Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae)

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Abstract

Family level molecular phylogenetic analyses of cichlid fishes have generally suffered from a limited number of characters and/or poor taxonomic sampling across one or more major geographic assemblage, and therefore have not provided a robust test of early intrafamilial diversification. Herein we use both nuclear and mitochondrial nucleotide characters and direct optimization to reconstruct a phylogeny for cichlid fishes. Representatives of major cichlid lineages across all geographic assemblages are included, as well as nearly twice the number of characters as any prior family-level study. In a strict consensus of 81 equally most-parsimonious hypotheses, based on the simultaneous analysis of 2222 aligned nucleotide characters from two mitochondrial and two nuclear genes, four major subfamilial lineages are recovered with strong support. Etroplinae, endemic to Madagascar (*Paretroplus*) and southern Asia (*Etroplus*), is recovered as the sister taxon to the remainder of Cichlidae. Although the South Asian cichlids are monophyletic, the Malagasy plus South Asian lineages are not. The remaining Malagasy lineage, Ptychochrominae, is monophyletic and is recovered as the sister group to a clade comprising the African and Neotropical cichlids. The African (Pseudocrenilabrinae) and Neotropical (Cichlinae) lineages are each monophyletic in this reconstruction. The use of multiple molecular markers, from both mitochondrial and nuclear genes, results in a phylogeny that in general exhibits strong support, notably for early diversification events within Cichlidae. Results further indicate that Labroidei is not monophyletic, and that the sister group to Cichlidae may comprise a large and diverse assemblage of percomorph lineages. This hypothesis may at least partly explain why morphological studies that have attempted to place Cichlidae within Percomorpha, or that have tested cichlid monophyly using only "labroid" lineages, have met with only limited success.
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Cichlidae is a species-rich clade of perciform fishes that has attracted much attention from systematists, particularly the "species flocks" of the East African lakes (Kornfield and Smith, 2000), which have been the focus of numerous micro- and macroevolutionary studies (e.g., Seehausen et al., 2003; Verheyen et al., 2003; are but two recent studies investigating the origin of the Lake Victoria cichlids). The current distribution of cichlids is essentially Gondwanan and they have a fossil record extending to the Eocene of Africa (~46 Ma; Murray, 2000). Interestingly, when compared with basal African lineages such as *Heterochromis* and *Tylochromis*, these Eocene fossils are found to share derived features with the remaining African lineages; they appear to be nested well within the African clade (Murray, 2000, 2001), suggesting a significantly older origin for the family.

Despite the great deal of attention that has been focused on this group, we still know little about either the diversification of the family or its placement within Percomorpha. There have been several attempts to reconstruct the familial-level relationships of cichlid fishes using nucleotide characters (see Sparks, 2004a, for a partial list of such studies), but only a single study has

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combined characters from both mitochondrial and nuclear genes (Farias et al., 2000). Although taxonomic sampling was extensive within the Neotropical assemblage, the study of Farias et al. (2000) exhibited limited sampling across all other geographic assemblages [e.g., no Malagasy cichlids were included in their analysis combining morphological and molecular evidence (Farias et al., 2000, fig. 4)]. Taxonomic sampling was limited, partly due to an absence of combinable data sets; many included species were sequenced for either mitochondrial or nuclear genes, not both, with the result that the data sets were considered not wholly combinable by the authors. Farias et al. (2000, fig. 4) contend that their results support a "robust phylogenetic hypothesis for the family", however, outside of the Neotropical assemblage, taxonomic sampling is extremely restricted and many recovered clades are not strongly supported.

Sparks (2004a) only utilized nucleotide characters from mitochondrial genes in a study that focused on recovering relationships among the Malagasy and South Asian cichlid lineages. In that study, a comprehensive familial-level data set could only be assembled using the 16S fragment, which proved insufficient for robustly recovering early intrafamilial divergences; relationships among the major geographic lineages of cichlid fishes were only weakly supported. Sparks (2004a) recovered a monophyletic Malagasy-South Asian assemblage (with only weak support), and the South Asian cichlids were not monophyletic, based solely on the analysis of 16S nucleotide characters. The African and Neotropical lineages were each monophyletic, although the African clade received only weak support (Sparks, 2004a, figs 2,3).

Molecular (and morphological) studies with a focus on recovering early divergences within Cichlidae—those between major Gondwanan assemblages—have met with limited success. All but one study (Farias et al., 2000) have relied on one or two molecular markers, which has proven to be insufficient for robustly recovering higher-level intrafamilial relationships (e.g., Zardoya et al., 1996; Streelman et al., 1998; Farias et al., 1999, 2001; Sparks, 2004a). The combined molecular and total evidence phylogenies of Farias et al. (2000, figs 3, 4) do not include a single ptychochromine cichlid (*Oxylapia*, *Ptychochromis*, and *Ptychochromoides*), and include only two and one etropline (*Paretroplus* and *Etroplus*) species, respectively. No family level hypothesis of cichlid intrarelationships to date, based on equitable family wide taxonomic sampling, has shown strong support for a transoceanic sister-group relationship between major geographic assemblages of cichlid fishes (e.g., African-Neotropical), less the well-supported sister-group relationship that is consistently recovered between the Malagasy (*Paretroplus*) and South Asian (*Etroplus*) etropline lineages (Sparks, 2004a).

Recovering the cichlid sister group has also been problematic, which is as much a result of dogmatic thinking (e.g., assuming monophyly of Labroidei and only including labroid lineages in molecular systematic studies) as it is due to the overall morphological similarity of many percomorph lineages (noted by many researchers, e.g., Stiassny, 1981; Johnson and Patterson, 1993; Johnson, 1993). Monophyly of the suborder Labroidei, an assemblage comprising Cichlidae, Pomacentridae, Embiotocidae, Odacidae, Labridae, and Scaridae, has been hypothesized on the basis of several features of the pharyngeal jaw apparatus (Kaufman and Liem, 1982; Stiassny and Jensen, 1987). Monophyly of the suborder has nevertheless been questioned (e.g., Johnson, 1993; Streelman and Karl, 1997), given that some of these traits are reported to occur outside of the labroid lineages, others are lacking in all members of the assemblage, and outside of features of the pharyngeal jaw apparatus, additional corroborative morphological evidence for their monophyly is lacking (Stiassny and Jensen, 1987; Johnson, 1993; Streelman and Karl, 1997). Results of a recent molecular phylogenetic study likewise suggest that Labroidei is not monophyletic (Streelman and Karl, 1997). Only Stiassny (1982) has looked extensively at non-labroid lineages in the context of placing Cichlidae within Perciformes, and no molecular study of cichlid fishes has presented a robust test of their monophyly, usually limiting outgroups to pomacentrids and embiotocids, or has attempted to place the family within Perciformes. Surprisingly, no family level molecular study of cichlids to date has included a non-perciform outgroup, and almost none have sampled outside of "Labroidei".

