Mitochondrial Phylogeny of the Endemic Mouthbrooding Lineages of Cichlid Fishes from Lake Tanganyika in *Eastern Africa*[1]

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Of the three cichlid species flocks in eastern Africa, Lake Tanganyika harbors the oldest species assemblage, which is also the most diverse morphologically and behaviorally. For 12 species (20 individuals) of 12 genera of the tribe Ectodini, 852 bp from two segments (cytochrome *b* and control region) of the mitochondrial genome were sequenced. In addition, orthologous sequences were obtained from eight species (11 individuals) representing other mouthbrooding lineages from Lake Tanganyika. Comparisons of sequence divergences revealed that the single Tanganyikan tribe Ectodini appears to be approximately five times older than the whole Lake Malawi cichlid species flock, suggesting that the radiation of the Tanganyikan mouthbrooding lineages took place long before the species flocks of Lakes Malawi and Victoria evolved. Seven of nine surveyed tribes of Tanganyikan cichlids appear to be approximately equally divergent, and this seems to corroborate the hypothesis of a rapid simultaneous formation of lineages at an early stage in the history of the Lake Tanganyika species flock. The close genetic relationship between the endemic *Tropheus* lineage and a nonendemic "Haplochromine," *Astatotilapia burtoni,* indicates that members of the tribe Tropheini may be the sister group of the cichlid flocks of Lakes Malawi and Victoria. The phylogenetic analyses demonstrate the monophyly of the Ectodini and identify the Cyprichromini as their sister group among the Tanganyikan cichlids. Within the tribe Ectodini the molecular data suggest both a branching pattern different than that previously proposed and a subdivision of the Ectodini into four clades, instead of the two originally described. The previously postulated model of morphological transformations believed to be responsible for the drastically different types of ecological specialization found among the Ectodini might therefore be in need of reinterpretation. Characters immediately related to foraging and nutrition seem to be particularly prone to homoplasy, even among members of a single lineage of cichlid fishes.

Introduction

There have been three explosive radiations of cichlid fishes in the eastern African Lakes Victoria, Malawi, and Tanganyika, with each lake containing hundreds of endemic species. With an estimated age of 9–20 Mya, Lake Tanganyika is the second oldest lake in the world (Tiercelin and Mondeguer 1990; Cohen et al., accepted) and is substantially older than the other two large eastern African lakes. Comparisons of sequence divergences suggest that the endemic Tanganyikan genus *Tropheus* is approximately twice as old as the whole Lake Malawi cichlid species flock and approx-

1. Key words: Ectodini, polymerase chain reaction, cytochrome *b*, control region, adaptive radiation, species flocks.

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Mol. Biol. Evol. 10(4):751–768. 1993.

0737-4038/93/1004-0003$02.00

imately six times older than the entire Lake Victoria flock (Sturmbauer and Meyer 1992). These data corroborate that the rate of morphological evolution has not been constant during the evolution of this species flock and that it differs substantially between lineages of cichlid fishes. Furthermore, genetic data from the *Tropheus* lineage suggest that large historic fluctuations in the lake level (Scholz and Rosendahl 1988, 1990) affected the distribution of species and speciation (Sturmbauer and Meyer 1992).

Cichlids vary tremendously in shape, yet this variation is largely due to allometric differences rather than to de novo evolution of characters (Strauss 1984). The differentiation of cichlids often proceeds through "evolution via concertina" (Stiassny 1991, p. 3). Hence, few morphological characters are available for cladistic analyses of cichlid relationships, and homoplasies seem to be commonplace.

Unlike those of Lakes Malawi and Victoria (Meyer et al. 1990; but see Greenwood 1983*a*), the Tanganyikan cichlid flock is believed to be of polyphyletic origin (Fryer and Iles 1972, p. 501; Poll 1986; Nishida 1991), and some lineages seem to be related to species from the Zaire River drainage system and to the species flocks of Lakes Malawi and Victoria (Fryer and Iles 1972, p. 501; Poll 1986; Meyer et al. 1990, 1991; Coulter 1991). The diversity of the Tanganyikan cichlid species flock is more extreme in terms of morphological and behavioral specializations than are the flocks of Lakes Malawi and Victoria (Fryer and Iles 1972, p. 500; Greenwood 1984). Their lack of intermediate morphotypes interrelating the sharply demarcated lineages has hindered phylogenetic analysis and the recognition of sister-group relationships (Greenwood 1984). Curiously, the Lake Tanganyika flock appears to have the fewest number of species: 171 (Poll 1986) compared with the 200–300 species for Lake Victoria (Witte et al. 1992) and the 500–1,000 species for Lake Malawi (Ribbink 1984; Lewis et al. 1986, p. 12). Numerous geographical races, however, have been reported from several Tanganyikan species (Brichard 1989).

The analysis of allozyme data on 20 Tanganyikan species resulted in a partial resolution of the relationships among lineages of the species flock (Nishida 1991). At least seven different lineages (*Tylochromis, Oreochromis, Boulengerochromis, Trematocara, Bathybates,* the *Lamprologus* lineage, and the "*Haplochromis*" lineage) are recognized. The phylogenetic relationships within the "Haplochromis" lineage, comprising 7 of the 12 Tanganyikan tribes described by Poll (1986), remain unresolved (see Nishida 1991, fig. 3).

Approximately two-thirds of the endemic cichlids of Lake Tanganyika brood their eggs in the buccal cavity (Poll 1986); this form of parental care is termed "mouthbrooding." While all endemic cichlids of Lakes Malawi and Victoria are mouthbrooders of a single type (only females incubate eggs and fry), the cichlids of Lake Tanganyika encompass a remarkable diversity of breeding behaviors of presumably more primitive but also seemingly highly derived patterns (Yanagisawa and Nshombo 1983; Yanagisawa 1985, 1986; Kuwamura 1986; reviewed in Barlow 1991; and Keenleyside 1991), pointing to the possibility that mouthbrooding may have evolved independently and repeatedly (Fryer and Iles 1972, p. 156; Barlow 1991).

Of the 171 cichlids endemic to Lake Tanganyika, the tribe Ectodini comprises 30 species assigned to 13 genera (Poll 1986) that are highly diverse ecologically, living in both sandy and rocky littoral zones. Five different foraging types are recognized: sand-dwelling species are zoobenthos "diggers" (benthic invertebrates and mollusks; Brichard 1989, p. 303) or pelagic invertebrate feeders; and rock-dwelling species feed on detritus or on epilithic algae (termed "Aufwuchs"; Yamaoka 1991). The Ectodini are probably the ecologically, morphologically, and behaviorally most diverse tribe of

Tanganyikan cichlids. All Ectodini are maternal mouthbrooders (Poll 1986), yet some have derived breeding patterns involving either monogamy and biparental guarding or complex lekking behavior combined with polygyny (Yanagisawa 1986; A. Rossiter, personal communication). Table 1 summarizes some features of the biology of the species considered here.

