Mitochondrial Phylogeny of the Lamprologini, the Major Substrate Spawning Lineage of Cichlid Fishes from Lake Tanganyika in Eastern Africa

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Lake Tanganyika harbors the oldest, morphologically and behaviorally most diverse flock of cichlid species. While the cichlids in Lakes Malawi and Victoria breed their eggs exclusively by buccal incubation (termed "mouthbrooding"), the Tanganyikan cichlid fauna comprise mouthbrooding and substrate-spawning lineages (fish spawn on rocks, and never orally incubate eggs or wrigglers). The substrate-spawning tribe Lamprologini appears to occupy a key position that might allow one to elucidate the origin of the Tanganyika flock, because five riverine (therefore nonendemic) species from the Zaire River system have been assigned to this tribe, in addition to the lake's endemic species, which make up almost 50% of all 171 species known from this lake (Poll 1986). From 16 species (18 individuals) of the tribe Lamprologini, a 402-bp segment of the mitochondrial cytochrome *b* gene was sequenced, and, from 25 lamprologine species (35 individuals), sequences from the mitochondrial control region were obtained. To place the Lamprologini into a larger phylogenetic framework, orthologous sequences were obtained from eight nonlamprologine Tanganyikan cichlid species (13 individuals). The Lamprologini are monophyletic, and a clade of six Tanganyikan lineages of mouthbrooders, representing five tribes (Poll 1986), appears to be their sister group. Comparisons of sequence divergences of the control region indicate that the Lamprologini may be older than the endemic Tanganyikan tribe Ectodini, and short basal branches might suggest a rapid formation of lineages at an early stage of the Tanganyika radiation. It is interesting that three analyzed riverine members of the tribe form a monophyletic group; however, they are not the most ancestral branch of the Lamprologini. This might indicate that they are derived from an endemic lamprologine ancestor that left Lake Tanganyika by entering the Zaire River system. These riverine species may not have seeded the Tanganyikan radiation, as currently thought, but may have recently recolonized the river after a long period of isolation, as soon as the lake was connected to the Zaire River again about 2 Mya. *Neolamprologus moorii,* endemic to Lake Tanganyika, appears to represent the most basal clade of the Lamprologini. Complex breeding behavior, involving the usage of gastropod shells and associated with dwarfism, is likely to have evolved in parallel in several lineages among the Lamprologini. The tribe Lamprologini may be in need of revision, since several genera appear to be polyphyletic.

Introduction

With an estimated age of 9–20 Myr, Lake Tanganyika is the oldest of the three eastern African Great Lakes (Tiercelin and Mondeguer 1991; Cohen et al. 1993; see fig. 1). The cichlid fish species flock of Lake Tanganyika is by far the morphologically, ecologically, and behaviorally most complex assemblage of cichlids in the world (Fryer and Iles 1972; Greenwood 1984). Unlike the monophyletic species flocks of Lakes Victoria and Malawi (Meyer et al. 1990, 1991), the cichlid fauna of Lake Tanganyika is believed to be of polyphyletic origin with affinities to the cichlid faunas of other African regions (Poll 1946, 1956, 1974, 1986; Fryer and Iles 1972, p. 501; Nishida 1991; Kocher et al. 1993). It comprises almost 200 cichlid fish species assigned to 49 endemic genera and 12 tribes, 8 of which are endemic (Poll 1986). These tribes are assemblages of genera that seem to represent well-defined phylogenetic lineages (Poll 1986; Nishida 1991; Sturmbauer and Meyer 1993; Meyer 1993*a*, 1993*b*).

The tribe Lamprologini comprises species endemic to Lake Tanganyika, as well as species from the Zaire River. Some "haplochromine" cichlids from Lake Tanganyika, of the tribes Tropheini and Haplochromini (i.e., *Tropheus* and *Astatotilapia*), appear to be the sister group to the species flocks of Lakes Malawi and Victoria (Meyer et al. 1990, 1991; Nishida 1991; Meyer 1993*b*; Sturmbauer and Meyer 1993). The single endemic Tan-

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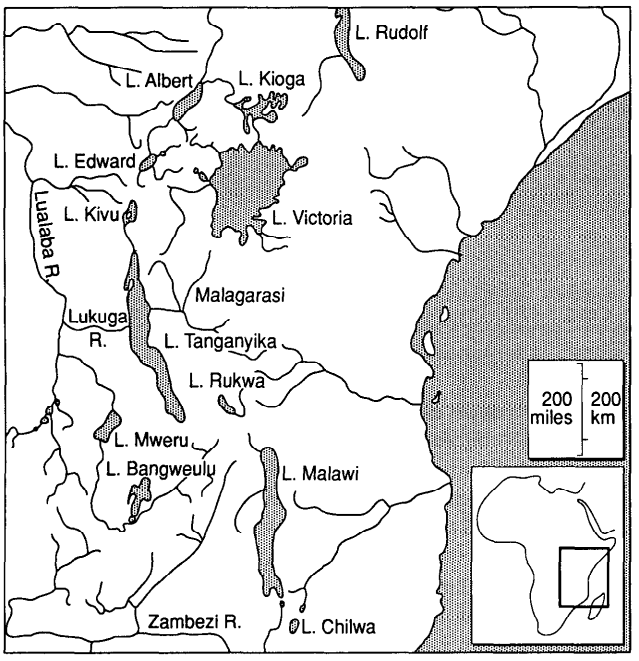

FIG. 1.—Map of eastern Africa, showing the locations of the lakes and the major river systems. (Redrawn after Fryer and Iles 1972.)

ganyikan species of the tribe Tylochromini appears to have its closest relatives in central and western Africa (Stiassny 1990; authors' unpublished results). The Tanganyikan cichlid fauna hence can be viewed as an evolutionary reservoir of ancient African cichlid lineages (Nishida 1991), and understanding its evolution might allow one to elucidate the origin, biogeography, and interrelationships between all the endemic flocks of the large African lakes and the cichlids living in the rivers of Africa.

The ages of the Tropheini and the Ectodini of Lake Tanganyika were recently estimated (Sturmbauer and Meyer 1992, 1993). The genus *Tropheus* appears to be about twice as old as the entire cichlid species flock from Lake Malawi and six times older than the Victoria flock (Sturmbauer and Meyer 1992), while the Ectodini, represented by 12 endemic genera, seem to be approximately twice as old as *Tropheus* and five times older than the Malawi flock (Sturmbauer and Meyer 1993).

Species of the Lamprologini dominate the endemic Tanganyikan cichlid flock, comprising almost 50% of the lake's species (Loiselle 1985). This proportion rises to about 75% if the littoral cichlid community is considered separately from the pelagic and benthic ones. They are morphologically, ecologically, and behaviorally highly diversified and have colonized all lacustrine habitats of Lake Tanganyika but most often live in the littoral zone (Hori 1983; Nagoshi 1983; Brichard 1989, p. 325). Most lamprologines live close to the substrate. These species are further subdivided into three categories inhabiting the sandy bottom, the muddy bottom, or rocky habitats (Brichard 1989, p. 325). Lamprologines are the only 1 of the 12 Tanganyikan cichlid tribes that exhibit levels of anatomic innovation sufficiently high that they might allow a morphology-based cladistic analysis (M. L. J. Stiassny, personal communication).

Unlike all other Tanganyikan cichlid lineages (except for *Boulengerochromis microlepis,* presently assigned to the tribe Tilapiini; Poll 1986), which incubate their eggs orally (termed "mouthbrooding"), the Lamprologini are exclusively substrate breeders, which form pairs that spawn in their territory, on a solid substrate where both parents usually guard the offspring. Among all cichlids, the Lamprologini probably evolved the most complex patterns of parental care (Keenleyside 1991; Kuwamura 1986; Barlow 1991; Gashagaza 1991). Interspecific variation in clutch size among lamprologines ranges from 3,000 eggs/spawn in the open-water species *Lepidiolamprologus cunningtoni* to about 12 in the rock-dwelling *Neolamprologus savoryi* (Brichard 1989, p. 363). Mating strategies cover a wide scope, from monogamy to serial polygamy and harem polygamy (Loiselle 1980; Hori 1983; Taborsky and Limberger 1981; Yanagisawa 1987; Barlow 1991; Gashagaza 1991; Keenleyside 1991). Nesting sites range widely and include flat rocks, crevices under rocks, and even the inside of gastropod shells. Breeding of different species in a single territory, formed by piling of gastropod shells by a male *L. callipterus,* have also been described (Brichard 1989, p. 337; authors' personal observation). Participation of the offspring of previous spawns as "helpers" in communal parental care has been reported for *N. brichardi* (Taborsky and Limberger 1981; Limberger 1983; Taborsky 1984, 1985; Taborsky et al. 1986). This ecological and behavioral diversity makes the Lamprologini particularly attractive for comparative evolutionary work.