Our primary objective was to use multiple nuclear and mitochondrial genes, with markedly different rates of evolution, to recover a well-supported family level phylogeny for Cichlidae. A broad range of both perciform and non-perciform outgroups were included in order to provide a robust test of ingroup monophyly and place Cichlidae within Perciformes, as well as to attempt to recover the cichlid sister group (Table 1). Within the context of this phylogeny, we addressed explicit macroevolutionary questions regarding the diversification and distribution of cichlids. Our approach was explicitly to provide robust tests of the monophyly of the Neotropical or African assemblages by including all major lineages within each respective continental assemblage. It was not our intention to resolve generic-level relationships within these clades, which is a task well beyond the scope of this analysis. Many comprehensive phylogenetic studies have been published that focus specifically on major lineages within these species-rich clades. However, by establishing a robust phylogenetic framework for Cichlidae, finer-scale studies aimed at sorting out the intrarelationships within these geographic assemblages will

Table 1
Collection localities, sources/original citations, and GenBank accession numbers for taxa included in this study

Species or Clade	Locality/Source	16S	COI	Tmo-4C4	Histone H3
Polymixiiformes (Root)					
Polymixia lowei	Smith and Wheeler, 2004	AY538966	AY662744	AY539382	AY539175
Beryciformes					
Hoplostethus mediterraneus	Smith and Wheeler, 2004	AY538968	AY662745	AY539384	AY539177
Non-labroid Perciformes					
Anabantidae					
Ctenopoma acutirostre	Aquarium Trade	AY662702	AY662749	AY662802	AY662878
Badidae					
Badis badis	Aquarium Trade	AY662699	AY662746	AY662799	AY662875
Grammatidae					
Gramma loreto	Smith and Wheeler (2004)	AY539053	AY662751	AY539461	AY539268
Haemulidae					
Haemulon plumieri	Smith and Wheeler (2004)	AY539057	AY662752	AY539465	AY539266
Kyphosidae					
Hermosilla azurea	Los Angeles, California	AY662703	Unavailable	AY662803	AY662879
Moronidae					
Morone saxatilis	Smith and Wheeler (2004)	AY538941	AY662754	AY539454	AY539255
Nandidae					
Polycentropsis abbreviata	Aquarium Trade	AY662705	AY662756	AY662805	AY662881
Pristolepis fasciata	Aquarium Trade	AY662706	AY662757	AY662806	AY662882
Percidae					
Perca flavescens	Smith and Wheeler (2004)	AY539055	AY662755	AY539463	AY539264
Plesiopidae					
Calloplesiops altivelis	Aquarium Trade	AY662701	AY662748	AY662801	AY662877
Polycentridae					
Monocirrhus polyacanthus	Aquarium Trade	AY662704	AY662753	AY662804	AY662880
Serranidae					
Diplectrum formosum	Smith and Wheeler (2004)	AY539048	AY662750	AY539456	AY539257
Sparidae					
Calamus penna	Mid-Atlantic Bight	AY662700	AY662747	AY662800	AY662876
Non-cichlid Labroidei					
Embiotocidae					
Cymatogaster aggregata	Mission Bay, California	AY662711	AY662762	AY662811	AY662887
Embiotoca jacksoni	Mission Bay, California	AY662712	AY662763	AY662812	AY662888
Pomacentridae					
Amblyglyphidodon leucogaster	Australia	Tang unpub.	AY662764	AY662813.	Tang unpub.
Amphiprion polymnus	Aquarium Trade	AY666170	Unavailable	AY662814	AY662889
Abudefduf saxatilis	Tang, 2001	AF285942	AY662765	AY662815	Tang unpub.
Labridae					
Lachnolaimus maximus	Belize	AY662709	AY662760	AY662809	AY662885
Tautoga onitis	Mid-Atlantic Bight	AY662710	AY662761	AY662810	AY662886
Odacidae					
Haletta semifasciata	Australia	AY662708	AY662759	AY662808	AY662884
Scaridae					
Cetoscarus bicolor	Aquarium Trade	AY662707	AY662758	AY662807	AY662883
Cichlidae					
Etroplinae—South Asia					
Etroplus canarensis	Aquarium Trade	AY662713	AY662766	AY662816	AY662890
Etroplus suratensis	Sparks, 2004a	AY263829	AY263870	AY662817	AY662891
Etroplus maculatus	Sparks, 2004a	AY263830	AY263858	AY662818	AY662892
Etroplinae—Madagascar					
Paretroplus dambabe	Sparks, 2004a	AY263822	AY263851	AY662819	AY662893
Paretroplus damii	Sparks, 2004a	AY263827	AY263856	AY662820	AY662894
Paretroplus kieneri "Kinkony"	Sparks, 2004a	AY263827	AY263854	AY662821	AY662895
Paretroplus kieneri "Amparimenidrino"	Lake Amparimenidrino	AY662714	AY662767	Unavailable	AY662896
Paretroplus kieneri "Ravelobe"	Lake Ravelobe	AY263825	AY263855	AY662822	AY662897
Paretroplus kieneri "Amboaboa"	Amboaboa River	AY662715	AY662768	AY662823	AY662898
Paretroplus maculatus	Sparks, 2004a	AY263820	AY263872	AY662824	AY662899
Paretroplus maromandia	Sparks, 2004a	AY263821	AY263852	AY662825	AY662900
Paretroplus menarambo	Sparks, 2004a	AY263823	AY263853	AY662826	AY662901
Paretroplus nourissati	Sparks, 2004a	AY263828	AY263857	AY662827	AY662902
Paretroplus polyactis "North"	Maroansetra	AY662718	AY662771	AY662831	AY662906