Liem (1981) first postulated the monophyly of five genera of the Ectodini as the "*Ophthalmotilapia* assemblage," on the basis of comparative osteology and myology. Greenwood (1983*b*) distinguished two "subassemblages" within the Ectodini, on the basis of different levels of intestinal complexity and prolongation. He defined the genera *Asprotilapia, Grammatotria, Xenotilapia, Callochromis, Aulonocranus,* and *Ectodus* as the *Asprotilapia* subassemblage; all have relative intestinal lengths less than three times the standard length. His *Ophthalmotilapia* subassemblage comprises *Lestradea, Cyathopharynx, Ophthalmotilapia,* and *Cunningtonia,* with higher relative intestinal lengths of up to six times standard length.

Poll (1986) added two genera, now comprising *Asprotilapia, Aulonocranus, Callochromis, Cardiopharynx, Cunningtonia, Cyathopharynx, Ectodus, Enantiopus, Grammatotria, Lestradea, Microdontochromis, Ophthalmotilapia,* and *Xenotilapia.* Liem (1981) considered the invertebrate feeder *Ectodus descampsi* to be the most generalized and as a prototype of the ancestral lineage. However, no sister-group relationships to any other Tanganyikan cichlid lineage could be established, either on

Table 1
Characterization of the Studied Cichlid Species from Lake Tanganyika

Species	Tribe	n[a]	Habitat[b]	Diet[c]
Oreochromis tanganiae	Tilapiini	1	Ubiquitous	Detritus
Bathybates ferox	Bathybatini	1	Pelagic	Carnivorous
Perissodus straeleni	Perissodini	1	Rock	Fish scales
Limnochromis auritus	Limnochromini	1	Bottom	Carnivorous
Tanganicodus irsacae	Eretmodini	2	Rock	Invertebrates
Tropheus duboisi	Tropheini	3	Rock	Aufwuchs
Astatotilapia burtoni	Haplochromini	1	Swamps	Invertebrates
Paracyprichromis brieni	Cyprichromini	1	Pelagic	Zooplankton
Grammatotria lemarii	Ectodini	2	Sand	Zoobenthos
Callochromis pleurospilus ..	Ectodini	1	Sand	Zoobenthos
Xenotilapia ochrogenys	Ectodini	1	Sand	Zoobenthos
Asprotilapia leptura	Ectodini	1	Rock	Aufwuchs
Microdontochromis tenuidentata	Ectodini	2	Sand/rock	Zoobenthos
Ectnodus descampsi	Ectodini	1	Sand	Invertebrates
Aulonocranus dewindti	Ectodini	2	Sand	Invertebrates
Lestradea perspicax	Ectodini	1	Sand	Invertebrates
Cunningtonia longiventralis	Ectodini	2	Sand/rock	Aufwuchs/detritus
Cardiopharynx schoutendeni	Ectodini	2	Sand	Aufwuchs/detritus
Ophthalmotilapia ventralis ..	Ectodini	2	Rock	Aufwuchs/detritus
Cyathopharynx furcifer	Ectodini	3	Sand/rock	Aufwuchs/detritus

NOTE.—Nine of the 12 Tanganyikan tribes that breed by buccal incubation of eggs and wrigglers were analyzed. The table shows that all littoral types have been colonized by the Ectodini and that five different foraging types are distinguishable.

[a] No. of individuals sequenced.

[b] Characterized according to Brichard (1989). Aufwuchs = filamentous and unicellular epilithic algae and attached detritus.

[c] According to Yamaoka (1991) and personal observations by C.S. [see Sturmbauer et al. (1992)].

the basis of comparative morphological data (Liem 1981; Greenwood 1983*b*) or on the basis of allozyme data (Nishida 1991).

The present paper attempts to resolve the phylogeny and to study phenotypic evolution within this tribe by means of mitochondrial DNA (mtDNA) sequences. Similar methodology has been the basis for our previous inferences about other aspects of the evolutionary history of the African cichlids (Meyer et al. 1990, 1991; Sturmbauer and Meyer 1992).

Material and Methods

Species, Tissues, and Genes Sequenced

Mitochondrial gene segments from 12 species of 12 genera from the tribe Ectodini were sequenced (see table 1). In addition, orthologous sequences from eight other Tanganyikan species, each representing a different tribe, were analyzed to test the monophyly of the Ectodini and to create a larger phylogenetic framework for Tanganyikan cichlids (table 1). From all species, a 402-bp segment of the mitochondrial cytochrome *b* gene was sequenced. In addition, sequences were obtained, from all species, from a 450-bp segment of the major mitochondrial noncoding region (control region, 367 bp), including part of the threonine tRNA gene (12 bp), and the proline tRNA gene (71 bp).

DNA Extraction and Nucleotide Sequencing

DNA was extracted from white muscle tissue of frozen and ethanol-preserved specimens (Kocher et al. 1989). Amplifications via the polymerase chain reaction (PCR) were conducted in 25 μl of Tris buffer (67 mM, pH 8.8) containing 2 mM $MgCl_2$, 1 mM of each dNTP, 1 μM of each primer, 1–1,000 ng template DNA, and *Taq* polymerase (0.5 unit; Cetus). The primers used for sequencing the cytochrome *b* gene segment were L14724 and H15148 (Kocher et al. 1989; Meyer et al. 1990), and L15926 and H16498 were used for the tRNAs and a segment of the control region (Kocher et al. 1989; Meyer et al. 1990). PCR was performed for 30 cycles for the double-stranded PCR (45 s at 92°C, 60 s at 52°C, and 90 s at 72°C). An aliquot of 5 μl of the double-stranded PCR products was gel purified on a minigel containing 2.5% NuSieve agarose in Tris-borate-EDTA buffer (0.1 M, pH 7.2), stained with ethidium bromide. The target product was excised and dissolved in 200–1,000 μl H_2O. Asymmetric PCR was conducted for 35 cycles (Gyllensten and Erlich 1988), with the primers L14724 and L15926, respectively, in limited concentration (0.01 μM; 40 s at 92°C, 60 s at 55°C, and 90 s at 72°C). The primer H 16498 was also diluted to a final concentration of 0.2 μM, for the asymmetric PCR. The single-stranded amplification products were ultrafiltrated three times with 300 μl H_2O in spin columns (Millipore 30,000) before direct sequencing (Sequenase Version 2.0; U.S. Biochemical; Sanger et al. 1977). The sequences were electrophoresed on two complementary runs using 6% acrylamide-urea gels in TBE buffer (45 mM, pH 8.0) on a wedge and a straight gel.

Age Estimates

Our age estimates rest on the assumption of comparable relative rates of molecular divergence among eastern African cichlids. Relative ages were estimated, by the comparison of maximum observed sequence divergences between clades, from a 450-bp segment of the control region and a 402-bp segment of cytochrome *b*. For the largely noncoding segment, sequence divergences were calculated from transversions and

indels only and were corrected for multiple substitutions, by the one-parameter model (Jukes and Cantor 1969; Jukes 1980) and by assuming equal probabilities of change between the three character states (purine, pyrimidine, and indels). In cytochrome *b*, Jukes-Cantor distances were calculated from all observed substitutions (Jukes and Cantor 1969).