The monophyly of the tribe Lamprologini (Poll 1986) was recently corroborated by a morphological cladistic study (Stiassny 1991). However, the phylogenetic relationships within the tribe Lamprologini and the monophyly of its genera remain untested (Colombe and Allgayer 1985; Poll 1986). Also, the relationships of the Lamprologini to the other major African cichlid lineages are tentative. The most recent classification of the Lamprologini was established by Poll (1986) and restricts the genus *Lamprologus* Schilthuis 1891 to five fluvial and eight endemic Tanganyikan species. The remaining Lamprologini are assigned to six other endemic genera. The genus *Lepidiolamprologus* Pellegrin 1903 comprises six species; 32 species are encompassed by *Neolamprologus* Colombe & Allgayer 1985; *Telmato-*

chromis Boulenger 1898 and *Julidochromis* Boulenger 1898 each include 5 species, and 2 species each belong to *Altolamprologus* Poll 1986 and *Chalinochromis* Poll 1974.

We present a molecular phylogeny of this group of cichlid fishes that is based on mitochondrial DNA (mtDNA) sequences. On the basis of a phylogenetic estimate, we attempt to test the hypothesis of riverine ancestors giving rise to numerous endemic species. We also test the monophyly of reproductive strategies in this lineage of cichlid fishes.

Material and Methods

Sampled Species and Genes Sequenced

Sequences of mitochondrial gene segments from 25 species of nine genera (see table 1) from the tribe Lamprologini were obtained. Of these, from 16 lamprologine species (18 individuals) a 402-bp segment of the mitochondrial cytochrome *b* gene was sequenced, and from 25 species (35 individuals) sequences were obtained from a 452-bp segment of mtDNA, which includes part of the control region (372 bp), part of the threonine (Thr) tRNA gene (12 bp), and the proline (Pro) tRNA gene (71 bp). Orthologous gene segments from eight more distantly related Tanganyikan cichlid species (from six other tribes) were sequenced to place the Lamprologini into a larger phylogenetic framework (see table 2).

DNA Extraction and Nucleotide Sequencing

DNA was extracted from white muscle tissue of frozen and ethanol-preserved specimens (Kocher et al. 1989). Two amplifications (one double-stranded and one single-stranded) via the polymerase chain reaction (PCR) were conducted as described elsewhere (Sturmbauer and Meyer 1993). The primers used for the cytochrome *b* gene segment were L14724 and H15148, and L15926 and H16498 were used for the tRNAs and a segment of the control region (Kocher et al. 1989; Meyer et al. 1990). Single-stranded amplification products were ultrafiltrated three times with 300 μl H_2O in spin columns (Millipore 30,000) before direct sequenc-

Table 1
Ecological Characterization of the Studied Lamprologine Species from Lake Tanganyika

Species	Locality[a]	n[b]	Habitat[c]	Diet[d]
Neolamprologus moorii	Zambia	1/2	Rock	Aufwuchs
N. cylindricus	Tanzania	0/1	Rock	Zoobiocover
N. toae	Zambia	1/1	Rock	Invertebrates
Altolamprologus compressiceps	Burundi	0/1	Rock, rubble	Zoobiocover
A. calvus	Zambia	1/1	Rock	Zoobiocover
Lamprologus callipterus	Burundi	1/5	Rock, sand	Shrimp, fry
Lepidiolamprologus elongatus	Zambia	2/2	Rock	Shrimp, fish
N. caudopunctatus	?	0/1	Rubble, sand	Invertebrates
N. calliurus	Burundi	0/1	Sand, shells	Invertebrates
N. brevis	Zambia	0/1	Sand, shells	Invertebrates
Chalinochromis brichardi	Burundi	2/1	Rock, rubble	Aufwuchs
N. furcifer	Burundi	0/1	Cave dweller	Zoobiocover
N. longior	?	1/2	Rock	Zoobiocover
N. brichardi	Burundi	1/2	Rock	Shrimp
Lamprologus mocquardii	Zaïre	1/1	Riverine	Invertebrates
Lamprologus congoensis	Zaïre	1/1	Riverine	Invertebrates
Lamprologus werneri	Zaïre	1/1	Riverine	Invertebrates
Telmatochromis bifrenatus	Burundi	1/1	Rock	Zoobiocover
T. vittatus	?	1/1	Rock	Zoobiocover
Julidochromis regani	Burundi/Zambia	0/3	Rock	Zoobiocover
J. regani 'affinis'	Burundi	0/1	Rock	Zoobiocover
J. marlieri	Burundi	1/1	Rock	Zoobiocover
T. burgeoni	?	1/1	Rock	Aufwuchs
N. christyi	Burundi	1/1	Rock	Invertebrates
N. christyi 'affinis'	Tanzania	0/1	Rock	Invertebrates

NOTE.—The nomenclature follows Poll (1986). Data were compiled from Konings (1988) and Brichard (1989).

[a] Coastal region where sample was obtained.

[b] No. of individuals sequenced for cytochrome *b*/no. of individuals sequenced for the control region.

[c] Characterized according to Brichard (1989).

[d] According to Yamaoka (1991) and personal observations by C. S. (see Sturmbauer et al. [1992]). Zoobiocover = epilithic invertebrates (ostracods and copepodids); and Aufwuchs = epilithic algae, attached detritus, and epilithic invertebrates.

Table 2
Ecological Characterization of the Studied Outgroup Species from Lake Tanganyika

Species	Tribe	n^a	Habitat[b]	Breeding[c]
Oreochromis tanganicae	Tilapiini	1	Ubiquitous	Mouth
Boulengerochromis microlepsis	Tilapiini	2	Pelagic	Substrate
Astatotilapia burtoni	Haplochromini	1	Swamp	Mouth
Tropheus duboisi	Tropheini	3	Rock	Mouth
Paracyprichromis brieni	Cyprichromini	1	Pelagic	Mouth
Grammatotria lemarii	Ectodini	2	Sand	Mouth
Triglachromis otostigma	Limnochromini	1	Bottom	Mouth
Limnochromis auritus	Limnochromini	1	Bottom	Mouth

NOTE.—Six of the 12 Tanganyikan tribes were included in the analysis, including two members of the distantly related tribe Tilapiini (Poll 1986).

[a] No. of individuals sequenced.

[b] Characterized according to Brichard (1989).

[c] According to Kuwamura (1986).

ing (Sequenase version 2.0; U.S. Biochemical; Sanger et al. 1977). The sequences were electrophoresed on 6% acrylamide-urea gels in Tris-borate–ethylenediaminetetraacetate buffer (45 mM, pH 8.0).

Phylogenetic Analysis

The sequence data were entered and aligned in ESEE (version 1.09; Cabot and Beckenbach 1989) and were analyzed by the parsimony method using PAUP (version 3.0s; Swofford 1992). The large number of taxa analyzed made the application of heuristic search procedures necessary. In order to make the heuristic searches more reliable (D. L. Swofford, personal communication), the option "random addition of taxa" with 50 replications was chosen in PAUP.

In addition, to choose among equally parsimonious phylogenetic trees (Carpenter 1988), the successive-approximation approach to character weighting according to their "cladistic reliability" was performed (Farris 1969; Williams and Fitch 1989). Reweighting was based on both the maximum rescaled consistency index (RC; Farris 1989) of all equally parsimonious trees found in parsimony analysis and a base weight of 1,000.

