

The Dynamics of Endemic Diversification: Molecular Phylogeny Suggests an Explosive Origin of the Thiarid Gastropods of Lake Tanganyika

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I. SUMMARY

The endemic gastropod fauna of Lake Tanganyika is remarkable not only for its great species richness, but also for its unusually ornate and heavily calcified shell morphologies that are convergent with diverse marine forms. The origin and intralacustrine radiation of these thiarid gastropods have been debated since the late nineteenth century, as they are perhaps the most dramatic lacustrine radiation of gastropods in the world. They parallel the endemic cichlid fish fauna of the African Great Lakes in their potential for providing information about the mechanisms of evolution.

This chapter presents the first molecular phylogenetic treatment of 12 of the 18 endemic gastropod genera of Lake Tanganyika, based on a mitochondrial

gene fragment of cytochrome oxidase I (*COI*). The endemic thiarid fauna of Lake Tanganyika was found to be paraphyletic, but a larger clade including *Cleopatra*, a thiarid genus widely distributed throughout East Africa, is monophyletic. The data reveal five robust clades within this larger monophyletic group: (1) ((*Reymondia*, *Cleopatra*) *Spekia*), (2) (*Stanleya*, *Tanganyicia*) as sister group to group 1, (3) the trochiform genera ((*Bathanalia*, *Chytra*) *Limnotrochus*) as a clade and (4) sister-taxon pairings for (*Lavigeria*, Nov. gen.) and (5) (*Anceya*, *Paramelania*).

Analyses using parsimony, neighbour-joining and maximum likelihood analyses agreed on sister-taxon relationships at terminal nodes, but were unable to resolve relationships among these Tanganyikan clades. This may be interpreted as an indication of rapid, burst-like radiation at the time of origin of this fauna. The term “superflock” (*sensu* Ribbink) may be used to describe the generic level radiation of Tanganyikan gastropods, as it preserves the information that this is a group of closely related endemics that have radiated *in situ*, but does not imply complete monophyly.

II. INTRODUCTION

Among biologists, the African Great Lakes have long been renowned for their species flocks of cichlid fishes. Lakes Malawi, Tanganyika and Victoria each host endemic flocks of several hundred morphologically, genetically and ecologically distinct cichlid species (e.g. Fryer and Iles, 1972; Greenwood, 1981; Seehausen, 1996; Turner, 1996; Kawanabe *et al.*, 1997). The advent of molecular systematics has provided a host of new characters from which to address questions of phylogenetic affinities among cichlids. These characters have shed light on several major questions. First, it is now known that the rates of molecular and morphological evolution in cichlids may be almost wholly decoupled (Meyer *et al.*, 1990; Sturmbauer and Meyer, 1992). Secondly, molecular studies have loaned support to the anatomically based findings of Stiassny (1981) that the striking morphological similarities between some Tanganyikan and Malawian cichlids is an example of convergence, and is not indicative of the cichlids of the African Great Lakes having once constituted a “superflock” in which sister taxa had dispersed to different lakes. Thirdly, evidence now exists that the Malawi and Victoria cichlid flocks are monophyletic and that both were probably derived from single lineages in the Tanganyikan flock (Kocher *et al.*, 1993; Meyer, 1993), a finding which again supports results from earlier, traditionally based taxonomic studies (Fryer and Iles, 1972). In short, the advent of molecular systematics has revolutionized how cichlid phylogenies are reconstructed and strengthened our understanding of evolutionary history and diversification in this group.

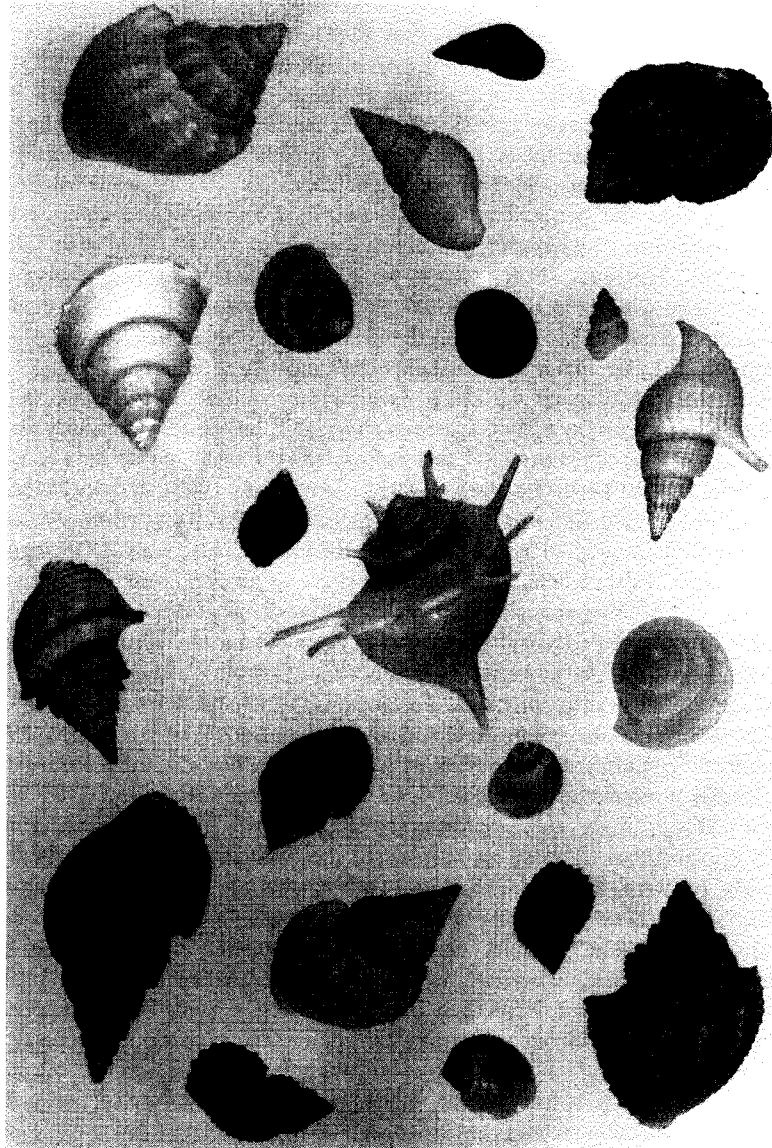
While cichlid species flocks have been the focus of intense study, the other species flocks in the African Rift Lakes have received comparatively little

attention from modern molecular techniques. The thiarid gastropods of Lake Tanganyika comprise a spectacular endemic radiation in their own right. Rich in species and diverse in form, the Tanganyikan thiarids (Figure 1) have unusually ornate and heavily calcified shells which look compellingly like marine shells (Moore, 1903), a similarity which stimulated the early scientific explorations of the lake (Fryer, this volume). Anatomical (Smith, 1904; Pelseener, 1906) and molecular (West, 1997) studies show, however, that this resemblance to marine shells is the result of convergent evolution and not phylogenetic affinities. Several different lines of evidence support the hypotheses that the ornate and heavily calcified Tanganyikan shells are an adaptive response to predation pressure (West *et al.*, 1991; West and Cohen, 1994, 1996). However, much work remains in analysing the patterns of diversification, phylogenetic relationships and mechanisms driving diversification in this group. This study of the molecular systematics of the thiarid gastropods of Lake Tanganyika explores some of these issues.

