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On the origin of the *Synodontis* catfish species flock from Lake Tanganyika

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Species flocks within Great Lakes provide unique insights into the factors affecting diversification. Lake Tanganyika (LT) is of particular interest because it contains many endemic groups for which general factors affecting diversification can be discerned. Here, we present the first phylogenetic study of the LT *Synodontis* (Siluriformes, Mochokidae) species flock using mtDNA sequence data. Our data reveal some previously unrecognized species diversity and indicate that the LT species flock is not monophyletic, and that two closely related clades of endemics may have independently colonized LT. Other comparable small species flocks are characterized by a single colonization event. Molecular date estimates of the timing of the initial within-lake diversification of the LT endemics, based on a fossil calibration, are comparable to those reported for other groups, suggesting that extrinsic factors maybe important common causes of clade diversification. The basal divergence in the sampled *Synodontis* reveals an East–West African faunal split seen in many terrestrial, but few aquatic groups, the timing of which coincides with East African rifting events.

Keywords: speciation; ancient lakes; diversification; molecular clock; cichlids

1. INTRODUCTION

The Great Lakes of East Africa are renowned for the rapid diversification of their endemic cichlid faunas. These adaptive radiations provide excellent systems for studying factors that influence speciation (Schluter 2000). Lake Tanganyika (LT) is the oldest (9–12 Myr old) of the Great Lakes, with a complex geological history and repeated fluctuations in lake level (Cohen *et al.* 1997) considered important for speciation (Sturmbauer *et al.* 2001). It is also distinctive in harbouring many endemic faunas besides cichlids (Coulter 1991) that make Tanganyika an excellent system for comparing radiations of different sympatric groups and investigating the extent to which these have been affected by their shared environmental history. However, reconstruction of the evolutionary histories of the non-cichlid species flocks has received comparatively little attention (West & Michel 2000). One overlooked LT species flock includes seven species of *Synodontis* catfish, six of which are endemic (Poll 1971), which is comparable to other small LT radiations (Coulter 1991). *Synodontis* (*ca* 116 described species) are distributed throughout tropical Africa, but of the other Great Lakes, only Lake Malawi (LM; 4–5 Myr old) harbours one (possibly two; Snoeks 2004) endemics, while Lake Victoria (LV; 12 400 kyr) has two non-endemics.

LT cichlids are both polyphyletic, implying independent colonizations of the lake, and paraphyletic, demonstrating that radiations within the lake have subsequently become a source for secondary seeding of rivers and younger lakes (Salzburger *et al.* 2002). In contrast, *Platytelphusidae* crabs (Marijnissen *et al.* 2006) and *Cleopatra* gastropods (West & Michel 2000) are monophyletic, consistent with single origins and no dispersal out of Tanganyika. A current phylogeny is lacking for East African *Synodontis* and thus it is not known whether the LT species flock is a single radiation. Molecular markers have undermined the traditional taxonomies of some other LT groups (e.g. Rüber *et al.* 1999), but have not yet been applied to *Synodontis*, so that the extent of phylogenetic diversity of the LT flock is unclear. Here, mtDNA data are used to test current species concepts and to infer a dated phylogeny for lacustrine and fluviatile species of primarily East African *Synodontis*. The phylogeny is used to test alternative hypotheses of the evolutionary history of *Synodontis* in East Africa, and in particular, whether the LT *Synodontis* constitute a single radiation. *Synodontis* catfish provide a parallel system that can be compared to other lake faunas to test the extent to which there are common patterns and processes of diversification in LT.

2. MATERIAL AND METHODS

Details of taxon sampling, voucher specimens and GenBank accession numbers are given in the electronic supplementary material. Polymerase chain reactions were used to amplify the complete cytochrome *b* (approx. 1200 bp) using published primers (see Hardman & Lundberg (2006) and references therein), with varied annealing temperatures (49–55°C). Following amplification and purification, the samples were cycle sequenced and analysed using an ABI 3730 sequencer.