Neighbor-joining analyses (Saitou and Nei 1987) were performed with NTSYS.PC (version 1.6; Applied Biostatistics), with distance matrices that were calculated according to the same weights as were used in parsimony analyses. Statistical analyses was done only for parsimony by means of the bootstrap method (Felsenstein 1985; 100 replications, heuristic search, and PAUP option "simple addition of taxa").

Nucleotide substitutions occur nonrandomly, because of different selective constraints acting on different genes or gene segments (Brown et al. 1982; Smith 1989; Kraus and Miyamoto 1991; Allard and Miyamoto 1992). Our analyses attempted to account for this fact by emphasizing the most infrequently observed base changes (transversions and indels), with separate weighting scales for cytochrome *b* and the control region.

The phylogenetic analysis was performed in two steps. A first analysis was performed in which both gene segments (857 bp) were first analyzed both separately and combined. To minimize the effect of multiple base substitutions, only inferred amino acid replacements and only transversions in first and third codon positions were considered in the coding sequence (Edwards et al. 1991; Irwin et al. 1991). For the tRNAs and the control region, the weights for transitions and transversions were calculated on the basis of their average frequency observed in a set of closely related taxa having an uncorrected sequence divergence of up to 5%. Because of the low frequency of indels, their frequency could not be estimated accurately, and they were weighted as were transversions. In cytochrome *b*, transversions and inferred amino acid replacements were given a weight of 3 in the combined analysis, making them equal to the weight of transversions and indels in the largely noncoding segment.

This analysis included 16 representatives of the Lamprologini and six species of five Tanganyikan tribes of mouthbrooders (tables 1 and 2). Two additional species of the more distantly related tribe Tilapiini (Poll 1986), one mouthbrooder (*Oreochromis tanganicae*) and one substrate breeder (*Boulengerochromis microlepis*), were chosen as outgroups on the basis of the analysis of all major eastern African cichlid lineages (authors' unpublished results).

A second analysis was performed to determine the branching order of the clades within the Lamprologini. Here, only the 455-bp largely noncoding segment was analyzed using the same set of weights as was used in

the first analysis. Of the total of 35 sequenced individuals, 28 were included in the analysis, so that each subspecies was represented. *Triglachromis otostigma* and *Limnochromis auritus* were used as outgroups, on the basis of the first analysis.

Age Estimates

Our age estimates rest on the prima facie assumption of comparable relative rates of molecular evolution among different lineages of eastern African cichlids. The relative ages were inferred from the comparison of maximum observed sequence distances between clades from the two mtDNA segments sequenced. In the control region, sequence distance was calculated as Kimura (1980) distances. In cytochrome *b*, distances were corrected for multiple substitutions by the one-parameter model (Jukes and Cantor 1969; Jukes 1980).

The sequence divergences among 24 species of the Lamprologini were compared with those found within the endemic Tanganyikan tribe Ectodini (12 species; Sturmbauer and Meyer 1993). The absolute age of the lineages was recently gauged on the basis of an estimate of the age of Lake Tanganyika, estimating the maximum age of the lacustrine habitat to be 9–12 Myr old (Cohen et al. 1993). Tiercelin and Mondeguer (1991) presented a three-stage model of origin for Lake Tanganyika. In the initial stage (20–14 Mya) the proto-river became meandric and progressively swampy and was probably connected to the Zaire hydrological system. The second stage (14–6 Mya) is characterized by the progressive formation of a mosaic of small, mostly isolated lakes. The third stage is characterized as the time after the fusion of the small sublakes and Lake Tanganyika became a truly lacustrine habitat, estimated at about 5 Mya (Tiercelin and Mondeguer 1991).

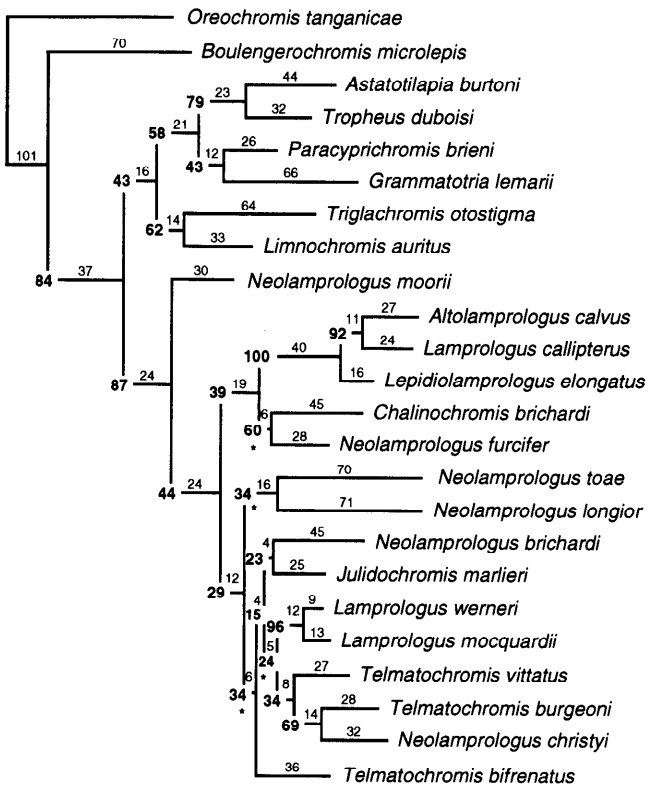

FIG. 2.—Single most parsimonious tree obtained for 16 species of the tribe Lamprologini and 6 species assigned to the "haplochromis lineage" by Nishida (1991), using two more distantly related species of the tribe Tilapiini, *Oreochromis tanganicae* and *Boulengerochromis microlepis*, as outgroup (weighted tree length = 1,271 steps; CI [Kluge and Farris 1969] = 0.75; CI excluding uninformative characters = 0.57; and RC [Farris 1989] = 0.50). Branch lengths are given above the branches. Bootstrap values are given on the branches. Asterisks indicate conflicts with the results from neighbor-joining analysis. The 402-bp segment of the mitochondrial cytochrome *b* and a 452-bp segment of part of the Thr tRNA, the Pro tRNA, and part of the control region were combined. For the cytochrome *b* all amino acid substitutions and only transversions in first and third codon positions were analyzed, given a weight of 3. In the tRNAs and the control-region, transversions and indels were weighted three times more than transitions.

Results

Age of Lamprologines

The average observed corrected sequence divergence among the Lamprologini amounted to 10.8% (standard deviation 3.3%, maximum 20%) in the control region. The average observed distance in the control region, among species of the endemic Tanganyikan tribe Ectodini, was 7.9% (standard deviation 2.1%, maximum 12.3%; Sturmbauer and Meyer 1993). The comparison of distances within one lacustrine clade comprising snail-dwelling *Neolamprologus brevis, N. calliurus,* and *Lamprologus callipterus;* the predatory *Lepidiolamprologus elongatus;* and the zooplanctivorous *N. caudopunctatus* and the two *Altolamprologus* species (see figs 2–4) yielded an average distance of 7.7% (standard deviation 1.2%, maximum 10.2%), similar to that observed among the Ectodini. This similarity might suggest that the lacustrine clades of the Lamprologini originated at about the same time as the Ectodini.

In cytochrome *b* the average distance was 5.7% (standard deviation 1.4%, maximum 8.5%) among the Lamprologini and 6.8% (standard deviation 2.2%, maximum 10.2%) among the Ectodini (Sturmbauer and Meyer 1993). Five inferred amino acid replacements were observed among the Ectodini, four among the Lamprologini.