The maximum sequence divergences among species of the Ectodini were compared with those found within the *Tropheus* lineage, a different lineage of Tanganyikan mouthbrooding cichlids (fig. 1). The genus *Tropheus* was represented by 54 individuals from 21 populations of all six described species (Sturmbauer and Meyer 1992). Further, we compared the molecular divergence within the Ectodini with that found in the cichlid species flock of Lake Malawi, represented by 24 species from 12 genera (Meyer et al. 1990; P. Reinthal and A. Meyer, unpublished data). Since no cytochrome *b* sequence differences were found among representative species from Lake Victoria (~0.2 Myr old), and since only two transversions were observed in the control region, they were not included in our comparisons.

The absolute age of the lineages was estimated on the basis of Banister and Clarke's (1980) approximation of the age of the Malawian species flock as being ~0.7 Myr

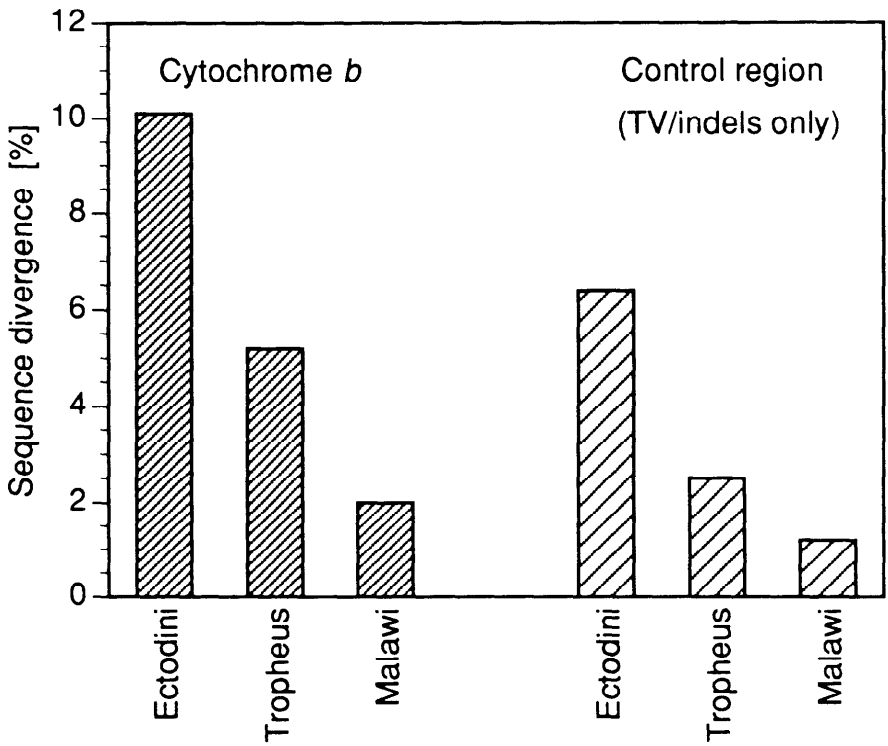

FIG. 1.—Maximum observed sequence divergence. Data are for the tribe Ectodini (12 species; n = 20), within the Tanganyikan genus *Tropheus* (six species; *n* = 54) and within 24 Malawian species from 12 genera (*n* = 45), representing the Malawi species flock. Genetic divergences were calculated for a 450-bp segment of the control region, including 83 bp of the Thr tRNA and the Pro tRNA, based on transversions (TV) and indels only. The sequence divergences of a 402-bp segment of the cytochrome *b* were calculated from all observed base substitutions equally weighted. The observed divergences for both segments have been corrected for multiple substitutions according to Jukes and Cantor's (1969) one-parameter model.

old. The geological age of Lake Malawi, however, may be considerably greater (Scholz and Rosendahl 1988, 1990; Tiercelin et al. 1989; Johnson and Ng'ang'a 1990).

Phylogenetic Analysis

The sequence data were entered and aligned in ESEE (version 1.09; Cabot and Beckenbach 1989) and were analyzed by means of the parsimony method using PAUP (Version 3.0s; Swofford 1992). In addition, to choose among equally parsimonious phylogenetic trees (Carpenter 1988), we performed a successive-approximation approach to weighting of characters according to their "cladistic reliability" (Farris 1969; Williams and Fitch 1989). Reweighting was based on both the mean rescaled consistency index of all equally parsimonious trees found in parsimony analysis and a base weight of 1,000.

Neighbor-joining analyses (Saito and Nei 1987) were always performed in parallel by means of NTSYS.PC (Version 1.6; Applied Biostatistics, 1990), using distance matrices that were calculated on the basis of the same weights as were used in parsimony analyses. The use of the same weights allowed comparison of the results from both methods and allowed the interpretation of corresponding results to be independent from the algorithm used.

Many studies have shown that different selective constraints act on different genes or gene segments, causing nonrandom occurrences of nucleotide substitutions (e.g., see Brown et al. 1982; Smith 1989; Hedges et al. 1990, 1991; Kraus and Miyamoto 1991; Allard and Miyamoto 1992). Our analyses accounted for different evolutionary rates in various gene segments and for different frequencies in the occurrence of transitions and transversions, by utilizing the most infrequently observed base substitutions to investigate more distant phylogenetic splits, whereas all substitutions were considered with equal weights to address more recent phylogenetic events between more closely related taxa (Fitch 1971). The phylogenetic analysis was performed in three steps.

The large number of taxa analyzed made application of heuristic search procedures necessary for parsimony analysis. To make the heuristic search more reliable (D. L. Swofford, personal communication), the option "random addition of taxa" with 50 replications was chosen in PAUP. Statistical analysis was done only for parsimony by means of the bootstrap procedure (Felsenstein 1985; 100 replications, heuristic search, and PAUP option "simple addition of taxa").

A first analysis was performed to place the Ectodini within the larger phylogenetic framework of the Tanganyikan mouthbrooding genera, to test the monophyly of the lineage, and to identify its sister group. Both gene segments (852 bp) were combined. To minimize the effect of multiple base substitutions, we considered only inferred amino acid replacements and only transversions in first and third codon positions in the coding sequence (Edwards et al. 1991; Irwin et al. 1991). In the tRNAs and the noncoding control region, only transversions and indels (given equal weights as were transversions) were utilized, as in transversion parsimony sensu Swofford and Olsen (1990). All 12 sequenced species from the Ectodini, as well as 8 mouthbrooding species, each representing a Tanganyikan tribe, were analyzed. *Oreochromis tanganicae,* a distantly related tilapiine cichlid endemic to the Tanganyika basin, was declared outgroup.

Once the sister-group relationships were estimated on the basis of the initial analysis, a second analysis was performed to determine the branching order of the clades within the Ectodini. This analysis was rooted by using its most ancestral species,

identified in the first analysis (*Grammatotria lemarii*) as outgroup. Both gene segments were combined, and the same set of weights as in the previous analysis was used.

In a third analysis, the clades found in the former analyses were analyzed separately, and the sister group of each assemblage was declared outgroup. Also, both gene segments were combined; all observed base substitutions and indels were weighted equally (Fitch 1971).