The thiarid gastropods of Lake Tanganyika include 18 endemic genera encompassing approximately 70 species. The high levels of endemism in this group suggest that they may have radiated within the basin, some time after the lake's origin, 9–12 Mya (Ebinger, 1989; Cohen *et al.*, 1993). It is perhaps the high levels of thiarid endemism in Lake Tanganyika that have led many people to assume that these gastropods constitute a species flock (Boss, 1978; Brown, 1994; Coulter, 1991; Michel, 1995; West, 1997). However, monophyly of this group has not been rigorously investigated to date, and in reality the taxonomy of Lake Tanganyika's thiarid gastropods is muddled and confused, the result of a century-long battle between taxonomic "lumpers" and "splitters". A recent taxonomic treatment of the Tanganyikan gastropods recognizes a total of 60 prosobranch and pulmonate species (Brown and Mandahl-Barth, 1987), but molecular studies indicate that, at least for the thiarids, these taxonomies significantly underestimate species-level diversity (E. Michel and K. West, unpubl.). Accordingly, these taxonomies are presently under revision.

It is unusual that in most taxonomies of the Tanganyikan thiarids, with the exception of Bourguignat (1885, 1890), the genera are either monotypic (e.g. *Chytra*, *Stormsia*) or highly speciose (e.g. *Lavigeria*, *Paramelania*, *Reymondia*). Perhaps the greatest difficulty encountered when studying this group is in defining and delineating species in these speciose clades. Morphological or anatomical studies alone have their limitations, and a more diverse approach, preferably one that incorporates modern biochemical tools, is necessary to address this problem. For example, through a combination of allozyme, conchological, soft-part anatomical, biogeographical and DNA sequence data, Michel (1995; this volume) was able to designate nine species of *Lavigeria*. Similar studies should be undertaken for the genera *Paramelania* and *Reymondia*.

Higher level systematic relationships among the Tanganyikan thiarid genera are also not clear. A recent contribution (Bandel, 1998) reassigned the Tanganyikan thiarids into three different families (the majority being placed



into the Pleuroceridae) and seven subfamilies therein. Because some of these groupings are contradicted by the present study and others (West *et al.*, in press; Michel and Todd, unpubl. data) and because the hypotheses of these relationships have not yet been tested through character analysis, this taxonomy (Bandel, 1998) has not been adopted in this study.

The present phylogenetic investigation focuses on generic-level relationships and aims to establish the coarse structure of the radiation of thiarid gastropods in Lake Tanganyika. While considerable work remains in delimiting taxonomic boundaries within the speciose genera, the taxonomic boundaries between genera, monotypic and speciose alike, are well established and provide confidence in the genus-level taxonomy (Brown, 1994) examined in this study. Of particular interest are the following questions. Was there a single thiarid invasion into Lake Tanganyika, with subsequent radiation forming the diversity seen today? Are the Tanganyikan thiarids monophyletic, or have they also given rise to non-Tanganyikan species? Within the radiation, do speciose clades give rise to other speciose clades, or do they arise independently from non-speciose clades? Are there any key morphological features associated with the speciose clades?

In order to establish a phylogeny with which to explore these questions, we analysed 640 base pairs (bp) of the cytochrome *c* oxidase subunit I (*COI*) region of the mitochondrial genome. This gene is associated with electron

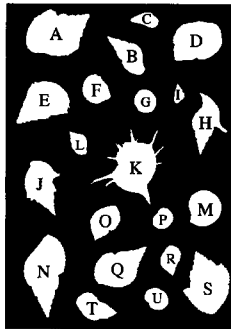


Fig. 1. Examples of the endemic thiarid gastropods of Lake Tanganyika. Note the heavily calcified and ornate shells. A, *Paramelania damoni*, form: typica; B, *Paramelania iridescens*; C, *Reymondia horei*; D, *Lavigeria grandis*; E, *Bathania howesii*; F, *Limnotrochus thomsoni*; G, *Spekia zonata*; H, *Paramelania iridescens*; I, *Mysorelloides multisulcata*; J, *Paramelania damoni*, form: imperialis; K, *Tiphobia horei*; L, *Lavigeria nassa*, form: typica; M, *Chytra kirki*; N, *Paramelania damoni*, form: mpalaensis; O, *Lavigeria nassa*, form: paucicostata; P, *Hirthis globosa*; Q, *Paramelania damoni*, form: crassigranulata; R, Nov. gen. n. sp.; S, *Paramelania damoni*, form: imperialis; T, Nov. gen. guillemei; U, *Tanganyica rufofilosa*. Scale: the maximum length of shell N, *P. damoni* form: mpalaensis, is 3.95 cm. Species identifications were based on Leloup (1953) and Brown (1994). However, the taxonomies of *Lavigeria* and *Paramelania* are currently under revision.

transport in cells (Frohlich *et al.*, 1996) and is one of the more conservative protein-coding genes of the mitochondrial genome (Brown, 1985). Many studies attest to the phylogenetic utility of *COI* at the genus, species and population levels across a variety of taxa (Crozier *et al.*, 1989; Folmer *et al.*, 1994; Frohlich *et al.*, 1996; Pederson, 1996; Michel, this volume). The differential rates of evolution of first, second and third position nucleotides also offer multiple levels of resolution within a study.

III. MATERIALS AND METHODS

A. Sample Collection and Preservation

Thiarid gastropods were collected from the Burundi and the Democratic Republic of Congo (formerly Zaïre) coastlines of Lake Tanganyika in 1992–1993 and the Tanzania and Zambia coastlines in 1995. Thiarid and non-thiarid outgroups were sampled from other East African lakes and rivers during this same time. Live specimens of the ingroup (Tanganyikan) and outgroup (non-Tanganyikan) taxa were obtained by snorkelling, SCUBA diving and/or dredging in 0.25–60 m of water. Sample lots (populations of a single species from the same locality) were randomly split into three sub-lots to form reference collections for conchological, anatomical and molecular studies, respectively. The shells of the gastropods reserved for molecular studies were cracked and peeled completely and the soft tissues were placed directly in 95% ethanol.

