10	5 / 10	0	12	1.0000	0 : 100	0 : 100	80.667 ± 11.171 64.0 - 98.0	79.417 ± 10.460 64.0 - 98.0	11.125 ± 9.684 1.0 - 34.0	12.708 ± 8.484 1.0 - 25.0	-1.583 ± 5.571 -11.5 - 9.0	23.833 ± 17.334 3.0 - 59.0	2.833 ± 2.855 0.0 - 8.0	3.778 ± 2.682 1.0 - 8.0
12	R	monomorphic	sandy	-	11 (3) / 14	11 (3) / 14	8 / 10	9 / 10	0	17	1.5455	0 : 100	0 : 100	78.727 ± 8.844 63.0 - 92.0	70.559 ± 12.240 50.0 - 90.5	10.735 ± 9.141 1.0 - 30.0	14.588 ± 12.412 1.0 - 42.5	-3.853 ± 7.295 -20.5 - 9.0	25.324 ± 20.544 2.0 - 65.0	1.818 ± 5.076 0.0 - 17.0	6.667 ± 8.963 1.0 - 17.0
	total R			**-**	**64 (38) / 84**	**64 (38) / 84**	**52 / 60**	**45 / 60**	**3**	**100**	**1.5625**	**0 : 100**	**0 : 100**	**78.047 ± 9.782 61.0 - 103.0**	**75.500 ± 11.618 50.0 - 102.5**	**7.595 ± 9.420 0.0 - 70.5**	**10.700 ± 10.421 0.0 - 64.0**	**-3.105 ± 7.344 -31.5 - 32.0**	**18.295 ± 18.459 2.0 - 109.0**	**4.656 ± 7.872 0.0 - 44.0**	**7.842 ± 8.937 1.0 - 44.0**
13	M	polymorphic	rocky	3 (1) / 7	4 (3) / 7	7 (4) / 14	7 / 10	3 / 10	0	10	1.4286	50 : 50	42 : 57	73.857 ± 6.012 70.0 - 84.0	78.400 ± 14.734 53.5 - 99.5	6.400 ± 3.414 3.0 - 15.0	6.650 ± 4.750 1.5 - 17.5	-0.250 ± 6.763 -12.0 - 13.5	13.050 ± 4.764 6.5 - 23.0	6.571 ± 7.458 0.0 - 20.0	11.500 ± 5.972 6.0 - 20.0
14	M	polymorphic	rocky	5 (4) / 7	4 (3) / 7	9 (7) / 14	4 / 10	1 / 10	0	5	0.5556	50 : 50	55 : 44	74.000 ± 4.717 67.0 - 80.0	76.400 ± 15.690 63.0 - 95.5	27.200 ± 10.474 12.5 - 37.5	21.600 ± 17.387 7.5 - 48.5	5.600 ± 11.518 -11.0 - 21.5	48.800 ± 26.293 20.0 - 86.0	3.444 ± 3.644 0.0 - 11.0	4.429 ± 3.552 1.0 - 11.0
15	M	polymorphic	rocky	6 (6) / 7	4 (1) / 7	10 (7) / 14	3 / 10	8 / 10	0	11	1.1000	50 : 50	58 : 42	80.400 ± 13.218 62.0 - 102.0	71.409 ± 11.256 59.0 - 90.5	17.455 ± 10.734 3.0 - 37.5	11.818 ± 11.660 3.5 - 42.5	5.636 ± 9.521 -13.0 - 26.0	29.273 ± 20.291 7.5 - 72.0	4.800 ± 7.084 0.0 - 23.0	6.857 ± 7.669 1.0 - 23.0