Table 1
Continued

Species or Clade	Locality/Source	16S	COI	Tmo-4C4	Histone H3
Paretroplus polyactis "South"	Sparks, 2004a	AY263826	AY263871	AY662828	AY662903
Paretroplus tsimoly	Kamoro River	AY662716	AY662769	AY662829	AY662904
Paretroplus sp. "Mahajamba"	Mahajamba	AY662717	AY662770	AY662830	AY662905
Ptychochrominae—Madagascar					
Oxylapia polli	Sparks, 2004a	AY263817	AY263881	AY662832	AY662907
Paratilapia cf. *bleekeri*	Sparks, 2004a	AY263819	AY263885	AY662833	AY662908
Paratilapia polleni "Nosy Be"	Nosy Be	AY662719	AY263886	AY662834	AY662909
Paratilapia polleni "Ravelobe"	Lake Ravelobe	AY662720	AY662772	AY662835	AY662910
Paratilapia sp. "East"	Sparks, 2004a	AY263818	AY263884	AY662836	AY662911
Paratilapia sp. "Ifasy"	Ifasy River	AY662721	AY662773	AY662837	AY662912
Ptychochromoides betsileanus	Sparks, 2004a	AY263815	AY263882	AY662838	AY662913
Ptychochromoides vondrozo	Sparks, 2004a	AY263816	AY263883	AY662839	AY662914
Ptychochromoides katria	Sparks, 2004a	AY263814	AY263880	AY662840	AY662915
Ptychochromis grandidieri	Sparks, 2004a	AY263811	AY263878	AY662841	AY662916
Ptychochromis inornatus	Sparks, 2004a	AY263812	AY263875	AY662842	AY662917
Ptychochromis oligacanthus "Nosy Be"	Sparks, 2004a	AY263813	AY263873	AY662843	AY662918
Ptychochromis oligacanthus "North-west"	North-west mainland	AY662722	AY662774	AY662844	AY662919
Ptychochromis sp. "Garaka"	Mahanara River	AY662723	AY662776	AY662845	AY662920
Ptychochromis sp. "Makira"	Makira Region	AY662724	AY662775	AY662846	AY662921
Ptychochromis sp. "Sofia"	Sofia River	AY662725	AY662777	AY662847	AY662922
Cichlinae—Neotropics					
Acarichthys heckelii	Aquarium Trade	AY662726	AY662778	AY662848	AY662923
Acaronia nassa	Sparks, 2004a	AY263835	AY263862	AY662849	AY662924
Apistogramma sp.	Aquarium Trade	AY662727	AY662779	AY662850	AY662925
Astronotus ocellatus	Sparks, 2004a	AY263832	AY263859	AY662851	AY662926
Chaetobranchopsis orbicularis	Aquarium Trade	AY662728	AY662780	AY662852	AY662927
Cichla temensis	Aquarium Trade	AY662729	AY662781	AY662853	AY662928
Cichlasoma bimaculatum	Sparks, 2004a	AY263836	AY263863	Unavailable	AY662929
Crenicichla alta	Sparks, 2004a	AY263837	AY263860	AY662854	AY662930
Dicrossus sp.	Aquarium Trade	AY662730	AY662782	AY662855	AY662931
Nandopsis ramsdeni	Aquarium Trade	AY662731	AY662787	Unavailable	AY662932
Pterophyllum scalare	Aquarium Trade	AY662732	AY662783	AY662856	AY662933
Retroculus xinguensis	Aquarium Trade	AY662733	AY662784	AY662857	AY662934
Satanoperca leucosticta	Sparks, 2004a	AY263838	AY263861	Unavailable	AY662935
Teleocichla sp.	Aquarium Trade	AY662734	AY662785	AY662858	AY662936
Tomocichla asfraci	Aquarium Trade	AY662735	AY662786	Unavailable	AY662937
Pseudocrenilabrinae—Africa					
Astatoreochromis alluaudi	Sparks, 2004a	AY263846	AY662788	AY662859	AY662938
Chalinochromis popelini	Sparks, 2004a	AY263844	AY263867	AY662860	AY662939
Diplotaxodon sp.	Sparks, 2004a	AY263843	AY263866	AY662861	AY662940
Etia nguti	Cross River	AY662736	AY662789	AY662862	AY662941
Gobiocichla ethelwynnae	Aquarium Trade	AY662737	AY662790	AY662863	AY662942
Haplochromis simpsoni	Sparks, 2004a	AY263848	AY662791	AY662864	AY662943
Haplochromis sp. "Silver Bullet"	Sparks, 2004a	AY263847	AY662792	AY662865	AY662944
Hemichromis guttatus	Aquarium Trade	AY662738	AY662793	AY662866	AY662945
Heterochromis multidens	Farias et al., 1999, 2000	AF048996	Unavailable	AF113060	Unavailable
Neolamprologus brichardi	Sparks, 2004a	AY263845	AY662794	AY662867	AY662946
Oreochromis esculentus	Aquarium Trade	AY662739	AY662795	AY662868	AY662947
Oreochromis mossambicus	Sparks, 2004a	AY263841	AY263864	AY662869	AY662948
Pelmatochromis nigrofasciatus	Aquarium Trade	AY662740	Unavailable	AY662870	AY662949
Pelvicachromis pulcher	Aquarium Trade	AY662741	AY662796	AY662871	AY662950
Pseudotropheus zebra	Sparks, 2004a	AY263842	AY263865	AY662872	AY662951
Steatocranus tinanti	Aquarium Trade	AY662742	AY662797	AY662873	AY662952
Tylochromis pulcher	Aquarium Trade	AY662743	AY662798	AY662874	AY662953

be feasible (e.g., outgroup choice is "simplified" and monophyly of various included groups is provisionally established).

This study represents the first molecular phylogenetic analysis of cichlid fishes to sample broadly across all major geographic assemblages (viz., the Malagasy and South Asian lineages). It is also noteworthy in that it includes all described species of Malagasy and South Asian cichlids, less *Paretroplus petiti*, which is known only from the formalin-fixed holotype, and

Ptychochromoides itasy, which is known from only four specimens collected nearly 100 years ago and is presumed to be extinct (Sparks, 2004b). Due to recent collecting efforts, we have been able to incorporate a number of Malagasy and South Asian taxa that were not examined by Sparks (2004a), including several new Malagasy species discovered over the past decade, many of which await formal description (Sparks and Stiassny, 2003). Thus, the recovered hypothesis of relationships also serves as a comprehensive, species-level phylogeny for the Malagasy and South Asian cichlid lineages.

Materials and methods

Acquisition of nucleotide sequences

To provide a robust test of cichlid monophyly, as well as to test the monophyly of the suborder Labroidei (*sensu* Kaufman and Liem, 1982), representatives of both perciform ($n = 22$) and non-perciform ($n = 2$) lineages were included as outgroups. In addition to members of all so-called labroid lineages (i.e., cichlids, pomacentrids, embiotocids, odacids, scarids, and labrids), perciform outgroups include families that have been found to share derived features with cichlids based on previous morphological studies (Stiassny, 1981; Sparks, 2001), including haemulids, sparids, moronids, kyphosids, percids, nandids, and plesiopids. The topology is rooted with the polymixiiform, *Polymixia lowei*.

The 65 cichlid taxa analyzed in this study include representatives of all major cichlid lineages that have been recognized in previous family-level phylogenetic analyses based on morphological evidence (e.g., Cichocki, 1976; Oliver, 1984; Stiassny, 1991; Kullander, 1998; Sparks, 2001), as well as all Malagasy and Indian/Sri Lankan (South Asian) species, except *Paretroplus petiti* and *Ptychochromoides itasy*. Based on relationships recovered in morphological and molecular phylogenetic analyses of cichlid fishes (Cichocki, 1976; Stiassny, 1982, 1987, 1990, 1991; Oliver, 1984; Kullander, 1998; Farias et al., 1999; Sparks, 2001, 2003, 2004a; Schliewen and Stiassny, 2003), the lineages included were expressly chosen to provide a rigorous test of the monophyly of the assemblages found on each of the Gondwanan landmasses where cichlids occur (Table 1).

