Ancient lakes as evolutionary reservoirs: evidence from the thalassoid gastropods of Lake Tanganyika

Anthony B. Wilson[1*], Matthias Glaubrecht[2] and Axel Meyer[1]

[1]*Department of Biology, University of Konstanz, Konstanz 78457, Germany*
[2]*Museum für Naturkunde, Institute of Systematic Zoology, Humboldt University, Invalidenstraße 43, Berlin 10115, Germany*

Ancient lakes are often collectively viewed as evolutionary hot spots of diversification. East Africa's Lake Tanganyika has long been the subject of scientific interest owing to dramatic levels of endemism in species as diverse as cichlid fishes, paludomid gastropods, decapod and ostracod crustaceans and poriferans. It is the largest and deepest of the African rift lakes, and its endemic fauna has been presented with a stable inland environment for over 10 Myr, offering unique opportunities for within-lake diversification. Although astonishing diversification has been documented in the endemic cichlid fauna of the lake, similar patterns of rapid diversification have long been assumed for other groups. In contrast to this hypothesis of rapid speciation, we show here that there has been no acceleration in the rate of speciation in the thalassoid gastropods of the lake following lake colonization. While limited within-lake speciation has occurred, the dramatic conchological diversity of gastropods presently found within the lake has evolved from at least four major lineages that pre-date its formation by as much as 40 Myr. At the same time, a widespread group of African gastropods appears to have evolved from taxa presently found in the lake. While Lake Tanganyika has been a cradle of speciation for cichlid fishes, it has also been an important evolutionary reservoir of gastropod lineages that have been extirpated outside the basin.

Keywords: ancient lakes; morphological stasis; gastropod phylogeny; Tanganyika

1. INTRODUCTION

Speke and Burton's legendary expeditions in search of the source of the Nile resulted in the discovery of the East African Lake Tanganyika in 1858 (Burton 1860). Even before the first published accounts of the lake's discovery appeared, Speke's collections of gastropods from Tanganyika attracted considerable scientific interest (Woodward 1859), and further collections highlighted the spectacular diversity (25–70 species; Brown 1994; West & Michel 2000; 62% endemism) and thalassoid (marine-like) form of the gastropods of the lake (figure 1; Moore 1897; Smith 1906). The identification of marine-like gastropods, with spines, strong shells and morphologies uncommon among freshwater snails, led to the suggestion that Lake Tanganyika was once directly connected to the ocean (Moore 1898, 1903; Nicolas 1898). However, more recent geological data indicate that the lake was formed by rifting in the African subcontinent and could not have had an oceanic origin (Tiercelin & Mondeguer 1991; Cohen *et al.* 1993, 1997).

The cichlid fishes of the East African Great Lakes have long been recognized as key model organisms in the study of rapid evolution in freshwater (Meyer *et al.* 1990), and, although virtually nothing is known about the evolution of the other members of the rich aquatic fauna, these lakes have often been viewed collectively as 'evolutionary hot spots' (Coulter 1991; Rossiter 1995). Although it is clear that Lake Tanganyika is home to a disproportionately large number of freshwater endemics (Coulter 1991),

understanding the evolutionary history of these animals is critical to clarify the relative importance of the lacustrine environment and intrinsic properties of the animals themselves in their evolution.

Striking changes in gastropod external morphology may occur rapidly as an ecophenotypic response to predation pressures and/or changes in the environment without leading to speciation (Palmer 1985; Trussell & Smith 2000). The results of recent predation experiments on the gastropods of Tanganyika are consistent with a predator–prey model of coevolution, where the diversification of molluscivoran Cichlidae and Crustacea is believed to have led to a corresponding evolution of marine-like gastropods from a freshwater ancestor (West & Cohen 1994). While ecological models of coevolution are invaluable, a phylogenetic framework is necessary to clarify the pace and mode of evolution and to elucidate coevolutionary processes (Huelsenbeck & Rannala 1997; Thompson 1999). The decoupling of conchological and molecular diversity evident in strombid gastropods (Roy *et al.* 2001) supports the view that morphological disparity may not always reflect the evolutionary history of the organisms involved.

In this study, we present a combined molecular and conchological investigation of diversification in Lake Tanganyika's endemic gastropods. We use a molecular phylogeny based on mitochondrial DNA to investigate the hypothesis of a within-lake radiation of gastropod species and measure patterns of morphological diversity, as assessed by Fourier-transformed shell outlines, which differ markedly from estimates based on molecular data. Our data suggest that, in contrast to the commonly held view of ancient lakes as evolutionary hot spots, the long-term persistence of the gastropods of Lake Tanganyika may reflect the importance of this basin as an evolutionary reservoir.

*Author and address for correspondence: Genetics and Evolution, Conservation Biology Division, Northwest Fisheries Sciences Center, 2725 Montlake Boulevard East, Seattle, WA 98112, USA (tony.wilson@noaa.gov).