16	M	polymorphic	sandy	4 (3) / 7	5 (4) / 7	9 (7) / 14	10 / 10	8 / 10	0	18	2.0000	50 : 50	44 : 55	79.889 ± 5.011 71.0 - 89.0	78.556 ± 13.885 57.0 - 107.0	7.972 ± 7.480 0.5 - 25.5	8.417 ± 6.274 0.0 - 23.5	-0.444 ± 5.418 -14.5 - 7.0	16.389 ± 12.700 0.5 - 49.0	8.444 ± 9.838 0.0 - 31.0	10.857 ± 9.924 2.0 - 31.0
17	M	polymorphic	sandy	6 (6) / 7	4 (4) / 7	10 (10) / 14	5 / 10	2 / 10	0	7	0.7000	50 : 50	60 : 40	76.000 ± 10.985 59.0 - 95.0	82.143 ± 16.525 62.5 - 101.0	19.714 ± 7.588 8.5 - 32.5	9.929 ± 3.359 4.5 - 15.0	9.786 ± 9.827 -6.5 - 23.0	29.643 ± 6.414 23.5 - 42.0	6.600 ± 6.022 1.0 - 15.0	6.600 ± 6.022 1.0 - 15.0
18	M	polymorphic	sandy	3 (2) / 7	3 (3) / 7	6 (5) / 14	5 / 10	3 / 10	0	8	1.3333	50 : 50	50 : 50	82.500 ± 5.612 78.0 - 93.0	76.429 ± 15.034 59.5 - 103.0	9.250 ± 6.840 1.0 - 20.0	4.625 ± 2.888 1.0 - 9.0	4.625 ± 9.330 -8.0 - 19.0	13.875 ± 4.816 8.5 - 21.0	2.167 ± 1.722 0.0 - 5.0	2.600 ± 1.517 1.0 - 5.0
	total M			**27 (22) / 42**	**24 (18) / 42**	**51 (40) / 84**	**34 / 60**	**25 / 60**	**0**	**59**	**1.1569**	**50 : 50**	**52 : 48**	**77.667 ± 8.856 59.0 - 102.0**	**77.164 ± 13.937 53.5 - 107.0**	**12.669 ± 10.051 0.5 - 37.5**	**9.534 ± 8.956 0.0 - 48.5**	**3.136 ± 8.619 -14.5 - 26.0**	**22.203 ± 16.975 0.5 - 86.0**	**5.490 ± 6.655 0.0 - 31.0**	**7.000 ± 6.779 1.0 - 31.0**
	TOTAL L + R (monomorphic only)			**47 (28) / 84**	**64 (38) / 84**	**111 (66) / 168**	**108 / 120**	**93 / 120**	**6**	**207**	**1.864865**	**-**	**-**	**79.432 ± 9.030 61.0 - 103.0**	**74.236 ± 11.589 50.0 - 104.0**	**8.275 ± 7.674 0.0 - 70.5**	**8.314 ± 9.185 0.0 - 64.0**	**-0.039 ± 7.659 -31.5 - 32.0**	**16.589 ± 15.094 1.0 - 109.0**	**4.459 ± 7.260 0.0 - 44.0**	**7.500 ± 8.126 1.0 - 44.0**
	TOTAL L + R + M (mono- & polymorphic)			**74 (50) / 126**	**88 (56) / 126**	**162 (106) / 252**	**142 / 180**	**118 / 180**	**6**	**266**	**1.641975**	**-**	**-**	**78.877 ± 8.993 59.0 - 103.0**	**74.877 ± 12.174 50.0 - 107.0**	**9.250 ± 8.440 0.0 - 70.5**	**8.585 ± 9.132 0.0 - 64.0**	**0.665 ± 7.975 -31.5 - 32.0**	**17.835 ± 15.677 0.5 - 109.0**	**4.784 ± 7.071 0.0 - 44.0**	**7.311 ± 7.616 1.0 - 44.0**

