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Evolution

Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika

(Eretmodini/phylogeny/adaptive radiation)

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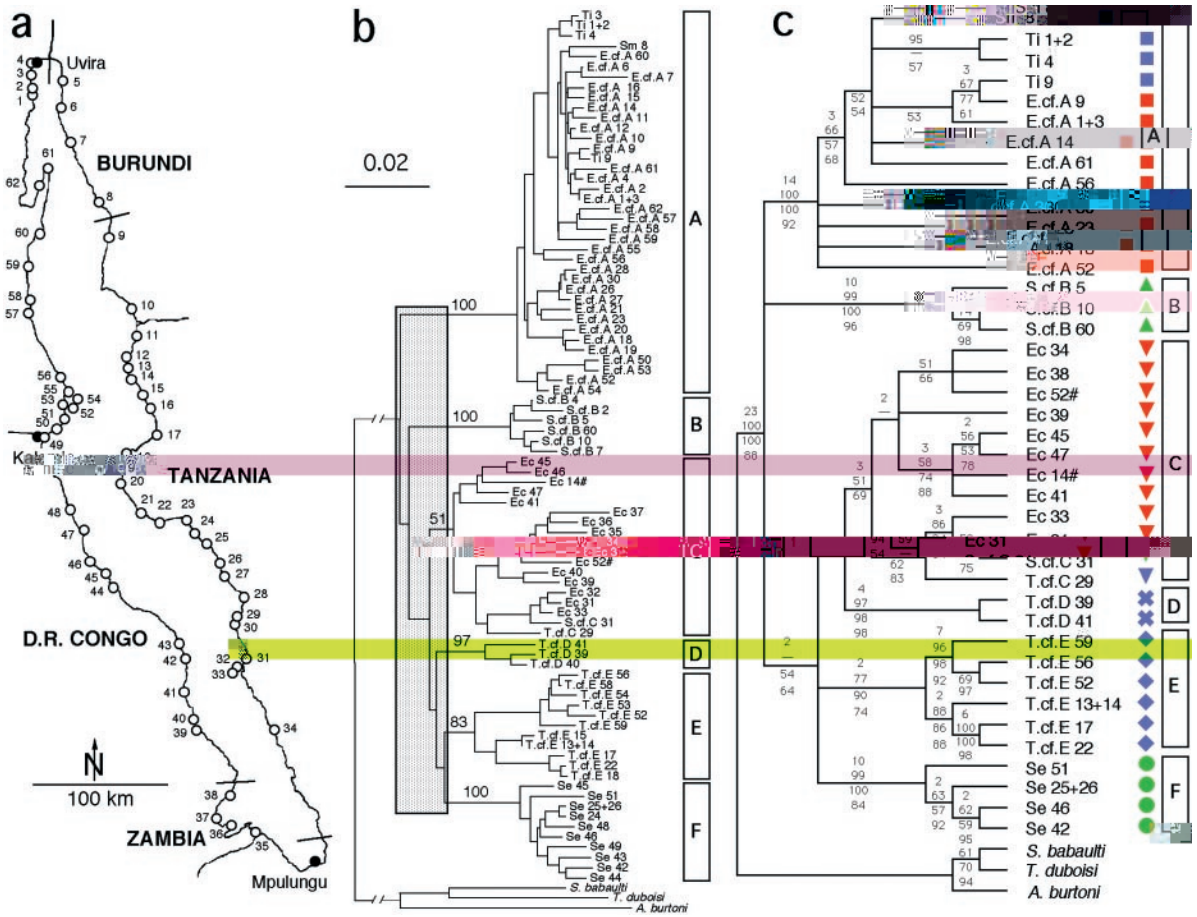