Results

Age of the Ectodini

The comparison of the maximum observed corrected sequence divergences (fig. 1) indicates that the Ectodini may be approximately twice the age of the genus *Tropheus* and might be more than five times the age of the Lake Malawi cichlid flock. The same relative ages were obtained independently from both gene segments. If an approximate age of the Malawi species flock of ~0.7 Myr (Banister and Clarke 1980) is accepted, the Ectodini may be ~3.7 Myr old, on the basis of the control region. This age is also supported by cytochrome *b*, where ≥10.1% of corrected sequence divergence between members of the Ectodini was found, compared with <2% in Lake Malawi (Meyer et al. 1990), also resulting in an estimated age of ~3.7 Myr.

The results of the comparisons of sequence divergences among different lineages of the Ectodini suggest that they originated rapidly and nearly simultaneously. This is reflected in their similar relative genetic divergences in the control region and in cytochrome *b*. However, in the comparison of sequence divergences with the two more distantly related species (*Bathybates* and *Oreochromis*), only cytochrome *b* seems to maintain the same relative rates of substitution, and the control region appears to become saturated by multiple substitutions. Table 2 summarizes the maximum

Table 2
Maximum Observed Corrected Genetic Divergences among the Ectodini and from Other Tanganyikan Genera, for Cytochrome *b* (above Diagonal) and Control Region (below Diagonal)

	Ore	Bat	Tro	Abu	Lim	Cyp	Gra	Cal	Asp	Oph
Ore ...		13.9	12.1	12.7	10.2	11.0	11.8	12.1	12.4	12.7
Bat	7.6		13.3	12.7	11.0	12.1	14.5	13.3	14.5	15.1
Tro ...	7.5	8.8		6.8	8.7	8.5	9.8	10.7	10.2	10.9
Abu ...	8.5	10.3	2.5		7.6	7.9	9.6	10.2	9.9	10.4
Lim ...	7.7	8.2	5.8	7.1		6.3	6.8	7.9	8.5	10.2
Cyp ...	7.2	8.6	3.7	5.0	5.3		8.7	9.8	8.7	9.8
Gra ...	9.9	10.0	5.3	6.1	7.5	3.9		8.5	7.3	10.1
Cal	11.8	11.5	7.5	8.4	8.7	5.5	5.3		8.5	8.7
Asp ...	11.0	11.2	7.2	8.1	8.0	5.3	5.1	5.3	2.7	9.6
Oph ...	11.3	10.8	7.5	8.4	9.2	6.9	6.1	6.4	6.4	4.0

NOTE.—Above the diagonal are distances (substitutions/100 bp) of a 402-bp segment of the cytochrome *b* gene, based on all observed differences, corrected for multiple substitutions (Jukes and Cantor 1969). Below the diagonal are Jukes-Cantor distances of a 450-bp segment of the control region, including 83 bp of the Thr tRNA and the Pro tRNA, based on transversions and indels only. On the diagonal (underlined), the maximum corrected divergences found within the *Xenotilapia* and within the *Ophthalmotilapia* lineage are given, based on transversions and indels in the 450-bp segment of the tRNA and control region. Ore = *Oreochromis tanganicae;* Bat = *Bathybates ferox;* Tro = *Tropheus duboisi;* Abu = *Astatotilapia burtoni;* Lim = *Limnochromis auritus;* Cyp = *Paracyprichromis brieni;* Gra = *Grammatotria lemarii;* Cal = *Callochromis pleurospilurus;* Asp = *Asprotilapia subassemblage* (three species sequenced); and Oph = *Ophthalmotilapia* subassemblage (seven species sequenced).

observed corrected divergences, of the two sequenced gene segments, both from other Tanganyikan lineages and among clades of the Ectodini.

Intraspecific Variation

We obtained sequences for more than one individual, from each of 7 of the 12 species of the Ectodini and, in addition, from *Tanganicodus irsacae* and *Tropheus duboisi* (table 1). Sequences from individuals of the same species differed only very little from each other. In the control region, at most three transitions and one transversion were observed between the two individuals of *Ophthalmotilapia ventralis* from two different populations (Kasanga and Mpulungu; for map, see Sturmbauer and Meyer 1992). In cytochrome *b*, most individuals from each species were identical; the two individuals of *Ophthalmotilapia ventralis* differed by one transition, and two *Cyathopharynx* individuals (also from two different localities) differed by two transitions. The branching order was never affected, depending on which individual sequence was used in the analyses.

Phylogenetic Position and Monophyly of the Ectodini

The initial analysis of all 12 taxa representing the Ectodini and of eight Tanganyikan mouthbrooding species, each representing one tribe (Poll 1986), resulted in 10 equally parsimonious trees, based on 118 phylogenetically informative positions. The weighted tree length is 268 substitutions, the consistency index is 0.75 (Kluge and Farris 1969), the consistency index excluding uninformative characters is 0.63, the retention index is 0.80, and the rescaled consistency index is 0.60 (Farris 1989). The topology obtained from the neighbor-joining analysis based on the corresponding distance matrix was identical to 1 of the 10 most parsimonous trees (fig. 2). The reweighting procedure reached a stable topology after two replications. It differed from the tree favored by neighbor joining in that (*a*) *Limnochromis* branched after the *Tanganicodus-Tropheus-Astatotilapia* lineage and (*b*) *Aulonocranus* and *Ectodus* were not resolved as clade but as separate branches. These branches, however, were obtained in <50% of the bootstrap replications, in both the first and the second analysis.

The monophyly of the tribe Ectodini is supported by 95% of the bootstraps (fig. 2), confirming the morphological analysis of Greenwood (1983*b*) and Poll (1986). Although relatively few base substitutions have been fixed during the speciation of most Tanganyikan lineages, indicating a truly explosive radiation, the Cyprichromini, represented by *Paracyprichromis brieni,* can be identified as the sister group of the Ectodini (fig. 2).

This analysis suggests that the Ectodini comprise four clades. The most ancient split within the tribe is observed between *Grammatotria lemarii* and all other taxa, albeit at a low bootstrap value of 55% (fig. 2). *Callochromis* branched next, forming another separate clade. The third clade comprises the genera *Xenotilapia, Microdontochromis,* and *Asprotilapia;* it shares a common ancestor with a fourth subassemblage, comprising the genera *Ectodus, Aulonocranus, Cunningtonia, Lestradea, Cardiopharynx, Cyathopharynx,* and *Ophthalmotilapia.*

Relationships of Genera within the Ectodini

The analyses of all 12 representatives of the Ectodini, with *G. lemarii* (on the basis of the initial analysis), as outgroup resulted in four equally parsimonious trees, based on 57 phylogenetically informative sites (weighted tree length = 114; consistency index = 0.86; consistency index excluding uninformative characters = 0.83; retention