Supplementary Table 2:

A
Q1

glmer(attack_strategy ~ experimental_population + habitat + (1|cage), data = missing_scales_binomial, family = "binomial")

B
Q2.1

glmmadmb(feeding_event ~ experimental_population_mixture + (1|cage), data = stomach_scales_binomial, family = "binomial")

Q2.2
glmmadmb(intestinal_scale_count ~ experimental_population_mixture + SL + offset(log(prey_predator_ratio) + (1|cage), data = subset(intestinal_scale_count_numeric > 0), family = "truncnbinom1")
glmmadmb(intestinal_scale_count ~ experimental_population_mixture + SL + (1|cage), data = subset(intestinal_scale_count_numeric > 0), family = "truncnbinom1")

Discussion

The major aim of my thesis was to improve the current state of knowledge on East African cichlid fishes in the thematic framework of comparative ecology (part one and three) and to point out the importance of phylogeographic studies of riverine cichlid taxa in the region and across Africa (part two).

In **part one** of my thesis (Comparative Ecology) I deal with the concept of convergent evolution where similar phenotypes arise in divergent lineages as a result of similar ecological and hence selective forces acting. This concept had been put forward as a major indicator for adaptive radiations (McGhee 2007) since convergent cases strongly support the ecological 'adaptiveness' in the diversity of a given trait (Osborn 1902). Further support is gained by theoretical work predicting convergence to be common in species rich communities such as adaptive radiations (Scheffer 2006, Terhorst 2010).

In **Chapter 1** (Muschick et al. 2012) we present the to date most extensive analysis of a cichlid adaptive radiation. We discover multiple instances of convergent morphologies both in body shape and trophic morphology. We also show that similar morphologies are related to a connected specific habitat and/or resource use. We show that convergent forms of distantly related species do temporally and spatially coexist. We conclude that such ecological overlap might be a possible explanation for the extremely elevated taxonomic diversity in East African cichlid species flocks.

In **Chapter 2** (Colombo et al. 2012) we applied phylogenetic, demographic, geometric morphometric, ecological and comparative gene expression data to study a case of convergent evolution in the thick lip trait of cichlid fishes. Opposed to studies focusing on species within adaptive radiations, we here compare two species from independent radiations on different continents to investigate the degree of convergence and its ecological and developmental implications between less related species belonging to separate radiations, but with a stronger focus on the convergent trait itself.
We report that very similar morphological (body shape) and ecological (preference for hard shelled food) adaptations are associated with the thick-lipped phenotype. Further comparative Illumina RNA sequencing yielded a strong set of candidate genes showing similar expression patterns in the two species, indicating similar developmental pathways, which could be independent of phylogenetic background.

Ecological background data of natural systems is a vital part of investigating adaptive radiations and convergent evolution (Gavrilets and Losos 2009). This is especially crucial if one wants to investigate radiations and speciation in general on a theoretical basis, as modelling of such events requires detailed ecological system information.

In **Chapter 3** (Dittmann et al. 2012) we infer estimates of population sizes by means of line transect surveys in two small crater lakes in Nicaragua which each harbour their own adaptive radiation with convergent forms. We found the total number of fishes to be much higher for Laguna Xiloa than Laguna Apoyo. We discuss the ecological implications of this finding, and suggest that these differences could be due to a higher trophication level or increased ecological opportunity as result of different habitat structure.The abundance and density estimates resulting from our data are a valuable source of information for future studies that aim at modelling ecological speciation and adaptive radiation.

In **part two** (Phylogeography) I combine studies emphasizing the importance of understanding the phylogeographic patterns of the cichlid fishes of the East African river system on the way to fully understand the history and extent of the stunning African cichlid diversity.

In **Chapter 4** (Meyer, Indermaur et al. 2014) we investigate the phylogenetic relationship of a newly discovered species of cichlid fish form the southern Lake Tanganyika basin. Using an extensive backbone of published sequences from a wide array of haplochromine species we were able to concisely place the species/lineage at the base of the lake Victoria Region Super Flock in sister-group position to some of the Lake Kivu species. This is surprising considering that the large radiations are thought to be of monophyletic origin and not to share any species i.e. independently evolved genetic variation among each other. A recent study based on SNP data (Loh et al. 2012) reported large amounts of genetic variation being shared across major cichlid radiations and put forward the concepts of ancient shared polymorphism or the recently popular idea of transporter species which might maintain overall genetic diversity through sequential dispersal events (Schluter & Conte 2009)
On the basis of a network analysis we reconstruct the hypothetical pathway the lineage used for it's dispersal throughout East African river systems originating well within the Lake Victoria basin and reaching Lake Tanganyika basin. To qualify, as a true transporter dispersing species must exhibit the possibility of introgression in to other systems e.g. species flocks. In the present case this question is not fully resolved but there is evidence that reproductive isolation between the lineages is not strong enough (Stelkens *et al.* 2010) qualifying the present case as valid candidate for the further study of the transporter hypothesis.