Age of the Zaire River *Lamprologus*

It is interesting that the three *Lamprologus* species outside Lake Tanganyika do not appear to represent a basal lineage, as had been suggested. Furthermore, they

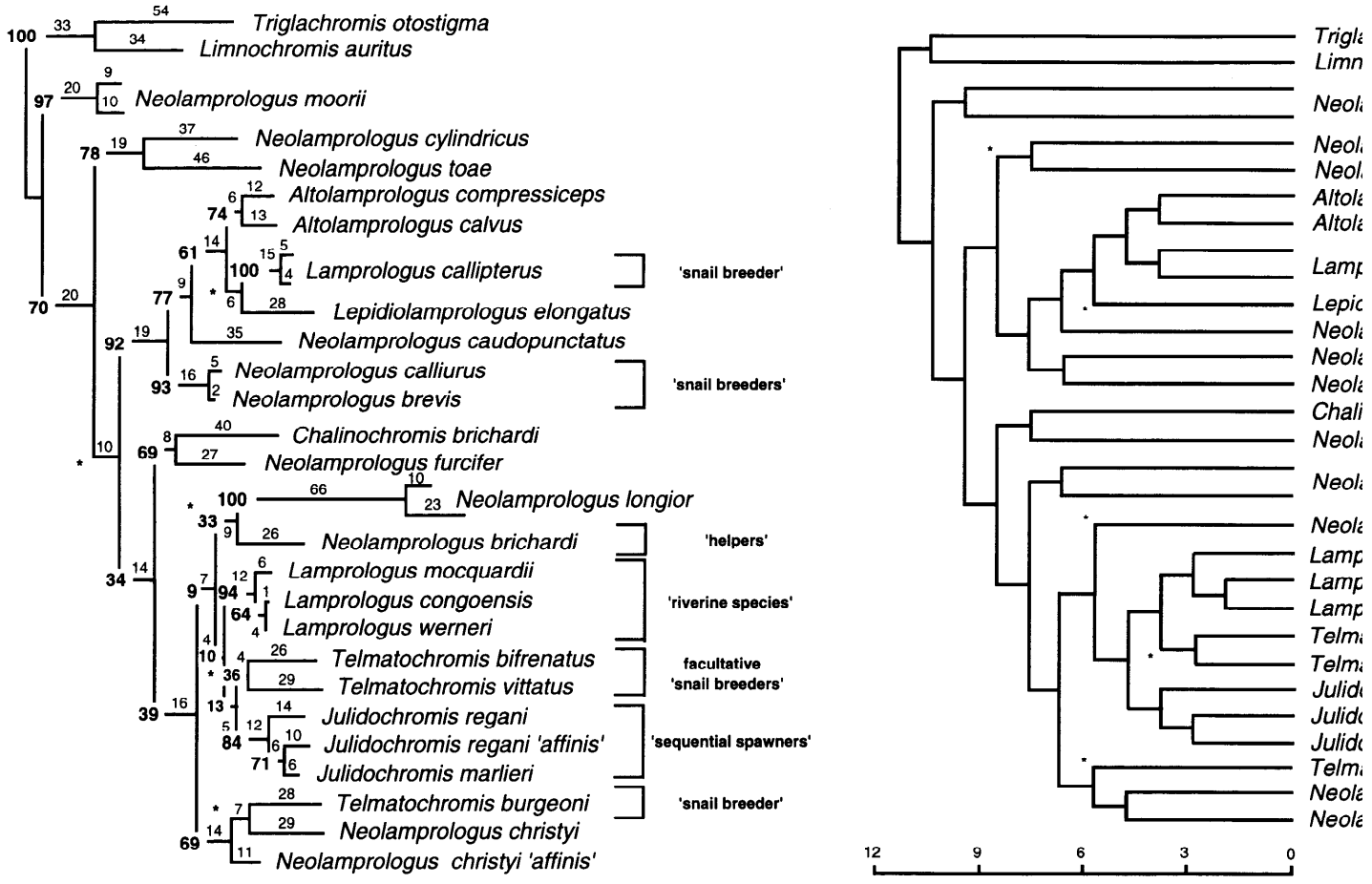

FIG. 3.—Phylogeny of 24 species (25 taxa) of the tribe Lamprologini, for a 452-bp segment of part of the Thr tRNA, the Pro tRNA, and part of the contr declaring *Triglachromis otostigma* and *Limnochromis auritus* as outgroup. *Left,* Single most parsimonious tree obtained by parsimony (weighted tree length length = 541 steps; CI [Kluge and Farris 1969] = 0.51; CI excluding uninformative characters = 0.44; and RC [Farris 1989] = 0.30). Transversions and inc more than transitions, according to their average observed frequency between taxa of <5% nucleotide differences. Branch lengths are given above the branch on those branches that did not conflict with the bootstrap consensus tree. *Right,* Neighbor-joining tree of 25 species of the tribe Lamprologini, for a 450-bp segi the Pro tRNA, and part of the control region. Distances were calculated as *p*-distances according to the weights used for parsimony, so that transversions a times more than transitions. Asterisks symbolize discrepancies to the corresponding parsimony analysis.

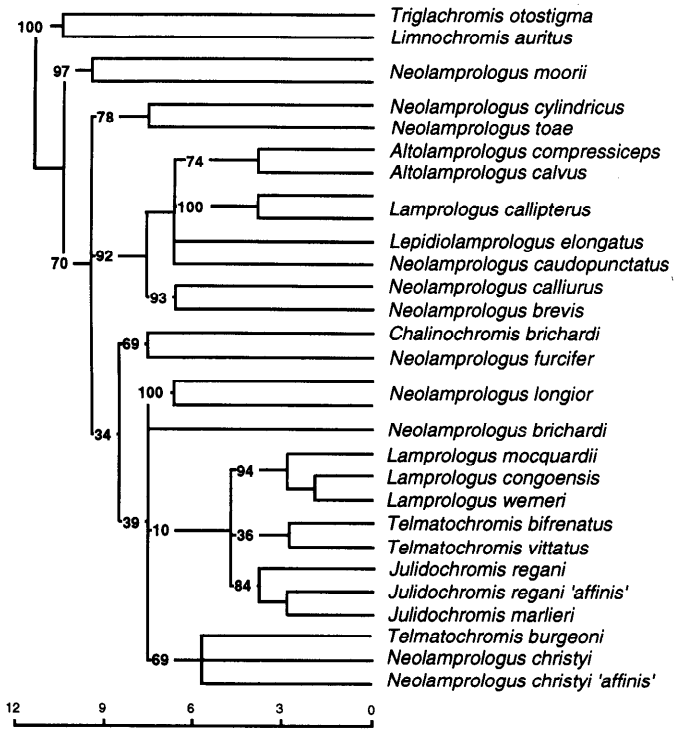

FIG. 4.—Strict-consensus tree of the corresponding parsimony and neighbor-joining analyses: 25 species of the tribe Lamprologini, for a 450-bp segment of part of the Thr tRNA, the Pro tRNA, and part of the control region. The cladogram is rooted declaring *Triglachromis otostigma* and *Limnochromis auritus* as outgroup. Parsimony bootstrap values are given on branches that correspond in both analyses.

form a monophyletic group (fig. 3) with an average distance, in the control region, of 1.1% (standard deviation 0.6%, maximum 1.6%). Considering that genetic distances in the control region varied from 1.2%, among five individuals of *Lamprologus callipterus,* up to 5.1%, between two geographically separated populations of *N. christyi,* the three Zairean species might be younger than some endemic Tanganyikan species (Sturmbauer and Meyer 1992). Because of small morphological differences in the type specimen, these populations of *N. christyi* may prove to be different species.

Analysis of Cytochrome *b* and Control Region

When cytochrome *b* was analyzed separately, 792 equally parsimonious trees (tree length 47 steps) were obtained. The strict consensus of those trees was completely unresolved (data not shown). However, the application of the successive reweighting, starting from all most parsimonious trees with the highest RC, yielded four equally parsimonious trees after three replications (weighted tree length 76,872 steps). The "stable" topology corroborated the monophyly of the Lamprologini, placing the six Tanganyikan mouthbrooders as their sister group (data not shown).

Parsimony analysis of the control region only resulted in a single most parsimonious tree (weighted tree length 1,101 steps), which was also obtained as a "stable" topology after reweighting. As with cytochrome *b*, after reweighting the topology corroborated the monophyly of the Lamprologini, placing the six Tanganyikan mouthbrooders as their sister group (data not shown).