Proc. R. Soc. Lond. B (2004) **271**, 529–536
DOI 10.1098/rspb.2003.2624

529

© 2004 The Royal Society

2. MATERIAL AND METHODS

(a) *Sample preparation and DNA amplification*

Molecular and conchological analyses were undertaken on samples of gastropods collected from sites located in the southern basin of Lake Tanganyika and neighbouring Lake Mweru in 1999 and 2000. Outgroup selection followed the taxonomic treatment of Glaubrecht (1999), which recognized a sister-group relationship between the Paludomidae and the Thiaridae. Specimens were collected by hand using SCUBA, sorted and cracked and preserved in 70% ethanol. DNA was extracted from ethanol-preserved foot tissue using a CTAB protocol (Winnepenninckx *et al.* 1993). Both the large-subunit mitochondrial ribosomal gene (16S) and the mitochondrial cytochrome oxidase subunit I gene (COI) have proven to be powerful tools in the elucidation of gastropod relationships at the genus, family and superfamily levels (Reid *et al.* 1996; Marko 1998; Lydeard *et al.* 2002). A PCR was used to amplify a 944 bp segment of the 16S mitochondrial ribosomal gene and a 658 bp fragment of the mtDNA COI gene; details of the protocol and the primer sequences for amplifying COI have been published elsewhere (Folmer *et al.* 1994). Long-range PCR (using the expand long template PCR system; Boehringer Mannheim) was used with previously published primers 12SA (Kocher *et al.* 1989) and 16SBr (Palumbi *et al.* 1991) to amplify a 1967 bp fragment from a subset of gastropod individuals. These sequences were used to design an internal primer pair with widespread applicability among Tanganyikan gastropods (16SF: 5′-CCGCACTAG TGATAGCTAGTTTC-3′; H3059: 5′-CCGGTYTGAACT CAGATCATGT-3′). Sequences have been deposited in Gen-Bank under accession numbers AY456529–AY456636.

(b) *Phylogenetic analyses*

The orthologous DNA sequences obtained were aligned, using default settings, by CLUSTAL W (Thompson *et al.* 1994) and optimized by eye. Third-codon positions of cytochrome oxidase were saturated at distance values greater than 0.15 and were eliminated from further analyses.

Neighbour-joining distance and maximum-parsimony analyses were performed on the combined dataset of 1021 bp using PAUP v. 4b10 (Swofford 2000), with indels coded as missing data. Bootstrapped neighbour-joining trees (500 replicates) were constructed under a GTR + G + I model of substitution (where GTR is general-time-reversible, G is gamma and I is invariable sites) with parameters estimated by MODELTEST v. 5.0 (Posada & Crandall 1998). Maximum-parsimony trees were constructed with full heuristic searches (500 bootstrap replicates) using random addition (10 replicates), the tree–bisection–reconnection (TBR) branch-swapping algorithm and the MULPARS option. Phylogenetic relationships were also approximated following a Bayesian method of phylogenetic inference as implemented by MRBAYES v. 2.01 (Huelsenbeck & Ronquist 2001). Posterior probabilities of phylogenetic trees were estimated by a 100 000 generation Metropolis-coupled Markov chain Monte Carlo simulation (four chains, chain temperature = 0.2) under the GTR + G + I model of substitution, with parameters estimated from the dataset. A 50% majority-rule consensus tree was constructed following a 20 000 generation burn-in to allow likelihood values to reach stationarity.

The overall maximum-likelihood (ML) tree was constructed following a heuristic search on starting-tree topologies generated by distance, parsimony and Bayesian analyses. Initial branch lengths were estimated using a Rogers–Swofford approximation and optimized following Newton–Raphson optimization (100

branch-length smoothing passes, $\Delta = 0.000\,006$). Branch swapping followed the TBR algorithm. The optimal ML tree was identical in each of the three replicates.

(c) *Molecular-clock analysis*

While significant rate heterogeneity was observed for the COI gene fragment across multiple lineages (branch-length χ^2-test), a test of branch length as implemented by LINTREE (Takezaki *et al.* 1995) highlighted only two taxa that deviated from a molecular clock based on the 16S dataset. Following the elimination of these taxa, genetic distances were recalculated for the 16S dataset with a molecular-clock constraint (Takezaki *et al.* 1995), and a molecular calibration for transitions and transversions of 16S rDNA of littorinid gastropods (Reid *et al.* 1996) was used to estimate a molecular clock for Tanganyikan snails. Combining the littorinid dataset (Reid *et al.* 1996), the aligned dataset for the Tanganyikan species and various caenogastropods representing deeper level phylogenetic relationships within the superfamily (Lydeard *et al.* 2002) failed to reveal any significant deviation from a constant rate of molecular evolution within the Caenogastropoda based on a branch-length χ^2-test (see electronic Appendix C available on The Royal Society's Publications Web site).

(d) *Modelling lineage diversification*

A lineage-through-time plot was constructed from the linearized 16S phylogenetic tree (figure 2). While the *Lavigeria* species group exhibited an accelerated rate of cladogenesis ($p < 0.05$) based on the relative cladogenesis statistic (Nee *et al.* 1994*b*), the remainder of the phylogeny did not exhibit significant variation in speciation and extinction rates among lineages. A Monte Carlo constant-rates test (Pybus 2000) failed to reject the null hypothesis of constant speciation and extinction rates through time for the phylogeny (critical value of γ (at 5% level) for 21 species randomly sampled from a phylogeny containing 100 species (25 000 replicates) = 3.182 860 (Pybus & Harvey 2000); γ-statistic for phylogeny shown in figure 2 = -0.181; $p > 0.95$).