Chapter 5 (Theis et al. 2014) investigates the degree of ecological divergence of a riverine cichlid species, which are found in affluent rivers of Lake Tanganyika as well as the river deltas and suited parts of the lake proper. Along this ecological gradient several populations of *Astatotilapia burtoni* are investigated in four different replicated river systems. Genetic relations of the sampled populations are assessed and we find patterns of isolation by distance along the lakeshore while this pattern cannot be found along the rivers. Further, we find ecological divergence in body shape (streamlined body shape in relation to elevated water current) ingested foods and related trophic morphology comparing lacustrine and riverine populations. The lack of correlation between genetic and morphological traits indicates a high degree of ecological adaptation of the species to the respective habitats. Along the same lines divergence in the different river systems could be seen as first steps in convergent ecological evolution (McGhee 2007).

In **Chapter 6** (Egger et al. 2015) we studied a recently discovered population of the basal haplochromine cichlid *Pseudodrenilabrus cf. philander* from Lake Chila in northern Zambia. We discovered two distinct and phylogenetically distant mitochondrial lineages, which are neither divergent with respect to nuclear genetics nor in morphological traits (body coloration). For further examination we placed the species in a broader phylogeographic framework by greatly expanding species and location sampling from existing studies (Katongo et al. 2005, Koblmüller et al. 2012). We do not find any nuclear genetic divergence within the Lake Chila population. By observing the fish we hypothesize this lacustrine population to be ecologically divergent compared to the otherwise mainly riverine populations of *P. cf. philander*. This idea seems to be strengthened by other studies reporting considerable ecological divergence in other lake habitats colonized by *P. cf. philander* (Stelkens & Seehausen 2009)

In **part three** of my thesis I deal with a special case of morphological diversity, namely asymmetrical polymorphisms.

Chapter 7 (Indermaur et al. in preparation) is concerned with a special case of morphological variation within natural populations – asymmetry. Asymmetries are a widely studied phenomenon and are fairly common in fish (Palmer 1996, 2010, Nakajima 2004). Particularly peculiar cases of asymmetry are the endemic scale eating cichlids of Lake Tanganyika, which exhibit a lateralized feeding behaviour where individuals feed from the flanks of prey species in an optimized way by attacking only the side to which their mouth is tilted. The maintenance of this polymorphism was emphasized as a strait forward example of negative frequency dependent selection by Hori (1993) and hence gained a lot of attention. Later studies discussed dissasortative mating as mechanisms of polymorphism maintenance (Takahsasi & Hori 2008) and an array of other studies focused mainly on the morphological and ontogenetic properties of the trait putting forward the rather uni- than bimodal distribution of the trait and fact that innate handedness of young via plastic development seems to be the basis of the trait rather then genetic determination (Kusche et al 2012, Van Dooren et al. 2010).
On the background of such extensive work in the system it seems surprising that so far nothing is known about the emergences of this polymorphism. In a field experiment using the highly specialized scale-eater *P. microlepis* we here compare predator-prey dynamics of purely left respectively right morph populations against mixed morph populations. We show that mixed morph populations have greater feeding success. This finding indicates the selective advantage of a polymorphic population and thus might be the force enabling the emergence of such a symmetrical polymorphism, which is then maintained by either frequency dependent selection or dissasortative mating or both.

Summarizing the above in my thesis, I believe I was able to make substantial advancements to the understanding of the exceptionally high diversity of the East African cichlid species flocks and cichlid fishes in general.

The main findings being:

- I identified the study of convergent evolution as a mean to understand the exceptionally high species number of the East African great lakes in the light of ecological exclusion.

- I added to the understanding of the importance of riverine cichlid lineages for the patterns of past and future evolution in East African cichlid assemblages.

- I contributed to the knowledge on the selective regimes of asymmetrically polymorphic scale-eater populations from Lake Tanganyika.

