Copeia, 1994(2), pp. 274–288

Molecular Systematics and Radiation of the Haplochromine Cichlids (Teleostei: Perciformes) of Lake Malawi

PAUL MORAN, IRV KORNFIELD, AND PETER N. REINTHAL

Mitochondrial DNA (mtDNA) restriction fragment length polymorphisms were assayed among 40 species of Lake Malawi haplochromines (Cichlidae) including representatives of ecologically divergent genera. Six distinctive mtDNA lineages were distinguished, two of which were major clades, represented by a large number of species. The other four lineages were each represented by a single species with a divergent mtDNA haplotype. One of the two major clades was composed of the shallow-water, rock-dwelling mbuna species, whereas the other included a diverse array of sand-dwelling and pelagic species. A number of taxa, found to be firmly embedded within the mbuna clade, are quite distinct in morphology and generally inhabit deeper, sediment-rich areas rather than the rocky habitats typical of other mbuna. The mbuna group is generally thought to be a monophyletic assemblage, but these results suggest that it is actually paraphyletic. In contrast to the high morphological diversity among Malawi haplochromine species, mtDNA sequence divergence was found to be remarkably low. This finding underscores the unprecedented rapidity of speciation and evolutionary plasticity in this fish species flock.

Beginning with the earliest collections and descriptions of Malawi haplochromines, taxonomists have been challenged and frustrated by attempts to reconstruct the phylogenetic history of this fauna. Two primary obstacles have confounded this reconstruction. First, a paucity of shared derived traits often prevented identification of sister-group relationships (though many autapomorphic traits are present). Second, an abundance of parallelism has made it difficult to assure that shared traits are actually synapomorphic (Eccles and Trewavas, 1989). Early work on the Malawi ichthyofauna revealed a large number of new spe-

cies (Günther, 1864; Boulenger, 1908; Regan, 1922). Christy made extensive collections in 1925 and 1926, which Trewavas included in a synopsis of the Malawi haplochromines (1931, 1935). Based on these studies, it became clear that Lake Malawi contained one of the most species-rich vertebrate faunas known. Because of the lack of clear synapomorphic characters, a large number of the Malawi species were originally placed in the genus '*Haplochromis.*' The 1935 synopsis did not completely describe all the members of '*Haplochromis*' in Malawi, and it was widely recognized that this genus was polyphyletic. The name of that species complex was subsequently changed to '*Cyrtocara*' to reflect its apparently discrete evolutionary path relative to the Lake Victoria fauna in which *Haplochromis* was first described (Greenwood, 1979).

A recent monograph by Eccles and Trewavas (1989) described 22 new genera in Lake Malawi, bringing to 21 the number of genera from the former '*Haplochromis*' complex. The genus *Cyrtocara* is now monotypic containing only *C. moorii.* These new descriptions were based on phenotypic characters, principally melanin patterning, which were judged to be evolutionarily conserved. With these new descriptions, the total number of haplochromine genera in Lake Malawi is now 49.

The diverse and species-rich East African cichlid fauna presents an excellent model system for critical examination of the processes of speciation and adaptive radiation (Futuyma, 1986; Greenwood, 1991; Kornfield, 1991). In less than two million years, the haplochromine cichlids of Lake Malawi have radiated to fill almost every conceivable trophic niche. This assemblage includes a diverse array of pelagic planktivores, piscivores, detritivores, molluscivores, paedophages, scale eaters, and insectivores (Fryer and Iles, 1972). The species that comprise these trophic groups fill niche space that might be occupied by 10 or more fish families in other great lakes of the world. A group of species of particular interest to evolutionary biologists is the rock-dwelling mbuna (Trewavas, 1935; Ribbink et al., 1983; Reinthal, 1990). This assemblage includes an estimated 200 species in 10 genera and has radiated over an extremely short period of time (Kornfield, 1978). The mbuna are morphologically distinct from the other haplochromines in Lake Malawi and are presumably monophyletic (Regan, 1922; Trewavas, 1935).

To provide additional perspective on the phylogenetic systematics of the Malawi ichthyofauna, we have used restriction enzyme analysis of mitochondrial DNA (mtDNA) to examine an ecological cross-section of species. MtDNA analysis is particularly amenable to systematic studies of closely related taxa (Wilson et al., 1985; Avise et al., 1987; Moritz et al., 1987) because of its primarily maternal, haploid, and nonrecombining mode of inheritance (but see Gyllensten et al., 1991). Moreover, mtDNA evolves more rapidly and shows greater sensitivity to historical biogeographic events than does single copy nuclear DNA (Brown et al., 1979). This study examined 40 species representing 32 genera in Lake Malawi. These genera included representatives of the most morphologically divergent lineages, as well as seven of the 10 mbuna genera and 11 of the newly described nonmbuna genera (Eccles and Trewavas, 1989). Representatives of four genera from Lake Victoria and two from Lake Tanganyika were examined as outgroups.

Materials and Methods

Restriction enzyme analysis of mtDNA was used to estimate genetic relationships among haplotypes. Estimates were made using both phenetic and cladistic techniques. Haplotypes were defined by a unique composite of all restriction profiles. MtDNA isolated from fresh and frozen fish tissue was purified by density gradient ultracentrifugation (Lansman et al., 1981; Dowling et al., 1990). Restriction digests were carried out per manufacturers' instructions (GIBCO BRL, Gaithersburg, Maryland; Boehringer Mannheim, Indianapolis, Indiana; New England Biolabs, Beverly, Massachusetts); visualization of fragments was achieved by rapid end-labeling (Drouin, 1980) and autoradiography. Fragment sizes were estimated by comparison to size standards included on each gel [1 kilobase (kb) ladder, GIBCO BRL].

To identify a suite of enzymes providing multiple restriction sites consistently resolvable by agarose gel electrophoresis (0.5–2.0%), an initial screening was conducted using 29 restriction endonucleases (data not shown). Subsequently, 55 individuals were examined using 18 restriction enzymes. Our final analysis included 40 species representing 32 Malawi genera. This sample represented most of the morphologically divergent taxa in the lake, including seven of 10 mbuna genera and 13 of 15 nonmbuna genera recognized prior to the recent redescription of the Malawi haplochromines (Eccles and Trewavas, 1989). In all, we have examined representatives of over half (63%) of the 47 endemic cichlid genera in Lake Malawi. Collec-

tion locations and species are provided in Materials Examined; restriction enzymes are listed in the Appendix. Voucher specimens have been deposited with the American Museum of Natural History (AMNH).

Cladistic analyses were conducted using Wagner parsimony and strict consensus with the tree-bisection-reconnection algorithm of PAUP Ver. 3.0 (Swofford, 1989). Characters for cladistic analysis consisted of the presence or absence of individual restriction fragments. Replicated bootstrap analyses were used to evaluate the relative strength of cladistic groupings using PHYLIP 3.1 (Felsenstein, 1988). The number of bootstrap replicates was limited to 200, which required 36.1 h of mainframe CPU time (IBM 3090). A limited number of bootstrap replicates may limit the power of statistical inference (Hedges, 1992). In spite of the controversy surrounding the use of bootstrapping and its statistical legitimacy, we feel that these results are useful as a relative measure of the strength of specific groupings.

Two primary criticisms have been advanced against restriction fragment length polymorphism (RFLP) analysis of mtDNA in favor of site mapping. First, the use of fragments as characters in cladistic analysis violates a fundamental Hennigian assumption, i.e., that the character state changes are independent. It is argued that single site changes are weighted more heavily than they should be, because one site change results in three fragment state changes (Swofford and Olsen, 1990). This is true in principle; however, when a large number of character state changes separate major clades, the error induced at each state change becomes relatively small. The critical issue in cladistic analysis is the ratio of synapomorphic characters to convergent characters (signal to noise). Because shared fragment states have equal weights whether they are synapomorphic or convergent, no directional bias is introduced. Randomly distributed errors would not be expected to consistently give the same incorrect phylogenetic topology when a large number of characters is examined. A second criticism of fragment analysis is that unique fragments of the same size may be incorrectly scored as homologous. This certainly happens although, when taxa are closely related, and alternative restriction profiles differ by only one or two sites at a given enzyme, the chance of error is minimal. Moreover, when virtually every restriction profile is visualized on the same gel with every other profile, the probability of such error is further diminished. Because we used the conservative approaches of strict consensus and bootstrapping, we expect that the relationships presented here will be robust to further analysis, including site mapping and DNA sequencing.