(e) *Conchological analyses*

Shell outlines were digitized in standard orientation using TPSDIG v. 1.31 (Rohlf 2001; 250 coordinates per object). Size-standardized outlines were Fourier transformed using HANGLE (Crampton & Haines 1996) following two smoothing passes (20 Fourier harmonics) and normalized for orientation and starting position using HMATCH (Crampton & Haines 1996). Principal-component analysis using a covariance matrix was conducted using JACKIE v. 1.20 (Cavalcanti 2001). The relative volume of morphospace occupied by each of the four major groups of Tanganyikan gastropods was calculated from the geometric mean of the ranges of each of the 38 principal components. Pairwise Euclidean distances of principal-component scores were calculated and morphological distances were used to construct a neighbour-joining tree. This tree based on external morphology was compared with the overall ML tree based on molecular data using a Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999).

3. RESULTS AND DISCUSSION

(a) *Molecular phylogeny indicates multiple origins of Tanganyikan gastropod lineages*

Analysis of a combined 16S and COI mitochondrial DNA dataset supports four major clades of Tanganyikan
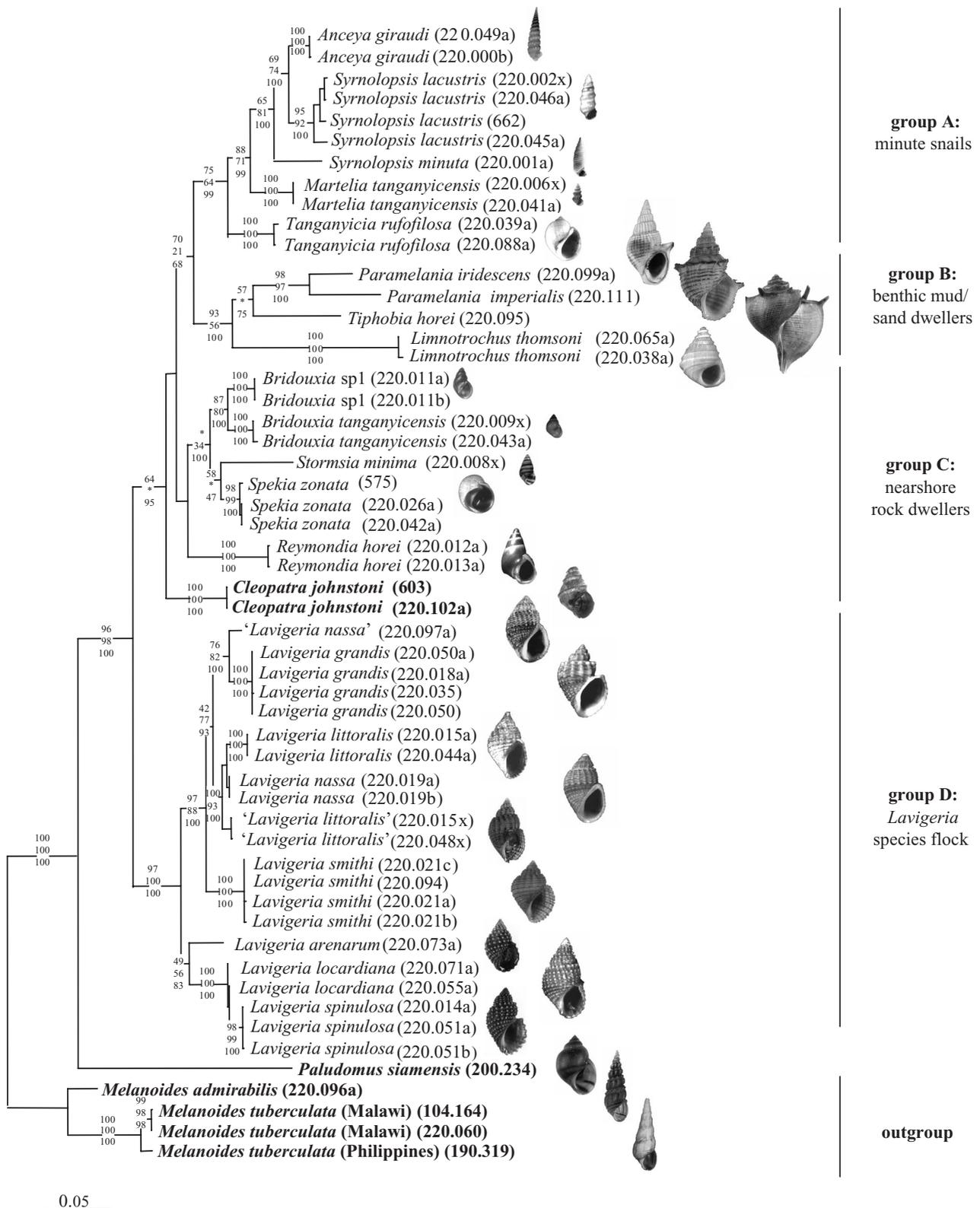