When both segments were combined, parsimony analysis resulted in a single most parsimonious tree with a length of 1,271 steps (fig. 2). The consistency index (CI; Kluge and Farris 1969) of this tree was 0.75, the CI excluding uninformative characters was 0.57, and the RC (Farris 1989) was 0.50. The successive-reweighting procedure resulted, after two replications, in a stable topology that was identical to the single most parsimonious tree (fig. 2).

The neighbor-joining analysis (data not shown) agreed with the parsimony tree, in that the Lamprologini appear as monophyletic, placing the six Tanganyikan mouthbrooders as their sister group. Again, *N. moorii* appeared as the sister group to all other Lamprologini; *A. calvus, Lamprologus callipterus* and *Lepidiolamprologus elongatus* were resolved as a clade; the two species from the Zaire River system were placed as a clade, as were *Telmatochromis vittatus, T. burgeoni,* and *N. christyi;* and, finally, *Chalimochromis brichardi* formed a clade with *N. furcifer.* However, the branching order among these clades differed between the parsimony and neighbor-joining analyses (differences are indicated by asterisks in fig. 2). The positions of *N. toae* and *N. longior* also differed between the parsimony and neighbor-joining analyses (see fig. 2). In parsimony analysis *T. bifrenatus* was never identified as sister group to its apparent morphological sister species *T. vittatus* (fig. 2); however, in the neighbor-joining analysis it was.

Analyses of the Control Region

The second analysis of the largely noncoding mtDNA segment included eight additional lamprologine species, for a total of 25; *Limnochromis auritus* and *Triglachromis otostigma* were outgroups. A single most parsimonious tree with a weighted length of 955 steps was obtained (fig. 3, *left*). The tree length with all equal weights was 541 steps, the CI based on equal weights was 0.51, the CI excluding uninformative sites was 0.44, and the RC was 0.30.

The successive-reweighting procedure reached a stable topology (one tree) at the third replication (weighted tree length 178,047 steps; CI = 0.76; CI excluding uninformative characters = 0.60; and RC = 0.58). The topology after reweighting (not shown) is in agreement with the shortest parsimony tree, with *N. moorii* being the sister group to all other Lamprologini. Also, *N. brevis, N. calliurus, N. caudopunctatus, Lepi-*

diolamprologus elongatus, Lamprologus callipterus, and the two *Altolamprologus* species form a clade. Furthermore, *C. brichardi* formed a clade with *N. furcifer,* as did *N. toae* with *N. cylindricus;* the *Lamprologus* species from the Zaire River are monophyletic groups; and *Telmatochromis burgeoni* joined *N. christyi* and *N. christyi* 'affinis.' However, the clade of *N. toae* and *N. cylindricus* branched on the basis of the clade containing *N. brevis, N. calliurus, N. caudopunctatus, Lepidiolamprologus elongatus, Lamprologus callipterus,* and the two *Altolamprologus* species. While *Telmatochromis bifrenatus* was placed as sister group to its apparent morphological sister species *Telmatochromis vittatus* in parsimony, reweighting resolved them differently. The stable topology also differed from the shortest parsimony tree, in that *Lepidiolamprologus elongatus* now formed a separate clade, between the clade of *N. brevis* and *N. calliurus* and the clade of *Lamprologus callipterus* and the two *Altolamprologus* species.

The corresponding neighbor-joining analysis (fig. 3, *right*) is in agreement with the stable topology after reweighting, in that *Lepidiolamprologus elongatus* was a separate clade between the two clades of snail dwellers, and in the placement of the clade containing *N. toae* and *N. cylindricus.* The clade of *C. brichardi* and *N. furcifer* was consistently resolved on the basis of the Zaire lamprologines, *Julidochromis, Telmatochromis, N. christyi,* and *N. brichardi.* As in the single most parsimonious tree, *Telmatochromis bifrenatus* formed a clade with *Telmatochromis vittatus,* in the neighbor-joining analysis. The agreements of the parsimony and neighbor-joining analyses are summarized in a strict-consensus tree (fig. 4) based on the single most parsimonious tree and the neighbor-joining tree.

Discussion

Monophyly and Origin of the Lamprologini

Although the monophyly of the tribe Lamprologini (first described as genus *Lamprologus* Schilthuis 1891) is widely accepted, the monophyly of this assemblage of genera was corroborated only recently, on the basis of a cladistic morphological analysis (Stiassny 1991). The choice of outgroups had been problematic, because the precise placement of the Lamprologini within the African Cichlidae could not be inferred on the basis of morphology. The Zairean genus *Teleogramma* Boulenger 1899 has been recently suggested to be a close relative or even a member of the Lamprologini, on the basis of similarities in dentition (M. L. J. Stiassny, personal communication). For our analyses, we selected the outgroups on the basis of an extended mitochondrial phylogeny (cytochrome *b* and control region) that included representatives of several African lineages and all 12 Tanganyikan tribes, using the neotropical species *Cichlasoma citrinellum* as outgroup (authors' unpublished data). To place the Lamprologini within a larger phylogenetic framework and to test their monophyly, we selected two members of the more distantly related tribe Tilapiini as outgroups for the first analysis and included six Tanganyikan mouthbrooders representing five endemic tribes (Poll 1986). Whether *Teleogramma brichardi* is related to the Lamprologini remains to be tested.

The present analyses of two mitochondrial genes support the monophyly of the Lamprologini (87% and 100% of the bootstraps, respectively; see figs. 2 and 3, *left*), independent of the outgroups chosen. *Boulengerochromis microlepis,* the only endemic substrate-breeding cichlid other than the lamprologines, does not seem to be closely related to the Lamprologini. A group of six Tanganyikan lineages representing five tribes (Poll 1986) appears to be sister group to the Lamprologini. They are part of the "Haplochromis-lineage" in Nishida's (1991) analysis of allozymes. All six mouthbrooding lineages seem to be equally distantly related to the Lamprologini, implying a radiation in which the mouthbrooders evolved and radiated in parallel with the substrate spawners. Within the mouthbrooders, two species considered ancestral in their form of mouthbrooding seem to represent the most basal split: in *Limnochromis* and *Triglachromis,* both parents participate in the incubation of eggs and wrigglers, implying monogamy at least for the breeding period (Konings 1988, p. 218; Brichard 1989, p. 383). This fact may support the hypothesis that the lamprologines and these Tanganyikan mouthbrooders shared a common ancestor who was a substrate-breeding species and that mouthbrooding evolved *after* the split of those two major lineages.

Relationships between the Zaire Lamprologines and the Tanganyikan Endemics

Clearly, the three riverine species of lamprologines from the Zaire River form a monophyletic group that is not basal to all Lake Tanganyika lamprologines. This is corroborated both by genetic distances and by the branching order of the phylogenetic hypothesis. They had long been assumed to be the sister group to the Tanganyikan endemics. When the Zaire lamprologines were placed as the most ancestral branch in MacClade (Maddison and Maddison 1992), the tree length increased by 30 steps, according to the weights used in PAUP (eight transversions and six transitions). The Zaire Lamprologini seem to be close to some endemic Tanganyikan genera, with an average distance in the largely noncoding segment of 6.2% (standard deviation 1.2%) to *Julidochromis, Telmatochromis, Neolamprologus christyi,* and *N. brichardi,* and more distantly re-

lated to the clade of specialized lacustrine lamprologines mentioned above (average distance 11.5%, standard deviation 1.4%). The average Kimura distance among the three Zaire species is 1.1% (standard deviation 0.6%), suggesting that their common ancestor might have left the lake and speciated recently in the Zaire River. Two evolutionary scenarios may explain why the lineage of riverine Lamprologini does not constitute the most ancestral lineage.

The first hypothesis assumes that the initial radiation of the Lamprologini covered both the Zaire basin—which at that time was an internal drainage system surrounding a large lake (Cahen 1954)—and the early Tanganyikan basin. The subsequent isolation of Lake Tanganyika during the Pliocene was then followed by the extinction of all except the only extant lamprologine lineage in the Zaire River.

