

What, if Anything, is a Tilapia?—Mitochondrial ND2 Phylogeny of Tilapiines and the Evolution of Parental Care Systems in the African Cichlid Fishes

Vera Klett and Axel Meyer

Department of Biology, University of Konstanz, Germany

We estimated a novel phylogeny of tilapiine cichlid fish (an assemblage endemic to Africa and the Near East) within the African cichlid fishes on the basis of complete mitochondrial NADH dehydrogenase subunit 2 (ND2) gene sequences. The ND2 (1,047 bp) gene was sequenced in 39 tilapiine cichlids (38 species and 1 subspecies) and in an additional 14 nontilapiine cichlid species in order to evaluate the traditional morphologically based hypothesis of the respective monophyly of the tilapiine and haplochromine cichlid fish assemblages. The analyses included many additional cichlid lineages, not only the so-called tilapiines, but also lineages from Lake Tanganyika, east Africa, the Neotropics and an out-group from Madagascar with a wide range of parental care and mating systems. Our results suggest, in contrast to the historical morphology-based hypotheses from Regan (1920, 1922), Trewavas (1983), and Stiassny (1991), that the tilapiines do not form a monophyletic group because there is strong evidence that the genus *Tilapia* is not monophyletic but divided into at least five distinct groups. In contrast to this finding, an allozyme analysis of Pouyaud and Agnèse (1995), largely based on the same samples as used here, found a clustering of the *Tilapia* species into only two groups. This discrepancy is likely caused by the difference in resolution power of the two marker systems used. Our data suggest that only type species *Tilapia sparrmanii* Smith (1840) should retain the genus name *Tilapia*. One particular group of tilapiines (composed of genera *Sarotherodon*, *Oreochromis*, *Iranocichla*, and *Tristramella*) is more closely related to an evolutionarily highly successful lineage, the haplochromine cichlids that compose the adaptive radiations of cichlid species flocks of east Africa. It appears that the highly adaptable biology of tilapiines is the ancestral state for all African cichlids and that the more stenotypic lifestyle of the haplochromine cichlids is derived from this condition. We reconstructed the evolution of the highly variable parental care systems on the basis of the most inclusive composite phylogeny to date of the African, Neotropical, and Madagascan cichlids with special emphasis on a group of tilapiines comprising the substrate-spawning genus *Tilapia*, and the mouthbrooding genera *Sarotherodon* and *Oreochromis*. We demonstrate several independent origins of derived mouthbrooding behaviors in the family Cichlidae.

Introduction

Cichlid fishes have received wide attention from evolutionary biologists for more than 100 years because of their extremely diverse morphology, behavior, and ecology (e.g., reviews in Fryer and Iles 1972; Liem and Osse 1975; Greenwood 1978; Trewavas 1983; Oliver 1984; Meyer et al. 1990; Keenleyside 1991; Meyer 1993; Meyer, Montero, and Spreinat 1994; Stiassny and Meyer 1999). This perciform fish family is one of the most species-rich families of vertebrates with at least 1,300 and perhaps as many as 1,870 species (Kullander 1998). Members of this family are found chiefly in the fresh waters of South and Central America, in Africa (except for desert regions and the extreme south), in Madagascar, along the south Indian coast and Sri Lanka, in the Levant and in one northern tributary to the Persian Gulf (Nelson 1994).

The geographic centers of the astonishing diversity of cichlid fish are the east African rift lakes (reviewed in Fryer and Iles 1972; Meyer 1993; Stiassny and Meyer 1999). In Lakes Victoria, Malawi, and Tanganyika, the morphological diversification and speciation of some cichlid lineages occurred extremely rapidly, and many new species arose within only a few thousand genera-

tions, explosively forming adaptive radiations of several hundred species that are endemic to each of these three lakes (Sage et al. 1984; Meyer et al. 1990; Owen et al. 1990; Meyer, Montero, and Spreinat 1994; Johnson et al. 1996; Meyer, Montero, and Spreinat 1996). Recent molecular phylogenetic studies have established that these adaptive radiations are derived from only a single or a few founder lineages (Meyer et al. 1990; Meyer, Kocher, and Wilson 1991; Kocher et al. 1993; Meyer 1993; Albertson et al. 1999). Interestingly, the large east African cichlid species flocks are almost, without exception, composed of only a subgroup of cichlids, the haplochromine cichlids, those that belong to the so-called H-lineage of Nishida (1991). The Tanganyikan cichlid species flock is the most complex assemblage of cichlids with respect to morphology, ecology, and behavior in the world (e.g., Greenwood 1984; Brichard 1989). The cichlid fauna of Lake Tanganyika is believed to be of polyphyletic origin, with affinities to other African regions (Fryer and Iles 1972; Nishida 1991) and is considered as an evolutionary reservoir of ancient African cichlid lineages (Nishida 1991). It comprises about 200 cichlid fish species that are assigned to 12 tribes. Eight of these tribes, the Bathybatini, Cyprichromini, Ectodini, Eretmodini, Limnochromini, Perissodini, Trematocarini, and Tropheini, are endemic (Poll 1986; Nishida 1991; Sturmbauer and Meyer 1993). Some haplochromine cichlids of Lake Tanganyika (Tropheini, Haplochromini) are the sister group to the species flocks of Lakes Malawi and Victoria (Meyer et al. 1990; Nish-

Key words: NADH dehydrogenase subunit 2, cichlids, tilapiine, phylogeny, parental care, mating systems.

Address for correspondence and reprints: Axel Meyer, Department of Biology, University of Konstanz, 78457 Konstanz, Germany. E-mail: axel.meyer@uni-konstanz.de.

Mol. Biol. Evol. 19(6):865–883. 2002

© 2002 by the Society for Molecular Biology and Evolution. ISSN: 0737-4038

ida 1991; Sturmbauer and Meyer 1993). In contrast to the species flocks of Lake Malawi and Lake Victoria, which exclusively consist of mouthbrooding cichlids (Barlow 1991; Keenleyside 1991), the cichlid fauna of Lake Tanganyika comprises both mouthbrooding and substrate-brooding lineages.

The other major group of African cichlids, the tilapiine cichlids, have also repeatedly formed smaller species flocks of typically less than 10 species, for example, in some crater lakes in Cameroon, but they have not speciated to any noticeable degree like the cichlids of their putative sister group, the haplochromine cichlids (Trevawas 1972, reviewed in Stiassny, Schliewen, and Dominey 1992; Schliewen, Tautz, and Pääbo 1994).

In contrast to the highly specialized species of the famous haplochromine cichlid species flocks of Lakes Malawi and Victoria, the tilapiines have a more general body plan and are often river inhabitants, whereas most haplochromines live in lakes. Evolutionary biologists dealing with the relationships of cichlids have often assumed a basal position of the tilapiines within the African clade because of their generalized morphology and ecological diversity (e.g., Stiassny 1991). Because of this less specialized morphology, tilapiines are highly adaptable to diverse ecological habitats and are presumably less prone to extinction. This is perhaps one reason why these presumed generalist species have persisted to this day, although they are confined to the rivers and have been largely unable to colonize lakes that are occupied by the more specialized haplochromine cichlids.

Teleost fish show a wide array of different parental care behaviors where one or both parents defend and care for the eggs or the young after hatching. Gittleman (1981) suggested that in fishes the evolutionary transitions in the sex of the caregiver occurred in discrete pathways: from no care to male-only, to biparental, to female-only, to no care. Evolutionary changes requiring that both sexes change at once (e.g., from no care to care) are not expected to occur because two adaptive mutations are not likely to arise simultaneously (Lewontin 1974, reviewed in Gittleman 1981). Gittleman's hypothesis (1981) was supported by a cost-benefit analysis of Gross and Sargent (1985) of externally fertilizing fishes. In this study one benefit, the "increased survivorship of young," and three costs, a "mating cost," an "adult survivorship cost," and a "future fertility cost," were taken into account. Goodwin, Balshine-Earn, and Reynolds (1998) recently carried out a comparative method approach of the family Cichlidae. Cichlids offer a great opportunity for comparative studies of the evolution of behavioral patterns, such as mating systems and parental care patterns, because in this family there is more behavioral diversity than in any other family of fishes (Gross and Sargent 1985), and even closely related species exhibit a wide range of behavioral strategies (e.g., Brichtard 1989).

Previous research on cichlids has included all aspects of their biology, including behavior, ecology, and evolutionary biology (reviews in Keenleyside 1991). The recently increased knowledge of their phylogenetic relationships (e.g., Oliver 1984; Stiassny 1991; Kocher

et al. 1993; Meyer 1993; Zardoya et al. 1996; Kullander 1998; Streelman et al. 1998; Farias et al. 1999) permitted the study of the evolution of their diverse ecology and varied mating and parental care behaviors (e.g., Goodwin, Balshine-Earn, and Reynolds 1998).

Until recently, the investigation of phylogenetic relationships among cichlids was restricted to morphological characters (Stiassny 1991; Kullander 1998). However, although cichlids are a morphologically extremely diverse group, there are only few morphological characters which can be used in a cladistic approach to investigate the intrafamilial relationships (Stiassny 1991). One large and evolutionarily important group of cichlids that has been characterized solely by morphological features so far is the tilapiines (Seegers, Sonnenberg, and Yamamoto 1999). The members of this group are found throughout Africa, in the eastern Mediterranean region, in Israel, and in Iran (Trevawas 1983).

The significance of the tilapiines for human religion and consumption predates their modern scientific investigation, e.g., *Oreochromis niloticus*, a geographically widespread tilapiine cichlid, was already known to the early Egyptian cultures and played a significant role in their lives, as indicated by their frequent occurrence in ancient Egyptian art (Fryer and Iles 1972). The tilapiine cichlids continue to have great economic importance because some of them are an important source of protein in human diets, particularly in Africa, and increasingly, through aquaculture, worldwide (Pullin and Lowe-McConnell 1982; Trewavas 1982b). Widespread exploitation and aquaculture of tilapias has led to their introduction in all tropical regions around the globe, often adversely impacting the natural ichthyofauna.

Regan's (1920) important systematic subdivision of the family Cichlidae into two main groups—later known as the tilapiine and the haplochromine cichlids—was based on morphological differences of the neurocranial apophysis for articulation of the upper pharyngeal bones. According to Regan (1920), the *Tilapia*-type apophysis consists exclusively of the parasphenoid bone, and the *Haplochromis*-type apophysis includes also a portion of the basioccipital bone. One Neotropical genus, *Cichla*, was noted to have a *Haplochromis*-type apophysis, whereas the remaining American cichlids were observed to have a *Tilapia*-type apophysis (Regan 1920).

The phylogenetic reliability of this morphological criterion and the resulting subdivision of cichlid fishes have been repeatedly challenged during the last 80 years (Regan 1920, 1922; Greenwood 1978; Stiassny 1991; Kullander 1998) (table 1). Even the African cichlid monophyly and the Neotropical cichlid monophyly are questioned by some modern workers (Stiassny 1991; Kullander 1998), although the reciprocal monophyly of the hundreds of species of the Neotropical and African cichlids is supported by large-scale molecular phylogenetic studies (Zardoya et al. 1996; Streelman et al. 1998; Farias et al. 1999, 2000, 2001).

Greenwood (1978) analyzed the pharyngeal apophyseal morphology in greater detail and arrived at a classification of four types, all of which occur in the

Table 1
Tilapiine (“Tilapia”) Groups According to the Criteria of Regan (1920, 1922), Trewavas (1983), and Stiassny (1991)

Regan (1920, 1922)	Trewavas (1983)	Stiassny (1991)
<i>Asprotilapia</i>		
<i>Boulengerochromis</i>		
<i>Chilochromis</i>		
<i>Cunningtonia</i>		
<i>Cyathopharynx</i>		
<i>Cyphotilapia</i>		
<i>Gephyrochromis</i>	<i>Danakilia</i>	<i>Danakilia</i>
<i>Heterochromis</i> (<i>Paratilapia multidentis</i>) ^a		
	<i>Iranocichla</i>	<i>Iranocichla</i>
	<i>Konia</i>	<i>Konia</i>
<i>Limnochromis</i>		
<i>Limnotilapia</i>		
<i>Lobochilotes</i>		
<i>Neotilapia</i> (<i>Oreochromis tanganyicae</i>) ^a	<i>Myaka</i>	<i>Myaka</i>
<i>Ophthalmotilapia</i>	<i>Oreochromis</i>	<i>Oreochromis</i>
<i>Parachromis</i> (<i>Tristramella sacra</i>) ^a	<i>Tristramella</i>	<i>Tristramella</i>
<i>Pelmatochromis</i>	<i>Pelmatochromis</i>	
<i>Perissodus</i>		
<i>Petrochromis</i>		
<i>Simochromis</i>	<i>Pungu</i>	<i>Pungu</i>
	<i>Stomatepia</i>	<i>Stomatepia</i>
	<i>Steatocranus</i>	
	<i>Pterochromis</i>	
<i>Tilapia</i> (<i>Coptodon</i> , <i>Heterotilapia</i> , <i>Tilapia</i> , <i>Sarotherodon</i>) ^a	<i>Tilapia</i>	<i>Tilapia</i>
	<i>Sarotherodon</i>	<i>Sarotherodon</i>
<i>Tropheus</i>		
<i>Tylochromis</i> (<i>Pelmatochromis jentinki</i>) ^a		

^a The names of the type species used for classification in this group is shown in parentheses in the cases where the genus name and the first name of this species are not the same.