The taxa examined in the present study, along with their region of occurrence and GenBank accession numbers corresponding to the gene fragments sequenced, are listed in Table 1. All of the Malagasy specimens were obtained from field collections made by the authors, Peter Reinthal (UA), Melanie Stiassny (AMNH), Paul Loiselle (New York Aquarium/WCS), Laif Demason, Jean-Claude Nourissat, and Patrick de Rham. The remaining tissue samples were obtained from various collectors, researchers, the aquarium trade, and institutions, as acknowledged in Table 1.

Fragments of two mitochondrial [~530 bp from the large ribosomal subunit (16S) and ~649 bp from cytochrome *c* oxidase subunit I (COI)] and two nuclear genes (~334 bp from the histone H3 fragment and ~506 bp from the Tmo-4C4 fragment) were sequenced. These four genes were chosen specifically because of their markedly different rates of substitution. The two more slowly evolving nuclear (protein coding) genes, histone H3 and Tmo-4C4, were purposely chosen to recover and test higher-level inter- and intrafamilial relationships, whereas the more quickly evolving mitochondrial genes were selected to increase resolution and support for more recent events within Cichlidae.

Fish tissues were preserved in either 70–95% ethanol or stored frozen at −70 °C prior to the extraction of DNA. In a few cases, DNA was extracted from dried specimens (*Astatoreochromis alluaudi* and *Haplochromis simpsoni*). Total genomic DNA was extracted from muscle, liver, or fin clips via the use of a Qiagen Tissue Extraction Kit (QIAamp, QIAquick, or DNeasy Tissue Extraction Kit) following the manufacturer's protocol. PCR was used to amplify the four target segments. Double-stranded amplifications were performed in either 25 or 50 µL volumes containing $1 \times$ PCR buffer, 2 mM MgCl$_2$, 0.2 mM of each dNTP, 0.2–0.5 µL of each primer, 10–1000 ng of genomic DNA (1–2 µL), and 1 µL of Taq polymerase, or a 25 µL volume containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.25 µL of each primer, and 2–5 µL of genomic DNA. To amplify and sequence the 16S fragment, the primers 16S ar-L 5′-CGCCTGTTTATCAAAAACAT-3′ and 16S br-H 5′-CCGGTCTGAACTCAGATCACGT-3′ (Kocher et al., 1989; Palumbi, 1996) were used. To amplify and sequence the cytochrome *c* oxidase subunit I (COI) fragment, the primers LCO1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al., 1994) or Pros1Fwd 5′- TTCTCGACTAATCACAAAGACATYGG-3′ and Pros2Rev 5′-TCAAARAAGGTTGTGTTAGGTTYC-3′ (P. Chakrabarty, pers. comm.) were used. To amplify and sequence the histone H3 fragment, the primers H3-L 5′-ATGGCTCGTACCAAGCAGACVGC-3′ and H3-H 5′-ATATCCTTRGGCATRATRGTGAC-3′ (Colgan et al., 1998) were used. To amplify and sequence the Tmo-4C4 fragment, the primers Tmo-f1 5′-CCTCCGGCCTTCCTAAAACCTCTC-3′, Tmo-f2 5′-ATCTGTGAGGCTGTGAACTA-3′, Tmo-f3 5′-ATCCCCTCAGGAGATTCTGC-3′, Tmo-r1 5′-CATCGTGCTCCTGGGTGACAAAGT-3′, and Tmo-r2 5′- TCCACGTCAAACTCCATCAC-3′ (Streelman and Karl, 1997; Lovejoy, 2000) were used. Amplifications for all fragments were carried out in 30–40 cycles according to the following temperature profile: initial denaturation for 6 min at 94 °C, denaturation for

45–60 s at 94 °C, annealing for 45–60 s at 45–55 °C, and extension for 1–2 min at 72 °C, with an additional terminal extension at 72 °C for 6 min. Double-stranded amplification products were either desalted and concentrated using Qiagen Quick-Spin PCR Purification Columns or an ArrayIt PCR Product Purification Kit (TeleChem International Inc.) using a Beckman BIOMEK 2000 laboratory automated pipetting workstation. When multiple bands were amplified, individual fragments were isolated on 1% agarose gels, excised under UV light, and extracted using a Qiagen Gel Extraction Kit. Both strands of the purified PCR fragments were used as templates and directly cycle-sequenced using the original amplification primers and an ABI Prism Dye Terminator Reaction Kit. The sequencing reactions were electrophoresced on ABI 377, ABI 3700, and ABI 3730xl automated DNA sequencers.

Phylogenetic analyses

For the phylogenetic analysis, 2222 equally weighted nucleotide characters [based on the implied alignment (Wheeler, 2003b)] from the four gene fragments were simultaneously analyzed under the optimality criterion of parsimony. Because we were not able to obtain a tissue sample for *Heterochromis multidens,* we were unable to amplify the COI and histone H3 loci for this taxon. Additionally, we were unable to amplify the Tmo-4C4 locus in four Neotropical cichlids (*Satanoperca leucosticta, Cichlasoma bimaculatum, Nandopsis ramsdeni,* and *Tomocichla asfraci*) and one Malagasy cichlid (*Paretroplus kieneri* "Amparimenidrino"), as well as the COI fragment in *Pelmatochromis nigrofasciatus* (Cichlidae), *Hermosilla azurea* (Kyphosidae), and *Amphiprion polymnus* (Pomacentridae). Base positions corresponding to missing gene fragments are treated as missing data in the parsimony analyses. Missing gene fragments are designated as "unavailable" in Table 1.

The parsimony analysis was conducted using direct optimization (Wheeler, 1996) as implemented in the program POY (Wheeler et al., 2003), and run on the American Museum of Natural History Parallel Computing Cluster. The method of direct optimization was used to avoid any potential biases inherent in standard sequence alignment procedures (e.g., manual alignment), which may not necessarily result in the most-parsimonious topology (Slowinski, 1998). Unlike standard multiple sequence alignment, which is divorced from the search for optimal tree topologies, direct optimization combines alignment and tree-search into a single procedure (i.e., nucleotide homology is dynamic) to produce globally most-parsimonious trees. This is achieved by including insertions and deletions, in addition to transitions and transversions, as forms of character transformation during optimization.

The analysis began by generating 12 random addition sequences (RAS) per random replicate for 17 replicates. These 204 RAS were improved with TBR branch swapping during the searches, an additional round of TBR branch swapping of all trees within 0.5% of the shortest tree(s) found per replicate, and 340 parsimony ratchet replicates (Nixon, 1999; 20 rounds in each of the 17 replicates with ratchetpercent 20 and ratchetseverity 2 or 4). In addition to TBR branch swapping and ratcheting within each replicate, all resulting trees within 1.0% of the shortest trees were examined in an additional round of TBR branch swapping. The random replicates from these initial searches resulted in five equally most-parsimonious trees. These five trees were submitted to POY for further tree searching using the commands iterative pass (Wheeler, 2003a) and exact (Wheeler et al., 2003). This second step of the analysis began by tree fusing (Goloboff, 1999) the five submitted topologies and 20 additional RAS. The resulting trees were submitted to additional analyses including 100 rounds of parsimony ratcheting (ratchetpercent 20, ratchetseverity 2 or 5), and a final round of tree fusing and TBR branch swapping.