FIG. 1. (*a*) Mp of Lke Tngnyik showing the loclities studied. Circles in bold indicte type loclities: Uvir, *T. irsacae* nd *S. marlieri*; Mpulungu, *E. cyanostictus*; Klemie, *S. erythrodon.* Fishes from lineges where the distribution does not include the type loclity re referred to s: *genus name* cf. *species name.* (*b* nd *c*) Phylogenetic nlyses using combined dt set of prtil cyt *b* nd control region sequences. Loclity numbers re given behind species nmes tht re bsed on the current txonomy (9). Ec, *E. cyanostictus*; E.cf.A., *E.* cf. *cyanostictus* (linege A); Se, *S. erythrodon*, S.cf.B or C, *S.* cf. *erythrodon* (lineges B nd C respectively); Sm, *S. marlieri*; Ti, *T. irsacae*; T.cf.C, D, or E, *T.*cf. *irsacae* (lineges C, D, or E, respectively). Ec (14)# nd Ec (52)# indicte distinct tx with n *Eretmodus*-like dentition thn E.cf.A (14) nd E.cf.A (52). They differ in colortion (33) nd in the number of tooth groups nd teeth per group (15). Published sequences (cyt *b*/control region) from *Tropheus duboisi* (Z12039/Z12080), *Simochromis babaulti* (Z12045/U40529), nd *Astatotilapia burtoni* (Z21773/Z21751) were used s outgroups (34–36). The ssignments to the six mjor lineges (A–F) re given in boxes. (*b*) NJ phylogrm of the 90-tx dt set. Bootstrp vlues re shown only for the six mjor lineges (A–F). Shded box highlights the time window in which the six eretmodine lineges originted. Br scle indictes the inferred number of nucleotide substitutions. (*c*) Strict consensus tree of the MP nd the NJ nlyses using the 44-tx dt set. Bootstrp vlues ≥50% for the MP nlysis nd decy indices >1 re shown bove brnches. Bootstrp vlues ≥50% for the NJ nlysis nd qurtet-puzzling support vlues re shown below brnches. Different symbols follow the ssignments to lineges A–F (red, *Eretmodus*-; green, *Spathodus*-; nd blue, *Tanganicodus*-like dentition type).

PCR nd direct sequencing of two mtDNA gene frgments by using stndrd methods (13). The two primer pirs used to mplify portion of the cytochrome *b* (cyt *b*) gene nd of the proline tRNA with segment of the control region re given in refs. 17 nd 18.

Phylogenetic Reconstruction and Hypotheses Testing. A totl of 338 bp of the control region nd 363 bp of the cyt *b* were ligned nd combined for ll further nlyses. Gps in the control region were treted s missing dt. We conducted the nlyses in two steps. First, we constructed neighbor-joining (NJ; ref. 19) tree with ll 90 specimens by using TREECON Version 1.3b (20). Second, we used smller dt set with representtive subset of 44 specimens from 34 loclities. This dt set ws nlyzed with the mximum prsimony (MP; ref. 21) nd NJ methods by using PAUP* Version 4.0d64 (21). Heuristic serches (TBR brnch swpping, MULPARS option effective, nd rndom stepwise ddition of tx with 10 replictions) were used to find the most prsimonious trees. NJ ws performed bsed on Kimur two-prmeter corrected distnces (22) s in the first step of the nlysis. In ddition, heuristic mximum likelihood (23) tree serch procedure ws performed by using the qurtet-puzzling lgorithm in PUZZLE Version 3.1 (24) by using the defult options with 1,000 puzzling steps.