African cichlids. The simplest structure is called the *Tylochromis* type and corresponds to Regan’s description of the *Tilapia* type, whereas Greenwood redefined the *Tilapia* type to also include the prootic bones. Greenwood concluded that different apophyseal structures could occur within a single genus and recommended the rejection of the character for relationship studies. Most Neotropical taxa have the *Tylochromis* type of apophysis. However, Kullander (1998) found additional morphological types inside the Neotropical assemblage, including those that can be classified as *Tilapia* type and *Haplochromis* type, and suggested that the particular morphology of *Cichla* is not homologous with the *Haplochromis* type.

Wickler’s (1963) comparison of the behavioral and secondary sexual characteristics of the African members of the different African groups also disagreed with Regan’s original dichotomy. Although it is clear that a simple tilapiine-haplochromine dichotomy of the African cichlids is no longer acceptable (Stiassny 1991), there are no alternative morphological characters known to diagnose the postulated major evolutionary lineages of the African cichlids. Trewavas (1983) insisted on the *Tilapia*-type apophysis as a conclusive criterion, but Greenwood (1986) questioned the reliability of this particular character.

Stiassny (1991) diagnosed the tilapiines by two synapomorphies, namely, two prominent foramina at the back of the lower pharyngeal jaw and a median ridge on the dorsal aspect of the keel of the lower pharyngeal jaw. Stiassny’s (1991) morphological diagnosis resulted in an elimination of *Pelmatochromis*, *Steatocranus*, and *Pterochromis*, which were included in the tilapiines by Trewavas (1983) (table 1). Tilapiines, by Stiassny’s definition, are a more reduced grouping compared with the taxonomic entity characterized by *Tilapia*-type apophysis by Regan (1920) and corresponds well with the traditional view of the genus *Tilapia*. *Tilapia* was split by Trewavas into several genera, including *Tilapia*, *Oreochromis*, and *Sarotherodon* (Trewavas 1983 and references therein).

Tilapias exhibit a wide spectrum of ecological adaptations and tolerances (pH, temperature, salinity), but in spite of their astonishing ecological diversity and adaptability, which permitted some species of *Oreochromis* to colonize some highly saline environments in East Africa and even some coral reefs of the Hawaiian islands, their morphological diversity remained somewhat muted. This morphological relative “sameness,” compared with the legendary variation in haplochromine cichlids, complicates the estimation of their phylogenetic relationships (McAndrew and Majumdar 1984).

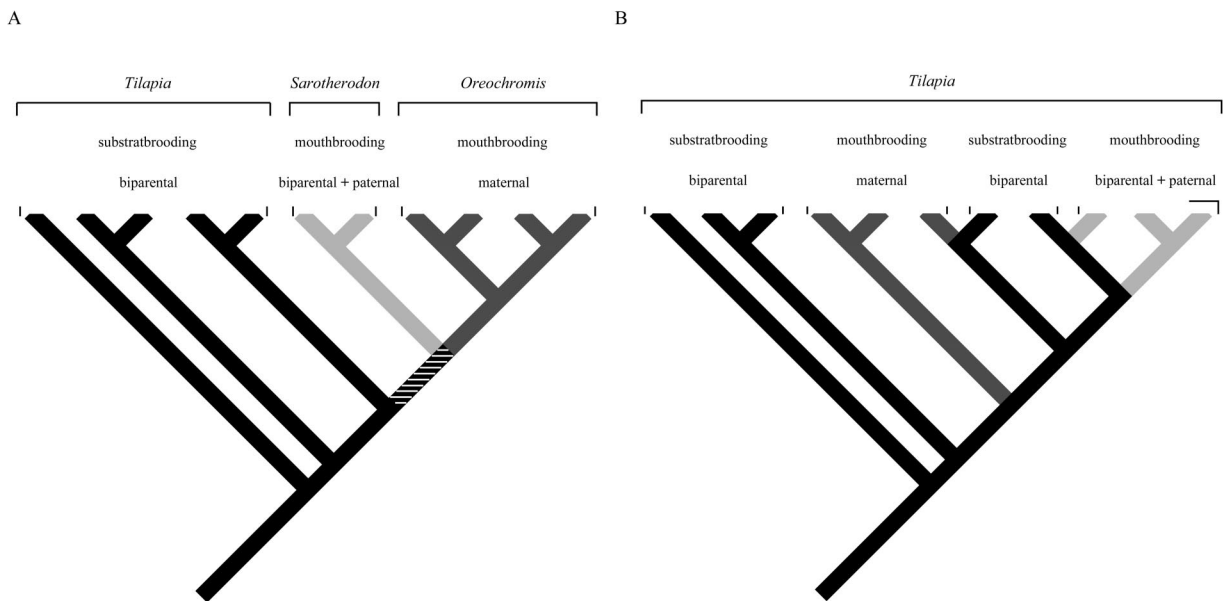


FIG. 1.—Schematic description of the evolution of parental care behavior in tilapiines. The evolution of mouthbrooding behavior according to (A) the single origin hypothesis of Trewavas (1980) and (B) the multiple speciation hypothesis of Peters and Berns (1978a, 1982). The shadings black, light, and dark gray symbolize biparental substrate-brooding, biparental or paternal mouthbrooding, and maternal mouthbrooding, respectively. The striped region is equivocal.

Tilapias are of special interest for ethologists and evolutionary biologists because they show a great diversity of parental care behavior. Some species are substrate-brooders, i.e., they take care of eggs and larvae in a crevice or nest, whereas others are mouthbrooders and incubate their eggs in their buccal cavities and call their young back into their mouths for protection. All mouthbrooding Tilapias are ovophile mouthbrooders, i.e., they take up their young into their buccal cavity already before hatching, except the “New Cross cichlid”—a heretofore undescribed species, which has been observed to be a larvophile mouthbrooding (J. Freyhof, personal communication).

In addition to the diversity of the form of parental care, the sex of the caregiver also varies in this group, which includes all possible modes of parental care that are found in the entire family Cichlidae: uniparental maternal-, uniparental paternal-, and biparental-brooding species. Among mouthbrooding cichlids, uniparental female care is most common (Fryer and Iles 1972). Substrate spawning by both parents is widely considered to be the ancestral state in cichlids (Lowe-McConnell 1959), and it is thought that mouthbrooding evolved several times independently in the family (Goodwin, Balshine-Earn, and Reynolds 1998).

Two models have been proposed for the evolution of mouthbrooding in Tilapias. Trewavas (1980) originally proposed that mouthbrooding in Tilapias evolved only once from a single substrate-brooding *Tilapia*-like ancestor. The mouthbrooding branch itself subsequently divided into two: a branch of maternal mouthbrooders and another of biparental and paternal mouthbrooders (fig. 1A). Later, Trewavas (1983) revised her hypothesis to suggest that there could have been an independent evolution of biparental and paternal mouthbrooding on

the one hand and maternal mouthbrooding on the other hand from close relatives. On the basis of parental care behavior and also morphologic, meristic, and biogeographic traits, Trewavas (1980, 1982a, 1982b, 1983) subdivided the Tilapias into three different genera or subgenera, respectively. She suggested that the substrate-spawning Tilapias should retain the name *Tilapia*, the biparental and paternal mouthbrooders should be grouped as *Sarotherodon*, and the maternal mouthbrooders as *Oreochromis*. Although *Sarotherodon caudomarginatus* is a maternal mouthbrooder (Stiassny 1996), this fact was unknown to Trewavas (1983), who classified this species into the genus *Sarotherodon* on the basis of its biogeographic distribution.

In contrast, according to the hypotheses of Peters and Berns (1978a, 1982), the evolution of mouthbrooding in Tilapias is not the result of a single or two events but rather the evolution of mouthbrooding behavior took place repeatedly (fig. 1B). They proposed that the phylogenetically older mouthbrooding species were maternal mouthbrooders and that the younger mouthbrooding species were biparental and paternal mouthbrooders (1982). The egg attachment system and larval headglands, which are important in substrate-spawning species, became superfluous after the evolution of mouthbrooding and were lost secondarily. The variable stages of regression of the egg attachment system and the larval headglands in various mouthbrooders were taken as evidence by Peters and Berns (1978a, 1978b, 1982) for their hypothesis. A subdivision of the species into different genera is, therefore, according to Peters and Berns (1982), not acceptable—all species should retain the genus name *Tilapia*. Previous work (McAndrew and Majumdar 1984) has failed to reject the hypotheses of Pe-

ters and Berns (1978a, 1982) as well as that of Trewavas (1980, 1982a, 1982b, 1983).

On the basis of the partial tilapiine phylogenies of Sodsuk (1993) and Schliewen, Tautz, and Pääbo (1994), a family wide reconstruction of the transitions in parental care behavior (Goodwin, Balshine-Earn, and Reynolds 1998) suggested that mouthbrooding evolved in this group only once, supporting Trewavas' hypothesis, but was then lost at least twice. Alternatively, it evolved within the tilapiine and Tanganyikan cichlid fishes at least three times independently.

We determined the DNA sequences of the mitochondrial NADH dehydrogenase subunit 2 (ND2) genes from a representative collection of all African cichlids to further examine behavioral (i.e., parental care) and morphological (apophysis and lower pharyngeal jaw) hypotheses concerning the evolution of the tilapiine cichlids. This gene was previously analyzed by Kocher et al. (1995) in an effort to elucidate taxonomic relationships among the east African cichlids of Lakes Malawi and Tanganyika. For reconstructing the history of parental care evolution in cichlids, we assembled the most complete composite phylogeny of any molecular and morphological studies.

Materials and Methods

We determined the complete DNA sequence of the ND2 gene in 39 presumed tilapiine cichlids (38 species and 1 subspecies) from nine genera (*Boulengerochromis* [1], *Iranocichla* [1], *Oreochromis* [13], *Pelvicachromis* [1], *Sarotherodon* [4], *Steatocranus* [2], *Stomatepia* [1], *Tilapia* [14], *Tristramella* [1]), in an additional 14 species of cichlids from Africa (representatives of all major lineages of the Lake Tanganyika cichlid species flock [11] and representatives of the main west African lineages [5]), and in one species from Madagascar. Additionally, we included previously published ND2 sequences of three Tanganyikan and one Neotropical cichlids in our analysis (Kocher et al. 1995) (table 2). The Madagascar genus *Oxylapia* was used as an out-group in the phylogenetic analysis because the position of Madagascar and Indian cichlids as an out-group for the African cichlids is firmly established (Zardoya et al. 1996; Farias et al. 1999, 2001). The genus *Amphilophus* was included as a representative of the monophyletic Neotropic cichlid clade (Farias, Orti, and Meyer 2000).

DNA was extracted from the muscle, liver, or fin clip tissue using standard protocols. The entire ND2 gene was PCR amplified using published primers in the flanking methionine ("ND2Met" 5'-CATACCCCAA-CATGTTGGT-3', internal primer number 2, Kocher et al. 1995) and tryptophan ("ND2Trp" 5'-GTSGSTTT-TCACTCCCGCTTA-3', internal primer number 7, Kocher et al. 1995) tRNA genes. In some cases, the gene was amplified in two steps, using ND2Met and the internal primer ND2B (5'-TGGTTTAATCCGCCTCA-3', internal primer number 10', Kocher et al. 1995) and a newly designed internal primer ND2.2A (5'-CTGACA-AAACTTGCCCCCTT-3') and ND2Trp.

Amplifications were carried out in 25- μ l reaction volumes in a Perkin-Elmer GeneAmp[®] PCR System 9700 (PE Applied Biosystems) using *Taq* DNA polymerase (0.125 μ l = 25 U/ml, Pharmacia). The PCR protocol was 92°C for 1 min, 47 to 55°C for 1 min, and 72°C for 2 min, for 35 cycles. The amplification product was purified with the QIAquick PCR Purification Kit (QIAGEN) and resuspended in 30 μ l ddH₂O. One to seven microliters (30–90 ng) was used as a template in a BigDye Terminator Ready Reaction Mix (Perkin-Elmer) and 1 μ l of primer (10 μ M) in 10- μ l reactions with the PCR protocol: 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, for 25 to 30 cycles. The cycle sequencing product was purified with an ethanol–ammonium acetate precipitation (3 M NaOAc, pH 4.6), the samples were dried and resuspended in 4 to 6 μ l of a 5:1 deionized formamide:blue dextran/EDTA (pH 8.0) solution, denatured at 90°C for 2 min, and loaded into 4% acrylamide gels. Gels were run on an ABI PRISM[®]377HT DNA Sequencer (PE Applied Biosystems).

Analyses of the ND2 Gene

To assess the degree of saturation of the transition and transversion mutations in each codon position, the number of substitutions (*Y*-axis) was plotted against Kimura-2-parameter distances (*X*-axis) of 1,680 pairwise comparisons of ND2 (fig. 2).

Phylogenetic Analyses

Maximum parsimony (MP), maximum likelihood (ML), and neighbor-joining (NJ) analyses were conducted using PAUP* 4.0b6 (Swofford 2001). Phylogenetic hypotheses with MP were reconstructed using the heuristic search option with simple stepwise addition of taxa and TBR branch swapping. Because of some degree of saturation (fig. 2), transitions at the third positions of fourfold degenerate amino acids were excluded from the MP analyses. Additionally, synonymous transitions of the first positions of the leucine codons were also excluded. Bootstrapping (Felsenstein 1985) was employed with 500 iterations. ML topologies were obtained by 25,000 puzzling steps, using the substitution model of Hasegawa, Kishino, and Yano (1985) with an empirically determined transition-transversion ratio (3.0), an estimated value for the gamma shape parameter (0.29), and a proportion of invariable sites (0.33) and with the empirical base frequencies determined by the program PUZZLE (Strimmer and Haeseler 1997). Topologies were generated from matrices of corrected genetic distances using the NJ algorithm (Saitou and Nei 1987). Genetic distances were corrected for multiple substitutions using Kimura's two-parameter method (1980) and the gamma parameter of 0.29.