References

- Colombo, M., Diepeveen, E. T., Muschick, M., Santos, M. E., Indermaur, A., Boileau, N., et al. (2012). The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. Molecular Ecology, n/a–n/a. doi:10.1111/mec.12029

- Dittmann, M. T., Rösti, M., Indermaur, A., Colombo, M., Gschwind, M., Keller, I., et al. (2012). Depth-dependent abundance of Midas Cichlid fish (Amphilophus spp.) in two Nicaraguan crater lakes. Hydrobiologia. doi:10.1007/s10750-012-1024-1

- Katongo, C., Koblmüller, S., Duftner, N., Makasa, L., & Sturmbauer, C. (2005). Phylogeography and speciation in the Pseudocrenilabrus philander species complex in Zambian Rivers. Hydrobiologia, 542(1), 221–233. doi:10.1007/s10750-004-1389-x

- Koblmüller, S., Katongo, C., Phiri, H., & Sturmbauer, C. (2012). Past Connection of the Upper Reaches of a Lake Tanganyika Tributary with the Upper Congo Drainage Suggested by Genetic Data of Riverine Cichlid Fishes. African Zoology, 47(1), 182–186. doi:10.3377/004.047.0115

- Gavrilets, S., & Losos, J. B. (2009). Adaptive Radiation: Contrasting Theory with Data. Science.

- Hori, M. (1993). Frequency-dependent natural selection in the handedness of scale eating cichlid fish. Science, 260, 216–219.

- Indermaur, A., Theis, A., Egger B., Salzburger, W. 2014 A field based assessment of attack strategy and feeding success in the scale eating cichlid Perissodus microlepis (Perciformes, Cichlidae); in preparation

- Indermaur A. Meyer B. Egger B. Salzburger W. 2014 Back to Tanganyika: a case of a recent immigration into a species-flock of East African cichlid fishes

- Kusche, H., Lee, H. J., & Meyer, A. (2012). Mouth asymmetry in the textbook example of scale-eating cichlid fish is not a discrete dimorphism after all. Proceedings of the Royal Society B: Biological Sciences, 279(1748), 4715–4723. doi:10.1111/j.1420-9101.2008.01539.x

- Loh, Y. H. E., Bezault, E., Muenzel, F. M., Roberts, R. B., Swofford, R., Barluenga, M., et al. (2013). Origins of Shared Genetic Variation in African Cichlids. Molecular Biology and Evolution, 30(4), 906–917. doi:10.1093/molbev/mss326

- McGhee, GR (2007). The geometry of evolution : adaptive landscapes and theoretical morphospaces. Cambridge, UK ; New York, Cambridge University Press.

- Muschick, M., Indermaur, A., & Salzburger, W. (2012). Convergent Evolution within an Adaptive Radiation of Cichlid Fishes. Current Biology, 22(24), 2362–2368. doi:10.1016/j.cub.2012.10.048

- Nakajima, M., Matsuda, H., and Hori, M. 2004 Persistence and Fluctuation of Lateral Dimorphism in Fishes. The American Naturalist , Vol. 163, No. 5 (May) , pp. 692-698

- Osborn, HF (1902). The law of adaptive radiation. American Naturalist 36: 353-363.

- Scheffer, M., and van Nes, E.H. (2006). Self-organized similarity, the evolutionary emergence of groups of similar species. Proc. Natl.
Acad. Sci. USA 103, 6230–6235. 13.

- Schluter D, Conte GL. 2009. Genetics and ecological speciation. Proc Natl Acad Sci U S A. 106:9955–9962.

- Stelkens, R. B., & Seehausen, O. (2009). Phenotypic divergence but not genetic distance predicts assortative mating among species of a cichlid fish radiation. Journal of Evolutionary Biology, 22(8), 1679–1694. doi:10.1111/j.1420-9101.2009.01777.x

- Rike B. Stelkens, R.B., Young, K.A. and Seehausen, O. 2010 The Accumulation of reproductive incompatibilities in African cichlid fish. Evolution vol. 64 (3) pp. 617-633

- Terhorst, C.P., Miller, T.E., and Powell, E. (2010). When can competition for resources lead to ecological equivalence? Evol. Ecol. Res. 12,843–854.