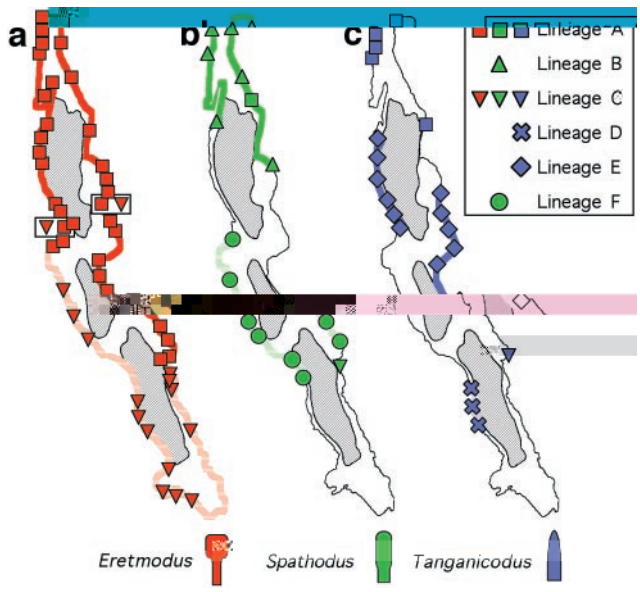
Phylogenetic reltionships were lso exmined by introducing different chrcter-stte weighting schemes for trnsitions nd trnsversions in the MP nlyses s well s by successive chrcter reweighting bsed on the rescled consistency index (25) by using the unweighted MP consensus tree s the strting tree. Robustness of the inferred MP nd NJ trees ws tested by using the bootstrp method (26) with 500 resmplings for the MP nlysis nd 500 nd 1,000 resmplings for the NJ nlyses of the 90 tx nd the 44 tx dt set, respectively. Decy indices (27) were clculted for the MP trees s n index of support (28) by using AUTODECAY Version 3.0.3 (29). Competing phylogenetic hypotheses were compred by using the Templeton (30) nd Kishino-Hsegw (31) tests s implemented in PAUP*. To exmine the evolution of trophic speciliztion in eretmodine cichlids, we mpped tooth shpes (treted s unordered chrcters with three sttes) onto phylogenetic hypotheses by using MACCLADE Version 3.06 (32).

RESULTS

Sequence Variation. Of 158 vrible sites (63 nd 95 for the cyt *b* nd the control region, respectively) identified mong the 40 different hplotypes from the combined cyt *b* nd control

Lineges A nd C re the only two lineges tht contin individuls with different trophic morphologies. In linege C, which is dominted by cichlids with n *Eretmodus*-like dentition, we found *Tanganicodus*-like dentition t loclity 29 (Fig. 1*a*) s well s few kilometers north of tht loction (13, 14) nd *Spathodus*-like dentition t loclity 31 (Fig. 1 *a*). The *Eretmodus*-like-dominted linege A contins the scrce species *S. marlieri*, which occurs in different, intermedite snd rock hbitts nd t greter depth thn other eretmodine species (38), nd *T. irsacae*, both of which show n berrnt tooth morphology for linege A nd re found only in the northernmost prt of the lke (Fig. 3). From these specimens, new tissue smples were tken nd resequenced to confirm their hplotypes.

The presence of multiple orl tooth shpes within single mtDNA linege s found in linege A nd C is not likely to result from phenotypic plsticity s response to different hbitt use. Although phenotypic plsticity in the lower phryngel jws hs been documented in cichlids (39–41), we re not wre of reported cses tht involve the shpe of orl jw teeth. Moreover, fishes with different tooth shpes lso differ



FIG

concomitntly in body shpe (L.R. nd D. C. Adms, unpublished dt), nd tnk-bred individuls kept on n identicl diet retin their orl tooth shpes (L.R., unpublished dt), indicting tht orl tooth shpe in eretmodine cichlids hs strong genetic component.

A second hypothesis to explin the occurrence of multiple orl tooth shpes within single mtDNA linege is hybridiztion. Experimentlly produced hybrids between two Lke Mlwi hplochromines tht differ in trophic morphology showed mosic of prentl, intermedite, nd unique ptterns of morphologicl expressions (42). All specimens from linege A nd C with either *Spathodus*- or *Tanganicodus*-like tooth shpe (Fig. 2) showed no morphologicl fetures of either linege A or C *Eretmodus*-like specimen. Therefore, it seems unlikely tht recent hybridiztion or pst introgression of mtDNA hplotypes into clde with different tooth morphology cn explin these results. Although unlikely, this possibility needs to be ddressed in future studies in which the morphology of hybrids is compred with tht of prentl species nd nucler mrkers re used to evlute whether hybridiztion hs hd n impct on the observed pttern.