Reconstruction of Parental Care Behavior in the Family Cichlidae

In order to elucidate the evolution of parental care behavior in the family Cichlidae, we assembled a composite phylogenetic tree based on diverse molecular and

Table 2
List of Species Examined in the ND2 Analysis with Information About the Source, Original Lab or Collection Number Individual, Locality, and GenBank Accession Number

Species	Source/Collector/ Original Number	Locality	Accession Number
<i>Amphilophus citrinellus</i>	Kocher et al. 1995	Nicaragua	U07246
<i>Astatotilapia burtoni</i>	Meyer	Lake Tanganyika	AF317266
<i>Bathybates</i> sp.	Kocher et al. 1995	Lake Tanganyika	U07239
<i>Benthochromis tricoti</i>	Meyer	Lake Tanganyika	AF317264
<i>Boulengerochromis microlepis</i>	Meyer	Lake Tanganyika	AF317229
<i>Chromidotilapia guentheri</i>	Meyer	—	AF317270
<i>Ctenochromis oligacanthus</i>	Meyer	Lake Tanganyika	AF416779
<i>Cyclopharynx fwaie</i>	Sturmbauer	Congo	AF317277
<i>Ectodus descampsii</i>	Meyer	Lake Tanganyika	AF317273
<i>Heterochromis multidens</i>	Meyer	Congo	AF317269
<i>Iranocichla hormuzensis</i>	Papenfuss	Iran	AF317278
<i>Lamprologus congoensis</i>	Meyer	Lake Tanganyika	AF317272
New cross cichlid.....	Schliwien/Stiassny	Congo River basin, Republic of Congo	AF416780
<i>Oreochromis amphilimas</i>	Seeger	—	AF317230
<i>Oreochromis andersonii</i>	Kullander/12761	Kafue River at Chanyanya	AF317231
<i>Oreochromis esculentus</i>	Balirwa/OE-L40	Lake Victoria basin	AF317232
<i>Oreochromis leucostictus</i>	Seehausen	Lab breed	AF317233
<i>Oreochromis macrochir</i>	Kullander/13358	Zaire drainage: Chambeshi River	AF317235
<i>Oreochromis mossambicus</i>	Spießer	Lab breed	AF317234
<i>Oreochromis mweruensis</i>	Kullander/13357	Zaire drainage: Lake Mweru-Wantipa	AF317236
<i>Oreochromis niloticus</i>	Balirwa/ON-V.I	Lake Victoria basin	AF317237
<i>Oreochromis niloticus vulcani</i>	Spießer	Lab breed	AF317242
<i>Oreochromis schwebischii</i>	Pouyaud/12	Congo River	AF317238
<i>Oreochromis tanganyica</i>	Kullander/12815	Zaire drainage: Lake Tanganyika	AF317240
<i>Oreochromis urolepis</i>	Seeger	—	AF317239
<i>Oreochromis variabilis</i>	Seehausen	Lake Victoria	AF317241
<i>Oxyapia polli</i>	Reinthal/Stiassny	Madagascar	AF317275
<i>Pelvicachromis pulcher</i>	Meyer	—	AF317271
<i>Perissodus polylepis</i>	Meyer	Lake Tanganyika	AF317265
<i>Sarotherodon caudomarginatus</i>	Stiassny	Sierra Leone	AF317243
<i>Sarotherodon galilaeus</i>	Spießer	Lab breed	AF317244
<i>Sarotherodon melanotheron</i>	Pouyaud/39	Kouilou Lagune Congo	AF317245
<i>Sarotherodon occidentalis</i>	Pouyaud/958	Guinea	AF317246
<i>Spathodus erythron</i>	Meyer	Lake Tanganyika	AF317267
<i>Steatocranus casuaris</i>	Sturmbauer	Lab breed	AF317247
<i>Steatocranus tinanti</i>	Seehausen	Lab breed	AF317248
<i>Stomatepia mariae</i>	Sturmbauer	—	AF317279
<i>Telmatochromis temporalis</i>	Kocher et al. 1995	Lake Tanganyika	U07266
<i>Thysochromis ansorgii</i>	Seehausen	Benin	AF317263
<i>Tilapia brevimanus</i>	Pouyaud/921	Kogon River, Guinea	AF317249
<i>Tilapia buettikoferi</i>	Pouyaud/927	Freetown, Sierra Leone	AF317251
<i>Tilapia busumana</i>	Pouyaud/925	Bia River, Ivory Coast	AF317250
<i>Tilapia cabrae</i>	Pouyaud/20	Kouilou River, Congo	AF317252
<i>Tilapia cessiana</i>	Pouyaud/934	Cavally River, Ivory Coast	AF317253
<i>Tilapia coffea</i>	Pouyaud/1028	St. Paul River, Ghana	AF317254
<i>Tilapia discolor</i>	Pouyaud/153	Pra River, Ghana	AF317255
<i>Tilapia guineensis</i>	Pouyaud/23	Kouilou River, Congo	AF317256
<i>Tilapia louka</i>	Pouyaud/942	Bourouma River, Guinea	AF317257
<i>Tilapia mariae</i>	Spießer	Lab breed	AF317258
<i>Tilapia rendalli</i>	Kullander/12805	Zambezi drainage: Kafue River	AF317259
<i>Tilapia sparrmanii</i>	Kullander/12807	Zambezi drainage: Kafue River	AF317260
<i>Tilapia walteri</i>	Pouyaud/949	Cess River, Liberia	AF317261
<i>Tilapia zillii</i>	Pouyaud/83	Lake Manzalla, Nile, Egypt	AF317262
<i>Trematocara unimaculatum</i>	Meyer	Lake Tanganyika	AF317268
<i>Tristramella simonis</i>	Balshine-Earn	Israel	AF317276
<i>Tropheus moorii</i>	Kocher et al. 1995	Lake Tanganyika	U07267
<i>Tylochromis leonensis</i>	Meyer	Lake Tanganyika	AF317274

morphological data. According to morphological (Stiassny 1991) and molecular (Zardoya et al. 1996; Farias et al. 1999; Farias, Orti, and Meyer 2000) studies, the Madagascan and Indian cichlids have been positioned basal to the monophyletic African and Neotropical cichlids. Phylogenetic relationships among Neotropical cichlids were recently determined by Farias et al.

(1999) on the basis of the 16S rRNA sequences and in a morphological study by Kullander (1998).

The west African genera were positioned according to our own results and studies by Greenwood (1985), Stiassny (1991), and van der Bank (1994). According to Meyer et al. (1990) the Tanganyikan cichlids were positioned in an ancestral position to the monophyletic

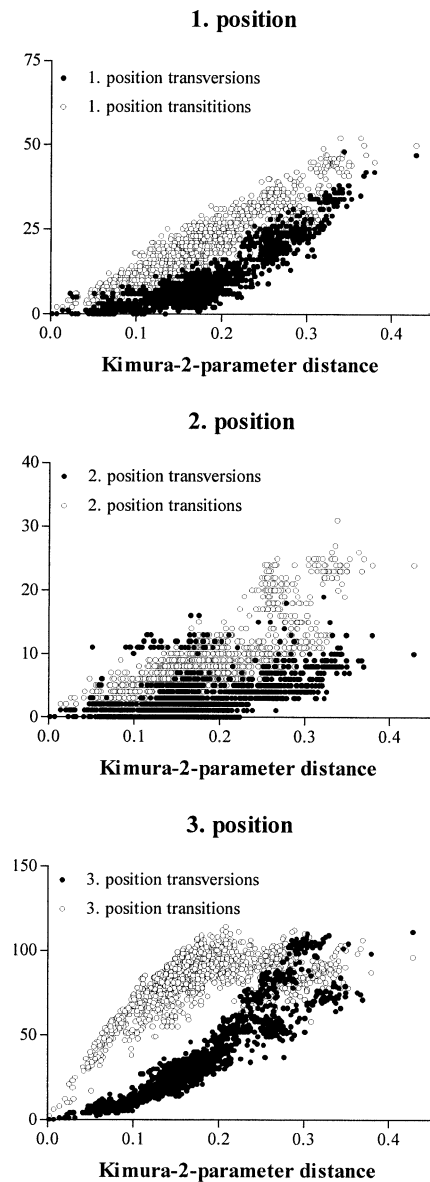


FIG. 2.—The number of transitions (open circles) and transversions (filled circles) (Y-axis) plotted against Kimura-2-parameter distances (X-axis) of 1,680 pairwise comparisons of the ND2 gene sequences for all three codon positions.

species flocks of Lake Victoria and Lake Malawi. Phylogenies of the monophyletic tribes Ectodini and Lamprologini have been adopted from Sturmbauer and Meyer (1993) and Sturmbauer, Verheyen, and Meyer (1994), respectively.

The tilapiine genera *Myaka*, *Pungu*, and *Konia* have been inserted at positions suggested by previous studies of Sodsuk (1993) and Schliewen, Tautz, and Pääbo (1994). Representatives of the genera *Iranocichla*, *Oreochromis*, *Tilapia*, *Sarotherodon* (except the crater lake species of Barombi-Mbo), *Stomatepia*, and *Tristramella* were placed according to the results from our MP analysis.

The evolution of parental care behavior (form of care and sex of caregiver) in the family Cichlidae was examined by mapping those states onto this composite

phylogeny using MacClade Version 3.06 (Maddison and Maddison 1992).

Reproductive Data

In the reconstruction of parental care behavior we considered the form of care (substrate-brooding and mouthbrooding) and the sex of the caregiver (biparental, female-only, and male-only). Delayed and immediate mouthbroodings have not been distinguished. As “biparental,” we defined those species where both the male and the female perform behavior which is likely to increase the chances of survival of the offspring (see also Keenleyside 1991). The assigned reproductive data are based on the results from Trewavas (1983), Koslowski (1985), Loiselle (1985), Staeck and Linke (1985), Linke and Staeck (1987), Richter (1989), Keenleyside (1991), Stiassny (1996), Kawanabe, Hori, and Nagoshi (1997), Kuwamura (1997), Stawikowski and Werner (1998), J. Freyhof (personal communication), and M. Stiassny (personal communication).

Analysis of Character Evolution

The composite phylogeny was assembled in MacClade 3.06 (Maddison and Maddison 1992). Multiple nodes were treated as unresolved “soft polytomies” rather than as multiple speciation events. Unordered character states were used, allowing any character state (form of care or sex of caregiver) to transform into any other using the Fitch parsimony option (Maddison and Maddison 1992). Where the reproductive behavior is unknown (shown by a lack of a box next to the taxon name), the program assigns the most parsimonious reproductive character to the branch. The numbers of transitions in character states was determined by hand because the trees included some equivocal branches. Typically, we report the transitions in a range—the minimum number shows the transitions that have been verified unambiguously in the trees, the maximum number shows all the possible changes.

Results

The ND2 gene reveals some degree of saturation of transition mutations in the third codon position at larger genetic distances (fig. 2).

All tree-generating methods result in similar topologies with respect to the relationships of tilapiine cichlids in the trees (figs. 3–5). The phylogram generated by the NJ analysis (fig. 5) reveals some long branches for the West African species *Chromidotilapia guentheri* and *Pelvicachromis pulcher* and also for the Tanganyikan species *Trematocara unimaculatum*, which might potentially result in a “pulling” of these species to more basal positions. Despite the long branches, their phylogenetic positions seem to be reasonable with respect to the biogeographic data.