- Takahashi, T., & Hori, M. (2008). Evidence of disassortative mating in a Tanganyikan cichlid fish and its role in the maintenance of intrapopulation dimorphism. Biology Letters, 4(5), 497–499. doi:10.1046/j.1365-294x.2000.00964.x

- Theis* A., Ronco* F., Indermaur A., Salzburger W. and Egger B. Divergence between lake and stream habitats in an East African cichlid fish Submitted to Axios Review, January 10th, 2014

Acknowledgements

These past years have been such an amazing and intense time, words can hardly describe my feelings…

First, and foremost I am very grateful to **Prof. Dr. Walter Salzburger** for introducing me to this lovely model system, which has become so dear to me, and for giving me the opportunity to do my PhD in such a warm and inspiring environment. I will never forget your support and your trust.
Secondly I am thankful to **Dr. Ulrich Schliewen (ZSM)** for taking the time co-examining my thesis and for all the great discussions we had and hopefully will have in the future.

I owe a great deal of gratitude to the entire Salzburger-lab, especially all my co-workers and co-authors over the years. I have learned so much from all of you and I will never forget the times in the laboratory, the field and the office. A special thank you to **Moritz Muschick, Britta Meyer, Bernd Egger, Anya Theis, Zuzana Musilová, Emília Santos, Astrid Böhne, Yuri Kläfiger, Michael Matschiner, Marco Colombo, Marius Rösti, Marie Dittmann, Corina Heule, Joost Raeymaekers, Hugo Gante, Florian Meury, Dario Moser and Eveline Diepeveen**. Without you, this thesis wouldn't have been possible. Merci beaucoup, aussi a **Nico Boileau** et **Brigitte Aeschbach** pour votre aide, collaboration et votre amitié!
For making field work all around the world such a great experience and a huge motivation, I thank Gilbert Tembo, Blessings Mengo, Charity Muwene and Lawrence Makasa in Mpulungu Zambia; Arnold Bitja Nyom, Ngando Jiku and Cyrille Dening in Cameroon and Marta Barluenga and Eric van den Berghe in Nicaragua.
I would like to express my gratitude for funding to the **European Research Council (ERC)**, the **Freiwillige Akademische Gesellschaft Basel (FAG)**, the **National Geographic Society (NGS), the Swiss Zoological Society (SZS)** and the **University of Basel,** without their support all this work would not have been possible.
A big cheers, goes to my "band" members for being great friends and providing a retreat from madness with great music, great discussions and the "occasional" beer. You Rock! Bernd "Speed Finger" Egger, Groovy Walter Salzburger and Blues Voice Yuri Klaefiger.
Many thanks also go out to **Dr. Dr. hc Heinz Büscher,** and **Angel M. Fitor,** two great friends, which have inspired me with their interesting discussions and love for nature's most divine creatures – fish! Thanks to **Zuzana Musilová** for taking me to Cameroon.

The biggest thank you, with a white unicorn on top goes to my office mates and best friends; **Anya Theis**, **Britta Meyer** and **Emília Santos**. I would have not made it through without your magic support, craziness and the massage training! E0.04 forever - You are awesome!

Last but certainly not least I am endlessly grateful to my parents, **Robert and Barbara** and my siblings, **Alex and Rebecca** for their great support on every level imaginable, all the cheering words and the freedom of leading my own live. Thank you so much - I love you!

Curriculum vitae

Adrian Urs Marius Indermaur

Born on:	January 31, 1984 (in Chur, CH)
Address (private):	Hammerstrasse 40, CH-4058 Basel
E-Mail:	a.indermaur@unibas.ch
Homepage:	http://evolution.unibas.ch/salzburger/team/aindermaur/
Phone, office:	061 267 03 04
Phone, privat:	077 440 12 17

Education

1991 - 2003	Primary School and High School in Rodels / Paspels (GR),Matura (2003); Kantonsschule Chur, Switzerland
2004 - 2007	Undergratuate studies in „Organismal Biology“ at University of Basel, Switzerland
2007 - 2009	**Master thesis:** „Evolution of genetic and morphological adaptations in the tribe Tropheini (Cichlidae, Perciformes)“, supervised by: Prof. Dr. Walter Salzburger at Zoology, University of Basel
2009 - 2014	**PhD thesis:** supervised by Prof. Dr. Walter Salzburger and funded by the Starting Grant “INGERGENADAPT”, European Research Council (ERC) at Zoology, University of Basel
2010	Education and Training as Person Conducting Animal Experiments; Institute for Laboratory Animal Science, University of Zürich; FELASA accreditation Nr. 027/08
2011	LTK Module 20E: Introductory Course in Laboratory Animal Science “Less usual species” (continuing education; 2 days)