Figure 1. Overall ML tree generated from concatenated 16S and COI data using starting trees generated by the neighbour-joining distance (Saitou & Nei 1987), maximum parsimony (Swofford & Berlocher 1987) and Bayesian phylogeny inference (Huelsenbeck & Ronquist 2001). Numbers on the branches represent bootstrap values (neighbour-joining and maximum-parsimony) and posterior probabilities (Bayesian analysis) of phylogeny (asterisks indicate collapsed branches). Archived samples (accession numbers listed above) and data on specimen collections are housed at the Museum für Naturkunde (Berlin). Non-Tanganyikan species are indicated in bold type. Images of type material (where available; Brown 1994); other images show archived samples used in the present study. The *Lavigeria* species group is currently under revision (E. Michel and J. Todd, unpublished data). See electronic Appendix A for more information on species identification.
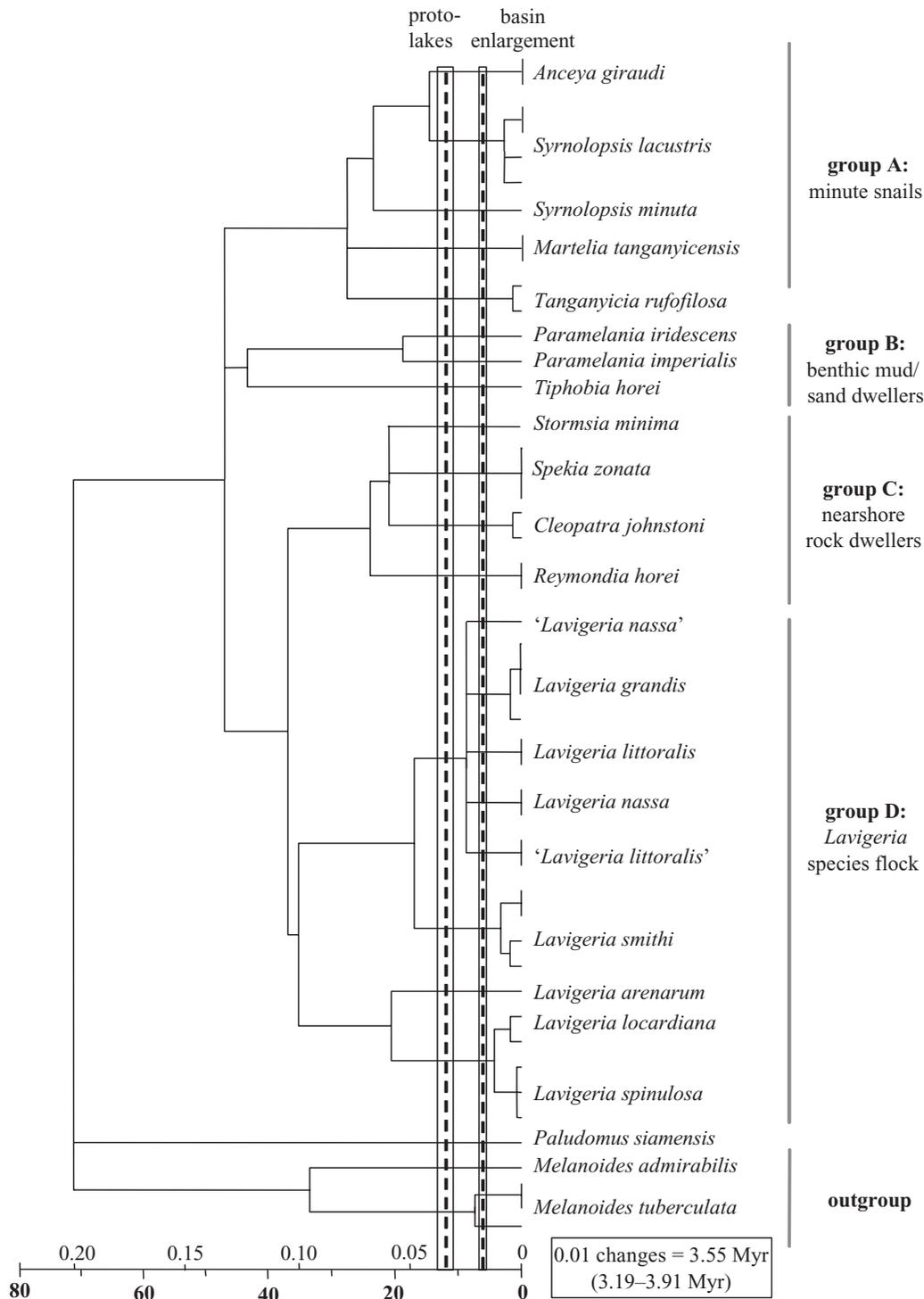
Figure 2. Linearized tree for 460 bp of 16S alignment based on Kimura two-parameter distances constructed using MEGA v. 2.1 (Kumar *et al.* 2001), using molecular-clock calibration for caenogastropods (Reid *et al.* 1996). Dashed vertical lines indicate lake formation and enlargement (error bars indicate standard errors associated with the molecular-clock analysis).