MP analyses, with the exclusion of transitions in fourfold degenerate third codon positions and transitions in first positions of leucine codons, yielded a most parsimonious tree with a tree length of 2,028 steps and a

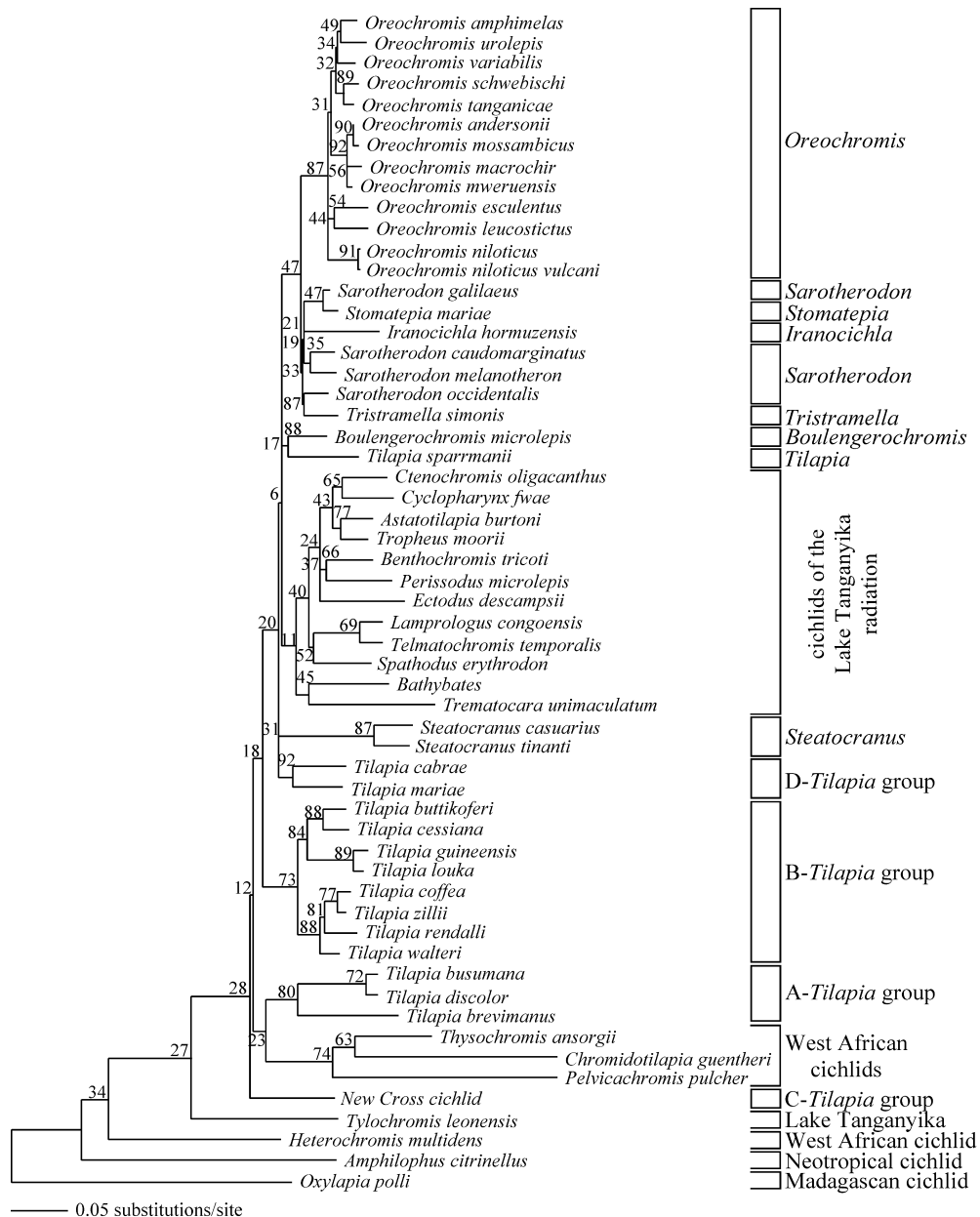


FIG. 3.—ML tree obtained by 25,000 puzzling steps, using the substitution model of Hasegawa, Kishino, and Yano (1985) with consideration of a transition-transversion ratio of 3.0, a gamma shape parameter of 0.29, a proportion of invariable sites of 0.33, and the empirical base frequencies.

consistency index (CI) of 0.2763 excluding the uninformative sites (Kluge and Farris 1969) (fig. 4).

In all the trees the genus *Tilapia* is split into five groups (figs. 3–5). The species composition of these five groups is identical in all analyses. The monophyly of the three west African *Tilapia* species, *T. brevimanus*, *T. discolor*, and *T. busumana*, hereafter designated as the “A-*Tilapia*-group,” is well supported (ML 80, MP 94, NJ 54). A more species-rich group of the *Tilapia* species that live in west Africa and a few species in east Africa, including *T. buettikoferi*, *T. cessiana*, *T. coffea*, *T. guineensis*, *T. louka*, *T. rendalli*, *T. walteri*, and *T. zillii*, is also clearly monophyletic (ML 73, MP 95, NJ 95), hereafter designated with “B-*Tilapia*-group” (figs.

3–5). The “New Cross cichlid” (C-*Tilapia*-group) from the middle Congo river (Lundberg et al. 2000) is always found solely in quite basal positions in all topologies, although in somewhat different positions—we designate this species to form the “C-*Tilapia*-group.” The remaining three *Tilapia* species, assigned into two groups, are placed in more derived groups. The two species *T. cabrae* and *T. mariae* are found to be sister species in all trees (D-*Tilapia*-group, ML 92, MP 59, NJ 66). Finally, *T. sparrmanii* is found to group with *Boulengerochromis microlepis* in all trees (ML 88, MP 23, NJ 34). The order of appearance among the first three (A-, B-, and C-*Tilapia*-groups) and between the last two *Tilapia* groups (D-*Tilapia*-group and *T. sparrmanii*) varies in

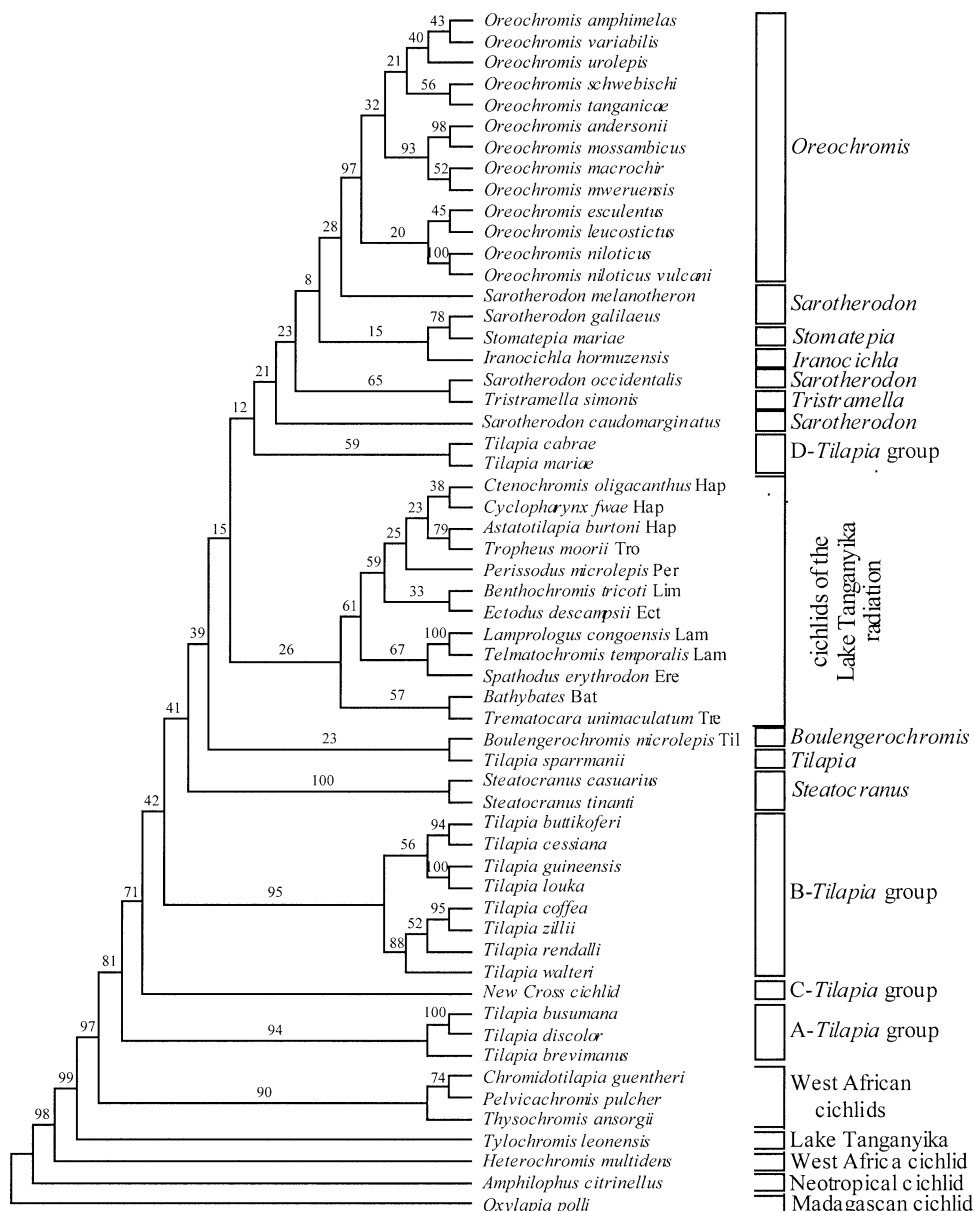


FIG. 4.—The phylogenetic tree obtained by the exclusion of transitions at the third positions of fourfold degenerate amino acids and synonymous transitions at first positions of leucine codons with MP of ND2 data set using PAUP* 4.0b6 by Swofford (2001). Numbers on the branches are bootstrap values (500 replicates). The present topology shows a tree length of 2,028 steps and a CI excluding uninformative sites of 0.2763 (Kluge and Farris 1969). The tree is rooted declaring the Madagascan species *Oxyapia polli* as outgroup.

the three tree-generating methods. In the ML analyses the D-Tilapia-group is found to cluster generally with the genus *Steatocranus* (fig. 3), in MP it is found to be basal to the mouthbrooding tilapiine genera *Iranocichla*, *Oreochromis*, *Sarotherodon*, *Stomatepia*, and *Tristramella* (fig. 4). In the NJ analyses the D-Tilapia-group is placed basal to the named mouthbrooding tilapiine genera and the Lake Tanganyika cichlids (except *Tylochromis*) (fig. 5). Also, the positions of *T. sparrmanii* and *Boulengerochromis microlepis* are found to be somewhat unstable. In ML they are found to be basal to the mouthbrooding tilapiine species, in MP they are basal to the mouthbrooding tilapiines and the species of Lake Tanganyika, and in NJ they occupy a position basal to the Tanganyikan cichlids (figs. 3–5). All alternative po-

sitions for the D-clade are statistically rather weakly supported. In this study all represented members of the genus *Oreochromis*, which includes *O. tanganyikae*, an endemic species from Lake Tanganyika, form a strongly supported monophyletic group (ML 87, MP 97, NJ 100). However, the so-called Soda tilapias, which inhabit highly alkaline and hot small lakes in east Africa, were not represented in this study. Originally, they were considered to represent a subgenus within the genus *Oreochromis* but have recently been assigned to a distinct genus *Alcolapia* (Seegers, Sonnenberg, and Yamamoto 1999). In contrast to *Oreochromis*, the genus *Sarotherodon* might be paraphyletic because the remaining mouthbrooding tilapiine genera *Iranocichla*, *Stomatepia*, and *Tristramella* are grouped within this genus. The re-

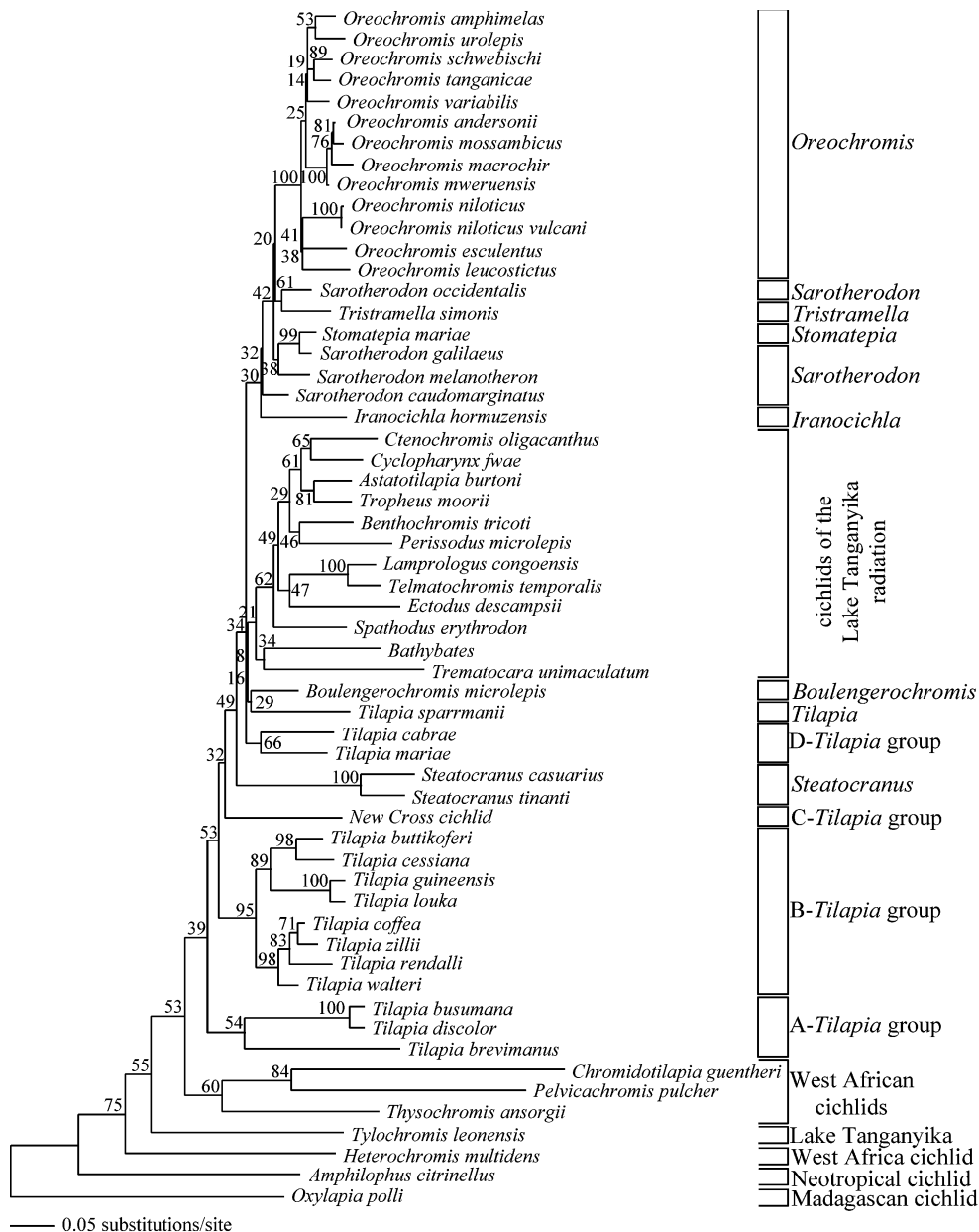


FIG. 5.—Phylogram generated from matrices of genetic distances corrected for multiple substitutions by Kimura's two-parameter method (1980) using the NJ algorithm (Saitou and Nei 1987) under consideration of a gamma parameter of 0.29. Numbers on the branches are bootstrap values (500 replicates).

relationships within the genus *Sarotherodon* and their relationship to the genera *Iranocichla*, *Tristramella*, and *Stomatepia* are not firmly established on the basis of our molecular data. Despite some differences in topology, all obtained phylogenies indicate a clear subdivision of the species that are currently assigned to the genus *Tilapia* into at least five distinct groups.