The length of the resulting implied alignment (Wheeler, 2003b) was verified in NONA (Goloboff, 1998) and PAUP* (Swofford, 2002). To estimate the "robustness" of the recovered phylogenetic hypotheses, Bremer supports (Bremer, 1988, 1995) were calculated using Tree Rot (Sorenson, 1999) in conjunction with PAUP*, and jackknife resampling analyses were performed using NONA (1000 replications, heuristic searches, 10 random additions per replication), via the WinClada interface (Nixon, 2000).

Results

A combined analysis of the four gene fragments resulted in 81 equally most-parsimonious trees with lengths of 8247 steps [842 phylogenetically informative base positions, consistency index (CI, Kluge and Farris, 1969) of 0.25, and retention index (RI, Farris, 1989) of 0.56 (when uninformative characters are retained)]. A strict consensus topology of these optimal trees (Fig. 1) showed that Cichlidae is monophyletic with strong support. Within Cichlidae, with the exception of the Malagasy cichlids (in their entirety), each of the major continental assemblages is monophyletic [i.e., Madagascar + India/Sri Lanka (etroplines, viz., *Etroplus* + *Paretroplus*); Madagascar (ptychochromines, viz., *Oxylapia, Ptychochromis,* and *Ptychochromoides* + *Paratilapia*); India/Sri Lanka (*Etroplus*); Africa; Neotropics] and receives strong support.

The strict consensus phylogeny has several additional noteworthy features. Etroplinae [*Paretroplus* (Madagascar) + *Etroplus* (India/Sri Lanka)] was recovered as the
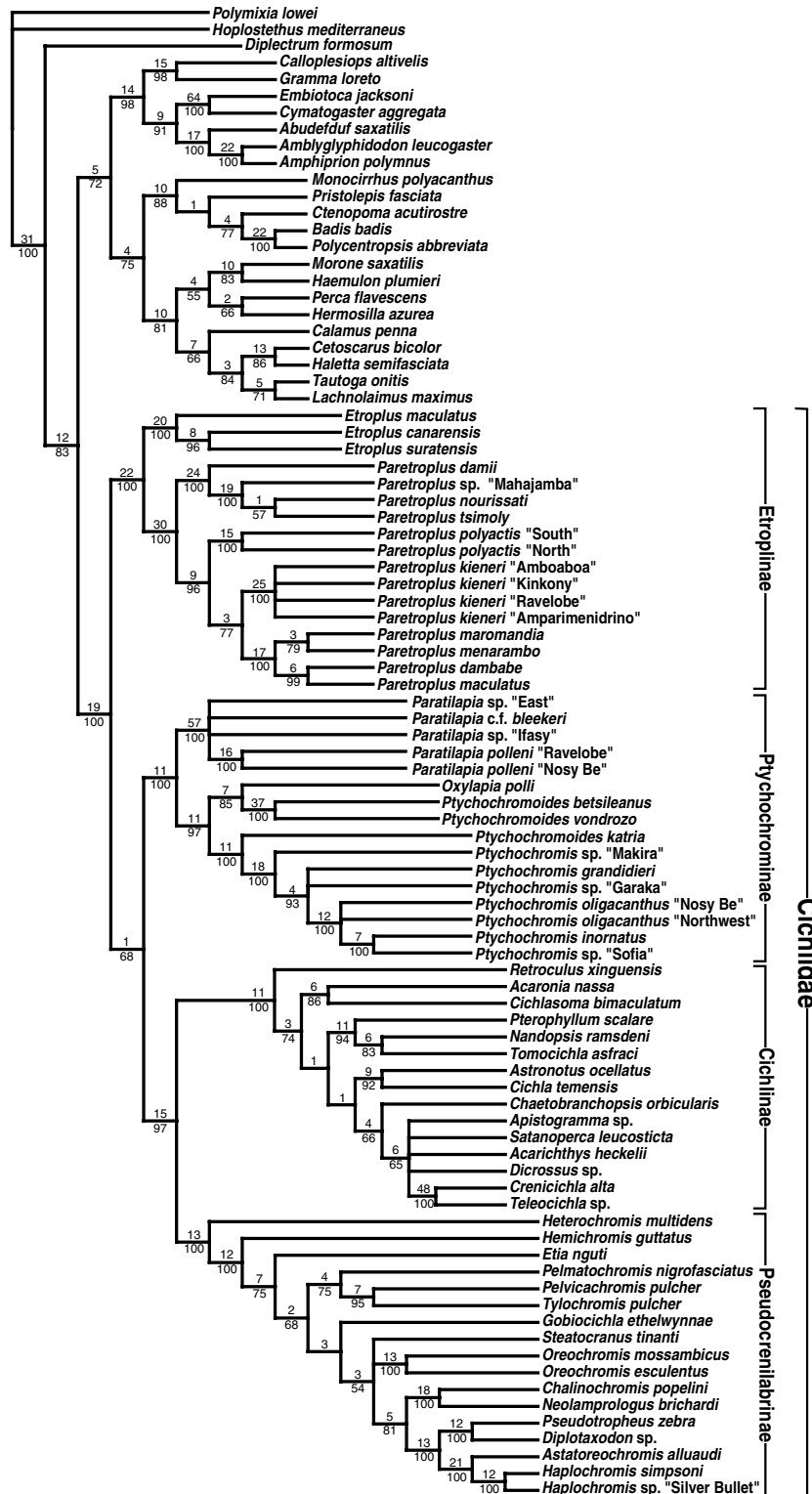
Fig. 1. Strict consensus cladogram of 81 equally most-parsimonious trees recovered (tree length = 8247 steps; CI = 0.25; RI = 0.56; uninformative characters retained) by direct optimization in POY (characters equally weighted) based on simultaneous analysis of mitochondrial 16S and COI, and nuclear histone H3 and Tmo-4C4 nucleotide characters. Numbers above branches represent Bremer support and those below represent jackknife resampling percentages (> 50%) for each recovered node.

sister taxon to the remainder of Cichlidae. As a result, the Malagasy-South Asian cichlids are not monophyletic, but instead they comprised two major (non-sister) clades, Etroplinae and Ptychochrominae (*Oxylapia*, *Ptychochromis*, *Ptychochromoides*, and *Paratilapia*), each of which receives strong support. The ptychochromine genera, including *Paratilapia*, are endemic to Madagascar.