Publications

Published

- Dittmann MT, Roesti M, **Indermaur A**, Colombo M, Gschwind M, Keller I, Kovac R, Barluenga M, Muschick M & Salzburger W (2012). *Depth-dependent abundance of Midas Cichlid fish (Amphilophus spp.) in two Nicaraguan crater lakes*. Hydrobiologia 686: 277-285.

- Colombo M*, Diepeveen ET*, Muschick M, Santos ME, **Indermaur A**, Boileau N, Barluenga M, Salzburger W (2012) *The ecological and genetic basis of convergent thicklip-phenotypes in cichlid fishes*. Molecular Ecology 22 670-684. **These authors contributed equally to this work*.

- Muschick M, **Indermaur A**, Salzburger W (2012) *Convergent Evolution within an Adaptive Radiation of Cichlid Fishes.* Current Biology, Volume 22, Issue 24, 18 December 2012, Pages 2362–2368.

- Musilová Z, **Indermaur A**, Nyom A R B, Tropek R, Martin C, & Schliewen U K (2014). Persistence of stomatepia mongo, an endemic cichlid fish of the Barombi Mbo crater lake, Southwestern Cameroon, with notes on its life history and behavior. Copeia, 2014(3), 556-560.

- Theis A, Ronco F, **Indermaur A,** Salzburger W, & Egger B (2014). Adaptive divergence between lake and stream populations of an East African cichlid fish. Molecular ecology, 23(21), 5304-5322.

- **Indermaur A**, Meyer B S, Ehrensperger X, Egger B, Banyankimbona G, Snoeks J & Salzburger, W. (2015). Back to Tanganyika: a case of recent trans-species-flock dispersal in East African haplochromine cichlid fishes. Royal Society open science, 2(3), 140498.

- Egger B, Klaefiger Y, **Indermaur A**, Koblmüller S, Theis A, Egger S, ... & Salzburger W (2015). Phylogeographic and phenotypic assessment of a basal haplochromine cichlid fish from Lake Chila, Zambia. Hydrobiologia, 748(1), 171-184.

- Boileau N, Cortesi F, Egger B, Muschick M, **Indermaur A**, Theis A, ... & Salzburger W (2015). A complex mode of aggressive mimicry in a scale-eating cichlid fish. Biology letters, 11(9), 20150521.

In Preparation

- **Indermaur A**, Theis A, Egger B, Salzburger W. A field based assessment of attack strategy and feeding success in the scale eating cichlid Perissodus microlepis (Perciformes, Cichlidae); in preparation

Meetings, Conferences and Communications

Participant

- Biology 2008, (University of Lausanne, Switzerland)
- Cichlidscience 2010, (University of Basel, Switzerland), organizing committee
- ESEB 2012, (University of Tübingen, Germany)
- Biology 2013 (University of Basel, Switzerland)

Talks

- Biology 09 (University of Bern, Switzerland), talk: Evolution of genetic and morphological adaptations in the tribe Tropheini (Perciformes; Cichlidae)

- SIAL 6 „Speciation in Ancient Lakes“ (2012), Classic concepts & new approaches (LIPI Research Center for Biology, Cibinong, Bogor, Indonesia) talk: „Back to Tanganyika – Migration between closed systems“

Posters

- Cichlidscience 2012 (University of Leuven, Belgium), poster: „Back to Tanganyika – Migration between closed systems“

- Exhibiton of the Center for Africa Studies 2012 (University of Basel, Switzerland), poster: „Back to Tanganyika – Migration between closed systems“

- Fifth International Conference of the Pan African Fish and Fisheries Association (PAFFA5) 2013 (Bujumbura, Burundi), poster: „Back to Tanganyika: a case of a recent immigration into a species-flock of East African cichlid fishes“