gastropods (figure 1). These major lineages as delineated by molecular data correspond closely to taxonomic groupings of Tanganyikan taxa based on internal morphology (E. E. Strong and M. Glaubrecht, unpublished data) and include:

(i) the minute Syrnolopsidae with *Syrnolopsis*, *Anceya* and *Martelia* (+ *Tanganyicia*) exhibiting strong affinities for shallow sandy habitats and rock surfaces;

(ii) benthic mud- and sand-dwelling taxa *Limnotrochus*, *Tiphobia* and *Paramelania*:

(iii) a nearshore rock-dwelling group including *Spekia*, *Bridouxia*, *Stormsia* and *Reymondia*; and

(iv) the *Lavigeria* species flock (Lavigeriinae), which is found in a diversity of nearshore habitats, including both rocky and sandy shores.
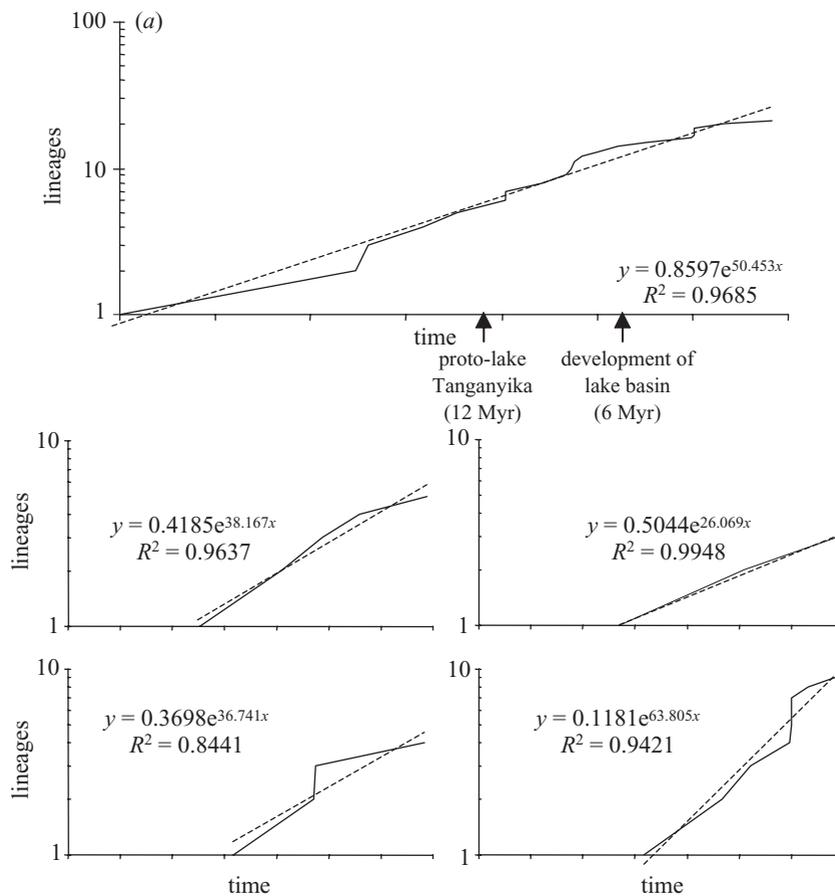
Representatives of both the Thiaridae *sensu stricto*

Figure 3. Lineage-through-time plots (Nee *et al.* 1992) for species of endemic Tanganyikan gastropods constructed from the pruned phylogenetic tree (figure 2). (*a*) All lineages; (*b*) group A: *Anceya*, *Martelia*, *Syrnolopsis* and *Tanganyicia*; (*c*) group B: *Paramelania* and *Tiphobia*; (*d*) group C: *Reymondia*, *Spekia* and *Stormsia*; and (*e*) group D: *Lavigeria* species flock. Dashed line represents best-fit curve ($p < 0.001$ for regression of combined analysis).

(*Melanoides* spp.) and Paludomidae were included in the phylogenetic analyses as outgroup species to address ongoing controversy about the taxonomic affinities of the Tanganyikan endemics (Brown 1994; Glaubrecht 1999; West & Michel 2000). Interestingly, although *Paludomus siamensis* (Thailand) clusters at the base of the Tanganyikan species, *Cleopatra johnstoni*, a paludomid found in nearby Lake Mweru, clusters within the Tanganyika group (figure 1). *Cleopatra ferruginea*, a cosmopolitan African species, has also been shown to cluster within the Tanganyikan taxa (West & Michel 2000). Taken together, these data suggest that *Cleopatra*, a genus that is now widely distributed in Africa, may have originated in Lake Tanganyika and reached its current range of distribution in as little as 8 Myr (see § 3b). Alternatively, the placement of a widespread African group within the Tanganyikan endemics may indicate that lineages currently restricted to Lake Tanganyika once had distributions extending beyond the Tanganyika basin (see § 3e). The position of *Cleopatra* within the Tanganyikan taxa supports recent work that suggests that the Tanganyikan gastropods are not Thiaridae *sensu stricto* as often assumed in the literature (Brown 1994; West & Michel 2000), but should instead be recognized as members of the Paludomidae, a distinct gastropod family with an oriental distribution and an exceptionally wide range of shell variation (Glaubrecht 1999; Strong & Glaubrecht 2002).

(b) *Molecular clock suggests multiple gastropod lineages pre-date lake formation*

Combined seismic reflection readings and radiocarbon-dating work indicate that the Tanganyika basin began to form as a series of swampy proto-lakes between 9 and 12 Myr ago and developed into a large lacustrine basin between 5 and 6 Myr ago (Tiercelin & Mondeguer 1991; Cohen *et al.* 1993, 1997). In an effort to clarify the importance of within-lake diversification, we assume that snails present in these proto-lakes 12 Myr ago gave rise to the present-day diversity of Tanganyikan gastropods. After calculating standard errors of the rates of molecular evolution for transitions and transversions of the 16S rDNA gene, all four major lineages still clearly pre-date the formation of proto-Lake Tanganyika by as much as 40 Myr (figure 2).