Presumed orthologous DNA sequences (lacking stop codons or frameshifts) were aligned manually. Bayesian inferences (BI) used Markov Chain Monte Carlo (MCMC) sampling from 10^6 generations, every 100 generations (four chains, temperature 0.2°C, burn-in 50 000 generations), under the general time-reversible (GTR)+I+Γ model (selected using the Akaike Information Criterion in MODELTEST v. 3.7; Posada & Crandall 1998) implemented in MRBAYES v. 3.1.1 (Ronquist & Huelsenbeck 2003). An additional partitioned analysis allowed the faster-evolving third codon positions a partition-specific model. Maximum likelihood (ML) was performed using PHYML v. 2.4.4 (Guindon & Gascuel 2003) with the available model (GTR) closest to that selected in MODELTEST. Maximum-parsimony (MP) analyses (500 replicates, TBR branch swapping) and neighbour joining (NJ, using ML distances with model parameters from MODELTEST) were implemented using PAUP* (Swofford 2002). Branch support was evaluated using non-parametric bootstrapping (1000 pseudoreplicates) and Bayesian posterior probabilities. PAUP* was used to search for optimal constrained trees consistent with, and to perform Kishino and Hasegawa (KH) tests of, *a priori* hypotheses of the monophyly of: (i) the LT flock; (ii) the LT endemics; (iii) East African species; and (iv) West and Central African species.

The earliest *Synodontis* is 21.8–16.6 Myr old (Burdigalian), but younger fossils cannot be reliably assigned to extant species

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(Pinton *et al.* 2006). Molecular divergence time estimates were calculated using a pruned tree (eliminating intraspecific data), assuming either 21.8 or 16.6 Myr as the age of *Synodontis*, and using the optimal smoothing parameter value, determined through cross-validation in penalized likelihood as implemented in r8s (Sanderson 2003). Confidence limits on age estimates were based on two MCMC chains sampling 500 generations and enforcing topological constraint.

3. RESULTS

All methods recover the same phylogeny (figure 1). NJ aside, branch support for species or putative species and their interrelationships is strong (table 1). Thus, the phylogeny appears to provide a good framework for discussing the evolutionary history of *Synodontis* and for inferring a molecular time-scale (figure 2). Sequence divergence (0.34–0.47% per million years per lineage) is comparable with some other catfish groups (Hardman & Lundberg 2006). Traditional taxonomy is supported except that *Synodontis petricola* (north) are widely separated from *S. af. petricola* (south), with the latter most likely an undescribed species. The ubiquitous brood parasite, *Synodonti multipunctatus*, displays little genetic variation or indication of population structure.

The basal divergence is of a Central African group (A) and East African clade (B), but neither strict regional grouping is monophyletic. Clade A began to diversify earlier than B (figure 2), most probably before the origin of LT. The origin and initial divergence of the East African *Synodontis* are compatible with isolation through vicariance during the major rifting events that took place between 25 and 10 Myr ago (Ma) (Vrba *et al.* 1995). The sampled Central African *Synodontis* are paraphyletic owing to *Synodontis afrofischeri*, which diverged and presumably arrived in East Africa more recently. The LT species flock is not monophyletic because LT non-endemic *Synodontis nigromaculatus* groups with the LM endemic *Synodontis njassae*. The endemic LT species are not monophyletic because *Synodontis victoriae* nests within the least inclusive group including all the LT endemics (D) as the sister species of the least diverse of two clades of endemics (E and F). The tree suggests either two independent origins of the LT endemics or a single origin and subsequent emigration of the *S. victoriae* lineage. The inferred time-scale is consistent with both scenarios because all of the three divergences corresponding to these possible origins occurred (at nodes D and F), or could have occurred (node B) after the origin of LT. While the divergence of *S. victoriae* (node F) predates the origin of LV, indicating the long persistence of its ancestral lineage elsewhere, origin of the Malawi endemic *S. njassae* (node C) may coincide with the origin of this lake.