The alternative hypothesis, which we favor, suggests that at least the three sampled Zairean Lamprologini (of the five described species) evolved from a single Tanganyikan lineage that was able to leave the lake and colonized the Zaire basin during the Miocene when intermittent connections existed between Lake Tanganyika and the Zaire River (Coulter 1991, p. 291). On the basis of both the "absolute" age for the major lineages of the Lamprologini and the assumption that the extinction of all but a single lineage of Lamprologini in the Zaire basin is less parsimonious, our data might be best explained by postulating that the extant Zaire Lamprologini are descendants of an endemic Tanganyikan lamprologine lineage that colonized the Zaire basin. The fact that an endemic lineage leaves a lake habitat and recolonizes a river seems highly unusual, and no evidence for this exists in other Tanganyikan tribes.

Age of the Lamprologini

The observation of particularly short internodes defining the arising lineages among all Tanganyikan tribes (see figs. 2 and 3) suggests that the initial radiation of the lamprologines may have been rapid. Similar patterns of "explosive" speciation have been suggested for other mouthbrooding lineages, e.g., the Tropheini (Sturmbauer and Meyer 1992), the Ectodini (Sturmbauer and Meyer 1993), and "haplochromine" cichlids form Lake Victoria (Meyer et al. 1990, 1991).

In order to test whether truly lacustrine Lamprologini radiated in parallel with the Ectodini, we selected a highly supported clade of seven Lamprologini (*N. brevis, N. calliurus, N. caudopunctatus, Lepidiolamprologus elongatus, Lamprologus callipterus,* and the two *Altolamprologus* species; see fig. 3). These occupy habitats that are not available in rivers, and three species breed in shells of endemic Tanganyikan gastropod species. The average Kimura distance among species of that specialized lacustrine clade corresponds to that found among 12 species of the Ectodini, which are also diversified in lacustrine habitats. Both radiations may have been induced by the formation of a lacustrine environment. As other clades of Tanganyikan endemics branched before these radiations, it seems possible that several consecutive radiations may have been triggered by environmental changes during various stages of the formation of Lake Tanganyika, according to the three-stage model of Tiercelin and Mondeguer (1991).

Intraspecific Variation

Several individuals of the same species (fig. 3) from different localities were included in the analysis of the mitochondrial control region (see also table 1). We also analyzed the "cline" of *Julidochromis* species from Burundi. Between localities inhabited by *J. regani* and localities inhabited by *J. marlieri* there exists a presumably transitional population, *J. regani* "affinis", showing features of both species. All conspecifics clustered with high bootstrap values (all more than 84%; see fig. 3, *left*), indicating that although intraspecific variation for this highly variable portion of mtDNA is large, it is not likely to obscure the interrelations among different species studied. All three closely related *Julidochromis* formed a clade, as did the two *Altolamprologus* species and two morphologically closely related snail dwellers, *N. brevis* and *N. calliurus* (fig. 3). The two older (Kimura distance 7.5%) morphological sister species *Telmatochromis bifrenatus* and *Telmatochromis vittatus* were placed as clade in some analyses; in others they were not.

Molecular Analysis

Only a few substitutions define the branching order of the arising lineages, particularly in slowly evolving genes. Even if slowly evolving genes may be less prone to homoplasy within a certain amount of time, the number of substitutions per unit time may not allow for the resolution of rapidly evolving lineages. Rapidly evolving genes might provide higher resolution for this question. In order to assess the patterns of homoplasy in cytochrome *b* and the control region, we compared the number of observed transitions and transversions in our data set used in the combined analysis. In cytochrome *b* a total of 25% of all positions were variable, compared with 41% in the control region. While only 18% of the observed substitutions were transversions in cytochrome b, we found 41% transversions in the control region. Only 6.7% of the first positions and 2.2% of the second positions varied. Of the third codon positions, 64% were variable, and 80% of these were scored as transitions. Fish tend to have highly biased base compositions (reviewed in Meyer 1993*a*), with third codon positions

often varying only by transversions (i.e., two character states), and hence may accumulate multiple substitutions more rapidly and become homoplasious rapidly. In the control region, 9% of the varied sites exhibited all four bases, and 29% exhibited three bases. While many genes have relatively few positions "free to vary" (Palumbi 1989) and exhibit a substitution bias that may dramatically cut down the "degrees of freedom" in the number of possible character states, the control region appears to contain a relatively high amount of less-biased variable sites.

The Evolution of Specialized Parental Care

In most substrate-breeding cichlids, only the female guards the eggs and performs "fanning" behavior to provide aerated water. Male parental care is usually limited to guarding the offspring during its free-swimming stage. This marked division of labor has been described for several substrate-brooding cichlids (Baerends and Baerends Van Roon 1950; Baerends 1984; Kuwamura 1986; Gashagaza 1991; reviewed in Barlow 1991; Keenleyside 1991; Stiassny and Gestner 1992). While most cichlid lineages are relatively uniform in terms of their breeding behavior, the Lamprologini evolved a high diversity from ancestral to highly derived patterns of parental care such as breeding in gastropod shells, polygyny, or the recruitment of the offspring as helpers for parental care (table 3).

Neolamprologus moorii also seems to be ancestral in its type of reproductive behavior, which is similar to that found in several substrate breeders. This species does not show sexual size dimorphism and attaches 200–500 eggs under an overhanging rock. Three of four sequenced species that breed in gastropod shells were placed in one clade (*N. brevis, N. calliurus,* and *Lamprologus callipterus*). Their branching order suggests that snail breeding is likely to have evolved in parallel within this clade. *Lamprologus callipterus* seems to have evolved snail breeding independently. One recently discovered new species of *Altolamprologus* from Sumbu Bay in southern Lake Tanganyika has also been reported to breed in snail shells (Konings 1988, p. 214). *Telmatochromis bifrenatus* and *Telmatochromis burgeoni* were also found to be facultative snail breeders (Konings 1988, p. 111; Brichard 1989, p. 207; authors' personal observations) and seem to represent other independent evolutions of this type of breeding.

Behavioral differences between *N. brevis* and *N. calliurus* and *Lamprologus callipterus* also seem to support the hypothesis of independent evolution of snail dwelling. While *N. brevis* and *N. calliurus* form pairs, males of southern populations of *Lamprologus callip-*

Table 3
Breeding and Parental Care of the Studied Lamprologini from Lake Tanganyika

Species	Nest Site	Mono/polygamous	Lekking	Guarding[a]
Neolamprologus moorii	Crevice	m + f	?	m + f
N. cylindricus	Crevice	m + f	No	m + f
N. toae	Crevice	m + f	No	m + f
Altolamprologus compressiceps	Crevice	m + f	No	m + f
A. calvus	Crevice	m + f	No	m + f
Lamprologus callipterus	Nest, shell	m + fff	Yes	m + fff
Lepidiolamprologus elongatus	Rock surface	m + f	No	m + f
N. caudopunctatus	Rock hole	m + f	?	m + f
N. calliurus	Gastropod shell	m + f	No	f
N. brevis	Gastropod shell	m + f	No	f
Chalinochromis brichardi	Crevice	m + f	No	m + f
N. furcifer	Overhanging rock	m + fff	No	f
N. longior	Sand among rocks	m + f	No	f
N. brichardi	Rock hole	m + f	Yes	m + f + j as helpers
Lamprologus mocquardii	Rock hole	?	No	?
Lamprologus congoensis	Rock hole	m + fff	No	f
Lamprologus werneri	Rock hole	m + fff	No	f
Telmatochromis bifrenatus	Crevice, gastropod shell	m + f	No	m + f
T. vittatus	Crevice	m + f	No	m + f
Julidochromis species	Crevice	m + f	No	m + f + j
T. burgeoni	Gastropod shell	m + f	No	m + f
N. christyi	Crevice	m + fff	No	m + f

SOURCES.—Kuwamura (1986), Konings (1988), Brichard (1989), Barlow (1991), Gashagaza (1991), Keenleyside (1991), and authors' unpublished data.
[a] m = male; f = female; fff = polygynous; and j = juveniles.

terus not only breed in gastropod shells but are also polygamous (Konings 1988, p. 198; authors' personal observation). Males accumulate piles of gastropod shells in their territory to breed with several females. A tendency toward polygyny has also been reported for *N. furcifer* (Yanagisawa 1987), *N. modestus, N. savoryi, N. toae, N. brichardi, Lamprologus attenuatus,* and *N. tetracanthus* (Limberger 1983; Kondo 1986). The most complex system of parental care among substrate-breeding cichlids may be the inclusion of the offspring from previous spawn as helpers in parental care, a behavior found in *N. brichardi* (Taborsky and Limberger 1981; Taborsky 1982, 1984; Taborsky et al. 1986).