(c) *No evidence of accelerated within-lake radiation of gastropod species*

Although phylogenetic trees reflect relationships among extant taxa, lineage-through-time plots offer a means of detecting differential proliferation of lineages through evolutionary time (Goldstein & Harvey 1999). A lineage-through-time plot of diversification in endemic Tanganyikan gastropods does not deviate significantly from that of a constant-rates process with no extinction, indicating that the unique environment of Lake Tanganyika has not led

to a significant acceleration in lineage splitting and speciation in its endemic gastropod fauna (figure 3). While additional taxa remain to be described from the Tanganyika basin (Michel 1994), theoretical models demonstrate that a lineage-through-time plot including as few as 10% of extant taxa may be sufficient to estimate speciation and extinction coefficients from phylogenetic data (Nee *et al.* 1994*a*). Interestingly, *Lavigeria*, the most species-rich genus within the lake, appears to have experienced a radiation following the development of the Tanganyikan basin (figure 2) and appears to be a species flock *sensu stricto* (Michel 2000). *Lavigeria* gastropods are a highly specialized group found predominantly in the nearshore rocky zone of the lake. Small-scale water-level changes may be continuing to play an important role in the isolation and diversification of populations of *Lavigeria* on a local scale, as has been shown for the eretmodine cichlids of the lake (Ruber *et al.* 1999).

(d) Differential rates of conchological evolution

Our molecular evidence indicates that a significant proportion of the genetic diversity of the endemic gastropods presently found in Lake Tanganyika pre-dates the formation of the lake basin and ancient diversification events correlate with the major ecological divisions presently found in the lake (figure 2). Conchological variation among Tanganyikan gastropods as measured by morphospace occupation has also accrued disproportionately in each of the four major lineages (figure 4) and extreme variation in shell shape within the benthic lineage of *Tiphobia*, *Paramelania* and *Limnotrochus* (group B; figure 1) does not reflect an increase in the speciation rate (figure 3). At the same time, the diversification of the *Lavigeria* gastropods within Lake Tanganyika (Michel 2000) has occurred in the absence of major external morphological change (figures 1 and 3*e*), strongly suggesting that conchological differentiation has not been a major engine of speciation in Tanganyikan gastropods. The correlation between conchological variation and molecular variation is not significantly greater than that expected by chance (figure 4*b*) and a Shimodaira–Hasegawa test rejects a tree based on Fourier-transformed outline data as significantly inferior to the overall ML tree based on molecular data (Δ ($-\ln L$) = 604.75, $p < 0.0001$; see electronic Appendix D for a tree based on external morphology). An increased level of conchological divergence in the benthic clade and a lack of relationship between molecular and morphological distances remain evident even after the spinuous *Tiphobia* is eliminated from the analysis (data not shown).

(e) Conclusions: Lake Tanganyika as an evolutionary reservoir

As Lake Tanganyika is believed to have been divided into at least three major basins during its history and has experienced repeated episodes of desiccation (Tiercelin & Mondeguer 1991; Cohen *et al.* 1997), allopatric speciation may at least partly explain within-lake diversification of the endemic gastropods, as has been demonstrated for cichlid fishes (Ruber *et al.* 1999). The identification of fossils of two now-endemic Tanganyikan genera (*Lavigeria* and *Neothauma*) outside the lake basin (Fuchs 1936; Cox 1939), coupled with the identification of new species affinities with *Cleopatra* (see § 3a), indicate that species that are
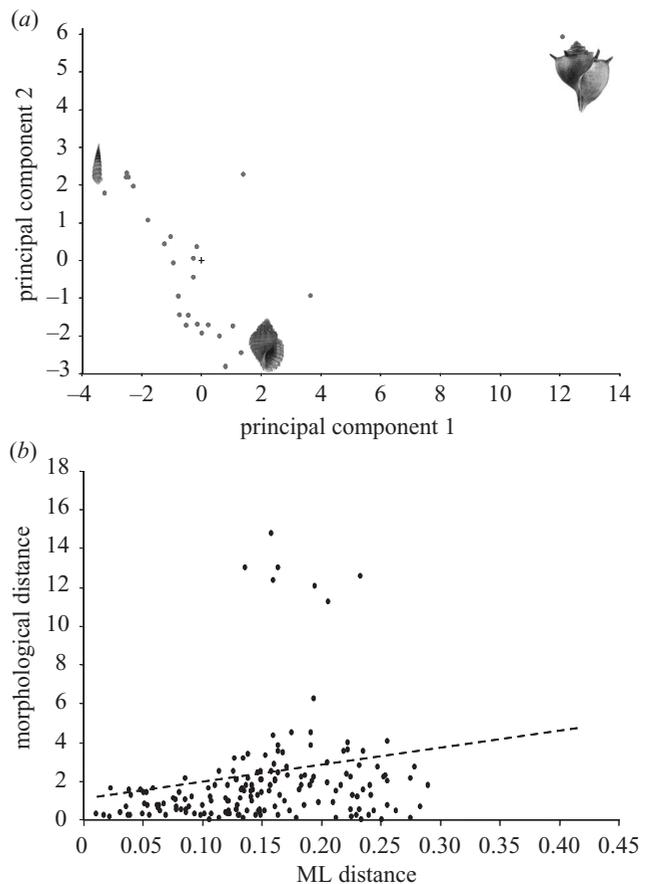
Figure 4. Analysis of conchological variation. (*a*) Distribution of shell shape among Tanganyikan gastropods along the first two principal components of Fourier-transformed shell shape. These two axes explain 56.3% of the overall variance. Illustrated are shell shapes that characterize specific regions of morphospace. Percentages of total morphospace volume occupied by major lineages: group A, 50.4%; group B, 65.4%; group C, 36.1%; group D, 50.3%. (*b*) Regression of pairwise ML-distance estimates against morphological Euclidean distances ($p = 0.342$; permutation test; 10 000 replicates). See figure 3 for group definitions.

now restricted to the lake once had distributions extending beyond the Tanganyika basin and suggest that Lake Tanganyika has played an important role as an evolutionary reservoir of ancient lineages, which have spread outside the lake to colonize much of Africa.