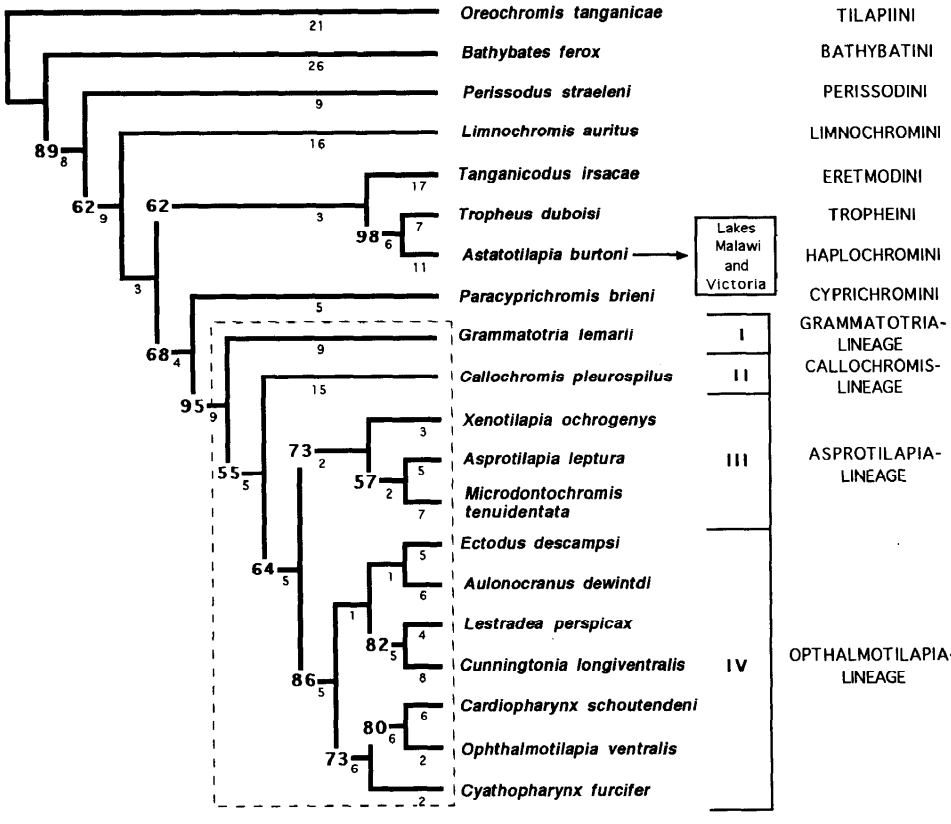

FIG. 2.—Phylogenetic tree obtained both by parsimony (1 of 10 most parsimonious trees) and by the neighbor-joining method. This tree has the major mouthbrooding lineages of Lake Tanganyika and 12 genera of the tribe Ectodini [tree length = 268 substitutions; consistency index (Kluge and Farris 1969) = 0.75; consistency index excluding uninformative characters = 0.63; and rescaled consistency index (Farris 1989) = 0.60]. Bootstrap values are given on those branches that were obtained in >50% of the replications. Small numbers under the branches are branch lengths inferred by parsimony. The 402-bp segment of the mitochondrial cytochrome *b* and a 450-bp segment of part of the Thr tRNA, the Pro tRNA, and part of the control region were combined. For cytochrome *b*, all amino acid replacements and only transversions in first and third codon positions were analyzed. In the tRNAs and the control region, only transversions and indels with equal weights were considered. The cladogram is rooted with *Oreochromis tanganicae*, a tilapiine cichlid, as outgroup. *Bathybates*, a member of the tribe Bathybatini, appears as the most ancestral lineage among the endemic mouthbrooding lineages of Lake Tanganyika. All other tribes appear to be more closely related to each other, as is also indicated by similar genetic divergences (table 2). Note the placement of the nonendemic "Haplochromine" *Astatotilapia burtoni* that was shown to be closely related to cichlids from Lake Victoria (Meyer et al. 1990, 1991). The species flocks of Lakes Malawi and Victoria are most closely related to the Tanganyikan tribe Tropheini. A more complete analysis of the three endemic species flocks will appear elsewhere (authors' unpublished data). The monophyly of the Ectodini (indicated by a dashed box) is supported by 95% of the bootstrap replications. The branching order obtained among the Ectodini (*dashed box*), with *Grammatotria lemarii* as the outgroup, was identical to that obtained with all 20 taxa. On the right the tribe represented is indicated.

index = 0.91; and rescaled consistency index = 0.78). The unweighted tree length of two of those four trees is 335 (rescaled consistency index = 0.38), and it is 339 (rescaled consistency index = 0.36) for the other two trees. One of these four most parsimonious trees (unweighted tree length = 335; and rescaled consistency index = 0.38) shows a branching order identical to that of the tree obtained from neighbor joining using the corresponding distance matrix. The topology of this tree is identical to the shortest

tree favored by neighbor-joining in the initial analysis with 20 taxa, shown in figure 2. The application of the successive weighting procedure (Farris 1969), starting from those four equally parsimonious trees, reached, after two replications, a stable topology nearly identical to that favored by neighbor joining: only *Ectodus* and *Aulonocranus* were not resolved as clade but were positioned as separate branches (unweighted tree length = 333; consistency index = 0.68; consistency index excluding uninformative characters = 0.55; and rescaled consistency index = 0.39).

The third series of analyses, in which the *Xenotilapia* clade and the *Ophthalmotilapia* clade were analyzed separately, resulted each in one most parsimonious tree (fig. 3). The topologies differ depending on whether (*a*) transitions were excluded (except for those coding for amino acid replacements in cytochrome *b*) or (*b*) all observed substitutions were weighted equally. While *Xenotilapia* appeared to be sister group to both *Microdontochromis* and *Asprotilapia,* when transitions were excluded (except for those coding for amino acid replacements in cytochrome *b*), *Microdontochromis* appeared to be sister group to *Asprotilapia* and *Xenotilapia* (fig. 2), when all substitutions were weighted equally, with *Callochromis* as outgroup (fig. 3A).

The length of the single most parsimonious tree is 123 substitutions (consistency index = 0.92; consistency index excluding uninformative characters = 0.64; and rescaled consistency index = 0.41). This topology was also obtained in the neighbor-joining analysis using the corresponding distance matrix. The application of Lake's (1987) method of phylogenetic invariants did not result in a significant support of a particular topology with *Grammatotria* and *Callochromis* as fourth taxon. The inclusion of more *Xenotilapia* species in the analysis may resolve this conflict.

The analysis of the *Ophthalmotilapia* lineage, with all equal weights and with *Asprotilapia* as outgroup, resulted in a different arrangement for the genera *Cyathopharynx, Ophthalmotilapia,* and *Cardiopharynx. Cardiopharynx* now appears to be the sister lineage of the other two genera (fig. 3B). The topology of the phylogeny obtained from the neighbor-joining analysis with the corresponding distance matrix was identical to that of the parsimony analysis. Again, the application of Lake's method did not unequivocally support a particular topology. This branching order must also remain unresolved, until more *Ophthalmotilapia* species or populations are included in the analysis.

Discussion

Two previous morphological studies suggested the monophyly of the Ectodini on the basis of at least five synapomorphies (fig. 4; Liem 1981; Greenwood 1983*b;* also see Poll 1986). However, no sister-group relationship to other Tanganyikan lineages could be established by morphological cladistic analyses (Liem 1981; Greenwood 1983*b*). A phylogeny derived from allozyme data also failed to identify a sister group (Nishida 1991).