B. Molecular Methods

Except for one taxon for which only a single individual was collected, two or more individuals from a representative species of each thiarid genus in Lake Tanganyika, and all of the outgroups, were prepared for molecular analyses (see Table 1 for taxonomic information and sampling locales). Following the molecular methods of T. Collins (pers. comm.) and Palumbi (1996), DNA was extracted from alcohol-preserved tissues using a CTAB protocol. In the larger specimens (body > 0.5 cm), the heart and kidney and portions of the gonad were dissected out and used to prepare the DNA extract. In the smaller specimens (body < 0.5 cm), the whole soft body was used. The mucopolysaccharides secreted by molluscs sometimes inhibit DNA amplification. Several problematic DNA templates were successfully amplified after being treated with one-half volume 8 M lithium chloride for 1 h at 65°C (Palumbi, 1996).

Exploratory analyses were conducted to identify an appropriate mitochondrial gene region for this study. In initial studies, the 16S to ND1, 16S RNA, 12S RNA and cytochrome *b* regions proved to be either less informative and/or more difficult to amplify or sequence than cytochrome *c* oxidase I. This gene fragment was readily amplified and sequenced from the several taxa used in the preliminary analyses.

Table 1
Gastropods (family Thiariidae) collected from Lake Tanganyika and its environs

Genus	Species	Author	Sample no.	Locality	Depth, substrate
<i>Ancya</i>	<i>giraudi</i>	Bourguignat 1885	95 KW 45	Gitaza, BR	8 m, rocks
<i>Bathanalia</i>	<i>howesi</i>	Moore 1898	95 KW 17	Cameron Bay, ZM	57 m, mud
<i>Bridouxia</i>	<i>giraudi</i>	Bourguignat 1885	95 KW 10	Wonye Pt., ZM	2 m, under rocks
<i>Chytira</i>	<i>kirki</i>	Smith (1880b)	93 KW 01	Gitaza, BR	12 m, sand or silt
<i>Cleopatra</i>	<i>ferruginea</i>	Lea and Lea 1850	93 KW 57	Kinango Dam, KN	0.25 m, silt or mud
<i>Hirthis</i>	<i>globosa</i>	Ancey 1898	95 KW 28	Mtossi, TZ	5 m, rocks
<i>Lavigeria</i>	cf. <i>nassa</i>	Woodward 1859	95 KW 01	Kasenga Pt. ZM	5 m, rocks
<i>Limnotrochus</i>	<i>thomsoni</i>	Smith (1880a)	95 KW 12	Myiamba, ZM	4–13 m, sand
<i>Martelia</i>	<i>tanganyicensis</i>	Dautzenberg 1907	93 KW 31	Rwaba, BR	8 m, rocks
<i>Melanooides</i>	<i>admirabilis</i>	Smith (1880b)	95 KW 42	Malagarasi R., TZ	1 m, mud
	<i>tuberculata</i>	Müller 1774	93 KW 55	Mazeras, KN	0.5 m, macrophytes
<i>Mysorelloides</i>	<i>multisulcata</i>	Bourguignat 1888	95 KW 31	Kala Bay, TZ	30 m, dredge
Nov. gen.	n. sp.	Michel (unpubl.)	93 KW 06	Gitaza, BR	12 m, rocks
<i>Parametania</i>	<i>damoni: imperialis</i>	Giraud 1885	93 KW 36	Nyanza-Lac, BR	30 m, sand and silt
<i>Reymondia</i>	<i>horei</i>	Smith (1880a)	95 KW 04	Kasenga Pt., ZM	5–9 m, under rocks
<i>Spekia</i>	<i>zonata</i>	Woodward 1859	93 KW 04	Gitaza, BR	< 1m, rocks
<i>Stanleya</i>	<i>neritinooides</i>	Smith (1880b)	95 KW 29	Msamba, TZ	5 m, silt and sand
<i>Stormsia</i>	<i>minima</i>	Smith 1907	95 KW 08	Wonye Pt. ZM	< 1m, rocks
<i>Synolopsis</i>	<i>minuta</i>	Bourguignat 1885	93 KW 15	Muguruka, BU	8 m, sand
<i>Tanganyicia</i>	<i>rufofilosa</i>	Smith (1880a)	95 KW 13	Myiamba, ZM	4–13 m, sand
<i>Tiphobia</i>	<i>horei</i>	Smith (1880a)	95 KW 21	Nkamba, Bay, ZM	30 m, mud

All samples were collected alive. Species author, sample identification number, locality (BR, Burundi; KN, Kenya; TN, Tanzania; ZR, Democratic Republic of Congo (formerly Zaire); ZM, Zambia), depth and substrate data are included.

Using the polymerase chain reaction (PCR) (Saiki *et al.*, 1988), an approximately 640 bp region of the mitochondrial *COI* gene region was amplified with universal primers developed by Folmer *et al.* (1994). Amplification reactions, including a negative control, were carried out following typical protocols (Palumbi, 1996). Amplified DNA products were separated in a 2% agarose gel in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA) and visualized with ethidium bromide. Bands, approximately 640 bp in length, were excised and purified using the UltraClean Kit (Mo Bio Laboratories) following the manufacturer's directions. Both strands of the resultant double-stranded DNA product were sequenced directly using slightly modified sequencing primers (J. Staton, pers. comm.). Sequencing reactions were carried out following the manufacturer's instructions for the ABI PRISM Cycle Sequencing Kit. Excess dye terminators were removed from cycle-sequenced product by purifying the product in Centri-Sep spin columns (Princeton Separations). The cycle-sequenced products were visualized with an Applied Biosystems 377 automated sequencer and supporting ABI Prism software.

Spectrogram sequences were proof-read by eye. Nucleotide sequences were translated into amino acid sequences using DNA Strider 1.2 (Marck, 1995) with the *Drosophila* mitochondrial genome genetic code and three-phase translation option, so as to identify any reading-frame violations. Sequences were readily aligned by eye in SeqApp (version 1.9a169; Gilbert, 1994).