As a phenetic measure of genetic distance, sequence divergence was estimated among haplotypes (nucleotide diversity, d_{ij}; Nei and Li, 1979; Nei, 1987). Phenetic relationships based on sequence divergence estimates were visualized by clustering taxa with the neighbor-joining algorithm (Saitou and Nei, 1987). Results of UPGMA analysis (not shown) were broadly concordant with neighbor-joining. We have chosen not to report these results and focus rather on neighbor-joining because UPGMA has been shown to be less reliable than other methods of phylogenetic analysis (Hillis et al., 1992). Because neither neighbor-joining nor parsimony analysis provide accurate portrayal of genetic distance, principal coordinates analysis was performed (NTSYS Ver. 1.6, Rohlf, 1990). Principal coordinates analysis maximizes the orthogonal separation of haplotypes and balances the tendency of agglomerative methods to produce clusters, regardless of the structure of genetic relationships. By employing both clustering and ordination, groups common to both techniques were identified; such groups are likely to be particularly robust (Rohlf, 1990). Input data files for all of the above analyses (binary code and d-value matrices) were generated using REAP (McElroy et al., 1992).

The principal coordinates projection is presented with a minimum spanning tree (MST) superimposed on the haplotypes. The MST is useful in that it can reveal local distortions in the principal coordinates projection by identifying pairs of haplotypes which appear to be similar on the first three principal axes but are more distant if additional dimensions are included (Rohlf, 1990).

Results

From the 29 restriction enzymes originally screened, 18 were selected for their ability to yield consistent cleavage and reliable labeling and to provide multiple restriction profiles with between one and 15 fragments. The mean number of restriction fragments per haplotype was 95, representing an average of 510 bases surveyed (approximately 3.14% of the 16,300 base pairs in the Malawi haplochromine mitochondrial genome). Some enzymes generated relatively few unique restriction profiles whereas others produced more than 30. All individuals, including sympatric conspecifics, demonstrated unique haplotypes, each separated from others by one or more restriction-site changes (Ap-

pendix). Where conspecifics differed by only one or two restriction-site changes, a single individual was selected arbitrarily to simplify analyses. Complete information on all haplotypes, including the estimated sizes of restriction fragments for each cleavage profile is available on request.

Genome size variation.—No cases of mtDNA heteroplasmy were detected nor were genome size polymorphisms found within lakes. However, all four haplochromine species from Lake Victoria were found to have an mtDNA genome approximately 1000 base pairs larger than the Malawi and Tanganyika genomes (17,300 base pairs, Fig. 1). *Serranochromis robustus*, a widespread East African species occurring in Lake Malawi, had the same genome size as other Malawi taxa and the species of Lake Tanganyika. Variation in genome size causes inflated estimates of genetic distance when calculated from fragment differences. This error occurs because fragment mobility shifts, resulting from the size difference, are interpreted as site changes produced by sequence divergence. Genome size variation also confounds cladistic analysis because fragments shared by all three groups (i.e., defined by the same restriction sites) appear to unite Malawi and Tanganyika species to the exclusion of those in Lake Victoria. For these reasons, and because full characterization of the genome size variation is beyond the scope of this study, the haplochromine species of Lake Victoria were excluded as outgroups in phylogenetic analyses of the Lake Malawi fauna.

Phylogenetic structure.—Although genome size differences preclude comparisons of sequence divergence between Lake Victoria lineages and those of Lakes Tanganyika and Malawi based on restriction fragments, comparisons within lakes and comparisons between Malawi and Tanganyika species are unbiased. The range of sequence divergence observed among species within Lake Victoria ranged from 1.0–1.3% (data not shown). Divergence between the two Tanganyikan species was substantially larger (5.4%) and was comparable to the divergence of 6.0% observed between Lakes Tanganyika and Malawi. Levels of mtDNA sequence divergence indicated that *Serranochromis robustus* was equidistant from the Malawi and Tanganyika haplochromines (6.38% and 6.35%, respectively). This relationship is further supported by Wagner parsimony analysis (see below).

Six distinct lineages were found within the Malawi haplochromines (Figs. 2–5). Two of these lineages contained a large number of diverse species. One of the two included all the mbuna species examined, whereas the other contained most of the nonmbuna haplochromines. These two major groups are referred to as A (nonmbuna) and B (mbuna), after Meyer et al. (1990; A, "sand dwellers"; B, "rock dwellers"). The level of divergence between these groups, as estimated by restriction analysis, was 3.3%, with 10 pairwise comparisons yielding estimates over

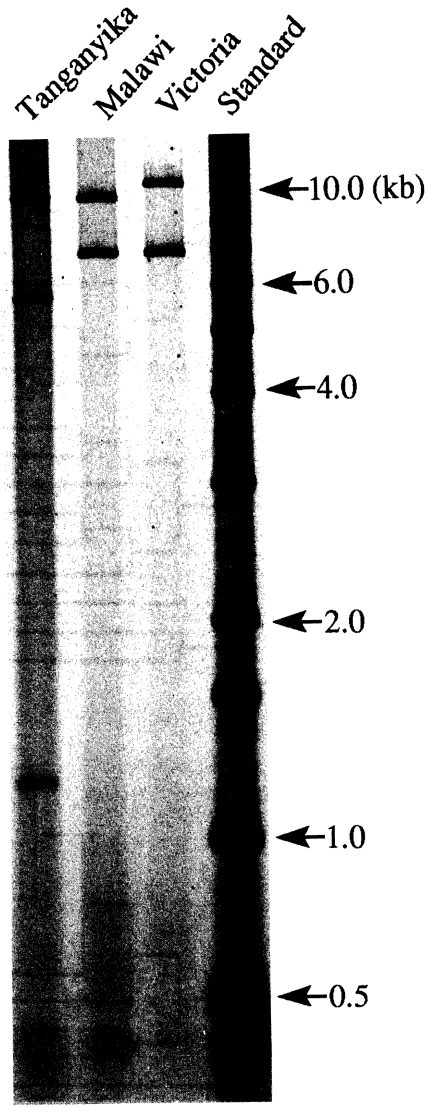

Fig. 1. *Sma*I digests of East African cichlid mtDNAs. This autoradiograph demonstrates the difference in mtDNA genome size between Lake Malawi and Lake Tanganyika versus Lake Victoria haplochromines. *Cyphotilapia frontosa* from Lake Tanganyika and *Copadichromis mloto* from Lake Malawi share a genome size of 16.3 kb. The mtDNA genome of *Neochromis nigricans* from Lake Victoria is approximately 1.0 kb larger.