Nishida (1991) showed that *Oreochromis, Trematocara,* and *Bathybates* represent three ancient and separate lineages among the Tanganyikan cichlids. This phylogeny of the Tanganyikan mouthbrooders was determined by using a representative of one of those ancient lineages, *Oreochromis tanganicae,* a member of the tilapiine cichlids, as outgroup. As is *Astatotilapia burtoni,* it is found in surrounding swamps and river estuaries and is not a truly endemic species of Lake Tanganyika. Our analysis, including representatives from nine different Tanganyikan tribes (Poll 1986), supports the monophyly of the Ectodini (95% bootstrap value). It tentatively identified the Tanganyikan tribe Cyprichromini (represented by *Paracyprichromis brieni*) as sister lineage

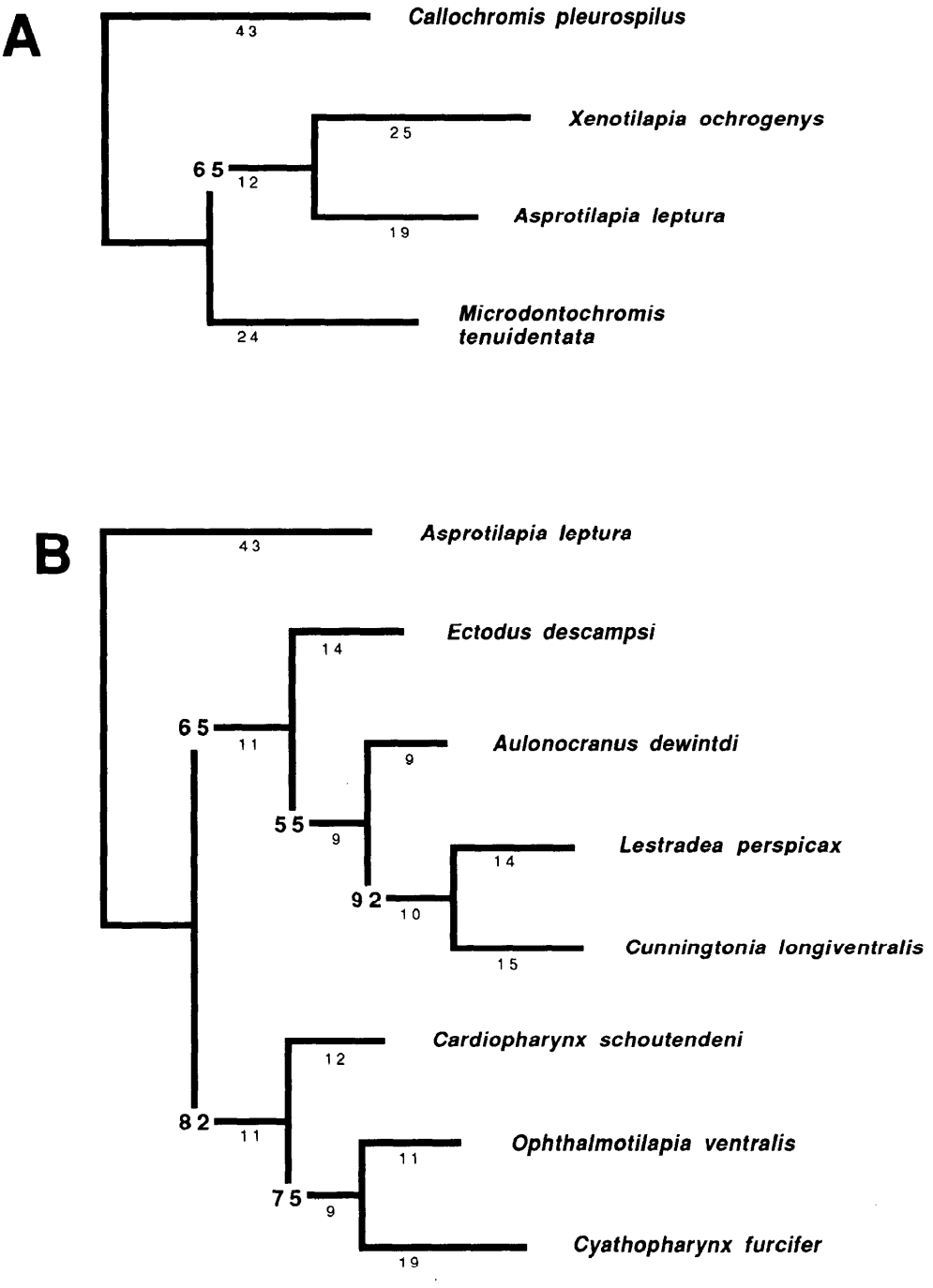

FIG. 3.—Phylogeny of the major two clades of the tribe Ectodini, obtained both by parsimony and by neighbor joining. Numbers on the branches are bootstrap values (100 replications). Small numbers under the branches are branch lengths inferred by parsimony. For parsimony, all observed substitutions were considered with equal weights; for neighbor joining, distance matrices were calculated on the basis of the weights used in parsimony. A, Analysis of the *Asprotilapia* lineage, with *Callochromis* as outgroup. (tree length = 123; consistency index = 0.92; consistency index excluding uninformative characters = 0.64; and rescaled consistency index = 0.41). B, Analysis of the *Ophthalmotilapia* lineage, with *Asprotilapia* as outgroup (tree length = 184; consistency index = 0.78; consistency index excluding uninformative characters = 0.63; and rescaled consistency index = 0.41).