C. Phylogenetic Analyses

As a means of measuring hierarchical signal in the data, the skewness of the distribution (i.e. the g_1 statistic) of 100 000 randomly generated trees was calculated (Hillis, 1991; Hillis and Huelsenbeck, 1992). Because the inclusion of even one set of closely related taxa can significantly skew a frequency distribution, thereby giving a false sense of confidence in the signal of the data, the skewness of distributions of randomly generated trees was explored iteratively, at first with the entire data set and subsequently with subsets of the data (Lara *et al.*, 1996).

Because the efficiency and fidelity of different methods may vary depending on intrinsic characteristics of the data set (Kim, 1993), the data were analyzed using a suite of phylogenetic methods. Phylogenetic reconstruction using methods of maximum parsimony, neighbour-joining (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981) were employed in phylogenetic analyses using the Phylogenetic Analysis Using Parsimony (PAUP) computer program, version 4.0 (Swofford, 1997). Support for specific nodes was assessed using both bootstrap analysis (Felsenstein, 1985) (with 1000 replicates each with 10 random addition sequences per replicate and two trees saved at each step) and decay indices (Bremer, 1988) calculated by Autodecay version 2.9.6

(Eriksson, 1997). Character evolution at the amino acid level was explored using the MacClade computer program (Maddison and Maddison, 1996).

IV. RESULTS

A. PCR Reaction Amplification and DNA Sequencing of Preserved Tissues

Despite successful extractions of high molecular weight DNA from several individuals, some taxa (including *Bridouxia*, *Hirthia*, *Martelia*, *Mysorelloides*, *Stormsia* and *Syrnolopsis*) consistently failed to amplify. Even after applying a wide variety of trouble-shooting measures,* no amplified product could be obtained. With the exception of *Hirthia*, taxa which failed to amplify were of very small body size (body < 0.5 cm). It is possible that digestive enzyme activity during preservation and the age of the tissues used in the analyses (2–5 years in preservative) are especially detrimental in small taxa because of high surface to volume ratios, and are perhaps ultimately responsible for PCR failure. Alternatively, these taxa may share a biochemical characteristic which inhibited DNA amplification.

In contrast, the *COI* region of many other taxa consistently amplified. In each of the following taxa, genomic DNA were successfully amplified, and both strands of the *COI* fragment were successfully sequenced from at least two individuals: ingroup taxa: *Anceya giraudi* (95KW45), *Bathanalia howesi* (95KW17), *Chytra kirki* (93KW01), *Lavigeria* (95KW01), *Limnotrochus thomsoni* (95KW12), Nov. gen. n. sp. (genus and species yet to be formally described; see Michel, this volume, 93KW06), *Paramelania damoni* form: *imperialis* (93KW16), *Reymondia horei* (95KW04), *Spekia zonata* (93KW04), *Stanleya neritinoidea* (95KW29), *Tanganyicia rufofilosa* (95KW13), *Tiphobia horei* (95KW21); outgroup taxa: *Cleopatra ferruginea* (93KW57), *Melanoides admirabilis* (95KW42), and *Bellamyia* sp., *Melanoides nodicincta*, *Melanoides tuberculata* and *Melanoides turritispira* (collected from Lake Malawi by S. Drill). Hereafter, these taxa will be referred to by their generic names. Sequences are available from the authors upon request.

B. Sequence Analyses

COI sequences from individuals of the same population did not vary. Because genetic haplotypes were identical for the two individuals examined from each

*Trouble-shooting measures included adjusting DNA template and/or magnesium chloride concentrations, adjusting thermal cycling parameters, reprecipitating total DNA in lithium chloride, gene-cleaning total DNA, extracting total DNA from different individuals and/or different populations, and extracting total DNA using the Chelex method.

lot, in subsequent analyses a single sequence was retained to represent each taxon. Two taxa were deleted from subsequent analyses. *Melanoides tuberculata* and *Melanoides turritispira* shared identical sequences, perhaps owing to an error in species identification. Resequencing produced a single sequence which was confirmed to be *M. tuberculata* and consequently *M. turritispira* was excluded from further analysis. The *M. nodocincta* sequence was also excluded because it was a distant outlier and behaved strangely in analyses, features which suggest contamination of the sample.

Aligned sequences of 640 bp of the *COI* gene, representing 12 Tanganyikan thiarid and four outgroup taxa, yielded an average nucleotide composition (along the coding strand) of 24.3% adenine, 18.5% cytosine, 20.5% guanine and 36.7% thymine. These taxa show an A–T bias, with 61% of the sites being adenine or thymine. Inspection revealed 337 invariant sites (48%) and 303 (52%) variable sites. Of the variable sites, 234 (39% of the total sites) were phylogenetically informative and 69 (13%) were phylogenetically uninformative (autapomorphic or symplesiomorphic for the ingroup). Variable sites were distributed across codons as follows: 69 first positions, 27 second positions and 207 third positions, yielding a ratio of 2.6:1:7.7 for variable sites across codon positions. This value differs slightly from the 2:1:5 ratio commonly reported for variable sites in mitochondrial protein coding genes.

Transition and transversion ratios for all pairwise comparisons of taxa were averaged. In these analyses using PAUP, transitions outnumbered transversions 1.43 to 1. This lower than expected ratio suggests that some kinds of substitution may be saturated (C. Marshall, pers. comm.). To explore further the degrees of transition and transversion saturation in the data set, the relationship between transitions and transversions at each codon position was plotted relative to maximum likelihood distances among taxa (Figure 2). For first and second positions, the relationships between either transitions or transversions and maximum likelihood distance are linear. At third positions, however, transitions and transversions both increase rapidly, with transitions reaching an asymptote, and probably saturation, at a maximum likelihood distance of approximately 0.2. Transversions, however, maintain a relatively linear increase (Figure 2).

Pairwise sequence divergence, estimated using the LogDet/paralinear distance model (Lockhart *et al.*, 1994; Lake, 1994) to correct for multiple substitutions, was 13–31% among ingroup taxa, except for the *Stanleya–Tanganyicia* comparison (3%) (Table 2).^{*} Divergences between ingroup and outgroup taxa ranged from 18 to 30% (Table 2).

^{*}West will argue elsewhere that the genus *Stanleya* should be synonymized with *Tanganyicia* based on a shared unique brooding system.