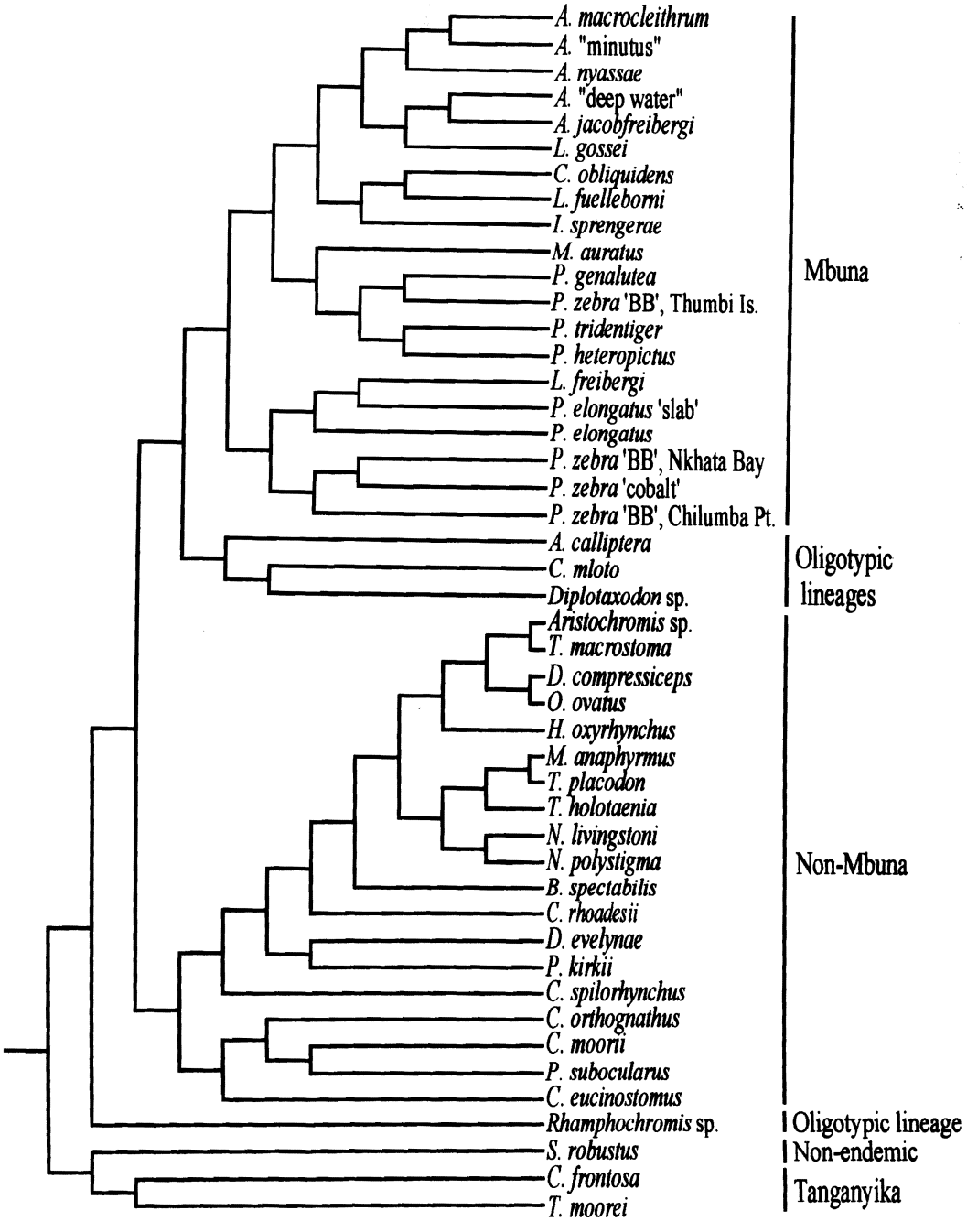

Fig. 2. Relationships among Malawi haplochromines based on estimated mtDNA sequence divergence (Nei and Li, 1979) produced by neighbor-joining (Saitou and Nei, 1987), two taxa from Lake Tanganyika were outgroups. The scale of values for the levels is arbitrary and only subset relationships are depicted. For full genus names, see Appendix. For relative genetic distances, see Figure 4.

4.0%. Meyer and co-workers found an average sequence divergence of approximately 2.5% between representatives of A and B haplotypes. Their analysis of 803 base pairs from two discrete mtDNA sequences included regions of the displacement loop, two tRNA genes, and the cytochrome B gene. The results presented here show a strong indication of structuring within the A and B lineages; both groups exhibit two species clusters (Figs. 2–4). However, the phy-

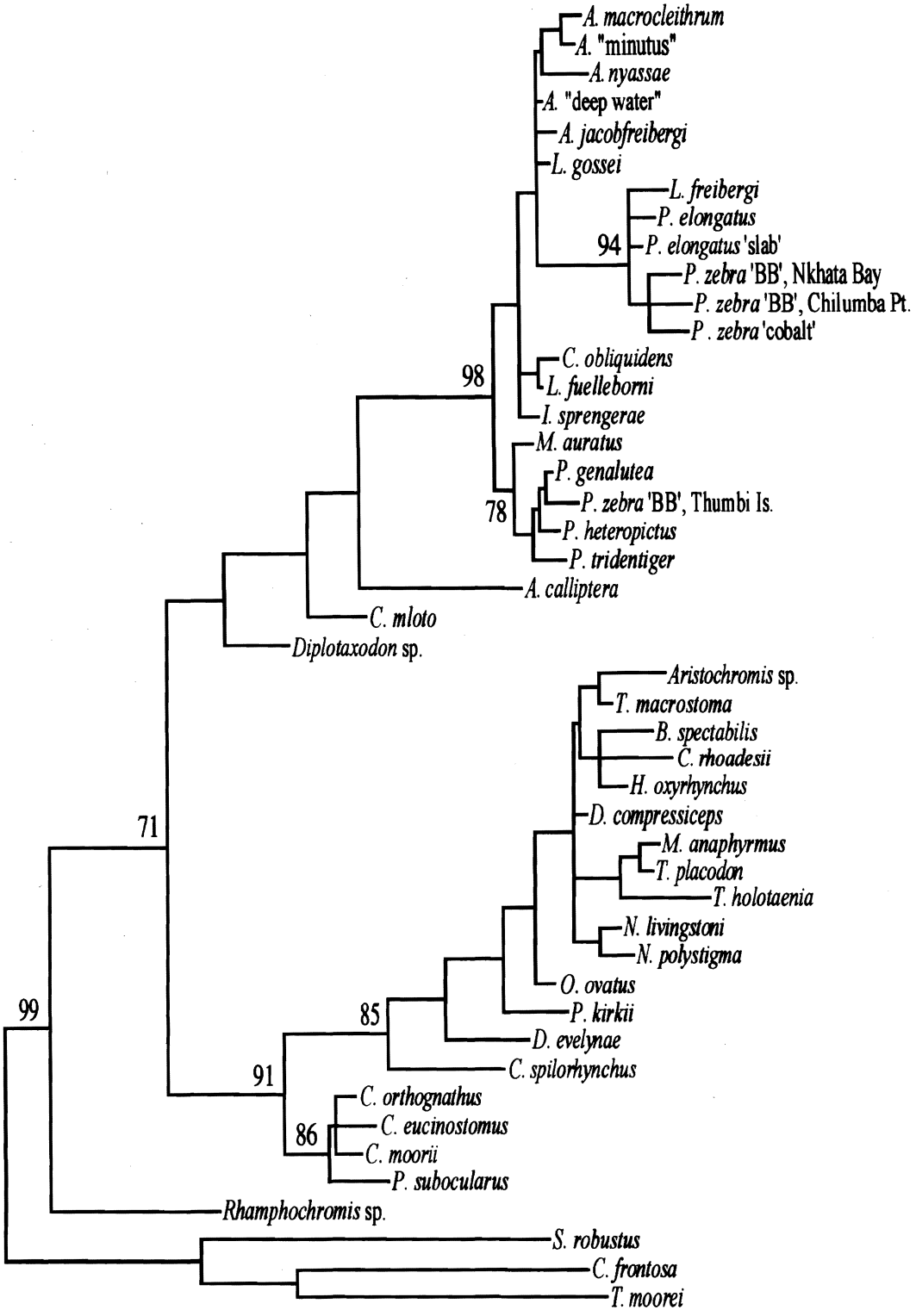

Fig. 3. Strict censuses cladogram based on Wagner parsimony. Forty-six trees of equal length (676 steps) were produced using tree-bisection-reconnection branch swapping (Swofford, 1989). The numbers at the nodes indicate the percentage of times the taxa in the indicated clade were united in 200 bootstrap resamplings (PHYLIP Ver. 3.1, Felsenstein, 1988). For full genus names, see Appendix.

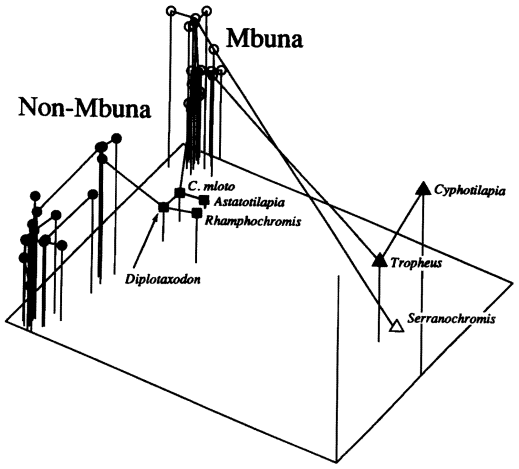

Fig. 4. Principal coordinates projection of sequence divergence among haplotypes with a minimum spanning tree superimposed (Rohlf, 1990). Oligotypic lineages are shown as squares, outgroup taxa as triangles. The filled triangles are Tanganyikan species.