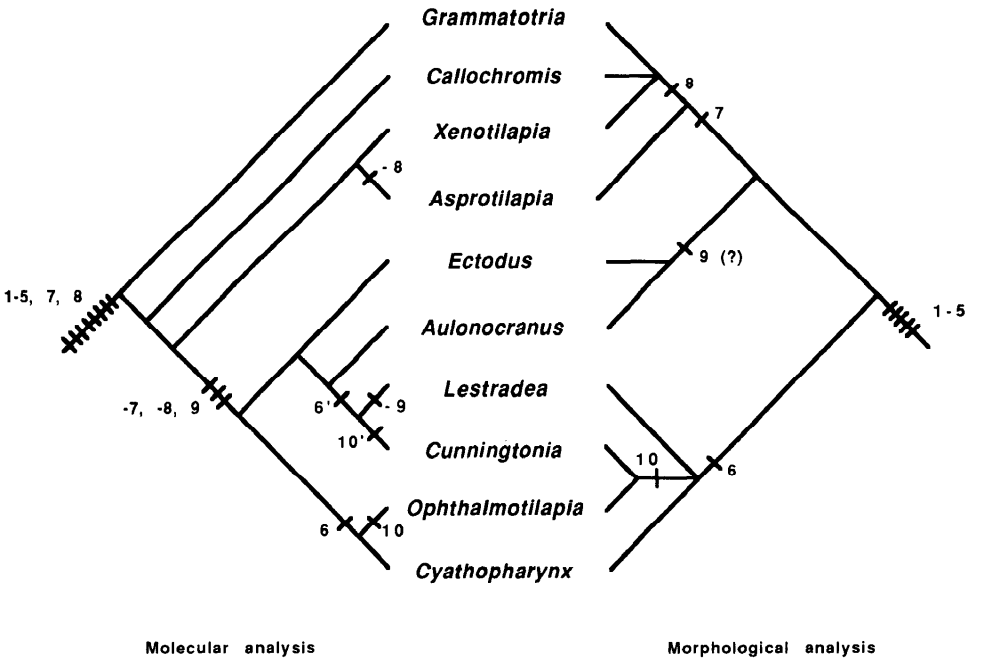

FIG. 4.—Comparison of the phylogeny suggested by mitochondrial DNA sequences (*left*) with that obtained by Greenwood (1983*b*) from morphological characters (*right*). The genera *Microdontochromis* and *Cardiopharynx* (synonymized to *Cyathopharynx*, by Greenwood) were omitted. The numbers on both cladograms refer to individual morphological characters listed below, according to Greenwood (1983*b*), and are discussed in detail by him on the pages noted below. The placement of these 10 characters on the molecular phylogeny indicates that 5 of the 10 characters may be homoplasious. A reversal of tree characters (characters 7–9, indicated by a minus symbol before the character number) is suggested. These characters have been shown, by Greenwood, to be homoplasious within African cichlids. Further, the placement invokes parallel evolution (indicated by a prime on the character number) of two characters: intestinal elongation (character 6) and the morphology of the sensory canal (character 10). Character 10 was discussed by Greenwood as being gradual. 1 = Palatopterygoid gap (p. 254); 2 = auricular process on the operculum (p. 259); 3 = morphological features of the palatine bone (p. 257); 4 = outline shape of the lachrymal, the first infraorbital bone, and the presence of six laterosensory canal pores (p. 259); 5 = adductor fossa on the lateral aspect of the anguloarticular bone (p. 261); 6 = intestine long and transversely coiled (p. 267); 7 = presence of a pharyngeal hanging pad and associated modifications to the gill-raker morphology (p. 265); 8 = dentary with a distinct step (p. 267); 9 = first branched pelvic fin rays produced [Greenwood's placement of a question mark indicates that this character also allowed alternative groupings (p. 272)]; and 10 = dorsal part of the flange behind the vertical part of the preopercular laterosensory canal not narrowing abruptly (p. 273).

of the Ectodini. The resulting branching order and the observed corrected sequence divergences (table 2) determined the Bathybatini to be the most divergent endemic Tanganyikan lineage, in agreement with Nishida (1991). All other lineages seem much more closely related to each other and have similar observed corrected divergences for both gene segments analyzed (table 2). This suggests that the radiation of those major mouthbrooding lineages may have happened within a short period of time and can be tentatively dated at 3.5–5.3 Mya. The age estimates rest on the assumption that the species flock of Lake Malawi is ~0.7 Myr old (Banister and Clarke 1980). However, our comparisons of the relative ages to the genus *Tropheus* and the Lake Malawi species flock demonstrate a much older age for the Ectodini and the Tanganyikan species flock than for the Lake Victoria and Lake Malawi flocks. In the com-

parison with the two more distantly related species (*Oreochromis* and *Bathybates*), only cytochrome *b* seems to maintain its relative rate of substitutions linear over time, whereas the control region appears to have become saturated (table 2).

When the breeding behavior is plotted (not shown) on the phylogeny (fig. 2), the position of the Perissodini seems critical for the interpretation of the evolution of different patterns of mouthbrooding. Perissodini perform a variation of mouthbrooding that is considered as transitional between substrate breeding (widely believed to be the ancestral condition; see Barlow 1991; Keenleyside 1991) and mouthbrooding (Yanagisawa and Nshombo 1983; Yanagisawa 1985). *Perissodus* forms pairs, and both parents take up the eggs, which had been attached to a substrate, to incubate them in their buccal cavities. Both parents guard the offspring. Our suggested phylogenetic placement of *Perissodus* supports the hypothesis of multiple origins of mouthbrooding in African cichlids (Barlow 1991), because the two ancestral branches, represented by *Oreochromis* and *Bathybates,* are more derived maternal mouthbrooders (Coulter 1991). The remaining lineages, sister groups to the Perissodini, also expressed various more derived forms of mouthbrooding. For several Tanganyikan mouthbrooders pair formation and biparental guarding have been described (Yanagisawa 1986; Brichard 1989, p. 383; Kuwamura et al. 1989; A. Rossiter, personal communication). This particular form of mouthbrooding appears to be intermediate between the ancestral breeding behavior expressed in the Perissodini and several other derived forms of maternal mouthbrooding found in the other lineages of Lake Tanganyika cichlids. Examples of such derived patterns may be the transfer of newly hatched wrigglers to the male, in some Eretmodini (Kuwamura et al. 1989). In the Cyprichromini a transition from spawning on hard substrate (*Paracyprichromis;* A. Rossiter, personal communication) to spawning without contacting solid substrate (*Cyprichromis;* Brichard 1989, p. 222; Coulter 1991, p. 189) has been observed. In *Tropheus,* mouthbrooding females feed during buccal incubation, to nourish themselves and their offspring (Yanagisawa and Sato 1990), and males of *Cyathopharynx furcifer* congregate in remarkable lekking arenas formed by multiple nest craters and polygynous spawning behavior (A. Rossiter, personal communication). No "haplochromine" cichlids, such as *Astatotilapia burtoni,* including all cichlids from Lakes Victoria and Malawi, show pair formation, and only females orally incubate eggs and fry (Wickler 1962).

Astatotilapia burtoni, a nonendemic "haplochromine" species with generalized morphology, seems closely related to the Lake Victoria cichlids (Meyer et al. 1991). Its placement as sister group to *Tropheus duboisi,* an endemic Tanganyikan Aufwuchs feeder, was also obtained from allozyme data, by Nishida (1991). This indicates the relative recency of the radiations of haplochromine cichlids from Lakes Victoria and Malawi (Meyer et al. 1990, 1991) and hints at the origin and phyletic relationships of the endemic Lake Victoria and Lake Malawi flocks to this particular lineage of Lake Tanganyika. Furthermore, it indicates that most of the Tanganyikan mouthbrooding lineages probably evolved prior to the radiation of haplochromine cichlids in eastern Africa, supporting the conclusion of Nishida (1991) that Lake Tanganyika may be an evolutionary reservoir of ancient lineages of eastern African cichlid fishes. Analyses of the relationships between the Tanganyikan mouthbrooders and riverine species indicate that *Oreochromis* and *Bathybates* form separate ancient African lineages and that *Orthochromis polyacanthus,* from the Zaire River, appears to be the sister group of other surveyed Tanganyikan mouthbrooders (authors' unpublished results).

The large ecological and morphological differences between the Tanganyikan lineages (Poll 1986; Coulter 1991) may indicate that major types of habitat were quickly occupied after the colonization of Lake Tanganyika and the initial radiation. Further speciation within each lineage rarely involved switching to other types of habitat. An exception is *Asprotilapia leptura,* which seems to have switched from sand dwelling to rock dwelling. It is closely related to *Xenotilapia* and *Microdontochromis,* both of which inhabit sandy areas (table 2).

In contrast to previous works (Liem 1981; Greenwood 1983*b*), our study suggests that the species originally assigned to the *Asprotilapia* subassemblage represent three separate clades. *Grammatotria lemarii* branched first, followed by the *Callochromis* lineage. The genera *Xenotilapia, Microdontochromis,* and *Asprotilapia* are sister groups of the *Ophthalmotilapia* lineage. However, we could not unambiguously resolve the branching order among these three genera. Additional *Xenotilapia* species may provide resolution of this branching order.