Our results llow us to sttisticlly reject the trditionl hypothesis (12) tht specimens with identicl trophic specilizations, such s the shpe of their orl jw teeth, re derived from single immedite common ncestor. None of the three tooth-shpe types (*Eretmodus*-, *Spathodus*-, nd *Tanganicodus*-like) ws resolved monophyleticlly (Tble 1), nd t lest eight evolutionry trnsitions between tooth shpe types occurred (Fig. 2).

Phylogeographic Patterns, the Geological History of Lake Tanganyika, and Morphological Differentiation. Eretmodine cichlids re restricted long shllow rocky nd pebble shores nd re unble to disperse cross open wter. Ech of the six eretmodine lineges shows limited distribution within the lke (Figs. 1 nd 3). The high degree of intrlcustrine endemism nd the pronounced phylogeogrphic structuring of eretmodines cn be prtly explined by the influence of mjor lke level fluctutions in the Pleistocene tht re generlly ssumed to hve hd strong influence on phylogeogrphic ptterns nd specition of rock-dwelling cichlids (34, 43). During this time, the single lke bsin of Lke Tngnyik split up into three isolted sub-bsins (shown in gry in Fig. 3; refs. 44 nd 45); this event is still reflected in the distribution of mtDNA lineges.

The northern nd southern shorelines of ech of these sub-lkes might hve permitted dispersl nd gene flow between cichlid popultions from western to estern cost lines or *vice versa.* The occurrence of some lineges on both opposite shores of the lke (e.g., linege E nd F; Fig. 3) cn best be explined by this route of gene flow (43). The formtion of the six distinct eretmodine lineges ppers to hve occurred within brief period of time (Fig. 1 *b*), probbly before the onset of the lke level fluctutions in the Pleistocene.

In ddition to the influence of lke level fluctutions on the geogrphic distribution of eretmodine mtDNA lineges, sev-

erl interesting ptterns emerge when distributions re viewed in conjunction with the phenotypes tht chrcterize certin lineges (Fig. 3). Eretmodine cichlids with identicl trophic morphologies from different mtDNA lineges in generl revel nonoverlpping distribution. Those with *Eretmodus*-like dentition (shown in red) from lineges A nd C hve complementry lke-wide distribution (Fig. 3 *a*). We found only two loclities where these morphologiclly nd geneticlly distinct *Eretmodus*-like specimens occur symptriclly (Fig. 1). Specimens with *Spathodus*-like dentition (green) from lineges B nd F show strict complementry distribution. Only *S. marlieri* from linege A is found within the distribution rnge of *S*. cf. *erythrodon* from linege B (Fig. 3 *b*). Specimens with *Tanganicodus*-like dentition (blue) from lineges A nd C–E lso show complementry distributions (Fig. 3 *c*).

In most prts of the lke, fish with two distinct tooth types from two different mtDNA lineges cn be found symptricly (Fig. 3). This is the cse for the rnge covered by lineges D–F. Not considering the *Spathodus*- nd *Tanganicodus*-like fishes from linege A, this pttern would extend nd include the distribution of linege B. The lloptric distributions of *S.* cf. *erythrodon* (linege B), *T.* cf. *irsacae* (linege E), *S. erythrodon* (linege F), nd *T*. cf. *irsacae* (linege D) re shown in Fig. 3 *b* nd *c*. These lineges re found symptriclly with either *E.* cf. *cyanostictus* from linege A or *E. cyanostictus* from linege C. In the southernmost prt of the lke (loclity 33–39, Fig. 1*a*) *E. cyanostictus* is the only eretmodine found (33).