Prices / Awards

- First price for African Fish Identification at the Fifth International Conference of the Pan African Fish and Fisheries Association (PAFFA 5) 2013 (Bujumbura, Burundi)

Fieldwork, Expeditions and Staying Abroad

- 1989/90: Residence stay in Sonoma, California; USA (15 months)
- 2004-12: Several independent travels to South-East Asia and Central Amerika (17 months in total)
- 2008: Sampling expedition to Mpulungu, Lake Tanganyika, Zambia (4 weeks)
- 2009: Student excursion to the Nicaraguan Crater Lakes, Nicaragua (3 weeks)
- 2010: Sampling expedition to Mpulungu, Lake Tanganyika, Zambia (4 weeks)
- 2011: Sampling expedition to Mpulungu with experimental fieldwork, Zambia (3 month)
- 2011: National Geographic expedition along the southern Tanzanian coast of Lake Tanganyika, Zambia (3 weeks)
- 2012: Experimental fieldwork in Mpulungu, Zambia (3 weeks)
- 2012: Extensive sampling in rivers throughout northern Zambia (5 weeks)
- 2013: Sampling expedition to Barombi Mbo, Western Cameroon (3 weeks)
- 2013: Sampling trip and assistance on student excursion in Mpulungu, Zambia (4 weeks)
- 2014: 2 Sampling expeditions to Western Cameroon (3 weeks each)
- 2014: Sampling trip to Mpulungu, Zambia (4 weeks)
- 2015: Sampling expeditions to Burundi, Tanzania and Zambia (16 weeks)

Teaching assitance and Organization

- Assistant to the student excursion of the Zoological Institute, University of Basel to the Nicaraguan Crater Lakes 2009 (4 weeks)

- Co-Organizer of the „Cichlid Science 2010“ - Meeting at the Zoological Institute, University of Basel 2010 (2 weeks)

- Teaching assistant to the student excursion of the Zoological Institute, University of Basel to southern Lake Tanganyika 2011 (4 weeks)

- Teaching assistant to the student excursion of the Zoological Institute, University of Basel to southern Lake Tanganyika 2013 (3 weeks)

- Project supervision „Blockurs: Zoology“: Laterality in scale eating cichlid *Perissodus microlepis*. University of Basel 2013; with Anya Theis (4 weeks)

Grants and fund raising

- 2008: Fieldwork in Zambia supported by: SCNAT+ travel grant, supporting MSc students:

1800 sFr.

- 2010: PhD Meeting (Cichlid Science 2010) supported by: Universität Basel, Nachwuchsförderung, Beiträge an Nachwuchsveranstaltungen und Albrecht'scher Reisefonds für Gastreferent / Gastreferentin im Rahmen von Graduierten-veranstaltungen:

8000 sFr.

- 2010: Fieldwork in Zambia supported by: SCNAT+ travel grant, supporting MSc students:

2000 sFr.

- 2012: SIAL meeting in Bogor Indonesia, supported by: Nachwuchsförderung der Universität Basel, Reisefonds:

1900 sFr.

- 2013: Abschlussfinanzierung des Doktorats, Freiwillige Akademische Gesellschaft Basel (FAG)

15000 sFr.

Additional skills

Labratory

- Basic Laboratory conduct (Rules and Behavior)
- Standard genetic sequencing and microsatellite methods (eg. PCR, Purifications, Sequencing etc.)
- Quantitative realtime PCR (qPCR)
- Advanced microscopy and binocular skills

Computer

- Advanced knowledge of basic applications (Excel, Pages, Powerpoint, Keynote, Adobe
- Illustrator and Photoshop... etc)
- Basic knowledge of comandline based applications such as Terminal, R and more)
- Generaly good knowledge and handling of IT applications

Fishkeeping

In depth knowledge of freshwater fishkeeping (development, maintenance and handling of large fish facilities)

Languages

German:	mothertongue	
English:	Spoken: fluently	Written: very good
French:	Spoken: good	Written: good
Spanish:	Spoken: basic	Written: basic
Italian:	Spoken: basic	Written: basic

Miscellaneous

- PADI Divemaster since 2010 (Nr: 264682)
- Swiss drivers license (Kat. B) since 2002