Taxonomy of the Lamprologini

The current generic classification within the Lamprologini is highly controversial. According to Greenwood (1981) and Stiassny (1991), the present classification into seven (Poll 1986) or nine (Colombe and Allgayer 1985) genera seems to be the result of morphological analyses that might have overemphasized highly variable and homoplasious morphological features (e.g., number of scales, anal spines, number and position of pharyngeal and mandibular teeth, and patterns in infraorbital ossification) that may not be considered to be unambiguously derived morphological features (Greenwood 1978, 1981; Jensen 1990; Stiassny 1991). For example, Colombe and Allgayer (1985) used "morphocline" patterns in the infraorbital ossification of the Lamprologini to restrict the genus *Lamprologus* to the type species *Lamprologus congoensis* and the other four fluvial species; the genera *Lepidiolamprologus* and *Variabilichromis* were defined on the basis of uncertain criteria (see Poll 1986).

The mtDNA phylogeny suggests that only two (*Altolamprologus* and *Julidochromis*) of the seven currently recognized genera appear to be monophyletic. Three of four representatives of the genus *Lamprologus* Schilthuis 1891 in our data set are grouped as a monophyletic assemblage. *Lamprologus callipterus,* the only lacustrine representative of this genus in our sample does not seem to be closely related to its fluvial congeners. The genus *Neolamprologus,* represented by eight species, seems to be a highly heterogeneous assemblage. The assumed close relationship between the genera *Julidochromis* and *Chalinochromis* (Poll 1974, 1986) is not supported by our data. Our results suggest a close relationship between *Chalinochromis brichardi* and *N. furcifer;* also Poll (1986) was unable to prove the monophyly of *Julidochromis* and *Chalinochromis* by comparative morphology.

Clearly there is a need for new morphology-based phylogenetic studies of this group of species, to further evaluate our molecular phylogeny. Such a cladistic analysis is underway, (M. L. J. Stiassny, personal communication).

Sequence Availability

The nucleotide sequences in this paper are available from EMBL and are as follows: for cytochrome *b*—*Oreochromis tanganicae* Z12046 (Swedish Museum of Natural History, NRM 12815; Sturmbauer and Meyer 1992, 1993); *Boulengerochromis microlepis* Z30076; *Astatotilapia burtoni* Z21773 (Sturmbauer and Meyer 1993); *Tropheus duboisi* Z12039, Z12040, and Z12041 (Sturmbauer and Meyer 1992, 1993); *Paracyprichromis brieni* Z21776 (Sturmbauer and Meyer 1993); *Grammatotria lemarii* Z21766 (Sturmbauer and Meyer 1993); *Triglachromis otostigma* Z30004; *Limnochromis auritus* Z21775 (Sturmbauer and Meyer 1993); *Neolamprologus moorii* Z30001; *Altolamprologus calvus* Z29989; *Lamprologus callipterus* Z29992; *Lepidiolamprologus elongatus* Z29994 and Z30184; *Chalinochromis brichardi* Z29990 and Z29991; *N. furcifer* Z29999; *N. toae* Z30002; *N. longior* Z30000; *N. brichardi* Z29997; *Julidochromis marlieri* Z30077; *Lamprologus werneri* Z29996; *Lamprologus mocquardii* Z29995; *Lamprologus congoensis* Z29993; *Telmatochromis vittatus* Z30003; *Telmatochromis burgeoni* Z30186; *N. christyi* Z29998; and *Telmatochromis bifrenatus* Z30185; and, for control region—*Triglachromis otostigma* Z30035; *Limnochromis auritus* Z21746 (Sturmbauer and Meyer 1993); *N. moorii* Z30029 and Z30030; *N. toae cylindricus* Z30025; *N.* Z30031; *Altolamprologus compressiceps* Z30005; *Altolamprologus calvus* Z30187; *Lamprologus callipterus* Z30010, Z30011, Z30012, Z30013, and Z30014; *Lepidiolamprologus elongatus* Z30016 and Z30017; *N. caudopunctatus* Z30024; *N. calliurus* Z30023; *N. brevis* Z30020; *Chalinochromis brichardi* Z30006; *N. furcifer* Z30026; *N. longior* Z30027 and Z30028; *N. brichardi* Z30021 and Z30022; *Lamprologus mocquardii* Z30018; *Lamprologus congoensis* Z30015; *Lamprologus werneri* Z30019; *Telmatochromis bifrenatus* Z30032; *Telmatochromis vittatus* Z30034; *J. regani* Z30008 and 30009; *J. regani* 'affinis' Z30079; *J. marlieri* Z30007; *Telmatochromis burgeoni* Z30033; *N. christyi* Z30188; and *N. christyi* 'affinis' Z30189.

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LITERATURE CITED

ALLARD, M. W., and M. M. MIYAMOTO. 1992. Testing phylogenetic approaches with empirical data, as illustrated with the parsimony method. Mol. Biol. Evol. **9**:778–786.

BAERENDS, G. P. 1984. The organisation of pre-spawning behaviour in the cichlid fish *Aequidens portalegrensis* (Hensel). Neth. J. Zool. **34**:233–266.

BAERENDS, G. P., and J. M. BAERENDS-VAN ROON. 1950. An introduction to the study of the ethology of cichlid fishes. Behaviour Suppl. **1**:1–242.

BARLOW, G. W. 1991. Mating systems among cichlid fishes. Pp. 173–190 *in* M. H. A. KEENLEYSIDE, ed. Cichlid fishes—behaviour, ecology and evolution. Chapman & Hall, London.

BRICHARD, P. 1989. Cichlids of Lake Tanganyika. T. F. H., Neptune City, N.J.

BROWN, W. M., E. M. PRAGER, A. WANG, and A. C. WILSON. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. J. Mol. Evol. **18**:225–239.

CABOT, E. L., and A. T. BECKENBACH. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comput. Appl. Biosci. **5**:233–234.

CAHEN, L. 1954. Géologie du Congo belge. Vaillant-Carmane, Liège.

CARPENTER, J. M. 1988. Choosing among equally parsimonious cladograms. Cladistics **4**:291–296.

COHEN, A. S., M. J. SOREGHAN, and C. A. SCHOLZ. 1993. Estimating the age of formation of lakes: an example from Lake Tanganyika, East-African rift system. Geology **21**:511–514.

COLOMBE, J., and R. ALLGAYER. 1985. Déscription de Variabilichromis, Neolamprologus et Paleolamprologus, genres nouveaux du lac Tangayika, avec redescription des genres Lamprologus Schilthuis 1891 et de Lepidiolamprologus Pellegrin 1904. Rev. Fr. Cichlidophiles **49**:9–28.

COULTER, G. W. 1991. The benthic fish community. Pp. 151–199 *in* G. W. COULTER, ed. Lake Tanganyika and its life. Oxford University Press, London, Oxford, and New York.

EDWARDS, S. V., P. ARCTANDER, and A. C. WILSON. 1991. Mitochondrial resolution of a deep branch in the genealogical tree for perching birds. Proc. R. Soc. Lond. [Biol.] **243**:99–107.

FARRIS, J. S. 1969. A successive approximations approach to character weighting. Syst. Zool. **18**:374–385.

———. 1989. The retention index and the rescaled consistency index. Cladistics **5**:417–419.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783–791.

FRYER, G., and T. D. ILES. 1972. The cichlid fishes of the Great Lakes of Africa. Their biology and evolution. Oliver & Boyd, Edinburgh.

GASHAGAZA, M. M. 1991. Diversity of breeding habits in the lamprologine cichlids in Lake Tanganyika. Physiol. Ecol. Japan **28**:29–65.