The ND2 data included in this study do not permit to unambiguously resolve the relationships among the tilapiine cichlids of the *Sarotherodon* group (i.e., genera *Sarotherodon*, *Iranocichla*, *Tristramella*, and *Stomatepia*), *Oreochromis*, *Boulengerochromis*, *Tilapia sparrmanii*, and the D-*Tilapia*-group and among the Lake Tanganyika tribes (*Tropheini*, *Limnochromini*, *Perisso-*

dini, *Ectodini*, *Bathybatini*, *Trematocarini*, and *Eretmodini*) and further representatives of the Lake Tanganyika radiation (*Lamprologus congoensis* [Lamprologini], *Astatotilapia burtoni* [Haplochromini], *Cyclopharynx fwaë* [Haplochromini]). The Lake Tanganyika radiation appears to be monophyletic with respect to the tilapiine lineages, except for *Boulengerochromis*, whose position at the base of the Lake Tanganyika endemic cichlids remains somewhat uncertain. Because there is a good resolution of both the basal and the very recent species relationships, the poor resolution in the trees after the branching-off of *Steatocranus* is likely to reflect a true biological phenomenon—the rapid origin of many cichlid lineages in east Africa within a short time span dur-

ing that part of the evolutionary history of cichlid fishes (figs. 3–5).

The Evolution of Parental Care in Cichlids

On the basis of the imposed composite phylogeny, the most parsimonious reconstruction of reproductive characters evolution reveals 13 evolutionary steps and indicates that mouthbrooding evolved from substrate-brooding 9 to 18 times with seven transitions in the Neotropical cichlids and 2 to 11 transitions to mouthbrooding in the African cichlids (fig. 6). Transitions in the reverse direction have been inferred in 1—the evolution of the substrate-brooding Lamprologini from the mouthbrooding ancestors of the Lake Tanganyikan species flock (Sturmbauer and Meyer 1993; Stiassny and Meyer 1999)—to 2 cases in the African clade.

The most parsimonious reconstruction of the evolution of biparental and uniparental care reveals a minimum of 25 evolutionary transitions in the cichlid family (fig. 7). The phylogeny indicates that biparental care is the ancestral state in cichlids, and uniparental care (especially female-only care) is a more derived behavior that evolved several times independently. In the Neotropical cichlids we found five to six transitions to female-only care, and in the African clade female-only care evolved 11 to 17 times. Male-only care evolved only twice in the cichlids. The tree indicates that evolution took place in the reverse direction—from uniparental to biparental care—4 to 12 times in the African cichlids and 0 to 2 times in the Neotropical cichlids (fig. 7).

Discussion

In order to determine if one of the previously proposed morphological criteria is suitable to describe a monophyletic tilapiine group, we undertook the most inclusive molecular phylogenetic study of the tilapiines to date. Our study includes a total of 39 putative tilapiine species (table 2). Apart from the tilapiines, we included several major west African cichlid lineages, which have been identified previously to be basal and paraphyletic to the east African lineages of the great lakes (Stiassny 1991; Zardoya et al. 1996; Farias et al. 1999). Additionally, many representatives of the Tanganyikan radiation, which do not strictly inhabit Lake Tanganyika but can be assigned to this lake phylogenetically (e.g., *A. burtoni* and *C. fuae* as members of the Haplochromine tribe; *L. congoensis* as a representative of the Lamprologines), were included in this study. Not all cichlid species that live in Lake Tanganyika can be traced back to a single ancestral lineage (e.g., *Boulengerochromis*); clearly, a few representatives of some lineages were able to leave the confines of this lake (e.g., *L. congoensis*, *A. burtoni*) or entered the lake late in its history; the clearest example for this is the endemic Lake Tanganyika tilapiine species *O. tanganicae*. Therefore, the Lake Tanganyika cichlid species flock is not strictly monophyletic, although the vast majority of species can be traced back to a single ancestral lineage that is probably related

to the tilapiine west African river cichlids such as *Steatocranus*.

Tilapia is Not a Monophyletic Group

Our study is the first to show that the tilapiine assemblage of the African cichlids (i.e., *Boulengerochromis*, *Iranocichla*, *Oreochromis*, *Pelvicachromis*, *Sarotherodon*, *Steatocranus*, *Stomatepia*, *Tilapia*, and *Tristramella*) is clearly not monophyletic. Likewise, the genus *Tilapia*, as currently defined, is also polyphyletic (figs. 3–5). We rather find evidence that some tilapiine species (e.g., *T. cabrae*, *T. mariae*, *T. sparrmanii*, genera *Oreochromis*, *Sarotherodon*, *Stomatepia*, *Tristramella*, and *Iranocichla*) are more closely related to genera and tribes that are traditionally considered to be nontilapiines (e.g., Tropheini, Lamprologini, and Perissodini) than to representatives of their own assemblage (representatives of the A- and B-*Tilapia*-groups), as previously defined by both Trewavas (1983) and Stiassny (1991) (table 1). That the genus *Tilapia* is probably not a phylogenetically cohesive group was already suggested by Stiassny, Schlieuwen, and Dominey (1992). In our study this genus is divided into at least five different groups, with three of them in basal positions in the phylogeny of the African cichlids (figs. 3–5). The presumably most basal *Tilapia* group (the A group), on the basis of the MP and NJ analyses, comprises the species *T. brevimanus*, *T. busumana*, and *T. discolor*, which are distributed in Ghana and Ivory Coast. The ND2 sequences of these species also differ diagnostically from all other *Tilapia* species by having a TAG instead of a TAA as the stop codon. Because of its V-shaped lower pharyngeal jaw, *T. busumana* has been thought to be a basal tilapiine (Greenwood 1987), and this presumed ancestral position of *T. busumana* as one of the most basal African lineages is confirmed in this molecular analysis.

The B-*Tilapia*-group includes *T. coffea*, *T. guineensis*, *T. louka*, *T. rendalli*, *T. walteri*, *T. zillii*, *T. buetikoferi*, and *T. cessiona*. It is interesting to note that the latter two species appear as sister species in our tree and are representatives of the subgenus *Heterotilapia* (Thys van den Audenaerde 1970). The other species of the B-*Tilapia*-group have been assigned to the subgenus *Coptodon* (Thys van den Audenaerde 1970), which on the basis of our data would appear to be paraphyletic.

The C-*Tilapia*-group is made up of a single, possibly biparental, mouthbrooding species New Cross cichlid from the Congo River. This species will be formally described shortly by U. Schlieuwen and M. Stiassny (personal communication).

Because the sister group relationship of *T. mariae* and *T. cabrae* is well supported in the ML, MP, as well as NJ phylogenetic analyses (figs. 3–5), we designated them as the D-*Tilapia*-group. Interestingly, the type species of the genus *Tilapia* *T. sparrmanii* Smith (1840) (Jordan 1963) is clustered in all tree topologies, albeit with a low bootstrap support, with *B. microlepis*. Because there is no well-supported monophyletic grouping with the other species, we suggest that *T. sparrmanii* is

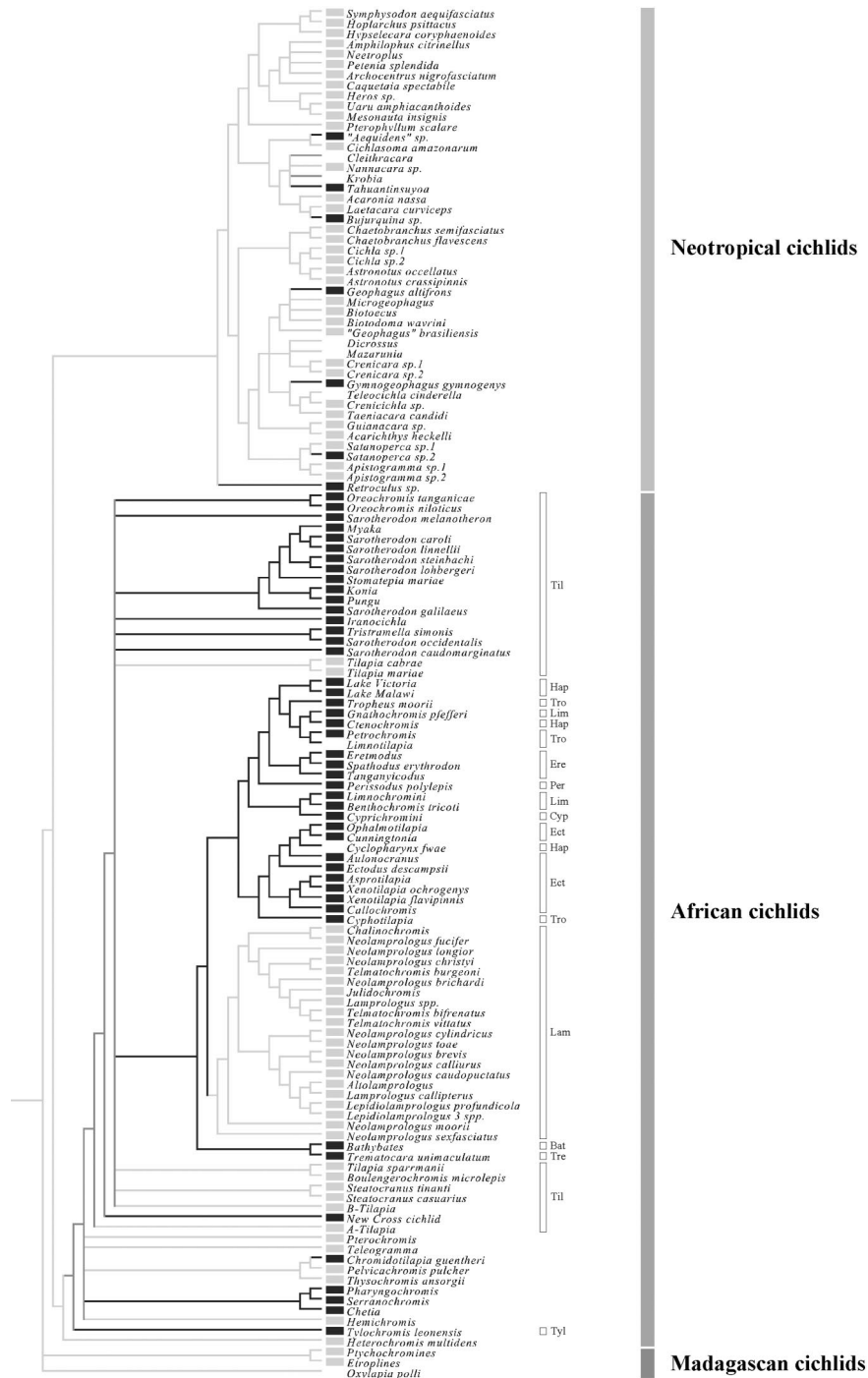


FIG. 6.—The phylogenetic reconstruction of the evolution of the form of parental care (substrate spawning: gray, mouthbrooding: black) in the family of cichlids. The composite tree has been assembled on the basis of several diverse morphological and molecular phylogenies. If the reproductive behavior is unknown this is indicated by a lack of a box next to the taxa name. Bat: Bathybatini; Cyp: Cyprichromini; Ect: Ectodini; Ere: Eretmodini; Hap: Haplochromini; Lam: Lamprologini; Lim: Limnochromini; Per: Perissodini; Til: Tilapiaiini; Tre: Trematocarini; Tro: Tropheini; Tyl: Tylochromini.

the only representative of the genus *Tilapia* in the present study. Thus, on the basis of these findings, only the type species is labeled with the genus name *Tilapia* in the boxes in our figures and might well be the only species that should remain taxonomically assigned to this genus. It is noteworthy that *T. sparrmanii* and *T.*

cabrae exhibit a much more southern distribution in Africa than any other *Tilapia* species, which are predominantly distributed throughout west Africa. Although *T. mariae* is found chiefly in west Africa, the southern range of its habitat (southern Cameroon) is contiguous with that of *T. cabrae*.