Within Etroplinae, *Paretroplus* is monophyletic and the sister taxon to *Etroplus*; both clades receive strong support. Within *Paretroplus*, three major clades are recovered and each is strongly supported. A clade comprising *P. damii*, *P. nourissati*, *P. tsimoly*, and an undescribed species known informally as *P.* sp. "Mahajamba" is recovered as the sister taxon to the remaining species of *Paretroplus*. A clade comprising four allopatric populations of *P. kieneri* is strongly supported and recovered as the sister group to a well-supported assemblage corresponding to the "deep-bodied clade" of Sparks and Reinthal (1999), and Sparks (2002, 2004a), which includes *P. maromandia*, *P. menarambo*, *P. maculatus*, and *P. dambabe*. *Paretroplus polyactis* is recovered as the sister group to a clade comprising *P. kieneri* and the "deep-bodied clade". Within the endemic South Asian genus *Etroplus*, *E. maculatus* is recovered as the sister taxon to the remaining two congeners, *E. suratensis* and *E. canarensis*.

Paratilapia is monophyletic and this clade receives strong support. *Paratilapia* is robustly recovered as the sister taxon to the ptychochromine lineages of Sparks (2003, 2004a). Within Ptychochrominae, *Oxylapia* is recovered as the sister taxon to a clade comprising *Ptychochromoides betsileanus* and *P. vondrozo*. *Ptychochromoides katria* is recovered as the sister taxon to a monophyletic *Ptychochromis*, which receives strong support. Thus, *Ptychochromoides* is not monophyletic. Relationships within *Ptychochromis* are not fully resolved. A clade comprising members of *Ptychochromis* restricted to north-western basins, *P. oligacanthus* + *P. inornatus* + *P.* sp. "Sofia", is recovered and is nested within a clade that includes members of the genus restricted to the lower to middle reaches of eastern basins, *Ptychochromis grandidieri* and two undescribed species, *P.* sp. "Garaka" and *P.* sp. "Makira".

A sister-group relationship is recovered between Pseudocrenilabrinae (Africa) and Cichlinae (Neotropics), and this clade receives strong support, whereas the node uniting the Malagasy ptychochromine cichlids and a clade comprising the African and Neotropical cichlids receives only weak support. Within Cichlinae, *Retroculus* is recovered as the sister taxon to the remaining Neotropical lineages. The cichlasomine cichlids (= Cichlasomatinae of Kullander, 1998) are not monophyletic. *Cichlasoma* and *Acaronia* comprise a clade that is recovered as the sister taxon to a clade

encompassing the remaining Neotropical lineages, less *Retroculus*. Within this latter clade, the heroine Cichlasomatinae (after Kullander, 1998), here represented by *Nandopsis*, *Pterophyllum*, and *Tomocichla*, are monophyletic. A clade comprising *Astronotus* and *Cichla* is recovered as the sister taxon to a clade comprising the chaetobranchine, geophagine, and crenicichline lineages. *Chaetobranchopsis* is recovered as the sister taxon to the geophagine + crenicichline clade. The crenicichline cichlids (*Crenicichla*, *Teleocichla*) are monophyletic, well supported, and are recovered within a clade that also includes the geophagine lineages, whose relationships remain unresolved.

Within Pseudocrenilabrinae, *Heterochromis* is recovered, with strong support, as the sister taxon to the remaining African lineages. *Hemichromis* is recovered as the sister taxon to the remaining African lineages, less *Heterochromis*, and *Etia*, recently described from West-Central Africa (Schliewen and Stiassny, 2003), is the sister taxon to the remaining African lineages, less *Heterochromis* and *Hemichromis*. *Pelmatochromis*, *Pelvicachromis*, and *Tylochromis* comprise a clade that is recovered as the sister taxon to a clade comprising *Gobiocichla* and the tilapiine, lamprologine, and haplochromine lineages. The lamprologine lineages are recovered as the sister taxon to the haplochromine lineages from lakes Malawi and Victoria that were included in this study.

In summary, all major geographic clades that were recovered [African, Neotropical, Etroplinae (Madagascar-India/Sri Lanka), Ptychochrominae (Madagascar), South Asian (*Etroplus*)] receive strong support, with Bremer supports ranging from 11 to 30 and jackknife percentages of 100%.

Discussion

Cichlid intrafamilial relationships

Based on the simultaneous analysis of all four gene fragments, four major Gondwanan assemblages of cichlid fishes were recovered with strong support: (1) the Malagasy and South Asian etroplines (*Paretroplus* + *Etroplus*); (2) the Malagasy ptychochromines (*Oxylapia*, *Ptychochromis*, *Ptychochromoides*, and *Paratilapia*); (3) the Neotropical lineages; and (4) the African lineages. Herein, we accord subfamilial rank to these four major assemblages (viz., Etroplinae, Ptychochrominae, Cichlinae, and Pseudocrenilabrinae, respectively; Fig. 1). Etroplinae is recovered as the sister taxon to the remaining cichlid lineages. Ptychochrominae is the sister taxon to a clade comprising Cichlinae and Pseudocrenilabrinae. No study to date has been able to simultaneously recover all of these major Gondwanan geographic clades, each with strong support.

A monophyletic African-Neotropical assemblage (Cichlinae + Pseudocrenilabrinae), inclusive of *Heterochromis*, is strongly supported (Fig. 1). Oliver (1984) was the first to recover this clade on the basis of morphological evidence, although neither the African nor Neotropical assemblages were monophyletic in that reconstruction (i.e., *Heterochromis* and *Cichla* formed a polytomy with a lineage comprising the remaining African and Neotropical cichlids). Stiassny (1991) advanced two morphological features in support of monophyly of the African-Neotropical lineage, less *Heterochromis*, which was recovered in a polytomy in that study with *Paratilapia*, Etroplinae, and a lineage comprising the remaining African and Neotropical cichlids. Sparks (2001) recovered a monophyletic African-Neotropical assemblage, which was supported by two unambiguously optimized features, a derived supraoccipital crest morphology and vomer-parasphenoid articulation, but stressed that neither feature was unique (to the African-Neotropical lineage) and unreversed. Likewise, in the reconstruction of Sparks (2001), neither the African nor Neotropical assemblage was monophyletic; *Heterochromis* was recovered as the sister taxon to the remaining African and Neotropical lineages that were examined.

Kullander (1998) partitioned the Neotropical cichlids into six subfamilial lineages, while assigning the African cichlids, less *Heterochromis*, to a single subfamily, Pseudocrenilabrinae. Whereas the study of Kullander (1998) focused on the South American cichlid lineages, and was comprehensive at that level, it did not test the monophyly of the Malagasy, South Asian, or African cichlids, which were poorly represented. Two of Kullander's (1998) proposed subfamilies are not monophyletic in any of his six shortest trees (Astronotinae and Cichlasomatinae); they only appear in his preferred (successively re-weighted) topology (Kullander, 1998, figs 7, 9). In the shortest trees recovered by Kullander (1998, fig. 7), we also note that two additional proposed subfamilies, the Malagasy-South Asian Etroplinae and African Heterochromidinae, are nested inside a non-monophyletic Neotropical assemblage; a monophyletic Neotropical assemblage is likewise not recovered in the preferred (re-weighted) topology (Kullander, 1998, fig. 9).