GREENWOOD, P. H. 1978. A review of the pharyngeal apophysis and its significance in the classification of African cichlid fishes. Bull. Br. Museum Nat. Hist. **33**:279–323.

———. 1981. The haplochromine fishes of the east African lakes. Kraus International, Munich.

———. 1984. African cichlids and evolutionary theories. Pp. 141–154 *in* A. A. ECHELLE and I. KORNFIELD, eds. Evolution of fish species flocks. University of Maine at Orono Press, Orono.

HORI, M. 1983. Feeding ecology of thirteen species of Lamprologus (Teleostei; cichlidae) coexisting at a rocky shore of Lake Tanganyika. Physiol. Ecol. Japan **20**:129–149.

IRWIN, D. M., T. D. KOCHER, and A. C. WILSON. 1991. Evolution of the cytochrome *b* gene of mammals. J. Mol. Evol. **32**:128–144.

JENSEN, J. S. 1990. Plausibility and testability: assessing the consequences of evolutionary innovation. Pp. 171–190 *in* M. H. NITECKI, ed. Evolutionary innovations. University of Chicago Press, Chicago.

JUKES, T. H. 1980. Silent nucleotide substitutions and the molecular evolutionary clock. Science **210**:973–978.

JUKES, T. H., and C. H. CANTOR. 1969. Evolution of protein molecules. Pp. 21–132 *in* H. N. MUNROE, ed. Mammalian protein metabolism. Academic Press, New York.

KEENLEYSIDE, M. H. A. 1991. Parental care. Pp. 191–208 *in* M. H. A. KEENLEYSIDE, ed. Cichlid fishes—Behaviour, ecology and evolution. Chapman & Hall, London.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. **16**:111–120.

KLUGE, A. G., and J. S. FARRIS. 1969. Quantitative phyletics and the evolution of anurans. Syst. Zool. **18**:1–32.

KOCHER, T. D., J. A. CONROY, K. R. MCKAYE, and J. R. STAUFFER. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. Mol. Phylogenet. Evol. **2**:158–165.

KOCHER, T. D., K. W. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, and A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA **86**:6196–6200.

KONDO, T. 1986. Feeding habits of *Lamprologus savoryi* (Teleostei: Cichlidae) with reference to its social behaviour. Physiol. Ecol. Japan **23**:1–15.

KONINGS, A. 1988. Tanganjika Cichliden. Lake Fish Movies, Herten, Germany.

KRAUS, F., and M. M. MIYAMOTO. 1991. Rapid cladogenesis among the pecoran ruminants: evidence from mitochondrial DNA sequences. Syst. Zool. **40**:117–130.

KUWAMURA, T. 1986. Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. J. Ethol. **4**:129–146.

LIMBERGER, D. 1983. Pairs and harems in a cichlid fish, Lamprologus brichardi. Z. Tierpsychol. **62**:115–144.

LOISELLE, P. V. 1980. Lamprologus, the different African cichlid. Fresh Water and Marine Aquatics Mag. **48548**:23–29, 79–83.

———. 1985. The cichlid aquarium. Tetra, Melle, Germany.

MADDISON, W. P., and D. R. MADDISON. 1992. MacClade: analysis of phylogeny and character evolution. Sinauer, Sunderland, Mass.

MEYER, A. 1993*a*. Evolution of mitochondrial DNA in fishes. Pp. 1–38 *in* P. W. HOCHACHKA and T. P. MOMMSEN, eds. The biochemistry and molecular biology of fishes. Vol. **2**. Elsevier, Amsterdam.

———. 1993*b*. Phylogenetic relationships and evolutionary processes in east African cichlid fishes. Trends Ecol. Evol. **8**:279–284.

MEYER, A., T. D. KOCHER, P. BASASIBWAKI, and A. C. WILSON. 1990. Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. Nature **347**:550–553.

MEYER, A., T. D. KOCHER, and A. C. WILSON. 1991. African fishes. Nature **350**:467–468.

NAGOSHI, M. 1983. Distribution, abundance and parental care of the genus Lamprologus (Cichlidae) in Lake Tanganyika. Afr. Study Monogr. **3**:39–47.

NISHIDA, M. 1991. Lake Tanganyika as an evolutionary reservoir of old lineages of east African cichlid fishes: inferences from allozyme data. Experientia **47**:974–979.

PALUMBI, S. R. 1989. Rates of molecular evolution and the fraction of nucleotide position free to vary. J. Mol. Evol. **29**:180–187.

POLL, M. 1946. Révision de la faune ichthyologique du lac Tanganyika. Ann. Musée Congo Belge, C Zool. ser. I, tome IV, fasc. **3**:141–364.

———. 1956. Poissons Cichlidae. Résult Sci. Expl. Hydrobiol. Lac Tanganyika (1946–1947) **3**:1–619.

———. 1974. Contribution à la faune ichthyologique du lac Tanganyika après les récoltes de P. Brichard. Rev. Zool. Afr. **88**:99–110.

———. 1986. Classification des cichlidae du lac Tanganika: tribus, genres et aspeces. Acad. R. Belg. Mem. Classe Sci., Coll. −8°–2° ser., tome XLV, fasc. **2**:1–163.

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

SANGER, F., S. NICKLEN, and A. COULSON. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.

SMITH, A. B. 1989. RNA sequence data in phylogenetic reconstruction: testing the limits of resolution. Cladistics **5**: 321–344.

STIASSNY, M. L. J. 1990. *Tylochromis,* relationships and the phylogenetic status of the African cichlidae. Novitates **2993**: 1–14.

———. 1991. Phylogenetic intrarelationships of the family Cichlidae: an overview. Pp. 1–35 *in* M. H. A. KEENLEYSIDE, ed. Cichlid fishes—behaviour, ecology and evolution. Chapman & Hall, London.

STIASSNY, M. L. J., and C. L. GESTNER. 1992. The parental care behaviour of Paratilapia polleni (Perciformes, Labroidei), a phylogenetically primitive cichlid from Madagascar, with a discussion of the evolution of maternal care in the family Cichlidae. Environ. Biol. Fishes **34**:219–233.

STURMBAUER, C., W. MARK, and R. DALLINGER. 1992. Ecophysiology of Aufwuchs-eating cichlids in Lake Tanganyika: niche separation by trophic specialization. Environ. Biol. Fishes **35**:283–290.

STURMBAUER, C., and A. MEYER. 1992. Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. Nature **359**:578–581.

———. 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes from Lake Tanganyika in eastern Africa. Mol. Biol. Evol. **10**:751–768.

SWOFFORD, D. L. 1992. Phylogenetic analysis using parsimony (PAUP), version 3.0s. Illinois Natural History Survey, Champaign.

TABORSKY, M. 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi:* their costs and benefits. Anim. Behav. **32**:1236–1252.

———. 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. Behaviour **95**: 45–75.

TABORSKY, M., E. HERT, M. V. SIEMENS, and P. STOERIG. 1986. Social behaviour of Lamprologus species: functions and mechanisms. Ann. Museum R. Afr. Cent. Sci. Zool. **251**:7–11.

TABORSKY, M., and D. LIMBERGER. 1981. Helpers in fish. Behav. Ecol. Sociobiol. **8**:143–145.

TIERCELIN, J. J., and A. MONDEGUER. 1991. The geology of the Tanganyika Trough. Pp. 7–48 *in* G. W. COULTER, ed. Lake Tanganyika and its life. Oxford University Press, London, Oxford, and New York.

WILLIAMS, P. L., and W. M. FITCH. 1989. Finding the minimal change in a given tree. Pp. 453–470 *in* B. FERNHOLM, K. BREMER, and H. JOERNFALL, eds. The hierarchy of life. Elsevier, Amsterdam.

YAMAOKA, K. 1991. Feeding relationships. Pp. 151–172 *in* H. M. A. KEENLEYSIDE, ed. Cichlid fishes—behaviour, ecology and evolution. Chapman & Hall, London.

YANAGISAWA, Y. 1987. Social organisation of a polygynous cichlid Lamprologus furcifer in Lake Tanganyika. Jpn. J. Ichthol. **34**:82–90.

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