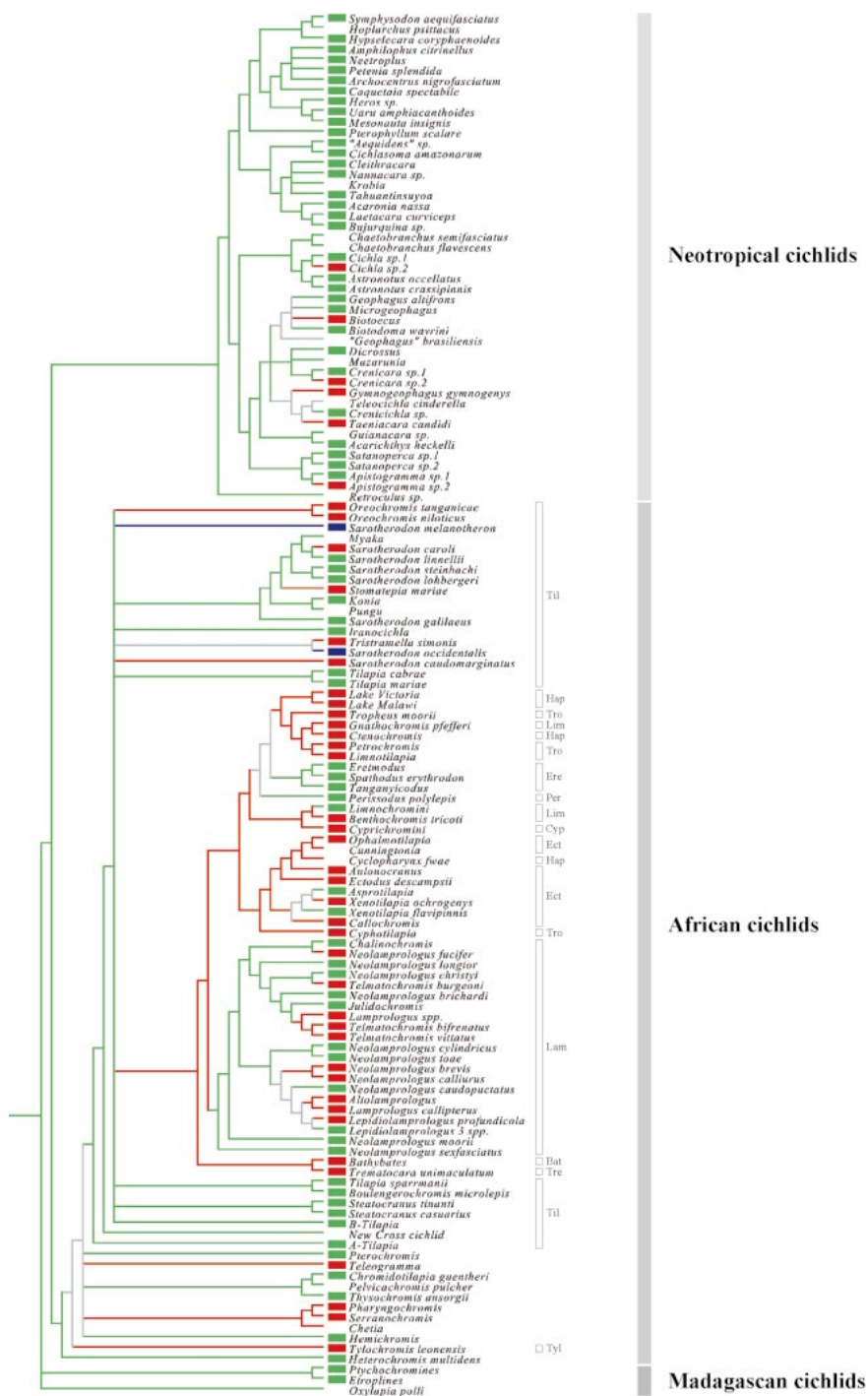


FIG. 7.—The phylogenetic reconstruction of the evolution of the sex of caregiver in the family Cichlidae (maternal: red; paternal: blue; biparental: green; equivocal: gray). Bat: Bathybatini; Cyp: Cyprichromini; Ect: Ectodini; Ere: Eretmodini; Hap: Haplochromini; Lam: Lamprologini; Lim: Limochromini; Per: Pterichromini; Til: Tilapiini; Tre: Trematocarini; Tro: Tropheini; Tyl: Tylochromini.

Although one of the two major groups identified in a previous allozyme study (Pouyaud and Agnès 1995) is composed of the same species as our B-Tilapia-group, the other group of this previous study is composed of species from three Tilapia groups as defined by us (A-, B-, and D-Tilapia-groups). This difference appears to be a consequence of the difference in the resolution power of the two marker systems (allozymes vs. mitochondrial

DNA sequences) because many samples from these two studies are identical.

Tilapia sparrmanii seem to be more closely related to the mouthbrooding species of the colloquially named Tilapias (Oreochromis and the Sarotherodon group comprising the genera Sarotherodon, Stomatopoma, Tristramella, and Iranocichla) and to several diverse non-tilapiine genera of Lake Tanganyika than to other rep-

representatives of its own genus that are found in the distantly related A-, B-, and C-*Tilapia*-groups (figs. 3–5).

Despite the saturation of the transition mutations in the third codon positions (fig. 2) which is mainly caused by synonymous substitutions in the fourfold degenerate codon positions, the relationships among the basal groups are well resolved. Hence, the sometimes short branches and, therefore, the often weak bootstrap support for relationships among members of the D-*Tilapia*-group, *T. sparrmanii*, the genera *Sarotherodon*, *Oreochromis*, and *Boulengerochromis*, and the Tanganyikan cichlids may reflect a rapid origin of these lineages early on in the history of diversification of the Lake Tanganyika cichlid species flock (Sturmbauer 1998). This period of rapid speciation would hence explain the limited resolving power of the phylogenetic relationships with any genetic marker.

What, if Anything, is a *Tilapia*—Systematics and Nomenclature of Tilapiine Cichlids

According to the nomenclature conventions, only species which form a natural (monophyletic) group with an explicitly designated type species share the genus name with this species. Because there is no proof of a monophyletic relationship of the type species of the genus *Tilapia*, *T. sparrmanii* Smith (1840) (Jordan 1963), with any other so-called *Tilapia* species, we will hereafter refer only to this species using the genus name *Tilapia* as the only “true *Tilapia* species.” We suggest that the representatives of the other four *Tilapia* groups (A-, B-, C-, and D-*Tilapia*-groups) should be renamed and be given new genera names that connote their strongly supported monophyly in the tree of the African cichlids. The continued use of the genus name *Tilapia* for all five diverse, and only relatively distantly related, groups of the tilapia-like African cichlids would falsely imply close or even monophyletic relationships that are clearly rejected by this study. However, because there are no rules for informal names, a continued use of the term “tilapiine” for tilapia-like fishes seems likely.

Our finding that the only true *Tilapia* species *T. sparrmanii* of our study is more closely related to some nontilapiines—the cichlids of Lake Tanganyika—than to other presumed *Tilapia* species (e.g., A- and B-*Tilapia*-group) affects the phylogenetic integrity of the traditional morphology-based tilapiine phylogenetic hypotheses presented by Regan (1920, 1922), Trewavas (1983), and Stiassny (1991). Morphology-based criteria proved to be insufficient to describe a monophyletic tilapiine group. The apophysis criterion used by Regan (1920, 1922) and Trewavas (1983) and perhaps also the two features of the lower pharyngeal jaw applied by Stiassny (1991) seem to be plesiomorphic characteristics of ancient cichlid fishes, which have been retained in different groups (e.g., D-*Tilapia*-group, *Sarotherodon*, *Oreochromis*, and *Tristramella*) and lost repeatedly in others (e.g., Haplochromini, Lamprologini, and Eretmodini). Hence, these morphological traits are homoplasious and, therefore, probably of limited phylogenetic value.

Boulengerochromis

The monotypic genus *Boulengerochromis*, which is the largest of all cichlid species, and endemic to Lake Tanganyika, has been classified by Greenwood (1978) as a tilapiine fish because of the morphology of its apophysis. This phylogenetic placement is quite remarkable because this genus has a clearly distinct ecology from all the other tilapiines; it is the largest species of all cichlids, reaching almost 90 cm in length. Whereas the tilapiines are typically slow swimming, primarily herbivorous fish, *Boulengerochromis* is a fast swimming piscivore. The designation of *Boulengerochromis* as tilapiine has been challenged repeatedly before because no species with intermediate habits and morphology are known (Fryer and Iles 1972). Although lepidological investigations (Lippitsch 1998), allozyme analyses (Nishida 1991, 1997), and ND2 sequence data (Kocher et al. 1995) all contradict the classification of Greenwood (1978) and Poll (1986), these studies were based only on a limited subset of tilapiines. In the present study *B. microlepis* typically groups closely with *T. sparrmanii* (figs. 3–5). Additionally, this species is found to group with the mouthbrooding tilapiines of the genus *Oreochromis* and the *Sarotherodon* group in ML analyses, albeit with only a low bootstrap support (fig. 3). This placement of *Boulengerochromis* would tend to support Greenwood’s (1978) and Poll’s (1986) classification of this species as a true tilapiine cichlid. In contrast, in NJ and MP, *Boulengerochromis* is found either to group within the Tanganyikan cichlids (NJ, fig. 5) or group basal to the mouthbrooding tilapiines of the genus *Oreochromis* and the *Sarotherodon* group (MP, fig. 4), respectively. Hence, we are currently unable to ascertain the phylogenetic position of this enigmatic fish.

Steatocranus, *Stomatepia*, *Tristramella*, and *Iranocichla*

The phylogenetic relationship of the riverine Congo genus *Steatocranus* to the tilapiines is controversial. Although Trewavas (1983) proposed that this genus is, according to its apophysial structure, a tilapiine fish, this classification is contradicted by Stiassny’s (1991) pharyngeal jaw criterion. In the MP and NJ analyses, *Steatocranus* branches off from the remaining African cichlids after the B- (MP, fig. 4) or C- *Tilapia*-group (NJ, fig. 5), respectively, and appears to be a sister taxon to an assemblage of *T. sparrmanii*, the D-*Tilapia*-group, *Oreochromis*, the *Sarotherodon* group, and the Tanganyikan lineages (except *Tylochromis*). Because the bootstrap support for all three topologies is rather weak, the relationship of this genus to the tilapiine cichlids of the genera *Iranocichla*, *Oreochromis*, *Sarotherodon*, *Stomatepia*, and *Tristramella* remains uncertain.

Because the genera *Iranocichla*, *Stomatepia*, and *Tristramella* are found to group within the genus *Sarotherodon*, we used the informal name *Sarotherodon* group for this assemblage (figs. 3–5). A close phylogenetic relationship between *Sarotherodon* and *Tristramella* has already been suggested in several previous studies (Trewavas 1942; Steinitz and Ben-Tuvia 1960,

reviewed in Peters and Berns 1982). Because of the well-supported close relationship of *S. galilaeus* with *S. mariae*, and *S. occidentalis* with *Tristramella simonis*, we conclude that the genus *Sarotherodon* is not monophyletic. Whether the genera *Tristramella*, *Stomatepia*, and *Iranocichla* should be assigned to this genus will require additional study. The informal usage of the name *Sarotherodon* group should, therefore, at this stage not imply monophyly.

Evolution of Parental Care Behavior in the Family Cichlidae

We reconstructed the evolution of parental care behavior by mapping these traits on a composite phylogeny of the family Cichlidae. Our composite tree comprises recent phylogenies of the Neotropical cichlids (Kullander 1998; Farias et al. 1999, 2001; Farias, Orti, and Meyer 2000) and, on the basis of our new findings of the ND2 data set, the relationships of the so-called tilapiines and their position in the African clade (figs. 6 and 7).

Substrate spawning by both parents is widely considered to be the ancestral state in cichlids (Lowe-McConnell 1959). A family wide reconstruction of the evolutionary changes in parental care behavior of cichlid fishes, carried out by Goodwin, Balshine-Earn, and Reynolds (1998), suggests that mouthbrooding has evolved from substrate guarding 10 to 14 times, zero to three reversals are inferred to have occurred, and transitions to mouthbrooding happened four to seven times in the African cichlid fishes and six to seven times in the Neotropical cichlid fishes. On the basis of our analysis, there is evidence that mouthbrooding evolved 2 to 11 times in the African clade and seven times in the Neotropical clade and that evolution in the reverse direction occurred in 1 to 10 occasions (fig. 6).

In agreement with the analysis of Goodwin, Balshine-Earn, and Reynolds (1998), we found that the sex of the caregiver has been more evolutionarily labile than the form of parental care. Minimally 16 (11 in African + 5 in Neotropical) and maximally 23 (17 in African + 6 in Neotropical) to female-only care transitions can be inferred from our reconstruction (fig. 7). We reconstructed only two transition to male-only care and 4 to 14 reversals back to biparental care compared with a total of 9 to 18 transitions from substrate spawning to mouthbrooding with only 1 to 10 reversals back to substrate spawning (figs. 6 and 7).

The reasons for the low number of transitions in the form of care compared with the sex of caregiver are probably related to the fact that this behavior is more dependent on the evolution of the complex morphological and physiological structures. For successful mouthbrooding, there is a need for a wide buccal cavity, high gill-raker numbers to prevent a loss of the eggs, and often the ability of the caregiver to fast during brooding (Fryer and Iles 1972; Keenleyside 1991). On the other hand, egg attachment systems and larval headlands are essential to facilitate substrate-brooding. Substrate- and mouthbrooders also differ in the size of their eggs and

their clutch size (Peters and Berns 1982) and in the duration of the developmental intervals during early ontogeny (Noakes and Balon 1982). Whereas substrate-brooders lay a lot of small eggs, mouthbrooders have only a few but big and yolky eggs. The evolution from one form of care to the other is, therefore, presumed to be a relatively slow process that involves evolutionary changes of several morphological and physiological systems. Transitions in the sex of the caregiver are less constrained and mostly influenced by ecological factors, like predation pressure, food abundance (Townshend and Wootton 1985), and the operational sex ratio (Balshine-Earn 1996). For example, high predation risk to the offspring would favor biparental care of the free-swimming fry, and a high probability of remating opportunities for the male because of a female-biased sex ratio would result in the desertion of the male. Goodwin, Balshine-Earn, and Reynolds (1998) found in their study of evolutionary transitions in parental care behavior that mouthbrooding evolved in tilapiine fish only once, supporting Trewavas' hypothesis, and that within a monophyletic cluster of different mouthbrooding tilapiine genera female-only care evolved four times and male-only care only once. According to their topology of cichlid relationships, Goodwin, Balshine-Earn, and Reynolds (1998) presumed that uniparental mouthbrooders evolved from biparental substrate-brooders and mouthbrooders, as hypothesized by Iles and Holden (1969), and female-only and biparental mouthbrooding evolved independently from biparental substrate-brooding (Lowe-McConnell 1959; Kraft and Peters 1963).