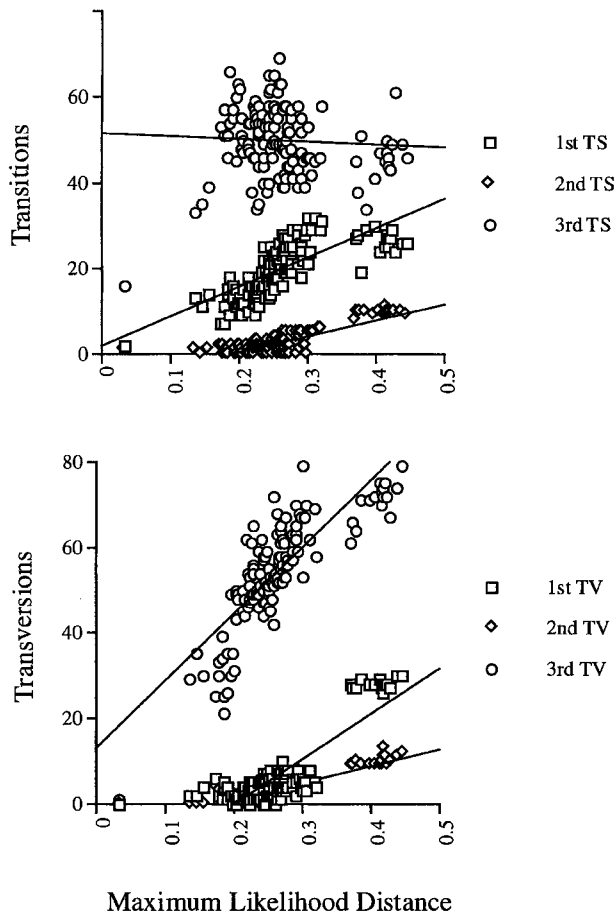


Fig. 2. Comparisons of cytochrome oxidase I sequences, showing relationships between the number of transitions (upper plot) and transversions (lower plot) at each codon position, and the maximum likelihood distance between all pairs. These plots serve as a visual estimate of nucleotide saturation levels.

C. Assessment of Phylogenetic Signal

The strength of phylogenetic signal in the data set was assessed by quantifying the skewness (g_1) of a distribution of the tree lengths of 100 000 randomly generated trees. When all taxa were considered, there was a significant negative skew in the tree length distribution ($g_1 = -0.9437$, $p < 0.01$), indicating the information content of the data to be significantly more structured than random (Hillis and Huelsenbeck, 1992). In addition, the shortest parsimony tree (1086 steps) was 371 steps shorter than the shortest random tree (1457

Table 2
 Estimated pairwise sequence divergence between taxa, calculated using the LogDet/paralinear distance model (Lockhart *et al.*, 1994; Lake, 1994) to correct for multiple substitutions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. <i>Anceya</i>	–															
2. <i>Bathanalia</i>	0.261	–														
3. <i>Chytra</i>	0.267	0.135	–													
4. <i>Lavigeria</i>	0.305	0.263	0.230	–												
5. <i>Limnotrochus</i>	0.265	0.156	0.146	0.215	–											
6. Nov. gen.	0.266	0.250	0.226	0.254	0.223	–										
7. <i>Paramelania</i>	0.232	0.249	0.252	0.274	0.214	0.267	–									
8. <i>Reymondia</i>	0.253	0.221	0.222	0.259	0.215	0.258	0.232	–								
9. <i>Spekia</i>	0.262	0.217	0.193	0.246	0.210	0.252	0.227	0.185	–							
10. <i>Stanleya</i>	0.260	0.227	0.213	0.260	0.200	0.272	0.230	0.212	0.199	–						
11. <i>Tanganyicia</i>	0.264	0.226	0.204	0.246	0.197	0.266	0.233	0.206	0.190	0.033	–					
12. <i>Tiphobia</i>	0.234	0.215	0.207	0.239	0.182	0.240	0.205	0.240	0.211	0.229	0.229	–				
13. <i>Cleopatra</i>	0.271	0.248	0.243	0.273	0.216	0.272	0.242	0.182	0.199	0.203	0.195	0.246	–			
14. <i>M. admirabilis</i>	0.289	0.282	0.287	0.261	0.253	0.293	0.264	0.279	0.266	0.252	0.259	0.272	0.283	–		
15. <i>M. tuberculata</i>	0.293	0.220	0.247	0.246	0.231	0.245	0.255	0.246	0.232	0.241	0.242	0.211	0.265	0.185	–	
16. <i>Bellamyia</i>	0.285	0.307	0.295	0.302	0.257	0.327	0.284	0.281	0.290	0.264	0.270	0.284	0.280	0.283	0.260	–

Taxa number 1–12 are genera endemic to Lake Tanganyika, 13–16 are outgroup taxa collected in East Africa, and 14 and 15 are from the genus *Melanoides*.

steps). However, the inclusion of a pair of very closely related taxa in the data sets can significantly skew the distribution of random tree lengths, and thereby produce an unrealistically high confidence level in the estimated phylogenetic signal (Lara *et al.*, 1996). To minimize this possibility, the skewness was measured repeatedly, after iteratively collapsing nodes with the highest bootstrap support. When this was done, in all permutations the distribution of random tree lengths remained significantly negatively skewed.

D. Phylogenetic Analyses

Phylogenetic analyses using maximum parsimony (specifically, a heuristic search with 1000 random addition replicates, 10 random addition sequences per replicate and two trees held at each step) produced a single tree 1086 steps in length (Figure 3a). Bootstrap and Bremer support values (Bremer, 1988) estimating support for various nodes were mapped on to the shortest tree (Figure 3a). Multiple heuristic searches with identical parameters except for the order of taxa in the data set yielded topologies identical to the shortest tree. Because of the high saturation rates in third position transitions, which accounted for much of the phylogenetic signal in the data set, phylogenetic analyses were conducted using first positions only, second positions only, first and second positions combined, and amino acids. These analyses produced topologies broadly similar to those obtained from analyses of the entire data set, but with considerably less resolution among the terminal nodes, which are less likely to be affected by saturation than deeper nodes. Consequently, all sites were retained in the analyses.

Neighbour joining (Saitou and Nei, 1987) using the LogDet/paralinear distance model (Lockhart *et al.*, 1994; Lake, 1994) produced the tree shown in Figure 3b, on to which bootstrap values were mapped. Maximum likelihood (Felsenstein, 1981) analyses, using the transition:transversion ratio calculated from the data, 1.43:1, produced the tree shown in Figure 3c. Bootstrap values were also mapped on to this tree.