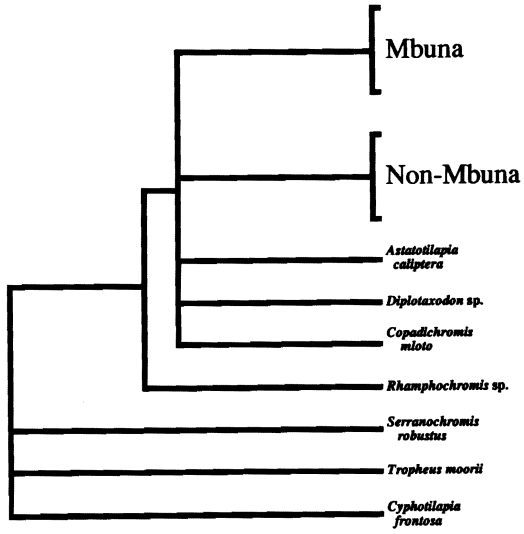

Fig. 5. Summary of relationships among Malawi haplochromines, produced as a composite of cladistic and phenetic analyses of mtDNA restriction fragments. This tree presents consensus results in a conservative manner, showing only the relationships supported by all methods of analysis.

logenetic importance of these observations may well be compromised by retained ancestral polymorphisms (Moran and Kornfield, 1993).

Within the A and B lineages, remarkably low sequence divergence was observed among haplotypes (1.7% and 1.0%, respectively). Six pairs of individuals representing discrete genera differed by less than 0.2%. Species as morphologically divergent as *Dimidiochromis compressiceps* and *Hemitilapia oxyrhynchus* differed by as few as three restriction-site changes, or 0.26% sequence divergence; *Cyathochromis obliquidens* differed from *Labeotropheus fuelleborni* by only 0.1% sequence divergence.

Representatives of four taxa produced haplotypes that were equidistant from the A and B linages; these were *Astatotilapia calliptera, Copadichromis mloto, Rhamphochromis* sp., and *Diplotaxodon* sp. We refer to these taxa as oligotypic lineages because they stood apart from the major A and B groupings and were only distantly related to each other. Other haplotypes may later be identified that are related to one or the other of these oligotypic linages, although it is unlikely that any of these four will be shown to be part of a group with as many species as A and B. Two patterns emerged for the relationships among the four species. First, *Rhamphochromis* was slightly more distant from the other three lineages than they were from each other (Fig. 4). Second, the remaining three species formed a loose cluster that excluded the other endemic species examined. In both neighbor-joining and Wagner parsimony analyses, *Rhamphochromis* occupied a position external to the A and B groupings, whereas the other oligotypic lineages were united with the A group to the exclusion of taxa in the B group (Figs. 2–3). However, bootstrap results indicated that the unity of the oligotypic lineages to the A lineage was not supported. Although support for the positioning of *Rhamphochromis* was not as strong as other relationships presented here, we have chosen to place it outside the major A and B groups (Fig. 5). Most of the bootstrap replicates supported this interpretation (Fig. 3) as did results of neighbor-joining (Fig. 2).

Six species in three nonmbuna genera (Eccles and Trewavas, 1989) consistently occurred within the mbuna group in all analyses (Figs. 2–4). Unlike most mbuna, the species *Lethrinops gossei, Alticorpus macrocleithrum, Aulonocara jacobfreibergi, A. nyassae,* and the undescribed species *Aulonocara "minutus"* generally inhabit deeper, sediment-rich areas and have distinctive morphologies. As an example of the unity of these six species to the mbuna, the deep-water species *Alticorpus macrocleithrum* differed from the nonmbuna haplochromines by at least 26 restriction-site changes, yet differed from the mbuna by an average of seven sites. Moreover, some of the mbuna are substantially closer to the deep-water species than they are to other members of the mbuna. For example, *Labeotropheus fuelleborni* was at least 10 restriction sites divergent from *Pseudotropheus zebra* "cobalt,"

yet only about three sites from *Aulonocara jacobfreibergi* and as few as two sites from *Aulonocara* "deep water." Thus, the mbuna is a paraphyletic group as currently recognized (Fryer and Iles, 1972; Ribbink et al., 1983; Eccles and Trewavas, 1989).

Clustering and maximum parsimony techniques provided broadly concordant results. Both phenetic and cladistic analyses were consistent in the separation of the A and B lineages and in the isolation of the four oligotypic lineages. Differences between analyses were limited to relationships of taxa within major clades and to the level of resolution of those relationships. Bootstrap resampling of cladistic characters further supported the distinctiveness of the A and B groups and the unity of the endemic haplochromines of Malawi (Fig. 3). The unity of the mbuna (B) group was supported in 98% of the bootstrap resamplings, whereas the nonmbuna (A) group formed a monophyletic clade in 91% of the replicates. When the oligotypic lineages were excluded from the analysis, both lineages became monophyletic in 100% of the replicates.

Sequence divergence among haplotypes projected on the first three principal axes revealed a pattern that further supported both clustering and parsimony techniques (Fig. 4). We provide the results of ordination primarily because neither neighbor-joining nor Wagner parsimony analysis allow accurate interpretation of relative genetic distances. Moreover, the concordance of cladistic, clustering and ordination approaches to phylogenetic analysis is consistent with the idea that these results provide an accurate view of the major phylogenetic relationships among Malawi haplochromines. A heuristic consensus tree for all methods of analysis is presented in Figure 5 as an estimation of these relationships.

Discussion

Three primary results emerged from this study. First, the distribution of sequence divergence among haplotypes was bimodal, forming the major clades A and B. Further, the B clade (which included all the mbuna examined) contained a number of species previously thought to be allied with other nonmbuna species. It appeared that these species were actually embedded within the mbuna rather than being a sister group. Therefore, the mbuna is a paraphyletic grouping. Second, remarkably low genetic differentiation was observed among the ecologically diverse Malawi haplochromines. For example, all of the mbuna species, including representatives of *Aulonocara*, *Lethrinops*, and *Alticorpus*, show an average sequence divergence of only 1.0%. Sequence divergence was only slightly greater among the nonmbuna haplochromines (1.7%). The levels of sequence divergence among Malawi genera are substantially less than values commonly observed within species of other freshwater fishes and, in some cases, less than values within populations. For example, in the southeastern United States, the sunfish species, *Lepomis punctatus*, showed population level divergence of 4.0% (Bermingham and Avise, 1986). Third, several relatively divergent oligotypic mtDNA lineages were represented by *Rhamphochromis* sp., *Diplotaxodon* sp., *Astatotilapia calliptera*, and *Copadichromis mloto*. These three primary results can be viewed both in the context of their systematic significance and also with respect to their implications for morphological diversification.

Mbuna are paraphyletic.—The molecular genetic data reported here provide new insight into the systematic relationships of the Lake Malawi haplochromines. With respect to the bimodal (mbuna/nonmbuna) distribution of haplotypes, the phylogenetic position of the genera *Aulonocara*, *Alticorpus*, and *Lethrinops* is unequivocal (Figs. 2–3). To enforce a topology placing any one of these species into the nonmbuna A clade would require over 20 restriction-site reversals. The unexpected affinity of these taxa with the mbuna indicates that a revision is required in the concept of the mbuna group if it is to be considered a monophyletic assemblage. It may be appropriate to construct a super-generic taxonomic category to recognize formally the phylogenetic systematic relationships among the Malawi haplochromines. Although these results alone cannot absolutely rule out a sister-group relationship between the mbuna and the deep-water species, we favor the explanation of paraphyly because some mbuna are three times more divergent from each other than from one or more of the deep-water species (6–10 sites compared to 2–4 sites).

Taxonomic revisions are required.—Two additional phyletic observations have significant taxonomic implications. First, *Copadichromis mloto* does not appear closely related to *C. eucinostomus*, suggesting that this genus is polyphyletic (Figs. 2–3). Additional members of this genus need to be examined to determine whether they are close to *C. mloto*, *C. eucinostomus*, or distinct from both of these species. Second, taxonomic revision is warranted for the genus *Astatotilapia*. Meyer et al. (1990) found that three *Astatotilapia* species of Lake Victoria were more closely re-

lated to the endemic haplochromines of that lake than to any of the Malawi taxa they examined. Our results suggest that the congener *A. calliptera* is embedded within the Malawi flock (Figs. 2–3). Although sequence divergence values between Victoria and Malawi haplotypes were not estimated because of the difference in mtDNA genome size, it is this very size difference that serves to unite *A. calliptera* with the Malawi haplochromines (both showing the smaller genome, whereas Victorian haplotypes had the larger). Furthermore, *A. calliptera* was less than 2.0% divergent from five of the mbuna species and less than 3.0% divergent from 26 others. Thus, these data strongly suggest that the genus *Astatotilapia* is polyphyletic. In addition to the restriction data, this relationship has been supported by subsequent mtDNA sequence analysis (A. Meyer, unpubl.). These results were not completely unexpected (Eccles and Trewavas, 1989) because the genus *Astatotilapia* was defined only on the basis of shared pleisiomorphic traits within the Victoria-Edward-Kivu flock (Greenwood, 1980).