KH tests allow rejection of the monophyly of the entire LT species flock and of strict Central and East African species groups ($p<0.015$), but not an alternative placement of *S. victoriae*, which leaves the endemic LT species monophyletic ($p=0.349$). The divergence (at node D) of the two main endemic LT clades (E and G) may coincide with the fusion of a single water body (5–6 Ma; see Coulter 1991). Whatever might be their origins, the time-scale suggests that initial diversification of the two main clades of endemic LT species was contemporaneous (figure 2).

Table 1. Support values calculated using phylogenetic methods (BI, ML, NJ, MP) correspond to labelled nodes in figure 1.

node	BI	ML	NJ	MP
A	1.00	1000	90	99
B	1.00	990	82	99
C	1.00	926	83	84
D	1.00	980	80	92
E	1.00	996	99	99
F	0.92	869	58	84
G	1.00	967	76	98
H	1.00	1000	95	100
I	1.00	1000	98	100
J	0.97	888	74	90
K	1.00	980	98	97
L	0.87	598	51	67
M	1.00	994	98	99
N	0.97	658	51	69
O	0.98	912	83	79
P	1.00	723	66	77
Q	1.00	1000	100	100
R	1.00	1000	100	100
S	1.00	1000	100	100

4. DISCUSSION

Environmental shifts in the history of LT are thought to have promoted the speciation of cichlids (e.g. Sturmbauer *et al.* 2001), but the extent to which their effects are more general remains untested. The recent divergence and subsequent diversification within the two main clades of LT endemic *Synodontis* are similar to those inferred for platythelphusid crabs (Marijnissen *et al.* 2006) and some cichlid groups (e.g. Duftner *et al.* 2005), and coincide with a period of aridification when lake levels dropped substantially (Cane & Molnar 2001). The simultaneous divergence of clades provides good evidence that extrinsic factors such as climatic shifts are responsible for promoting speciation across a wide variety of organisms. The inferred dates also indicate a relatively recent time frame for the diversification of different endemic faunas considering the age of LT.

LT radiations leading to secondary colonization of rivers are rare in LT cichlid tribes (Salzburger *et al.* 2002). The occurrence of a non-endemic *Synodontis* within the LT species flock might be similarly explained if the flock were monophyletic. The phylogeny does not support this, but it and the time-scale are consistent with *S. victoriae* having originated in this way. Thus, *Synodontis* may provide a second example of recolonization. It is tempting to suggest that the ability to recolonize rivers is correlated with mobility, and that *Synodontis* are more similar to cichlids than to benthic invertebrates, for which there is no evidence of recolonization (e.g. Marijnissen *et al.* 2006), in this respect. Unfortunately, the placement of *S. victoriae* is not compelling. We cannot reject the alternative hypothesis that the LT endemics are a single monophyletic radiation, which has not seeded any other