The long-term persistence of the Tanganyikan gastropod fauna may be attributed to the extreme depth and stability of this unique aquatic environment. If this is the case, then within-lake predator–prey coevolution (West & Cohen 1994) is unlikely to explain the present-day species-level diversity of the Tanganyikan gastropods. Alternatively, as major changes in gastropod external morphology need not necessarily reflect speciation (Palmer 1985), the unique ecological niches available in this large lacustrine environment may have contributed to the evolution of unique shell forms in the absence of a measurable increase in speciation rates, as exemplified by the benthic gastropods of Tanganyika. Our data suggest that, contrary to previous reports (Coulter 1991), the unique lacustrine environment has not fuelled a major species-level adaptive radiation (Schluter 2000) of gastropod species on the scale

of that observed in the cichlid fishes of the lake (Salzburger *et al.* 2002). While the Great Lakes of East Africa have served as evolutionary hot spots for the diverse flocks of cichlid fishes, Lake Tanganyika has also been an evolutionary reservoir of an ancient group of gastropods that have since been extirpated outside the lake. The extreme depth and stability of this inland sea have played an important role in the persistence of this ancient group of gastropods.

REFERENCES

Brown, D. S. 1994 *Freshwater snails of Africa and their medical importance*. London: Taylor & Francis.

Burton, R. F. 1860 *The lake regions of Central Africa*. New York: Harper & Brothers.

Cavalcanti, M. J. 2001 *JACKIE: Jackknifed interactive eigenanalysis*. Museu Nacional do Rio de Janeiro.

Cohen, A. S., Soreghan, M. J. & Scholz, C. A. 1993 Estimating the age of formation of lakes: an example from Lake Tanganyika, East African Rift system. *Geology* **21**, 511–514.

Cohen, A. S., Lezzar, K. E., Tiercelin, J. J. & Soreghan, M. 1997 New paleogeographic and lake-level reconstructions of Lake Tanganyika: implications for tectonic, climatic and biological evolution in a rift lake. *Basin Res.* **9**, 107–132.

Coulter, G. W. 1991 *Lake Tanganyika and its life*. London: Natural History Museum.

Cox, L. R. 1939 Mollusca from the quaternary deposits of Lake Rukwa (Tanganyika Territory). *Proc. Malacological Soc.* **23**, 242–252.

Crampton, J. S. & Haines, A. J. 1996 Users' manual for programs HANGLE, HMATCH, and HCURVE for the Fourier shape analysis of two-dimensional outlines. Report **96**/37, 1–28. Lower Hutt, New Zealand: Institute of Geological and Nuclear Sciences.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994 DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294–299.

Fuchs, V. E. 1936 Extinct Pleistocene Mollusca from Lake Edward, Uganda, and their bearing on the Tanganyika problem. *Zool. J. Linn. Soc.* **40**, 93–106.

Glaubrecht, M. 1999 Systematics and the evolution of viviparity in tropical freshwater gastropods (Cerithioidea: Thiaridae sensu lato)—an overview. *Courier Forschungsinstitut Senckenberg* **215**, 91–96.

Goldstein, D. B. & Harvey, P. H. 1999 Evolutionary inference from genomic data. *BioEssays* **21**, 148–156.

Huelsenbeck, J. P. & Rannala, B. 1997 Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science* **276**, 227–232.

Huelsenbeck, J. P. & Ronquist, F. 2001 MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl Acad. Sci. USA* **86**, 6196–6200.

Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. 2001 MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.

Lydeard, C., Holznagel, W. E., Glaubrecht, M. & Ponder, W. F. 2002 Molecular phylogeny of a circum-global, diverse gastropod superfamily (Cerithioidea: Mollusca: Caenogastropoda): pushing the deepest phylogenetic limits of mitochondrial LSU rDNA sequences. *Mol. Phylogenet. Evol.* **22**, 399–406.

Marko, P. B. 1998 Historical allopatry and the biogeography of speciation in the prosobranch snail genus *Nucella*. *Evolution* **52**, 757–774.

Meyer, A., Kocher, T. D., Basasibwaki, P. & Wilson, A. C. 1990 Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* **347**, 550–553.

Michel, E. 1994 Why snails radiate: a review of gastropod evolution in long-lived lakes, both recent and fossil. *Arch. Hydrobiol. Beiheft. Ergebnisse Limnol.* **44**, 285–317.

Michel, E. 2000 Phylogeny of a gastropod species flock: exploring speciation in Lake Tanganyika in a molecular framework. In *Ancient lakes: biodiversity, ecology and evolution* (ed. A. Rossiter & H. Kawanabe), pp. 275–302. London: Academic.

Moore, J. E. S. 1897 The fresh-water fauna of Lake Tanganyika. *Nature* **56**, 198–200.

Moore, J. E. S. 1898 On the zoological evidence for the connection of Lake Tanganyika with the sea. *Proc. R. Soc. Lond.* B **62**, 451–458.