Ecological Causes of Recurrent Parallel Evolution and Adaptive Radiations. The phylogenetic nlysis nd the phylogeogrphic distribution of mtDNA lineges refutes the ssumption tht the presence of similr pirs of trophic specilists (*Eretmodus*-like with either *Spathodus*- or *Tanganicodus*-like dentition type) evolved only once nd tht subsequently they colonized other costlines. The dt support the hypothesis tht lineges with identicl trophic morphology evolved independently nd concurrently in different prts of Lke Tngnyik. The multiple independent evolution of identicl tooth shpes, s indicted in Fig. 2, suggests recurrent prllel evolution of ecologiclly importnt morphologicl trits between closely relted species within the sme lke bsin nd chllenges the current pproch of cichlid txonomy, becuse it often relies, sometimes exclusively, on chrcters relted to feeding, such s dentition nd tooth morphology.

The phylogeogrphic distributions of the six mtDNA lineges nd the phylogenetic mpping of the morphologicl trits revel ptterns tht suggest tht not just vicrince events, such s mjor lke-level fluctutions, hve been responsible in shping the intrlcustrine distribution of eretmodine cichlids. Our dt show consistent pttern in morphologicl divergence in dentition of symptric species pirs. The lloptric distribution of geneticlly distinct lineges tht re chrcterized by similr trophic morphology strongly suggests tht ecologicl processes, such s competitive exclusion, tht cn ply centrl role in structuring communities (46) between two species (different mtDNA lineges) with the sme tooth morphology might be responsible for this pttern of species distributions. Moreover, over wide rnge of the lke's shores, symptriclly occurring eretmodine species pirs re found. In generl, species pir contins members of two distinct mtDNA lineges, nd in ddition, the species of such pir show consistent differences in orl tooth shpe, with one species hving n *Eretmodus*-like dentition nd the other either *Spathodus*- or *Tanganicodus*-like dentition. In different res of the lke, however, these morphologicl species pirs belong to different mtDNA lineges (Fig. 3).

Differences in trophic morphology, such s tooth shpe, in closely relted fishes or ecomorphs of the sme species re often correlted with trdeoffs for resource use (47, 48). The distinct tooth morphologies found in eretmodine cichlids re correlted with differences in diet (10, 11). The repeted formtion of morphologiclly distinct pirs of species in different prts of Lke Tngnyik suggests tht ecologicl diversifiction my be mjor driving force behind morphologicl differentition nd evolutionry divergence in these fishes. Similr ptterns hve been found in postglcil fishes inhbiting lcustrine environments tht hve led to ecologicl specition (2, 6). Further ecologicl studies might increse our understnding of the dptive vlue of orl tooth shpe in eretmodine cichlids (by evluting how species with different tooth shpes differ in hbitt use nd in efficiencies of trophic resource exploittion) nd how differentition in trophic morphology might hve fcilitted the coexistence of lineges. These ecologicl dt would lso provide informtion on the

Z97444; 45, Z97528/Z97442; 46, Z97529/Z97443; 48, Z97526/Z97441; 49, Z97522/Z97440; 51, Z97521/Z97439.

Ti: 1, Z97539/Z97450; 2, Z97540/Z97451; 3, Z97541/Z97452; 4, Z97542/Z97449; 9, Z97538/X90596.

T.cf.C: 29, Z97555/X90603.

T.cf.D: 39, Z97557/Z97459; 40, Z97556/Z97460; 41, Y15133/Y15134.

T.cf.E: 13, Z97549/X90597; 14, Z97550/X90598; 15, Z97551/X90628; 17, Z97552/X90599; 18, Z97553/X90600; 22, Z97554/X90601; 52, Z97546/Z97458; 53, Z97547/Z97456; 54, Z97548/Z97457; 56, Z97545/Z97455; 58, Z97544/Z97454; 59, Z97543/Z97453.

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