Autapomorphic mtDNA genome size in Lake Victoria haplochromines.—The difference in genome size identified here could have been quite misleading. If these results were examined in the absence of previous molecular studies, it might be concluded that the smaller mtDNA genome observed in the cichlids of Lake Malawi and Lake Tanganyika represents a synapomorphy uniting these faunas to the exclusion of Lake Victoria species. The pleisiomorphic condition may be the larger (17.3 kb) genome size, as represented by multiple mtDNA lineages in the tilapiine cichlid fishes (Seyoum, 1989; Seyoum and Kornfield, 1992). In contrast to the situation with *Astatotilapia*, a number of independent molecular studies support the unity of the Malawi and Victorian cichlid faunas and clearly indicate that the Tanganyika cichlids fall outside this group (Meyer et al., 1990; Kornfield, 1991; Nishida, 1991). Given the broad and independent support for the relationships among the haplochromine faunas of the three Great Lakes, and assuming that the large genome size is plesiomorphic, a deletion may have occurred in the mtDNA genome of the haplochromine ancestor relatively soon after its divergence from the tilapiine fishes. Subsequently, a duplication or insertion restored the large genome size to the progenitor of the Victoria haplochromine lineage. Regardless, however, the larger genome size found in the Victorian haplochromines appears to be an autapomorphic character.

An alternative less parsimonious explanation is that deletion events occurred independently in the lineage that gave rise to the Malawi haplochromine radiation and in the lineage supporting the Tanganyikan radiation. This explanation is unlikely because of the position of *Serranochromis robustus* (Figs. 2–4), which contains the small mtDNA genome. *Serranochromis robustus* appears to have diverged from a common haplochromine ancestor at approximately the same time as the progenitor of the Malawi radiation and the two Tanganyikan genera examined here. If *Serranochromis* and the Tanganyika species each form independent mtDNA lineages, then an additional deletion event would also have been required. The independent deletion scenario is further discredited because the South African haplochromine, *Pseudocrenilabrus philander*, was found to have an mtDNA genome size of 16,500 bp (de Villiers et al., 1992). Additional characterization of the mtDNA genome size differences observed here will require site mapping and sequencing. Such analyses are now in progress.

Rapidity of Malawi haplochromine radiation.—The mtDNA results presented here provide further evidence that the genetic divergence between morphologically distinct taxa is minute. Sequence divergence among genera was found to be quite low compared to other fish species (Avise et al., 1987) including estimates among tilapiine cichlids (Seyoum and Kornfield, 1992). Only the Victoria haplochromines are comparable to Malawi in having high levels of morphological divergence while lacking proportional genetic divergence (Dorit, 1990; Meyer et al., 1990). In Lake Malawi, the deep-water *Lethrinops gossei* is both ecologically and morphologically distinct from the shallow-water, algae grazing *Cyathochromis obliquidens*, yet the two species differ by less than 0.4% in mtDNA sequence. These dramatic shifts in morphology with very little genetic divergence are indications of the evolutionary plasticity that characterizes the haplochromine cichlids (Liem, 1973). This evolutionary plasticity underscores one of the most interesting questions of the haplochromine radiation in East Africa: how is such striking morphological diversity obtained with so little genetic divergence? The striking morphological divergence and parallelism, evident in the phylogenetic position *Alticorpus*, *Aulonocara*, and *Lethrinops* indicates that morphologic divergence is a poor indicator of genetic distance and evolutionary time even within the closely related members of the Malawi fauna.

In general, parallelism effectively obscures

phylogenetic patterns. This situation presents acute problems for phylogenetic analyses based on morphology alone, particularly for the determination of generic relationships. Indeed, the designation of genera in the East African Lakes has been highly problematic (Greenwood, 1980; Hoogerhound, 1984). Eccles and Trewavas (1989) emphasized the "problem of parallelism" and indicated that, in Lake Malawi, abundant parallelism, superimposed on an extensive radiation have "stretched to the limit" a cladistic approach to phylogenetic reconstruction using purely anatomical data. Although in principle, parallelism is less of a problem with mtDNA restriction data, the apparent rapidity of the radiation in Malawi (Kornfield, 1978) has resulted in mtDNA analysis being stretched to its limit as well. Although higher order relationships are clearly evident (e.g., between Malawi A and B lineages), the affinities of taxa within those major groups remain unclear because of limited genetic divergence.

Monophyly of Lake Malawi haplochromines?—Historically, there has been a consistent question regarding monophyly of the Lake Malawi haplochromines and whether multiple colonizations might explain some of the observed morphological diversity (Mayr, 1963; Fryer and Iles, 1972; Greenwood, 1979). There is now considerable evidence that the endemic Malawi species are monophyletic with respect to the other African Great Lakes (Kornfield et al., 1985; Meyer et al., 1990; Kornfield, 1991). Our mtDNA restriction data are consistent with this view because the taxa we examined as outgroups from Lakes Victoria and Tanganyika fell outside the Malawi flock. However, if a rate of sequence divergence of less than 1% per million years is assumed (Bentzen et al., 1989; E. Bermingham, unpubl.), the level of sequence divergence between the most distant mtDNA lineages within Lake Malawi (4.1%) would substantially predate the formation of the lake, estimated at 1–2 Ma (Banister and Clark, 1980). Either Lake Malawi was colonized by a polymorphic ancestor, or the major lineages identified here had diverged before their invasion of the Lake. Thus, depending on how the mtDNA "molecular clock" is calibrated (see below), the Malawi cichlids may not be monophyletic. Indeed, the possibility cannot be excluded that a sister group to one or more of the Malawi lineages remains to be found outside the lake. However, even if these lineages existed prior to the formation of Lake Malawi, it is clear that the vast bulk of the endemic cichlid radiation occurred relatively recently within the lake.

It must be recognized that the use of sequence divergence for the estimation of time is highly problematic (Moritz and Hillis, 1990). Rates of sequence divergence do not appear to be constant across distantly related taxa (Vawter and Brown, 1986). In the absence of four-point calibration in closely related taxa, conclusions drawn from estimated sequence divergence regarding time since reproductive isolation must be considered tentative. However, even in the absence of calibration, accurate estimates of relative divergence times can be obtained among closely related taxa (Hillis and Moritz, 1990). Among closely related taxa, the molecular genetic processes which control the rate of sequence evolution are likely to be similar. It is, therefore, reasonable to assume that the rate of sequence divergence is nearly constant, and that the topology of our phylogenetic reconstruction is unlikely to have been influenced by rate differences. Differing branch lengths in Figure 3 probably result from the stochastic distribution of character state changes rather than from real differences in the processes which underlie rates of sequence evolution.

An additional concern in the use of mtDNA for systematic studies is that stochastic extinction of mtDNA lineages may confound phylogenetic reconstruction. Typically, this occurs in groups of species that have been isolated recently from a polymorphic ancestor. This phenomenon may produce a gene tree which is substantially different than the "true" species tree (Avise et al., 1984; Nei, 1987). In such cases, it is inappropriate to use mtDNA lineages as proxies for species. Only when sibling species reach a state of reciprocal monophyly can a single DNA sequence (e.g., mtDNA) be used effectively for phylogenetic reconstruction (Moran and Kornfield, 1993).

It is highly unlikely that the phylogenetic position of *Alticorpus, Aulonocara,* and *Lethrinops* suggested in this study resulted from either stochastic lineage extinction or the retention of ancestral polymorphism. If these species had, in fact, been isolated from the mbuna for as long a period of time as have the nonmbuna species, it would be expected that sequence divergence between the deep-water species and the mbuna would be greater than the divergence within the mbuna. Clearly, this is not the case because divergence between many pairs of mbuna haplotypes is over 1.0%; yet many species of mbuna differ from the deep-water taxa by less than 0.4%. This pattern could still be explained by a series of very recent mtDNA lineage extinctions. Under this scenario, the shared ancestor of the mbuna and nonmbuna

would have been polymorphic for A and B mtDNA lineages. The A lineage would have been lost in all the mbuna species, the B lineage would have become extinct in all the nonmbuna species except the deep-water taxa, and, finally, the A lineage would have been lost in the deep-water species. We reject this hypothesis as less parsimonious and suggest instead that rapid and striking morphological divergence characterizes the species of *Alticorpus*, *Aulonocara*, and *Lethrinops*.