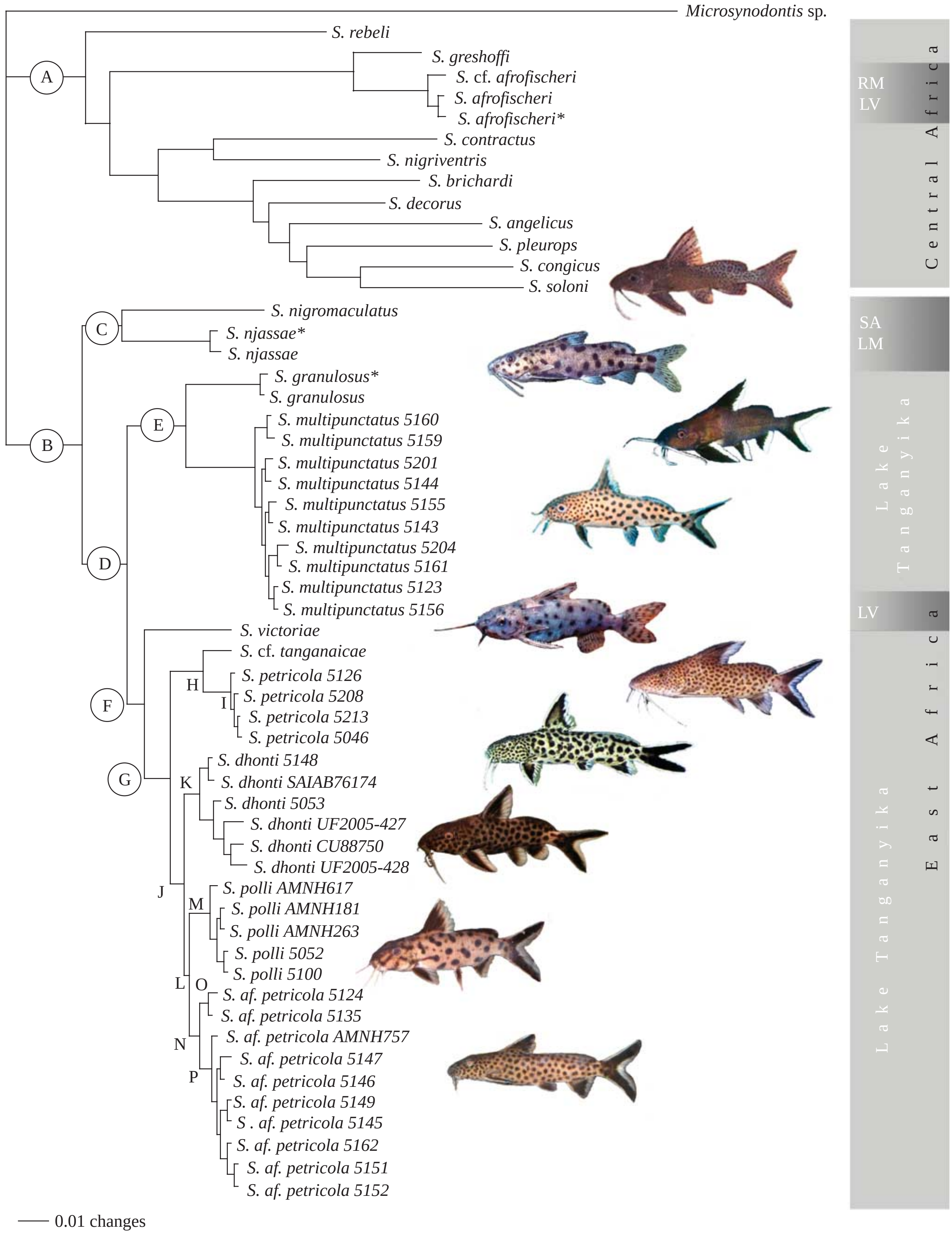
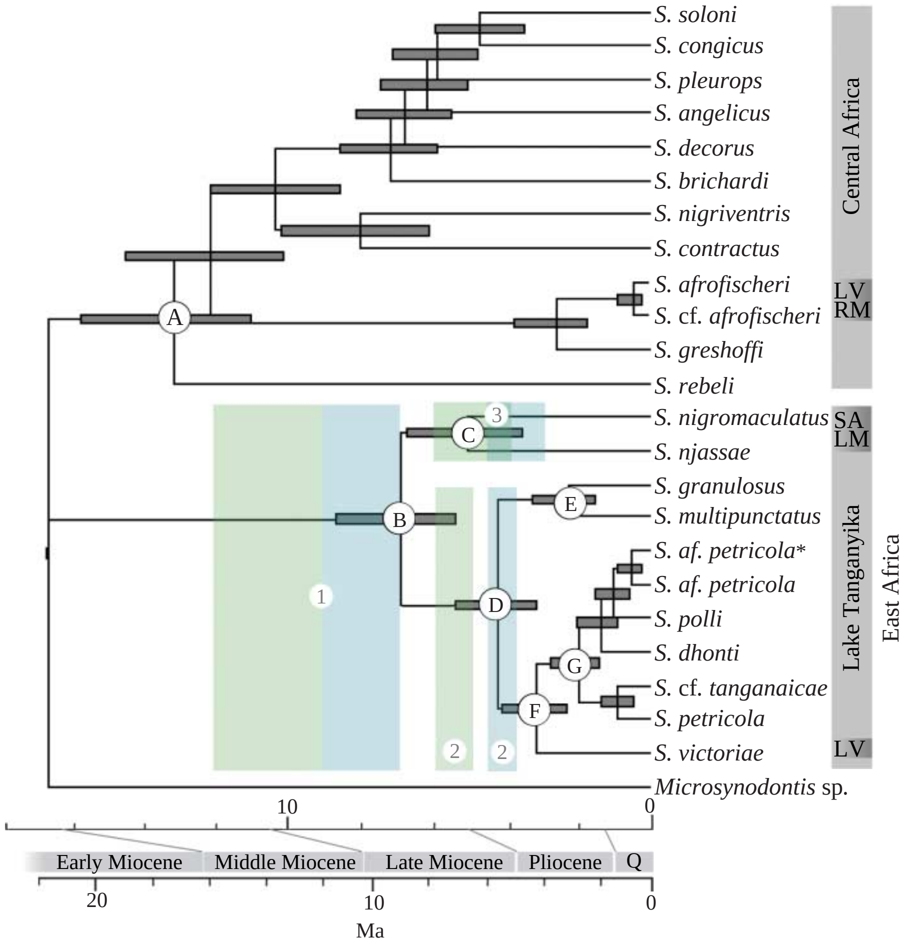