The high diversity of parental care behaviors in Tilapias has prompted many questions concerning its evolutionary history (Lowe-McConnell 1959; Iles and Holden 1969; Peters and Berns 1978a, 1982; Trewavas 1980, 1983; Goodwin, Balshine-Earn, and Reynolds 1998). In this group there are species that spawn and protect their eggs and larvae on the substrate (like sand or gravel) and others which brood them in their buccal cavity.

In addition, the sex of the caregiver also varies: in some species both parents (typically for the species of the A-, B-, and D-*Tilapia* groups but also in *S. galilaeus*), in others only the female (very common: all representatives of the genus *Oreochromis*, *T. simonis*, and *S. caudomarginatus*) or only the male (this form of care is rare and only known with certainty from *S. melanotheron* and *S. occidentalis*) take care of the offspring. In contrast to most of the fish families in which uniparental care by males is the predominate behavior (Gittleman 1981; Blumer 1982), the only known cichlid fish exhibiting paternal care are *S. melanotheron* (Lowe-McConnell 1959) and *S. occidentalis* (Loiselle 1985).

On the basis of our ND2 data, the two substrate-spawning species *T. cabrae* and *T. mariae* are found in relative phylogenetic proximity to the mouthbrooding genera *Iranocichla*, *Sarotherodon*, *Stomatepia*, *Tristramella*, and *Oreochromis* and the Lake Tanganyika cichlids in both the MP and NJ analyses (figs. 4 and 5). It is striking that these species exhibit morphological (such as the morphology of their buccal cavity and the number

of gill rakers) or behavioral characteristics that suggest that they have a common ancestry with the mouthbrooding tilapiines. It is assumed that oral transport of eggs was the first step in the evolution of mouthbrooding (Lowe-McConnell 1959; Baylis 1981). *Tilapia mariae* has been observed carrying eggs by mouth from one pit to another. The representatives of the genera *Sarotherodon* and *Oreochromis* typically have 13 to 28 gill rakers on the first gill arch, which prevent a loss of eggs during mouthbrooding. In contrast to the other substrate spawners, which have previously been considered as being *Tilapia* species, *T. mariae* and *T. cabrae* have 12 to 15 instead of only 6 to 12 gill rakers (Apfelbach 1969; Trewavas 1983). A further similarity to the mouthbrooding tilapiines is the wider head of *T. mariae* compared with other substrate spawners (Trewavas 1983). This is, by some workers, considered to be a preadaptation for mouthbrooding because it would permit for the larger buccal volume that is necessary for mouthbrooding behavior (Peters 1961). On the other hand, one has also to keep in mind that a wider head and the number of gill rakers could also be explained as an adaptation for the feeding method—most of the so-called *Tilapia* species are scrapers or pickers, whereas, e.g., the genus *Oreochromis* are filter feeders. Additional mouthbrooder characteristics of *T. mariae* are sexual dimorphism and contact behavior of the fry (Apfelbach 1969). All tree topologies show a clustering of biparental (*S. galilaeus*), paternal (e.g., *S. melanotheron*), and maternal (e.g., *S. caudomarginatus*, all representatives of the genus *Oreochromis*) mouthbrooding species, but the monophyly of these taxa is in all cases only weakly supported (ML 47, MP 21, NJ 30) (figs. 3–5). The albeit weakly indicated monophyly of the representatives of all tilapiine mouthbrooding taxa would confirm Trewavas' (1980) hypothesis of a single origin of the different mouthbrooding types and rule out Peters and Berns' (1978a, 1982) polyphyletic hypothesis. Allozyme analyses (McAndrew and Majumdar 1984; Sodsuk and McAndrew 1991; Pouyaud and Agnèsè 1995) and a SATA-satellite DNA study (Franck et al. 1994) have also found a close relationship between the mouthbrooding types, but the exact relationship remained unresolved in these studies also.

The relationships within the genus *Sarotherodon* and also its relationships to the genus *Oreochromis* remain somewhat uncertain. However, that the genus *Sarotherodon* is monophyletic seems questionable on the basis of this study and was already called into question by Schliewen, Tautz, and Pääbo (1994). There are several genera, including the maternal mouthbrooding genera *Tristramella* and *Stomatepia*, that are nested within the apparently paraphyletic genus *Sarotherodon* (figs. 3–5). Whereas Trewavas' (1980) hypothesis for the evolution of parental care in Tilapias did not consider the phylogenetic position of the Israeli genus *Tristramella*, our analyses strongly indicate that the maternal mouthbrooding species *T. simonis* is in fact a close relative of the genus *Sarotherodon*. Moreover, Schliewen, Tautz, and Pääbo (1994) showed that several *Sarotherodon* species and additional genera (e.g., *Konia*, *Myaka*, and

Pungu) of mostly biparental mouthbrooding species of endemic Cameroon tilapiine cichlids cluster with *Sarotherodon*.

Peters and Berns (1982) have suggested that the stronger atrophy of the egg attachment system and the larval headglands of the maternal mouthbrooders (*Oreochromis*) are a strong indication that these species are older than the biparental and paternal mouthbrooders (*Sarotherodon*), whose organs are less rudimentary. The ML analysis indicates that *T. simonis*, *Stomatepia mariae*, *Iranocichla hormuzensis*, and the genus *Sarotherodon*, which form an only weakly supported monophyletic group, appear as a sister group to the maternal mouthbrooders of the genus *Oreochromis* (fig. 3). This topology disagrees with Peters and Berns' (1978a, 1982) hypothesis because this sister group relationship indicates that both groups of species are of the same age. However, in the MP and NJ analyses there is no monophyletic grouping of these taxa—in contrast, in these tree topologies the *Sarotherodon* group is shown to be paraphyletic and basal to the genus *Oreochromis* (figs. 4 and 5). These topologies also contradict the hypothesis of Peters and Berns' (1978a, 1982) because it would imply that the biparental and paternal mouthbrooding *Sarotherodon* species are older than the maternal mouthbrooders of the genus *Oreochromis*. Because all tree topologies are only poorly resolved, they do not allow final conclusions about the evolution of the parental care within this group.

In our composite phylogeny of tilapiines the endemic species of the Barombi-Mbo lake were adopted from Schliewen, Tautz, and Pääbo (1994) and placed as a sister group to *S. galilaeus*. A reconstruction of the parental care behavior based on the ML analysis would indicate an evolution of the sex of caregiver from biparental substrate-brooding to maternal mouthbrooding and from maternal mouthbrooding to biparental mouthbrooding (fig. 3). That maternal mouthbrooding evolved directly from biparental substrate-brooding was also proposed by Goodwin, Balshine-Earn, and Reynolds (1998). In contrast to Goodwin, Balshine-Earn, and Reynolds (1998), we also included the maternal mouthbrooder (Stiassny 1996) *S. caudomarginatus*. Because of this, the MP-based reconstructions indicate that paternal mouthbrooding evolved at least in one case via a maternal mouthbrooding stage (fig. 4). This result would contradict the hypothesis of Iles and Holden (1969) who suggested that female-only and male-only care evolved independently from a biparental mouthbrooding stage. However, because of the fact that the relationships between the different species within the *Sarotherodon* group are only weakly resolved, a detailed reconstruction and final conclusions of the evolution of the sex of the caregiver is still wanting.

Acknowledgments

We are grateful to Jean-François Agnèsè John Balirwa, Sigal Balshine-Earn, Jörg Freyhof, Ted Papenfuss, Laurent Pouyaud, Peter Reinthal, Uli Schliewen, Lothar Seegers, Ole Seehausen, Hündrich Spiesser, Melanie

Stiassny, and Christian Sturmbauer and others listed in table 2 for providing samples. Some collections in Zambia were made by Erkki Schwank and Sven O. Kullander during a survey of Zambian Tilapias, financed by the Swedish Agency for Research Cooperation with Developing Countries, grant no S/5.11 SWE-88-019, and with permission from Dr. E. D. Muyanga, Department of Fisheries, Chilanga, Zambia. We would like to particularly acknowledge Erkki Schwank, who died as a consequence of fieldwork in Zambia. We thank Angelika Denk, Tomas Hrbek, Katharina Noack-Kunmann, and Edward Malaga for their technical support early in this study and Izeni Farias, Sven Kullander, Melanie Stiassny, Antony Wilson, and Rafael Zardoya for their helpful comments on the earlier versions of this article. Special thanks also to Melanie M. Hoefer for her tireless support. We gratefully acknowledge the financial support of the Deutsche Forschungsgemeinschaft (ME 1725/2-1, ME 1725/3-1, ME 1725/4-1, ME 1725/5-1, ME 1725/8-1), the U.S. National Science Foundation (DEB 9615178), the University of Konstanz, and the Fond der Chemischen Industrie to A.M. We dedicate this article to the late Ethelvynn Trewavas who conducted the groundbreaking work for all later work on tilapiine cichlids.

A publication of relevance to our study (Nagl, S., H. Tichy, W. E., Mayer, I. E. Samonte, B. J. McAndrew, and J. Klein. 2001. Classification and phylogenetic relationships of African Tilapiine fishes inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **20**: 361–374.) appeared while this article was under review.