Phylogenetic reconstruction may also be obscured by hybridization that results in the transfer of divergent mtDNA genomes between previously isolated taxa. However, hybridization is unlikely to explain the phylogenetic position of *Alticorpus*, *Aulonocara*, and *Lethrinops*; the first two species, in particular, have morphologies that are distinct from the mbuna (Eccles and Trewavas, 1989). The possession of such distinctive morphology along with mtDNA identical to the mbuna could occur if there had been repeated unidirectional back-crossing. However, this possibility appears extremely remote, because (1) in situ hybridization is undocumented among Malawi haplochromines, despite extensive study (Kornfield, 1991); and (2) all these species (except *Aulonocara jacobfreibergi* and *A. nyassae*) inhabit much deeper water and quite different habitat types than the mbuna. This physical separation would have provided, at best, very limited opportunity to interbreed.

Oligotypic lineages may not have radiated.—The fact that some divergent mtDNA haplotypes are restricted to a few taxa (e.g., *Rhamphochromis*, Fig. 4) suggests that some lineages in Malawi have radiated rapidly, producing large numbers of very diverse taxa (e.g., A and B mtDNA lineages) while others have not. It is not clear, however, whether the contrast between the species-rich A and B lineages and the oligotypic lineages represents a fundamental difference in the temporal pattern of radiation or whether it is simply the result of species extinction superimposed on homogeneous speciation rates (Stanley, 1979; Futuyma, 1987). For example, an oligotypic mtDNA lineage such as that carried by *Rhamphochromis* may be restricted to a few species simply because other species have been lost to extinction. *Rhamphochromis* may have been part of an earlier radiation which has since been pared down, leaving a few divergent haplotypes. Under this scenario, species of *Rhamphochromis* other than the one examined here might be expected to exhibit additional mtDNA haplotypes as distinct as the oligotypic lineages identified here.

The existence of the oligotypic mtDNA lineages could also indicate some basic biological difference between members of the clades that have radiated and those that have not. For example, Fryer and Iles (1972) indicated that another phyletically isolated taxon, *Copadichromis mloto* (Fig. 4) is the most pelagic of the utaka, the endemic, pelagic, plantivorous trophic group. Perhaps this life history is less conducive to cladogenesis than is more stenotopic behavior. An alternative possibility is that one or more of these taxa are relatively recent invaders of Lake Malawi and have not yet begun to radiate. If this were the case, it might be expected that sister groups to the oligotypic lineages could be discovered outside the lake.

Further investigation.—In phylogenetic systematics studies, tree topology may be dramatically influenced by the inclusion of additional taxa. However, we expect the results presented here to be relatively robust. We take this view based on the fact that representatives of the most ecologically and morphologically divergent lineages in the lake have been examined. An exception, however, might be the monotypic genus *Lichnochromis*, which was not examined. *Lichnochromis acuticeps* has a distinctive morphology and might indeed carry a unique and divergent mtDNA haplotype. Additional unique and divergent lineages may be found, but it seems unlikely that there are other major lineages that contain numbers of discrete taxa comparable to those observed in the A and B haplochromine groups.

Although a large number of characters are available using mtDNA analysis, their utility is limited because these characters are not independent. Because the mtDNA genome is inherited without recombination, mtDNA can be viewed as a single locus or tight linkage group with many alleles. Thus, if restricted to mtDNA, higher resolution techniques, such as extensive sequencing, are unlikely to provide substantially greater insight than is available through RFLP analysis. Because of the nonindependent nature of mtDNA polymorphism, further resolution of phylogenetic relationships within the Malawi haplochromines will require additional independent molecular markers. Appropriate target sequences might include nontranscribed ribosomal DNA (Federoff, 1979; Phillips et al., 1989; Hillis and Dixon, 1991), introns (IK, unpubl.) or single locus hypervariable sequences (Nakamura et al., 1987). These additional data will be required to obtain a more complete view of the true cladistic relationships among the Malawi haplochromines.

Materials Examined

Most collections were deposited as voucher specimens at the American Museum of Natural History. For three individuals, the entire specimen was used for mtDNA isolation, and there is no accession number. Where specimens were obtained through the aquarium trade, original collection locations (where available) are in parentheses. Six species marked with asterisks (*) are placed with the mbuna based on the results of mtDNA restriction analysis. Bootstrap resampling of cladistic characters indicated that these species were embedded within a monophyletic group containing all the mbuna species examined. Species are represented by a single individual unless otherwise noted.

Lake Malawi mbuna.—*Alticorpus macrocleithrum*—Maldeco Fishery, AMNH 98354; *Aulonocara* "deep water"—Maldeco Fishery, AMNH 98359; *A. jacobfreibergi*—Aquarium trade, AMNH 98364; *A. "minutus"*—Monkey Bay, AMNH 98366; *A. nyassae*—Thumbi Island W., AMNH 98357; *Cyathochromis obliquidens*—Thumbi Island W., AMNH 98353 (n = 2); *Iodotropheus sprengerae*—Aquarium trade (Chinyamwezi); *Labeotropheus fuelleborni*—Aquarium trade (Mbenji Island), AMNH 98345; *Labidochromis freibergi*—Thumbi Island W.; *Lethrinops gossei*—Monkey Bay, AMNH 92713 (n = 2); *Melanochromis auratus*—Aquarium trade (Mbenji Island), AMNH 98359; *Petrotilapia genalutea*—Thumbi Island W., AMNH 98350; *P. tridentiger*—Nkhata Bay, AMNH 98370; *Pseudotropheus elongatus*—Nkhata Bay, AMNH 98367; *P. elongatus* 'slab'—Thumbi Island W., AMNH 98358; *P. heteropictus*—Thumbi Island W., AMNH 92728; *P. zebra* 'BB'—Chilumba Point, AMNH 98369; Nkhata Bay, AMNH 98368; Thumbi Island W., AMNH 98344; *P. zebra* 'cobalt'—Nkhata Bay, AMNH 98371.

Lake Malawi nonmbuna.—*Aristochromis christyi*—Thumbi Island W., AMNH 92466; *Astatotilapia calliptera*—Thumbi Island W., AMNH 98356; *Buccochromis spectabilis*—Chimumbo Bay, AMNH 92645; *Caprichromis orthognathus*—Chimumbo Bay, AMNH 98343; *Champsochromis spilorhynchus*—Thumbi Island W., AMNH 98347; *Chilotilipia rhoadesii*—Chimumbo Bay, AMNH 92642; *Copadichromis eucinostomus*—Cape Maclear, AMNH 92666; *C. mloto*—Chimumbo Bay, AMNH 92717; *Cyrtocara moorii*—Cape Maclear, AMNH 98351; *Dimidiochromis compressiceps*—Aquarium trade, AMNH 98342; *Diplotaxodon* sp.—Monkey Bay, AMNH 92634; *Docimodus evelynae*—Thumbi Island, AMNH 98348; *Hemitilapia oxyrhynchus*—Cape Maclear, AMNH 92628; *Maravichromis anaphrymus*—Cape Maclear, AMNH 92626; *Nimbochromis livingstoni*—Monkey Bay, AMNH 92631; *N. polystigma*—Cape Maclear, AMNH 92629; *Otopharynx ovatus*—Aquarium trade, AMNH 98365; *Placidochromis subocularus*—Cape Maclear, AMNH 98352; *Protomelas kirkii*—Cape Maclear, AMNH 98355; *Rhamphochromis* sp.—Monkey Bay, AMNH 92714; *Serranochromis robustus*—Monkey Bay, AMNH 92729; *Taeniochromis holotaenia*—Chimumbo Bay, AMNH 92647; *Trematocranus placodon*—Cape Maclear, AMNH 92704; *Tyrannochromis macrostoma*—Thumbi Island W., AMNH 92721. Lake Tanganyika: *Cyphotilapia frontosa*—Aquarium trade, AMNH 98366; *Tropheus moorei*—Aquarium trade. Lake Victoria: *Neochromis nigricans*—Aquarium trade, AMNH 98363; *Psammochromis riponianus*—Aquarium trade, AMNH 98361; *Prognathochromis pellegrini*—Aquarium trade, AMNH 98362; *Pytochromis sauvagei*—Aquarium trade, AMNH 98360.