The branching order suggested by mtDNA sequences requires a different interpretation of the morphological transformation series among Ectodini cichlids than has previously been believed (Liem 1981; Greenwood 1983*b*). In figure 4 the alternative placement of Greenwood's (1983*b,* p. 277) morphological characters in the proposed molecular phylogeny is indicated. Greenwood used the presence of a pharyngeal hanging pad with its associated modifications of the gill rakers (character 7 in fig. 4) to suggest the monophyly of *Grammatotria, Callochromis,* and *Xenotilapia.* This character has to be interpreted as a synplesiomorphy of the Ectodini (see Greenwood 1983*b,* p. 277). A similar pharyngeal hanging pad was also found in other "sand digging" cichlids—namely, in the neotropical genus *Geophagus* and the primarily western African genera *Chromidotilapia* and *Tylochromis* (Greenwood 1983*b,* p. 267). Further, the suggested close relationship of *Asprotilapia* with *Xenotilapia* and *Microdontochromis* requires the reversal of a distinct "step" in the dentary (character 8 in fig. 4) in *Asprotilapia.* In contrast to *Xenotilapia* and *Microdontochromis,* which forage by "digging" for sand organisms, *Asprotilapia* browses epilithic algae mostly at vertical rock surfaces. The numerous autapomorphies in the morphology of *Asprotilapia leptura* might be related to both its switch to rocky habitat and its particular ecological specialization.

Greenwood (1983*b*) synonymized *Cardiopharynx* with the genus *Cyathopharynx.* We never obtained a topology in which *Cardiopharynx* and *Cyathopharynx* form a clade that is sister group to *Ophthalmotilapia,* even when our analyses resulted in two alternative topologies, depending on the weights used. This synonymization would also have to include the genus *Ophthalmotilapia.* However, in view of the considerably different cranial morphology and dentition of *Ophthalmotilapia nasuta,* we agree with Poll (1986), who did not follow Greenwood's synonymization, and suggest that separate generic status for all three lineages be retained.

Our data suggest the placement of *Ectodus descampsi* and *Aulonocranus dewindti* within the Ophthalmotilapia clade, and not within the *Asprotilapia* clade, despite their shorter relative intestinal length. This placement is corroborated by both mitochondrial segments. Together with *Lestradea perspicax* and *Cunningtonia longiventralis,* these two genera seem to share a common ancestor with *Cardiopharynx, Cyathopharynx,* and *Ophthalmotilapia.* Therefore, the tremendous intestinal prolongation, characterized as a synapomorphy of Greenwood's *Ophthalmotilapia* subassemblage (character 6 in fig. 4), may have evolved twice: once in *Cardiopharynx, Cyathopharynx,* and *Ophthalmotilapia* and a second time in *Lestradea* and *Cunningtonia,* who share a

common ancestor with *Aulonocranus* and, possibly, *Ectodus*. Intestinal prolongation, although indicative of specialization on diets with low nutritional value, such as those of epilithic algae and detritus, has been shown to be highly plastic (Sturmbauer et al. 1992). In *Tropheus moorii* the intestinal length of domestic fish measured only ~50% of the length found in wild individuals (Sturmbauer et al. 1992).

The discovery of similar morphological specializations among endemic species in different eastern African lakes led several scientists to postulate a common ancestry of these species (e.g., see Greenwood 1978, 1983*a*) and hence suggested a polyphyletic origin for all eastern African species flocks. Greenwood (1983*b*) found that the Malawian genus *Lethrinops* had several morphological features remarkably similar to those in the *Ophthalmotilapia* lineage. It seems as if convergent evolution in each of the lakes produced remarkably similar morphological solutions to similar ecological problems. The placement of morphological characters on the phylogeny derived from sequence data suggests that characters related to trophic specializations are prone to convergent evolution, even among the species from a single Tanganyikan lineage.

Sequence Availability

The nucleotide sequences in this paper are available from EMBL and are as follows: cytochrome *b*—*Oreochromis tanganicae* Z12046 (Swedish Museum of Natural History, NRM 12815, Sturmbauer and Meyer 1992), *Bathybates ferox* Z21774, *Perissodus straeleni* Z21777, *Limnochromis auritus* Z21775, *Tanganicodus irsacae* Z21778 and Z21779, *Tropheus duboisi* Z12039, Z12040, and Z12041 (Sturmbauer and Meyer 1992), *Astatotilapia burtoni* Z21773, *Paracyprichromis brieni* Z21776, *Grammatotria lemarii* Z21766 and Z21767, *Callochromis pleurospilus* Z21760, *Xenotilapia ochrogenys* Z21772, *Asprotilapia leptura* Z21758, *Microdontochromis tenuidentata* Z21769 and Z21770, *Ectodus descampsi* Z21765, *Aulonocranus dewindti* Z21759, *Lestradea perspicax* Z21768, *Cunningtonia longiventralis* Z21762, *Cardiopharynx schoutendeni* Z21761, *Ophthalmotilapia ventralis* Z21771 and Z21798, and *Cyathopharynx furcifer* Z21763 and Z21764; and control region—*Oreochromis tanganicae* Z21753 (Swedish Museum of Natural History, NRM 12815), *B. ferox* Z21752, *Perissodus straeleni* Z21755, *Limnochromis auritus* Z21746, *T. irsacae* Z21756 and Z21757, *Tropheus duboisi* Z12080, Z12081, and Z12083 (Sturmbauer and Meyer 1992), *Astatotilapia burtoni* Z21751, *Paracyprichromis brieni* Z21754, *G. lemarii* Z21743 and Z21744, *Callochromis pleurospilus* Z21735, *X. ochrogenys* Z21750, *Asprotilapia leptura* Z21732, *M. tenuidentata* Z21747, *E. descampsi* Z21742, *Aulonocranus dewindti* Z21733 and Z21734, *Lestradea perspicax* Z21745, *Cunningtonia longiventralis* Z21738 and Z21739, *Cardiopharynx schoutendeni* Z21736 and Z21737, *Ophthalmotilapia ventralis* Z21748 and Z21749, and *Cyathopharynx furcifer* Z21740 and Z21741.

Acknowledgments

For comments on the manuscript, we thank D. Dykhuizen, C. Lydeard, G. Orti, A. Rossiter, E. Verheyen, and P. Wilson. We thank M. Boniface Nyakageni, Republic of Burundi, and Prof. P. Bwathondi, Tanzanian Fisheries Research Institute, for the research permissions, and we thank S. Kullander, E. Verheyen, P. Henninger, and L. DeMasson for some of the fish samples. This study was supported by the Austrian Science Foundation through an Erwin Schrödinger postdoctoral fellowship to C.S., by a grant from the American Cichlid Association to C.S., by grant BSR-9107838

from the National Science Foundation to A.M., and by NATO collaboration grant CRG-910911 to A.M.

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JAN KLEIN, reviewing editor

Received November 4, 1992; revision received March 9, 1993

Accepted March 9, 1993