LITERATURE CITED

- ALBERTSON, R. C., J. A. MARKERT, P. D. DANLEY, and T. D. KOCHER. 1999. Phylogeny of a rapidly evolving clade: the cichlid fishes of Lake Malawi, East Africa. *Proc. Natl. Acad. Sci. USA* **96**:5107–5110.
- APFELBACH, R. 1969. Vergleichende quantitative Untersuchungen des Fortpflanzungsverhaltens Brutpflege mono- und -dimorpher Tilapien (Pisces, Cichlidae). *Z. Tierpsych.* **26**: 692–725.
- BALSHINE-EARN, S. 1996. Reproductive rates, operational sex ratios and mate choice in St. Peter's fish. *Behav. Ecol. Sociobiol.* **39**:107–116.
- 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.
- BAYLIS, J. R. 1981. The evolution of parental care in fishes, with reference to Darwin's rule of male sexual selection. *Environ. Biol. Fishes* **6**:223–251.
- BLUMER, L. S. 1982. A bibliography and categorization of bony fishes exhibiting parental care. *J. Linn. Soc. (Zool.)* **76**:1–22.
- BIRCHARD, P. 1989. Pierre Brichard's book of Cichlids and all the other fishes of Lake Tanganyika. T.F.H. Publications, Neptune City, NJ.
- FARIAS, I. P., G. ORTI, and A. MEYER. 2000. Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. *J. Exp. Zool.* **288**:76–92.
- FARIAS, I. P., G. ORTI, I. SAMPAIO, H. SCHNEIDER, and A. MEYER. 1999. Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and high genetic divergence of the Neotropical assemblage. *J. Mol. Evol.* **48**(6):703–711.
- . 2001. The cytochrome *b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. *J. Mol. Evol.* **53**:89–103.
- FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783–791.
- FRANCK, J. P. C., I. KORNFELD, and J. M. WRIGHT. 1994. The utility of SATA Satellite DNA sequences for inferring phylogenetic relationships among the three major genera of tilapiine cichlid fishes. *Mol. Phylogenet. Evol.* **3**(1):10–16.
- FRYER, G., and T. D. ILES. 1972. The cichlid fishes of the Great Lakes of Africa—their biology and evolution. Oliver and Boyd, Edinburgh.
- GITTLEMAN, J. L. 1981. The phylogeny of parental care in fishes. *Anim. Behav.* **29**:936–941.
- GOODWIN, N. B., S. BALSHINE-EARN, and J. D. REYNOLDS. 1998. Evolutionary transitions in parental care in cichlid fish. *Proc. R. Soc. Lond. B* **265**:2265–2272.
- GREENWOOD, P. H. 1978. A review of the pharyngeal apophysis and its significance in the classification of African cichlid fishes. *Bull. Br. Mus. Nat. Hist. (Zool.)*. **33**:297–323.
- . 1984. African cichlids and evolutionary theories. Pp. 141–154 in A. A. ECHELLE and I. KORNFELD, eds. *Evolution of fish species flocks*. University of Maine at Orono Press, Orono.
- . 1985. Notes on the anatomy and phyletic relationships of *Hemichromis* Peters, 1858. *Bull. Brit. Mus. Nat. Hist. (Zool.)* **48**:131–171.
- . 1986. The pharyngeal apophysis on the base of the skull in cichlid fishes. A reply to Trewavas (1985). *Neth. J. Zool.* **36**:562–564.
- . 1987. The genera of pelmatochromine fishes (Teleostei, Cichlidae). A phylogenetic review. *Bull. Br. Mus. Nat. Hist. (Zool.)*. **53**:139–203.
- GROSS, M. R., and C. R. SARGENT. 1985. The evolution of male and female parental care in fishes. *Am. Zool.* **25**:807–822.
- HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* **22**:160–174.
- ILES, T. D., and M. J. HOLDEN. 1969. Bi-parental mouth brooding in *Tilapia galilaea* (Pisces, Cichlidae). *J. Zool. Lond.* **158**:327–333.
- JOHNSON, T. C., C. A. SCHOLZ, M. R. TALBOT, K. KELTS, R. D. RICKETTS, G. NGOBI, K. BEUNING, I. SSEMMANDA, and J. W. MCGILL. 1996. Late Pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. *Science* **273**: 1091–1093.
- JORDAN, D. S. 1963. The genera of fishes and a classification of fishes. Stanford University Press, Stanford, Calif.
- KAWANABE, H., M. HORI, and M. NAGOSHI. 1997. Fish communities in Lake Tanganyika. Kyoto University Press, Kyoto, Japan.
- 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 rates of base substitutions 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., J. A. CONROY, K. R. MCKAYE, J. R. STAUFFER, and S. F. LOCKWOOD. 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. *Mol. Phylogenet. Evol.* **4**:420–432.
- KOSLOWSKI, I. 1985. Die Buntbarsche der Neuen Welt—Zwergcichliden. Meinders & Elstermann, Osabrück.
- KRAFT, A. V., and H. M. PETERS. 1963. Vergleichende Studien über die Oogenese in der Gattung *Tilapia* (Cichlidae, Teleostei). *Z. Zellforsch.* **61**:434–485.
- KULLANDER, S. O. 1998. A phylogeny and classification of the South American Cichlidae (Teleostei: Perciformes). Pp. 461–498 in L. R. MALABARBA, R. E. REIS, R. P. VARI, Z. M. LUCENA, and C. A. S. LUCENA, eds. *Phylogeny and classification of neotropical fishes. Part 5—Perciformes*. Porto Alegre, Brazil.
- KUWAMURA, T. 1997. The evolution of parental care and mating systems among Tanganyikan cichlids. Pp. 57–85 in H. KAWANABE, M. HORI, and M. NAGOSHI, eds. *Fish communities in lake Tanganyika*. Kyoto University Press, Kyoto, Japan.
- LEWONTIN, R. C. 1974. *The genetic basis of evolutionary change*. Columbia University Press, New York.
- LIEM, K. F., and J. W. M. OSSE. 1975. Biological versatility, evolution, and food resource exploitation in African cichlid fishes. *Am. Zool.* **15**:427–454.
- LINKE, H., and W. STAECK. 1987. *Amerikanische Cichliden I—Kleine Buntbarsche*. Tetra-Verlag, Melle, Germany.
- LIPPITSCH, E. 1998. Phylogenetic study of cichlid fishes in Lake Tanganyika: a lepidological approach. *J. Fish Biol.* **53**:752–766.
- LOISELLE, P. V. 1985. The care and breeding of *Sarotherodon occidentalis*. *Buntbarsche Bull.* **109**:14–24.
- LOWE-McCONNELL, R. H. 1959. Breeding behaviour patterns and ecological differences between *Tilapia* species and their significance for evolution within the genus *Tilapia* (Pisces: Cichlidae). *Proc. Zool. Soc. Lond.* **132**:1–30.
- LUNDBERG, G. J., M. KOTTELAT, G. R. SMITH, M. J. L. STIASSNY, and A. C. GILL. 2000. So many fishes so little time: an overview of recent ichthyological discovery in continental waters. *Ann. Miss. Bot. Gard.* **87**:26–62.
- MADDISON, W. P., and D. R. MADDISON. 1992. *MacClade: analysis of phylogeny and character evolution*. Sinauer Associates Sunderland, Mass.
- MCANDREW, B. J., and K. C. MAJUMDAR. 1984. Evolutionary relationships within three tilapiine genera (Pisces: Cichlidae). *J. Linn. Soc. (Zool.)* **80**:421–435.
- MEYER, A. 1993. Phylogenetic relationships and evolutionary processes in East African cichlids. *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.
- MEYER, A., C. MONTERO, and A. SPREINAT. 1994. Evolutionary history of the cichlid fish species of the East African great lakes inferred from molecular phylogenetic data. *Arch. Hydrobiol. Beih. Ergebn. Limnol.* **44**:409–425.
- . 1996. Molecular phylogenetic inferences about the evolutionary history of the East African cichlid fish radiations. Pp. 303–323 in M. L. J. STIASSNY, L. R. PARENTI, and G. D. JOHNSON, eds. *The interrelationships of fishes*. Academic Press, London.
- NELSON, J. S. 1994. *Fishes of the world*. John Wiley & Sons, New York.
- 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.
- . 1997. Phylogenetic relationships and evolution of Tanganyikan cichlids: a molecular perspective. Pp. 3–23 in H. KAWANABE, M. HORI, and M. NAGOSHI, eds. *Fish communities in Lake Tanganyika*. Kyoto University Press, Kyoto, Japan.
- NOAKES, D. L. G., and E. K. BALON. 1982. Life histories of tilapias: an evolutionary perspective. Pp. 61–82 in R. S. V. PULLIN and R. H. LOWE-McCONNELL, eds. *The biology and culture of tilapias*. ICLARM Conference Proceedings, Vol. 7. International Center for Living Aquatic Resources Management, Manila, Philippines.
- OLIVER, M. K. 1984. Systematics of African cichlid fishes: determination of the most primitive taxon, and studies on the haplochromines of Lake Malawi (Teleostei: Cichlidae). Yale University, Yale, Conn.
- OWEN, R. B., R. CROSSLEY, T. C. JOHNSON, D. TWEDDLE, I. KORNFIELD, S. DAVISON, D. H. ECCLES, and D. E. ENGTROM. 1990. Major low levels of Lake Malawi and their implication for speciation rates in cichlid fishes. *Proc. R. Soc. Lond. B* **240**:519–553.
- PETERS, H. M. 1961. Zur morphologischen Kennzeichnung der ethologischen Gruppen der Gattung *Tilapia* (Teleostei, Cichlidae). *Zool. Anz.* **166**:470–478.
- PETERS, H. M., and S. BERNS. 1978a. Über die Vorgeschichte der maulbrütenden Cichliden. I. Was uns die Haftorgane der Larven lehren. *Aquar. Mag. Stuttgart* **5**:211–217.
- . 1978b. Über die Vorgeschichte der maulbrütenden Cichliden. II Zwei Typen von Maulbrütern. *Aquar. Mag. Stuttgart* **7**:324–331.
- . 1982. Die Maulbrutpflege der Cichliden. Untersuchungen zur Evolution eines Verhaltensmusters. *Z. Zool. Syst. Evolutionforsch.* **20**:18–52.
- POLL, M. 1986. Classification des Cichlidae du lac Tanganika. Tribus, genres et espèces. *Acad. R. Belg. Mem. Classe Sci. Coll.-8°-2°ser.*, tome **XLV**:1–163.
- POUYAUD, L., and J.-F. AGNÈSE. 1995. Phylogenetic relationships between 21 species of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *J. Fish Biol.* **47**:26–38.
- PULLIN, R. S. V., and R. H. LOWE-McCONNELL. 1982. The biology and culture of Tilapias. ICLARM Conference Proceedings, Vol. 7. International Center for Living Aquatic Resources Management, Manila, Philippines.
- REGAN, C. T. 1920. The classification of the fishes of the family Cichlidae. I. The Tanganyika genera. *Ann. Mag. Nat. Hist.* **9**:33–53.
- . 1922. The classification of the fishes of the family Cichlidae. II. On African and Syrian genera not restricted to the Great Lakes. *Ann. Mag. Nat. Hist.* **9**:249–264.
- RICHTER, H. J. 1989. *Complete book of dwarf cichlids*. T.F.H. Publications, Neptune City, NJ.
- SAGE, R. D., P. V. LOISELLE, P. BASASIBWAKI, and A. C. WILSON. 1984. Molecular versus morphological change among cichlid fishes of Lake Victoria. Pp. 185–201 in A. A. ECHELLE and I. KORNFIELD, eds. *Evolution of fish species flocks*. University of Maine at Orono Press, Orono, Maine.
- SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**:406–425.
- SCHLIEWEN, U. K., D. TAUTZ, and S. PÄÄBO. 1994. Sympatric speciation suggested by monophyly of crater lake cichlids. *Nature* **368**:629–632.
- SEEGERS, L., R. SONNENBERG, and R. YAMAMOTO. 1999. Molecular analysis of the *Alcolapia* flock from Lakes Natron

- and Magadi, Tanzania and Kenya (Teleostei: Cichlidae), and implications for their systematics and evolution. *Ichthyol. Explor. Freshw.* **10**:175–199.
- SODSUK, P. 1993. Molecular genetics and systematics of tilapiine cichlids using allozymes and molecular characters. Ph.D. thesis, University of Stirling.
- SODSUK, P., and B. J. MCANDREW. 1991. Molecular systematics of three tilapiine genera *Tilapia*, *Sarotherodon* and *Oreochromis* using allozyme data. *J. Fish Biol.* **39**(Suppl. A): 301–308.
- STAECK, W., and H. LINKE. 1985. *Amerikanische Cichliden II—Grosse Buntbarsche*. Tetra-Verlag, Melle, Germany.
- STAWIKOWSKI, R., and U. WERNER. 1998. *Die Buntbarsche Amerikas*. Ulmer, Stuttgart, Germany.
- STEINITZ, H., and BEN-TUVIA, A. 1960. The Cichlid fishes of the genus *Tristramella*. *Ann. Mag. Nat. Hist. Ser. 3.* **III**: 161–175.
- STIASSNY, M. L. J. 1991. Phylogenetic intrarelationships of the family Cichlidae: an overview. Pp. 1–35 in M. H. A. KEENEYSIDE, ed. *Cichlid fishes—behaviour, ecology and evolution*. Chapman & Hall, London.
- . 1996. Notes on a little known cichlid from West Africa, *Sarotherodon caudomarginatus* (Boulenger, 1916), with some thoughts on the genus. Pp. 53–57 in *The Cichlid yearbook*, Vol. 6. Cichlid Press, St. Leon-Rot, Germany.
- STIASSNY, M. L. J., and A. MEYER. 1999. Cichlids of the Rift Lakes. *Sci. Am.* **280**:44–49.
- STIASSNY, M. L. J., U. K. SCHLIEWEN, and W. J. DOMINEY. 1992. A new species flock of cichlid fishes from Lake Bermin, Cameroon with a description of eight new species of *Tilapia* (Labroidae: Cichlidae). *Ichthyol. Explor. Freshw.* **3**: 311–346.
- STREELMAN, J. T., R. ZARDOYA, A. MEYER, and S. A. KARL. 1998. Multilocus phylogeny of cichlid fishes (Pisces: Perciformes): evolutionary comparison of microsatellite and single-copy nuclear loci. *Mol. Biol. Evol.* **15**:798–808.
- STRIMMER, K., and A. v. HAESELER. 1997. Likelihood mapping: a simple method to visualize phylogenetic content of a sequence alignment. *Proc. Natl. Acad. Sci. USA* **94**:6815–6819.
- STURMBAUER, C. 1998. Explosive speciation in cichlid fishes of the African Great Lakes: a dynamic model of adaptive radiation. *J. Fish Biol.* **53**(Suppl. A):1–12.
- STURMBAUER, C., and A. MEYER. 1993. Mitochondrial phylogeny of the endemic mouthbrooding lineages of cichlid fishes from Lake Tanganyika in eastern Africa. *Mol. Biol. Evol.* **10**:751–768.
- STURMBAUER, C., E. VERHEYEN, and A. MEYER. 1994. Mitochondrial phylogeny of the lamprologini, the major substrate spawning lineage of cichlid fishes from Lake Tanganyika in eastern Africa. *Mol. Biol. Evol.* **11**:691–703.
- SWOFFORD, D. L. 2001. PAUP*: phylogenetic analysis using parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, Mass.
- THYS VAN DEN AUDENAERDE, D. F. E. 1970. An annotated bibliography of *Tilapia* (Pisces: Cichlidae). *Doc. Zool. Mus. R. Afrique Centr.* **14**: xi + 406.
- TOWNSHEND, T. J., and R. J. WOOTTON. 1985. Variation in the mating system of a biparental cichlid fish, *Cichlasoma panamense*. *Behaviour* **95**:181–197.
- TREWAVAS, E. 1942. The Cichlid Fishes of Syria and Palestine. *Ann. Mag. Nat. Hist.* **9**:526–536.
- . 1980. *Tilapia and Sarotherodon*. *Buntbarsche Bull.* **81**: 1–6.
- . 1982a. Generic groupings of Tilapiini used in aquaculture. *Aquaculture* **27**:79–81.
- . 1982b. Tilapias: taxonomy and speciation. Pp. 3–13 in R. S. V. PULLIN and R. H. LOWE-McCONNELL, eds. *The biology and culture of tilapias*, Vol. 7. ICLARM Conference Proceedings, Manila, Philippines.
- . 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. *Br. Mus. Nat. Hist. Lond.*
- VAN DER BANK, F. H. 1994. Molecular systematics of fifteen Old World cichlid species using allozyme data. *Biochem. Syst. Ecol.* **22**:791–801.
- WICKLER, W. 1963. Zur Klassifikation der Cichlidae, am Beispiel der Gattungen *Tropheus*, *Petrochromis*, *Haplochromis* und *Hemihaplochromis* n. gen. (Pisces, Perciformes). *Senckenbergiana Biol.* **44**:83–96.
- ZARDOYA, R., D. M. VOLLMER, C. CRADDOCK, J. T. STREELMAN, S. KARL, and A. MEYER. 1996. Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). *Proc. R. Soc. Lond. B.* **263**:1589–1598.

MANOLO GOUY, reviewing editor

Accepted January 24, 2002