Acknowledgments

The cooperation and interest of the Malawi Department of Fisheries, Malawi Department of Parks, and the National Research Council of Malawi is gratefully acknowledged. S. Grant (Salima, Malawi) provided continuous assistance with fieldwork. We gratefully thank T. Dowling for introducing IK to mtDNA restriction analysis and for conducting preliminary work with African cichlids. J. Felsenstein and D. Swofford provided consultation regarding data analysis. W. Glanz, D. McElroy, and J. Ringo kindly commented on drafts of this manuscript. Preliminary communication of the results of this research was made at the ASIH annual meeting in New York, June 1991. This research was supported by the National Science Foundation (BSR 84-16131), the Smithsonian Oceanographic Sorting Center, and the University of Maine. Manuscript preparation was supported by National Marine Fisheries Service, Northwest Fisheries Science Center, Coastal Zone and Estuarine Studies Division.

Literature Cited

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489–522.

———, J. E. Neigel, and J. Arnold. 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. J. Mol. Evol. 20:99–105.

Banister, K. E., and M. A. Clarke. 1980. A revision of the large *Barbus* (Pisces, Cyprinidae) of Lake Malawi with a reconstruction of the history of the southern African Rift Valley lakes. J. Nat. Hist. 14: 483–543.

Bentzen, P., G. C. Brown, and W. C. Leggett. 1989. Mitochondrial DNA polymorphism, population structure, and life history variation in American shad (*Alosa sapidissima*). Can. J. Fish. Aquat. Sci. 46: 1446–1454.

Bermingham, E., and J. C. Avise. 1986. Molecular zoogeography of freshwater fishes in the southeastern United States. Genetics 113:939–965.

Boulenger, G. A. 1908. Diagnoses of new fishes discovered by Captain E. C. Rhoades in Lake Nyasa. Ann. Mag. Nat. Hist. (8), 2:238–243.

Brown, W. M., M. George, Jr., and A. C. Wilson. 1979. Rapid evolution of mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967–1971.

de Villiers, D. L., E. H. Harley, and A. J. Ribbink. 1992. Mitochondrial DNA restriction enzyme variation in allopatric populations of *Pseudocrenilabrus philander* (Pisces: Cichlidae). S. Afr. J. Sci. 88:96–99.

Dorit, R. L. 1990. The correlates of high diversity in Lake Victoria haplochromine cichlids: a neontological perspective, p. 322–353. *In:* Causes of evolution: a paleontological perspective. R. M. Ross and W. D. Allmon (eds.). Univ. of Chicago Press, Chicago, Illinois.

Dowling, T. E., C. Mortiz, and J. D. Palmer. 1990. Nucleic acids II: restriction site analysis, p. 250–317. *In:* Molecular systematics. D. M. Hillis and C. Moritz (eds.). Sinauer, Sunderland, Massachusetts.

Drouin, J. 1980. Cloning of human mitochondrial DNA in *Escherichia coli*. J. Mol. Biol. 140:15–34.

Eccles D. E., and E. Trewavas. 1989. Malawian cichlid fishes; the classification of some haplochromine genera. Lake Fish Movies, Herten, Germany.

Fedoroff, N. V. 1979. On spacers. Cell 16:697–710.

Felsenstein, J. 1988. PHYLIP (Phylogenetic Inference Package, computer software) Ver. 3.1. Univ. of Washington, Seattle.

Fryer, G., and T. D. Iles. 1972. The cichlid fishes

of the Great Lakes of Africa. Oliver Boyd, Edinburgh, Scotland.

FUTUYMA, D. J. 1986. Evolutionary biology, 2d ed. Sinauer, Sunderland, Massachusetts.

———. 1987. On the role of species in anagenesis. Amer. Nat. 130:465–473.

GREENWOOD, P. H. 1979. Towards a phyletic classification of the genus '*Haplochromis*' (Pisces, Cichlidae) and related taxa. Part I. Bull. Br. Mus. Nat. Hist. (Zool.) 35:265–322.

———. 1980. Towards a phyletic classification of the genus '*Haplochromis*' (Pisces, Cichlidae) and related taxa. Part II; the species from Lakes Victoria, Nabugabo, Edward, George and Kivu. *Ibid.* 39:1–101.

———. 1991. Speciation, p. 86–102. *In:* Cichlid fishes. Behaviour, ecology and evolution. M. H. A. Keenleyside (ed.). Chapman and Hall, New York, New York.

GÜNTHER, A. 1864. Report on a collection of reptiles and fishes made by Dr. Kirk in the Zambesi and Nyassa regions. Proc. Zool. Soc. London 1864:303–314.

GYLLENSTEN, U., D. WHARTON, A. JOSEFSSON, AND A. C. WILSON. 1991. Paternal inheritance of mitochondrial DNA in mice. Nature 352:255–257.

HEDGES, S. B. 1992. The number of replications needed for accurate estimation of the boostrap *P* value in phylogenetic studies. Mol. Biol. Evol. 9:366–369.

HILLIS, D. M., AND M. T. DIXON. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Quart. Rev. Biol. 66:411–453.

———, AND C. MORITZ. 1990. An overview of applications of molecular systematics, p. 502–515. *In:* Molecular systematics. D. M. Hillis and C. Moritz (eds.). Sinauer, Sunderland, Massachusetts.

———, J. J. BULL, M. E. WHITE, M. R. BADGETT, AND J. J. MOLINEUX. 1992. Experimental phylogenetics: generation of a known phylogeny. Science 255: 589–592.

HOOGERHOUND, R. J. C. 1984. A taxonomic reconsideration of the haplochromine genera *Gaurochromis* Greenwood, 1980 and *Labidochromis* Regan, 1920 (Pisces, Cichlidae). Netherlands J. Zool. 34: 539–565.

KORNFIELD, I. L. 1978. Evidence for rapid speciation in African cichlid fishes. Experientia 34:335–336.

———. 1991. Genetics, p. 103–128. *In:* Cichlid fishes behaviour, ecology and evolution. M. H. A. Keenleyside (ed.). Chapman and Hall, New York, New York.

———, K. R. MCKAYE, AND T. KOCHER. 1985. Evidence for the immigration hypothesis in the endemic cichlid fauna of Tanganyika. Isozyme Bull. 18:76.

LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA, AND J. C. AVISE. 1981. The use of restriction endonucleases to measure DNA sequence relatedness in natural populations. J. Mol. Evol. 17:214–226.

LIEM, K. F. 1973. Evolutionary strategies and morphological innovations: cichlid pharyngeal jaws. Syst. Zool. 22:425–441.

MAYR, E. 1963. Animal species and evolution. Belknap, Cambridge, Massachusetts.

MCELROY, D., P. MORAN, E. BERMINGHAM, AND I. KORNFIELD. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. 83:157–158.

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.

MORAN, P., AND I. KORNFIELD. 1993. Retention of an ancestral polymorphism in the mbuna species flock (Teleostei: Cichlidae) of Lake Malawi. Mol. Biol. Evol. 10:1015–1029.

MORITZ, C., AND D. M. HILLIS. 1990. Molecular systematics: context and controversies, p. 1–10. *In:* Molecular systematics. D. M. Hillis and C. Moritz (eds.). Sinauer, Sunderland, Massachusetts.

———, T. E. DOWLING, AND W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Ann. Rev. Ecol. Syst. 18:269–292.

NAKAMURA, Y., M. LEPPERT, P. O'CONNELL, R. WOLFF, T. HOLM, M. CULVER, C. MARTIN, E. FUJIMOTO, M. HOFF, E. KUMLIN, AND R. WHITE. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. Science 23:1616–1622.

NEI, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York, New York.

———, AND W.-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

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.

PHILLIPS, R. B., K. A. PLEYTE, L. M. VAN ERT, AND S. E. HARTLEY. 1989. Evolution of nucleolar organizer regions and ribosomal RNA genes in *Salvelinus*. Physiol. Ecol. Japan. Spec. Vol. 1:429–447.

REGAN, C. T. 1922. The cichlid fishes of Lake Nyasa. Proc. Zool. Soc. London 1921:675–727.

REINTHAL, P. N. 1990. The feeding habits of a group of herbivorous rock-dwelling cichlid fishes (Cichlidae: Perciformes) from Lake Malawi, Africa. Env. Biol. Fishes 27:215–233.

RIBBINIK, A. J., A. C. MARSH, C. C. RIBBINK, AND B. J. SHARP. 1983. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. S. Afr. J. Zool. 18:149–310.

ROHLF, F. J. 1990. NTSYS: numerical taxonomy and multivariate analysis system Ver. 1.6. Exeter, Setauket, New York.