Conversely, we feel that meaningful subfamilial designations for Cichlidae can only be made in the context of a robust, family level phylogeny, and that a regional study limited in taxonomic scope globally (e.g., Kullander, 1998) provides inadequate tests of the monophyly of poorly represented groups outside the region of focus. In contrast, the four subfamilies we recognize, Etroplinae, Ptychochrominae, Cichlinae, and Pseudocrenilabrinae, have been consistently recovered in recent family level studies based on molecular data (e.g., Farias et al., 2000, fig. 1; Sparks, 2004a, fig. 3; this study, Fig. 1). In addition to strong support from nucleotide characters for all four clades (Table 2), apomorphic morphological features have been presented to diagnose these subfamilies (e.g., Stiassny, 1990, 1991; Sparks and Reinthal, 2001; Sparks, 2001, 2002, 2004a; Stiassny et al., 2001), with the exception of a ptychochromine assemblage (Ptychochrominae) that includes *Paratilapia* and an African assemblage (Pseudocrenilabrinae) that includes *Heterochromis*. We note that these subfamilial names have previously been used in published systematic studies (e.g., Bonaparte, 1840; Fowler, 1934; Kullander, 1998; Sparks, 2002), in the aquarist literature, and in unpublished dissertations (e.g., Cichocki, 1976; Sparks, 2001). Herein, the lineages these subfamilial names encompass are revised in favor of a system based on a robust and stable family level phylogeny (i.e., they have a phylogenetic basis), and in each case they reflect a new usage. In addition, the

Table 2

Nucleotide transformations diagnosing subfamilial lineages of cichlid fishes. We report the diagnostic transformations for the mitochondrial 16S fragment followed by the total number of transformations for each subfamily based on the implied alignment of all four gene fragments. We have explicitly listed the diagnostic 16S transformations because it is the only fragment common to all 89 terminals analyzed, and it is sufficient to diagnose these subfamilial clades. Arrows leading to/from an empty space indicate deletion or insertion events, respectively. Nucleotide position numbers in boldface type indicate unique and unreversed transformations

Clade/transformations	16S nucleotide position and transformation that diagnose cichlid subfamilies									
Cichlinae	21	**138**	219	221	236	323	**358**	**516**		
8 16S transformations	C → T	A → T	A → G	A → T	C → T	G → A	T → C	T → A		
16 total transformations										
Etroplinae	224	**410**	**414**	437	446	455	**458**	516	600	626
10 16S transformations	C →	G →	C →	A → C	A → C	C →	T → C	T → C	C → T	G → A
30 total transformations										
Pseudocrenilabrinae	276	471	473	481	495	601	605	625		
8 16S transformations	A → C	C → T	C → G	T → C	C → T	C → T	C → T	G → A		
12 total transformations										
Ptychochrominae										
2 16S transformations	134	509								
18 total transformations	T → C	A → T								

subfamilial lineages we delimit are biogeographically informative, or in other words, they also have a biogeographical foundation or relevance.

Subfamily Etroplinae (Madagascar/India-Sri Lanka)

Etroplinae [= *Paretroplus* (Madagascar) + *Etroplus* (India/Sri Lanka)] is recovered as the sister taxon to the remainder of Cichlidae (Fig. 1). As a result, the Malagasy-South Asian cichlids are not monophyletic. Sparks (2004a) recovered a monophyletic Malagasy-South Asian assemblage, but support for this clade was not compelling. In the current study, the other Malagasy lineage, Ptychochrominae, is recovered as the sister taxon to the African-Neotropical clade, albeit with only weak support.

Congruent placement of Etroplinae was recovered by Streelman and Karl (1997), based on the analysis of Tmo-4C4 sequences, and Farias et al. (2000), based on the analysis of 16S sequences only, but not in an earlier study (Farias et al., 1999), which was also based only on 16S sequence data and which exhibited a similar taxonomic composition. In this earlier study (Farias et al., 1999), a monophyletic Malagasy-South Asian assemblage was recovered with only weak support. Based on the analysis of morphological features, Sparks (2001) also recovered a monophyletic, albeit weakly supported, Malagasy-South Asian assemblage. In the reconstruction of Sparks (2001), the Malagasy-South Asian cichlids are diagnosed by a single unique and unreversed apomorphic feature: the presence of enlarged exoccipital foramina. However, we note that these foramina are significantly more pronounced in Etroplinae, compared with Ptychochrominae (Stiassny, 1991; Sparks, 2001). Thus, our placement of Etroplinae as the sister taxon to the remainder of Cichlidae is not contradicted by overwhelming morphological evidence (Sparks, 2001).

Morphologically, the etropline cichlids are quite distinct, exhibiting numerous specializations that are absent in all other cichlid lineages (Cichocki, 1976; Sparks, 2001; Stiassny et al., 2001). These apomorphic features include complex paired anterior swim bladder chambers that are lodged in enlarged exoccipital recesses forming a unique otophysic connection, highly modified supraoccipital and exocippital bones of the neurocranium, and specialized ligaments associated with the suspensorium and oral jaws (Cichocki, 1976; Sparks, 2001; Stiassny et al., 2001).

Despite the inclusion of several additional taxa in this study, in general, relationships within Etroplinae are congruent with the hypothesis recovered by Sparks (2004a, fig. 2). *Etroplus*, endemic to southern India and Sri Lanka, is monophyletic and is robustly recovered as the sister taxon to a monophyletic *Paretroplus*, which is endemic to Madagascar. *Etroplus* is diagnosed by a number of apomorphic features including unique tricuspid oral dentition, an elevated number of anal-fin spines, and configuration of the anal-fin pterygiophore/hemal spine complex (Sparks, 2001; Stiassny et al., 2001). Within *Etroplus*, *E. suratensis* and *E. canarensis* are robustly recovered as sister taxa. Although these two species have not been examined in great detail morphologically, due historically to limited specimen availability, they nonetheless share a prominent lateral banding pattern that is absent in *E. maculatus*.