E. Phylogenetic Relationships of the Tanganyikan Thiarid Gastropods

The parsimony, neighbour-joining and maximum likelihood trees all divide the Tanganyikan thiarids into four clades that are identical, except for the placement of *Tiphobia*. All topologies support (i) the monophyly of ((*Reymondia*, *Cleopatra*) *Spekia*) with the (*Stanleya*, *Tanganyicia*) sister clade to this grouping, (ii) the monophyly of the trochiform taxa ((*Bathanalia*, *Chytra*) *Limnotrochus*), and (iii) the sister-taxon relationship between (*Lavigeria*, Nov. gen.) and (*Anceya*, *Paramelania*). Both neighbour-joining and maximum likelihood analyses place *Tiphobia* as the sister taxon to the (*Anceya*, *Paramelania*) clade.

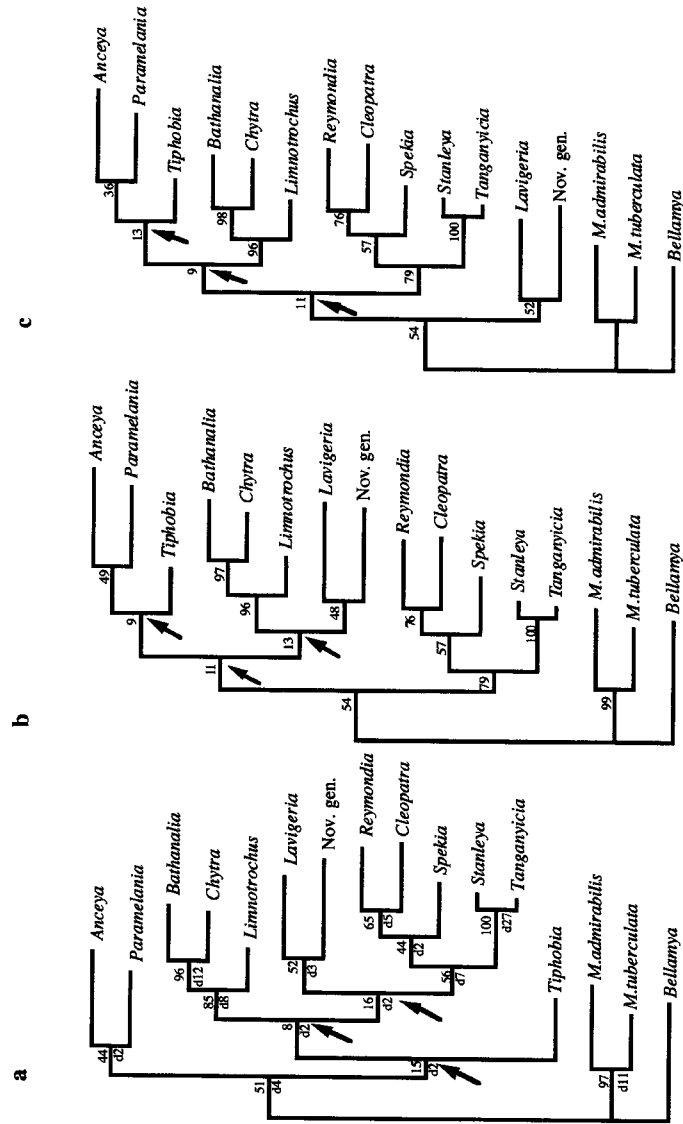


Fig. 3. (a) Parsimony, (b) neighbour-joining, and (c) maximum likelihood trees depicting relationships of Tanganyika thiarid gastropods. Bootstrap and Bremer support values, quantifying support for various relationships, are mapped on to phylogram nodes where appropriate. Arrows indicate nodes that are in discordance among the three topologies.

Although results from parsimony, neighbour-joining and maximum likelihood analyses show considerable agreement in generic sister-taxon relationships at the terminal nodes, and all support the monophyly of the Tanganyikan thiarids + *Cleopatra* clade (bootstrap values ranging from 54 to 100 and decay indices ranging from 4 to 27 for these relationships), they do not resolve the deeper branching order of these four clades concordantly (Figure 3). Parsimony analyses (Figure 3a) place the (*Lavigeria*, Nov. gen.) clade as the sister group to the ((*Reymondia*, *Cleopatra*) *Spekia*) (*Stanleya*, *Tanganyicia*) clade, with the trochiform taxa as the sister group to this larger clade, and the (*Anceya*, *Paramelania*) grouping basal among the Tanganyikan thiarids. Neighbour-joining analyses (Figure 3b), however, place the (*Lavigeria*, Nov. gen.) clade as sister group to the trochiform genera, with the (*Anceya*, *Paramelania*) *Tiphobia* clade sister to this grouping, and the ((*Reymondia*, *Cleopatra*) *Spekia*) (*Stanleya*, *Tanganyicia*) clade basal among the Tanganyikan thiarids. Maximum likelihood analyses (Figure 3c) produced a sister grouping of two larger clades, the trochiform + ((*Anceya*, *Paramelania*) *Tiphobia*) group, and the ((*Reymondia*, *Cleopatra*) *Spekia*) (*Stanleya*, *Tanganyicia*) clade, with the (*Lavigeria*, Nov. gen.) clade basal among the Tanganyikan thiarids. While these trees do differ, it is noteworthy that for all trees the internode branch lengths between these four clades are very short, and bootstrap and Bremer support for these deeper nodes is weak (bootstrap values ranging from 8 to 15 and decay indices of 2).

V. DISCUSSION

A. Rationale for Selecting Outgroups

Three thiarid outgroups, *Melanoides admirabilis*, *M. tuberculata* and *Cleopatra ferruginea*, were used in this study. The former species is endemic to the Malagarasi River, which presently drains into Lake Tanganyika and pre-dates the formation of the lake, whereas the latter two are distributed throughout southern Asia, the Middle East and eastern Africa (Brown, 1994). *Melanoides tuberculata* is known from lower Miocene (pre-rift) fossils in East Africa and from lake deposits (Schouteden, 1933; Gautier, 1970; Van Damme, 1984). *Cleopatra ferruginea* is known from lower Miocene deposits in East Africa and probably evolved prior to the splitting of Madagascar from the African continent (Fuchs, 1936; Gautier, 1970; Van Damme, 1984; Brown, 1994). Clearly, these three outgroup species have a long history in East Africa, and although none of these taxa is currently found within the lake proper, they are common in rivers and swamps fringing the lake. This, and the fact that they are the only cosmopolitan thiarid gastropods currently found within the Tanganyikan drainage basin, make them excellent candidates for outgroups to the endemic Tanganyikan thiarids.