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

SEYOUM, S. 1989. Stock identification and the evolutionary relationships of the genera *Oreochromis*, *Sarotherodon*, and *Tilapia* (Pisces: Cichlidae) using allozyme analysis and restriction of mitochondrial DNA. Unpubl. Ph.D. diss., Univ. of Waterloo, Waterloo, Ontario.

———, AND I. KORNFIELD. 1992. Taxonomic notes on the *Oreochromis niloticus* subspecies complex (Pisces: Cichlidae), with a description of a new subspecies. Can. J. Zool. 70:2161–2165.

STANLEY, S. M. 1979. Macroevolution: pattern and process. Freeman, San Francisco, California.

SWOFFORD, D. L. 1989. PAUP: phylogenetic analysis

using parsimony Ver. 3.0. Illinois Nat. Hist. Survey, Champaign, Illinois.

———, AND G. J. OLSEN. 1990. Phylogeny reconstruction, p. 411–500. *In:* Molecular systematics. D. M. Hillis and C. Moritz (eds.). Sinauer, Sunderland, Massachusetts.

TREWAVAS, E. 1931. A revision of the cichlid fishes of the genus *Lethrinops*, Regan. Ann. Mag. Nat. Hist. (10), 7:133–153.

———. 1935. A synopsis of the cichlid fishes of Lake Nyasa. *Ibid.* 10:65–118.

VAWTER, L., AND W. M. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. Science 234: 194–196.

WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, AND M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:375–400.

(PM, IK) DEPARTMENT OF ZOOLOGY AND CENTER FOR MARINE STUDIES, UNIVERSITY OF MAINE, ORONO, MAINE 04469; AND (PNR) DEPARTMENT OF BIOLOGY, EASTERN MICHIGAN UNIVERSITY, YPSILANTI, MICHIGAN 48197. PRESENT ADDRESS: (PM) NMFS/CZES, GENETICS, 2725 MONTLAKE BOULEVARD EAST, SEATTLE, WASHINGTON 98112-2097. Submitted 12 March 1992. Accepted 12 April 1993. Section editor: D. G. Buth.

APPENDIX. HAPLOTYPE DESCRIPTION OF MTDNA GENOTYPES OBSERVED IN LAKE MALAWI ENDEMICS AND OTHER EAST AFRICAN CICHLIDS. Descriptions are based on the composite of observed restriction profiles. Restriction endonucleases used included (left to right): *Apa*I, *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*I, *Bst*EII, *Dra*I, *Eco*RV, *Hinc*II, *Hin*dIII, *Hpa*I, *Nci*I, *Sma*I, *Sty*I, *Tha*I, *Xba*I, and *Xho*I. Six species marked with asterisks (*) were previously considered to be distinct from the mbuna. All individuals (including sympatric conspecifics) produced unique haplotypes.

Taxon	Haplotype description																	
Lake Malawi Mbuna																		
*Alticorpus macrocleithrum**	D	D	C	C	C	C	C	C	K	C	C	C	E	B	D	C	A	C
Aulonocara "deep water"*	D	D	C	C	C	C	C	C	C	C	C	C	E	B	D	C	C	C
*A. jacobfreibergi**	D	D	C	C	C	C	C	C	C	C	C	C	G	B	D	C	C	C
*A. "minutus"**	D	D	C	C	C	C	C	C	C	C	C	C	E	B	B	C	A	C
*A. nyassae**	D	B	I	C	C	C	C	C	C	C	C	C	E	B	D	K	M	C
Cyathochromis obliquidens	J	D	D	E	C	C	C	C	C	C	C	C	E	B	D	C	J	C
Iodotropheus sprengerae	D	D	C	E	C	C	C	C	C	C	G	C	E	B	D	C	C	C
Labeotropheus fuelleborni	J	D	D	E	C	C	C	C	C	C	C	C	E	B	D	C	C	C
Labidochromis freibergi	C	C	C	C	C	C	C	B	C	D	C	B	C	C	C	C	C	C
Lethrinops gossei	D	D	G	C	C	C	C	C	C	C	C	C	E	B	D	C	C	C
Melanochromis auratus	F	D	E	E	D	C	C	C	C	C	C	C	I	B	D	C	C	C
Petrotilapia genalutea	N	D	M	C	D	C	C	C	C	C	C	C	E	B	D	C	C	C
P. tridentiger	F	D	F	C	D	C	C	C	E	C	C	C	E	B	D	C	C	C
Pseudotropheus elongatus	C	C	C	C	E	C	C	B	C	C	C	C	C	C	C	C	C	C
P. elongatus 'slab'	C	C	C	C	C	C	C	A	C	C	C	C	C	C	C	C	C	C
P. heteropictus	F	D	M	C	D	C	C	C	C	C	D	C	E	B	D	C	C	C
P. zebra 'BB' Chilumba Point	C	C	C	C	C	C	C	B	D	C	C	C	C	C	G	C	C	C
P. zebra 'BB' Nkhata Bay	C	C	G	C	C	C	C	B	D	C	F	C	C	C	C	C	C	C
P. zebra 'BB' Thumbi Island	E	D	M	C	D	C	C	C	C	C	C	C	C	B	D	C	C	C
P. zebra 'cobalt'	G	C	H	C	C	C	C	B	D	C	C	C	F	C	C	C	C	C
Lake Malawi Nonmbuna																		
Aristochromis sp.	X	E	T	D	F	B	B	D	C	A	E	A	H	C	K	A	C	D
Astatotilapia calliptera	I	G	W	F	J	A	C	C	C	B	I	D	N	B	H	B	G	C
Buccochromis spectabilis	H	E	V	C	F	A	B	E	C	A	E	A	H	C	E	A	C	D
Caprichromis orthognathus	A	N	0	C	C	A	E	E	C	A	E	H	Q	C	F	A	C	C
Champsochromis spilorhynchus	P	O	1	C	C	A	E	C	C	F	M	E	Q	C	F	A	C	D
Chilotilipia rhoadesii	H	E	P	D	C	B	B	C	C	A	E	A	D	C	E	A	C	D
Copadichromis eucinostomus	R	N	5	C	C	A	E	F	C	A	E	H	C	C	Q	A	C	C
C. mloto	B	H	A	E	D	C	C	C	C	C	C	D	L	B	H	E	F	C
Cyrtocara moorii	R	N	5	C	C	A	E	E	C	A	E	H	H	C	P	A	C	C
Dimidiochromis compressiceps	I	E	Q	D	F	A	B	D	C	A	E	A	H	C	F	A	C	D
Diplotaxodon sp.	D	H	Y	E	I	A	C	C	C	C	E	D	L	B	I	A	D	C
Docimodus evelynae	M	E	Z	E	F	A	E	E	F	E	E	E	M	C	E	A	E	D
Hemitilapia oxyrhynchus	H	F	Q	D	F	A	B	D	C	A	E	A	H	C	E	A	C	D
Maravichromis anaphyrmus	I	E	Q	D	F	B	B	D	C	A	E	A	K	C	J	D	C	D
Nimbochromis livingstoni	I	E	Q	D	F	A	B	E	C	A	E	E	P	C	E	A	C	D
N. polystigma	I	E	J	D	F	A	B	E	C	A	E	E	V	C	E	A	C	D
Otopharynx ovatus	I	E	O	E	F	A	B	D	C	A	E	A	H	C	O	A	C	D
Placidochromis subocularus	Q	N	K	C	C	A	E	E	C	A	E	H	R	C	F	A	C	C
Protomelas kirkii	I	M	2	E	F	A	B	E	C	A	E	A	W	B	E	A	C	D
Rhamphochromis sp.	0	I	4	E	H	A	D	C	I	F	E	D	O	F	I	C	C	C
Serranochromis robustus	T	P	L	E	L	E	C	K	J	I	N	K	S	C	T	H	K	B
Taeniochromis holotaenia	I	E	P	D	G	D	E	F	C	A	E	F	K	C	F	A	C	D
Trematocranus placodon	I	E	U	D	F	B	B	E	C	A	E	A	K	C	E	D	C	D
Tyrannochromis macrostoma	O	E	B	D	F	A	B	E	C	A	E	A	H	C	E	A	C	D
Lake Tanganyika																		
Cyphotilapia frontosa	Z	A	$	C	M	I	D	L	B	L	B	J	T	C	U	I	B	E
Tropheus moorei	Y	Q	$	D	C	B	I	M	I	K	A	J	U	E	R	J	L	E