Moore, J. E. S. 1903 *The Tanganyika problem*. London: Hurst and Blackett.

Nee, S., Mooers, A. O. & Harvey, P. H. 1992 Tempo and mode of evolution revealed from molecular phylogenies. *Proc. Natl Acad. Sci. USA* **89**, 8322–8326.

Nee, S., Holmes, E. C., May, R. M. & Harvey, P. H. 1994*a* Extinction rates can be estimated from molecular phylogenies. *Phil. Trans. R. Soc. Lond.* B **344**, 77–82.

Nee, S., May, R. M. & Harvey, P. H. 1994*b* The reconstructed evolutionary process. *Phil. Trans. R. Soc. Lond.* B **344**, 305–311.

Nicolas, H. 1898 Origine marine de certains especes de mollusques, en cours de la transformation du lac Tanganyika. *Comptes Rendues de l'Academie des Sciences de Paris, Sciences de la Vie* **27**, 508–525.

Palmer, A. R. 1985 Quantum changes in gastropod shell morphology need not reflect speciation. *Evolution* **39**, 699–705.

Palumbi, S. R., Martin, A. P., Romano, S. L., McMillan, W. O. D., Stice, L. & Grabowski, G. 1991 *The simple fool's guide to PCR*. University of Hawaii.

Posada, D. & Crandall, K. A. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Pybus, O. G. 2000 *MCCRTest*. University of Oxford.

Pybus, O. G. & Harvey, P. H. 2000 Testing macro-evolutionary models using incomplete molecular phylogenies. *Proc. R. Soc. Lond.* B **267**, 2267–2272. (DOI 10.1098/rspb.2000.1278.)

Reid, D. G., Rumbak, E. & Thomas, R. H. 1996 DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. *Phil. Trans. R. Soc. Lond.* B **351**, 877–895.

Rohlf, F. J. 2001 TPSDIG v. 1.31. Stony Brook, New York: SUNY at Stony Brook.

Rossiter, A. 1995 The cichlid fish assemblages of Lake Tanganyika: ecology, behaviour and evolution of its species flocks. *Adv. Ecol. Res.* **26**, 187–252.

Roy, K., Balch, D. P. & Hellberg, M. E. 2001 Spatial patterns of morphological diversity across the Indo-Pacific: analyses using strombid gastropods. *Proc. R. Soc. Lond.* B **268**, 2503–2508. (DOI 10.1098/rspb.2000.1428.)

Ruber, L., Verheyen, E. & Meyer, A. 1999 Replicated evolution of trophic specializations in an endemic cichlid fish lineage from Lake Tanganyika. *Proc. Natl Acad. Sci. USA* **96**, 10 230–10 235.

Saitou, N. & Nei, M. 1987 The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425.

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.

Schluter, D. 2000 *The ecology of adaptive radiation*. Oxford University Press.

Shimodaira, H. & Hasegawa, M. 1999 Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**, 1114–1116.

Smith, E. A. 1906 Zoological results of the third Tanganyika expedition, conducted by Dr. W. A. Cunnington, 1904–1905. Report on the Mollusca. *Proc. Zool. Soc. Lond.* **1**, 180–186.

Strong, E. E. & Glaubrecht, M. 2002 Evidence for convergent evolution of brooding in a unique gastropod from Lake Tanganyika: anatomy and affinity of *Tanganyicia rufofilosa* (Caenogastropoda, Ceithioidea, Paludomidae). *Zool. Scripta* **31**, 167–184.

Swofford, D. 2000 *PAUP*: phylogenetic analysis using parsimony (* and other methods)*. Sunderland, MA: Sinauer.

Swofford, D. & Berlocher, S. H. 1987 Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. *Syst. Zool.* **36**, 293–325.

Takezaki, N., Rzhetsky, A. & Nei, M. 1995 Phylogenetic test of the molecular clock and linearized trees. *Mol. Biol. Evol.* **12**, 823–833.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. 1994 Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680.

Thompson, J. N. 1999 The raw material for coevolution. *Oikos* **84**, 5–16.

Tiercelin, J.-J. & Mondeguer, A. 1991 The geology of the Tanganyika trough. In *Lake Tanganyika and its life* (ed. G. W. Coulter), pp. 7–48. London: Natural History Museum.

Trussell, G. C. & Smith, L. D. 2000 Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. *Proc. Natl Acad. Sci. USA* **97**, 2123–2127.

West, K. & Cohen, A. 1994 Predator–prey coevolution as a model for the unusual morphologies of the crabs and gastropods of Lake Tanganyika. *Arch. Hydrobiol. Beiheft. Ergebnisse Limnol.* **44**, 267–283.

West, K. & Michel, E. 2000 The dynamics of endemic diversification: molecular phylogeny suggests an explosive origin of the Thiarid gastropods of Lake Tanganyika. In *Ancient lakes: biodiversity, ecology and evolution* (ed. A. Rossiter & H. Kawanabe), pp. 331–354. London: Academic.

Winnepenninckx, B., Backeljau, T. & DeWachter, R. 1993 Extraction of high molecular weight DNA from molluscs. *Trends Genet.* **9**, 407.

Woodward, S. P. 1859 On some new freshwater shells from Central Africa. *Proc. Zool. Soc. Lond.* **27**, 348–350.

Visit http://www.journals.royalsoc.ac.uk to see electronic appendices to this paper.