There is no *a priori* evidence to favour one of these outgroups over the other. In fact, both seem equally appropriate: *Melanooides* broods its young, whereas *Cleopatra* lays eggs, and both reproductive strategies are found among the Tanganyikan thiarids. In addition, *Bellamya*, a cosmopolitan viviparid gastropod, was added to the analyses to determine which of the more proximal outgroups, *Cleopatra* or *Melanooides*, was more distant. There are other potential outgroups with far more limited distributions which may be appropriate for inclusion in this investigation, notably *Potadamoides* and *Potadoma*, and future fieldwork will endeavour to sample these and other outgroup taxa.

B. Simultaneous Diversification of Major Tanganyikan Thiarid Lineages

There is relatively strong support for four distinct monophyletic clades of Tanganyikan thiarid gastropods with *Cleopatra* nested within (bootstrap values ranging from 54 to 100 and decay indices ranging from 4 to 27 for these relationships). There is little support, however, for resolved relationships among these four clades (bootstrap values ranging from 8 to 15 and decay indices of 2). The poor resolution of relationships among the four clades is partly due to the very high and similar levels of sequence divergence in the data set (Table 2). These equally high levels of sequence divergence across taxa can be explained in two ways.

The high levels of sequence divergence and weak support for deeper nodes may reflect the poor resolving power of *COI* for this systematic problem. Because third-position transitions are unconstrained at the amino acid level and occur rapidly, they would be the first type of substitution to record initial divergences. In the present data set, however, third-position transitions are saturated and therefore unable to contribute much meaningful phylogenetic information for the deeper nodes of the tree. Sequences from a more conservative gene, such as 16S or 12S RNA, and/or additional taxa could be used to assess whether *COI* has indeed evolved too quickly to be of use in resolving this systematic problem. However, it should be noted that *COI* has provided phylogenetically informative data in other studies at similar taxonomic levels with similar or considerably older divergence times (Crozier *et al.*, 1989; Folmer *et al.*, 1994; Frolich *et al.*, 1996; Pedersen, 1996), and therefore this explanation is considered unlikely.

An alternative explanation for the similar levels of sequence divergence seen in the data is that the four major Tanganyikan thiarid gastropod clades evolved simultaneously, or nearly so, such that *COI*, and probably any other molecule, could not capture the event(s). Rapid simultaneous or nearly simultaneous divergence would result in a true ("hard") polytomous relationship or star phylogeny. The following lines of evidence are consistent with a star radiation of the deeper nodes of the Tanganyikan thiarid gastropods.

First, while estimated pairwise sequence divergences are rather heterogeneous among terminal nodes on the tree (ranging from 3 to 18%), distances for each of the four major clades (averaged among clade members) to the *Cleopatra* outgroup were relatively uniform (ranging from 24 to 27%). Similar levels of sequence divergence are consistent with an evolutionary scenario of simultaneous divergence times from a common ancestor.

Secondly, poor resolution is concentrated in only one region of all of the trees, namely, in the relationships of the four major Tanganyikan thiarid clades. Nodes both terminal and basal to this region show greater resolution. If *COI* was completely saturated, one would expect to see progressively less support for the more basal nodes and the Tanganyikan thiarid clades perhaps pairing off with the *Melanoides* outgroup clade. In fact, low bootstrap or Bremer support values are concentrated where the four clades converge, but the monophyly of Tanganyikan thiarid + *Cleopatra* clade is comparatively well supported.

Finally, phylogenetic analyses of anatomical and allozyme data sets for many of these same taxa (West, 1991) produced strong support for species-level relationships at terminal nodes, which were united by long branches into unresolvable polytomies. Analyses of the *COI* sequences and congruent topologies from multiple independent data sets (West, 1991) strongly support the near-simultaneous diversification of several Tanganyikan gastropod clades.

C. Paraphyly of the Tanganyikan Thiarid Gastropods

Although strongly supported, it was none the less surprising to find *Cleopatra* positioned in the terminal branches of the phylogenetic trees. This positioning renders the endemic Tanganyikan thiarids paraphyletic. There is some support for the monophyly of *Cleopatra* + the endemic Tanganyikan thiarids. Bootstrap values of 51–54 for this clade jumped to 79 after deletion of the very distant outgroup *Bellamyia*. This node is also supported by an amino acid substitution, which is an otherwise rare event. Bootstrap values supporting relationships between the *Melanoides* taxa and the Tanganyikan thiarids were considerably weaker (10–20) and there are no amino acid substitutions supporting such pairings.

The non-monophyly of the Tanganyikan thiarid gastropods is also supported by other studies. West (1991) collected electrophoretic data across 30 presumptive genetic loci and examined 25 conchological characters for *Cleopatra*, *Melanoides* and 16 Tanganyikan species. Although the topologies differ somewhat from trees produced in the present study, these allozyme and conchological data sets, whether treated independently or combined, could not be resolved to yield a single monophyletic Tanganyikan thiarid clade (West, 1991).

In converting these cladograms (Figure 3a–c) into phylogenies (Figure 4a, b), there are two ways to interpret the position of *Cleopatra* and the paraphyly of the endemic Tanganyikan gastropods.