Figure 1. BI tree of *Synodontis* catfish. Voucher and field numbers (see the electronic supplementary material) are used to distinguish multiple specimens. LM, Lake Malawi; LV, Lake Victoria; RM, River Malagrasi; SA, South Africa. Photographs top to bottom: *S. nigromaculatus*, *S. njassae*, *S. granulosus*, *S. multipunctatus*, *S. victoriae*, *Synodontis* cf. *tanganaicae*, *S. af. petricola*, *Synodontis dhonti*, *Synodontis polli* and *S.* cf. *petricola*.

rivers or lakes. East–West African faunal divisions, consistent with continental-scale vicariance, are reported for many terrestrial (e.g. Matthee *et al.* 2004), but few aquatic groups. *Synodontis* phylogeny reveals an initial East–West split and subsequent dispersal between the regional faunas.



node	21.8 Ma	16.6 Ma
A	18.1 (20.6 – 14.4)	13.7 (15.6 – 11.0)
B	9.0 (11.4 – 7.0)	6.8 (8.6 – 5.4)
C	6.6 (8.7 – 4.7)	5.0 (6.7 – 3.5)
D	5.5 (7.0 – 4.1)	4.2 (5.4 – 3.1)
E	2.8 (4.2 – 1.9)	2.2 (3.2 – 1.5)
F	4.1 (5.3 – 3.1)	3.1 (4.1 – 2.3)
G	2.6 (3.5 – 1.8)	2.0 (2.7 – 1.4)

Figure 2. Chronogram, where *Synodontis* are dated at either 21.8 Ma (blue) or 16.6 Ma (green), with confidence limits calculated as 95% credible intervals conditional on the tree topology. Inset with ages (and confidence limits in parenthesis) for nodes A–G. 1, age of LT; 2, onset of deep water conditions in LT; 3, age of LM.

While the data presented here are from a single mtDNA marker, they are sufficient to provide a mostly well-supported dated phylogeny, reject several *a priori* hypotheses of monophyly based on distributions, evaluate traditional taxonomy and discern an outline of the evolutionary history of an unstudied LT species flock. The major uncertainties over the placement of *S. victoriae* and its implications for the origins of the LT species flock and the precision of the time-scale should be resolvable with additional data, including nuclear markers to avoid pitfalls associated with mtDNA (see Seehausen 2004) and re-evaluation of the fossil record, while expanded taxon sampling will provide additional tests of the monophyly of regional faunas. Ultimately, comparisons of different faunal radiations will be important in revealing the extent to which diversification can be explained in terms of common factors.

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