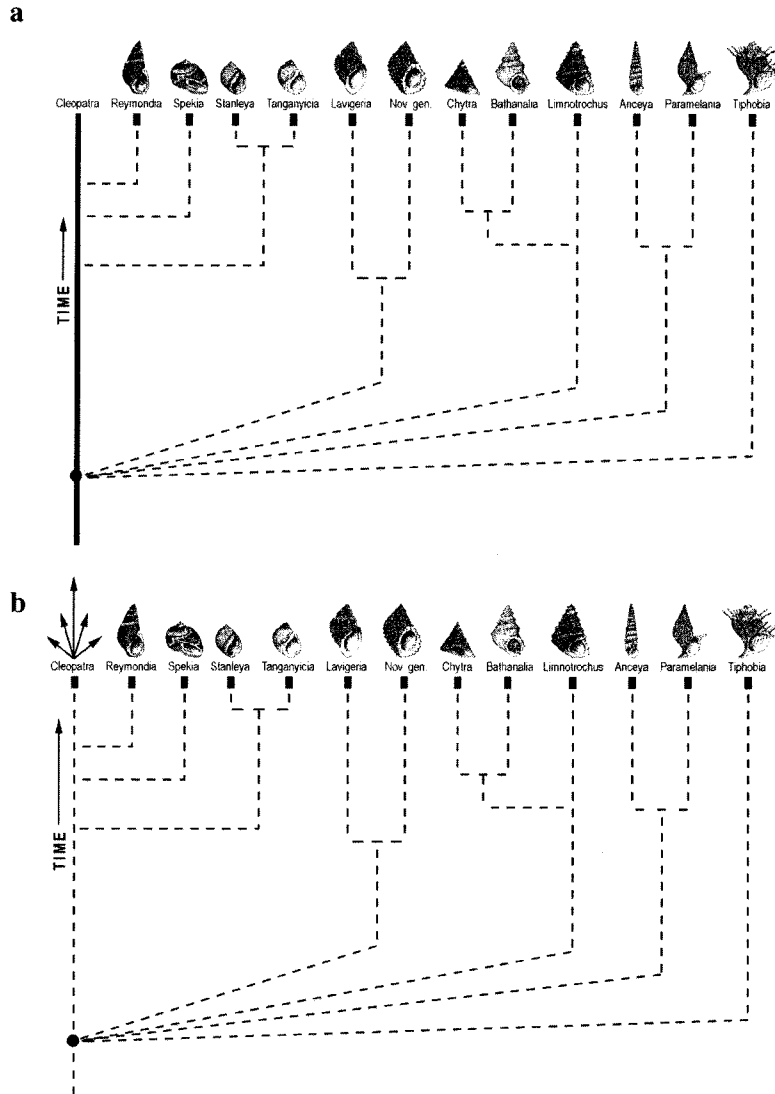


Fig. 4. Two different phylogenies consistent with a consensus of the parsimony, neighbour-joining and maximum likelihood cladograms derived from *COI* sequences. (a) A *Cleopatra*-like ancestor gives rise to the Tanganyika thiarid gastropods; (b) the cosmopolitan outgroup *Cleopatra* is the product of the Tanganyika thiarid radiation.

First, perhaps *Cleopatra* was the source from which all the Tanganyikan taxa were diverging (Figure 4a). In this model, a *Cleopatra*-like ancestor lived in or near to the proto-Lake Tanganyika and diverged four to seven times to produce the thiarid gastropod diversity in the lake. In this case, “*Cleopatra*” is a paraphyletic stem taxon. Early Miocene *Cleopatra* fossils (Van Damme, 1984) indicate that *Cleopatra* was established in the region before the formation of the lake, and thus provide anecdotal support for this scenario.

Alternatively, rather than the source, *Cleopatra* might be the product of the Tanganyikan thiarid radiation (Figure 4b). Perhaps, after diverging from a common ancestor with *Reymondia* in the lake, *Cleopatra* dispersed from the Tanganyika basin and colonized other African waters, from the Nile Valley to South Africa, and Senegal to Madagascar. This interpretation assumes that the *Cleopatra* species used in this study is not genetically related to fossils that have been attributed to this genus. Although some studies have demonstrated a decoupling of molecular and morphological evolution in gastropods (e.g. Palmer, 1985), it seems imprudent to rule out the considerable palaeo-record of this species.

To distinguish between these two different interpretations, further sampling of *Cleopatra* taxa throughout Africa is necessary. Monophyly of *Cleopatra* species positioned high in the tree would suggest that *Cleopatra* is the product of the Tanganyikan thiarid radiation, whereas paraphyly of *Cleopatra* species, with some species rooting basal to the Tanganyikan thiarid radiation, would suggest that this genus is the source of Tanganyikan thiarid diversity.

It is also possible that *Cleopatra* fossils are paraphyletic with respect to the Tanganyikan radiation. The earlier *Cleopatra* lineages may have gone extinct and the modern *Cleopatra* may have descended from a Tanganyikan ancestor; in such a case, *Cleopatra* would be monophyletic on the tree and unrelated to the fossils.

D. A Tanganyikan Thiarid Gastropod “Superflock”

Species flocks are monophyletic groups of organisms that are endemic to a geographically circumscribed area and possess unusual species richness or diversity relative to other members of the taxon (Greenwood, 1984). Species flocks can occur hierarchically, across several taxonomic scales (Ribbink, 1984). For example, in Lake Tanganyika one may consider the species flock of lamprologine cichlid fishes (an endemic, monophyletic tribe comprised of nine genera) or the flock of nine species of *Neolamprologus* (an especially rich genus in the lake), nested within the larger lamprologine flock (Sturmbauer *et al.*, 1994).

The thiarid gastropods of Lake Tanganyika, even at the generic level, are endemic to the Tanganyika basin, and are unusually rich and diverse compared to thiarids elsewhere. For these reasons many workers have assumed that they

constitute a species flock. A recent study lacking rigorous character analysis and phylogenetic treatment (Bandel, 1998) proposed polyphyletic origins for the Tanganyikan gastropods. Data in the present and other studies (West *et al.*, 2000; Michel and Todd, unpubl. data) support some of these findings, but conflict with others. A polyphyletic origin of the Tanganyikan gastropods remains to be convincingly demonstrated, pending character analysis and phylogenetic treatment with relevant outgroups.

However, the paraphyly of the Tanganyikan thiarid gastropods, established in this study, brings their status as a species flock into question. Because they are not monophyletic, should we refrain from referring to the larger radiation of thiarid gastropods in Lake Tanganyika as a species flock, reserving the term instead for lower level monophyletic components of the radiation, such as the *Lavigeria* species flock or the *Paramelania* species flock? Ribbink has argued that, in cases such as this, the term species flock should be maintained, for "it does not really matter whether the flock is the product of one or several ancestors entering the lake. The collection of closely related species within the confines of the lake has the qualities of a species flock" (Ribbink, 1984). The present authors disagree. Monophyly is a critically important aspect to the species flock concept. It is only after species have been parsed into monophyletic groupings (or true polytomies) that one can begin to explore the biotic and abiotic factors governing the diversification of these groups and thus begin to explore what, exactly, makes them so unique.

Before the striking morphological similarities between cichlids in Malawi and Tanganyika were shown to be due to convergence (Kocher *et al.*, 1993; Meyer, 1993; but see Stiassny, 1981), Greenwood (1983, 1984) used the term "superflock" to describe a larger cichlid species flock in which some sister taxa were dispersed to different lakes, thus circumventing the "endemic to a geographically circumscribed area" aspect of the species flock definition. Studies by the present authors indicate that the thiarid gastropods endemic to Lake Tanganyika diverged rapidly within the lake. The nature of their ancestor(s) remains to be resolved. In addition, the position of *Cleopatra*, nested among the ingroup taxa, renders the Tanganyikan thiarid gastropods paraphyletic, thus violating the monophyly requirement of the species flock definition. The term "superflock" could be resurrected to refer to the rapid paraphyletic radiation of Tanganyika thiarid gastropod genera.

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