Paretroplus is monophyletic and receives strong support. Numerous morphological synapomorphies corroborate the monophyly of the genus and include (Cichocki, 1976; Sparks, 2001, 2004a; Stiassny et al., 2001): major modifications to the posterior of the neurocranium; rigid, multi-chambered, anterior extensions of the swim bladder with narrow connections to the main chamber; a unique plate-like division of the lacrimal in which the second lateral line canal-bearing bone is elongate; and highly modified spatulate oral dentition. Congruent with the results of Sparks (2004a), a clade of elongate, primarily riverine *Paretroplus* (*P. damii*, *P. nourissati*, *P. tsimoly*, and an undescribed species known informally as *P. n.* sp. "Mahajamba") is recovered as the sister taxon to remaining members of the genus. The deep-bodied, disk-shaped, members of the genus [= "deep-bodied clade" of Sparks and Reinthal (1999), and Sparks (2002, 2004a)], *P. maromandia*, *P. menarambo*, *P. maculatus*, and *P. dambabe*, are monophyletic and this clade receives strong support. Although a suitable tissue sample for molecular studies was lacking, morphological evidence corroborates the placement of *P. petiti* within this assemblage (Sparks, 2002). These deep-bodied cichlids all exhibit restricted distributions in north-western Madagascar, where they typically occur in shallow, turbid oligotrophic floodplain lakes, and most species are threatened by extinction [e.g., *P. menarambo*, discovered and described in the 1990s, is presumed to be extinct as no specimens have been collected in several years (P. de Rham, pers. comm.)]. *Paretroplus polyactis* is the only member of the genus that occurs exclusively in eastern drainages (*P. damii* is widespread in northwestern Madagascar and has recently also been collected from northeastern basins) and is recovered as the sister taxon to a clade comprising *P. kieneri* and the "deep-bodied" *Paretroplus*.

At the species level, relationships within *Paretroplus* are completely resolved. It has been hypothesized that the widely distributed species, *P. kieneri*, comprises a number of "cryptic" species (de Rham and Nourissat, 2002). Slight phenotypic differences exist between the allopatric populations (J.S. Sparks, pers. obs.) of this species, however, based on the analysis of nucleotide characters from four genes, we were unable to resolve intraspecific relationships within *P. kieneri* (Fig. 1).

Subfamily Ptychochrominae (Madagascar)

Based on the results of morphological (Sparks and Reinthal, 2001) and molecular (Sparks, 2003) phylogenetic studies, the ptychochromine cichlids have been delimited to include *Oxylapia*, *Ptychochromis*, and *Ptychochromoides*, all of which are endemic to Madagascar (Sparks, 2004a). Herein, a clade comprising these three Malagasy lineages is also recovered with strong support (Fig. 1). This assemblage was diagnosed by Sparks (2001) and Sparks and Reinthal (2001) on the basis of several derived features which include: bilaterally symmetrical, bicuspid oral dentition in both inner and outer rows; supraneural morphology; and the presence of widely separated median frontal pores of the neurocranium.

To date, *Paratilapia* has not been included in the ptychochromine assemblage; placement of this lineage in previous molecular phylogenetic studies has been problematic (e.g., Sparks, 2004a). Placement of *Paratilapia* was equivocal depending on the gene fragment analyzed in a study using only mitochondrial nucleotide characters (Sparks, 2004a, figs 2, 3). Depending on whether morphological or molecular characters, or a combination of both, were analyzed, *Paratilapia* has been recovered as the sister taxon to the etropline or ptychochromine cichlids (Sparks, 2001; Sparks, 2004a), the sister taxon to a clade comprising *Oxylapia* and *Ptychochromoides* (Sparks, 2004a), the sister taxon to *Paretroplus* (Farias et al., 2001), and as the sister taxon to a clade comprising the etropline and ptychochromine lineages (Sparks, 2001).

Based on the simultaneous analysis of nucleotide characters from both mitochondrial and nuclear genes, in this study *Paratilapia* is robustly recovered as the sister taxon to the ptychochromine cichlids of Sparks (2003, 2004a), and it seems appropriate to include *Paratilapia* within Ptychochrominae (Fig. 1). The placement of *Paratilapia* as the sister group to the ptychochromine lineages, and not Etroplinae, is not contradicted by morphological evidence (Sparks, 2001). *Paratilapia* and the ptychochromine cichlids share morphological specializations (also shared with other cichlid lineages) that are absent in the etropline cichlids, including a derived configuration of the premaxillary-maxillary ligament(s) (Sparks, 2001). Although members of *Paratilapia* appear morphologically more similar to *Oxylapia*, *Ptychochromis*, and *Ptychochromoides* than to the highly modified etropline cichlids, no unique derived morphological features have been identified to unite *Paratilapia* with this ptychochromine lineage.

Relationships within the ptychochromine clade are, for the most part, congruent with results presented by Sparks (2003, 2004a). *Oxylapia* is recovered as the sister taxon to a clade comprising *Ptychochromoides betsileanus* and *P. vondrozo*. A tissue sample suitable for molecular studies was lacking, but based on morphological comparisons, *P. itasy* is hypothesized to be the sister taxon to *P. betsileanus* (Sparks, 2004b). This *Oxylapia* + *Ptychochromoides betsileanus* + *P. vondrozo* clade is in turn the sister taxon to a clade comprising *Ptychochromoides katria* and *Ptychochromis*. *Ptychochromoides katria* is recovered as the sister taxon to a monophyletic *Ptychochromis*, thus rendering *Ptychochromoides* paraphyletic. Establishing monophyly of *Ptychochromoides* based on morphological features has been problematic (Reinthal and Stiassny, 1997; Sparks and Reinthal, 2001), and so our results are not surprising. Sparks (2004a) discussed corroborative morphological evidence for the placement of *Ptychochromoides katria* as the sister taxon to *Ptychochromis*, including a derived pigmentation pattern and a laterosensory canal system on the mandible, preopercle, and neurocranium, characterized by markedly enlarged canals and expanded pores.

Within the clade comprised of *Oxylapia*, *Ptychochromis*, and *Ptychochromoides*, topological differences from results reported by Sparks (2003, 2004a) are restricted to intra-generic relationships within *Ptychochromis*. With the inclusion of additional species of *Ptychochromis* in this study, members of the genus that occur in eastern drainages [i.e., *P. grandidieri*, *P.* sp. "Garaka" (restricted to extreme NE Madagascar), *P.* sp. "Makira" (restricted to NE Madagascar, adjacent to the Masoala Peninsula)] are not monophyletic. Congruent with the results presented by Sparks (2003, 2004a), members of the genus restricted to western drainages (*P. oligacanthus*, *P. inornatus*, and *P.* sp. "Sofia") are monophyletic and this clade receives strong support. Relationships within *Paratilapia* are not fully resolved, and a clear east–west disjunction is not evident (i.e., *Paratilapia polleni* and *P.* sp. "Ifasy" are restricted to north-western basins, whereas *P.* sp. "East" and *P.* cf. *bleekeri* were collected from eastern drainages).

Subfamily Cichlinae (Neotropics)

In our reconstruction, the Neotropical cichlids (= Cichlinae) are monophyletic and this clade is strongly supported (Fig. 1). Nonetheless, corroborative morphological evidence to support monophyly of Cichlinae is lacking. The Neotropical cichlid lineages were not monophyletic in the study of Kullander (1998). In the shortest trees recovered in that study, Etroplinae, Ptychochrominae, and *Heterochromis* were recovered within the Neotropical assemblage (Kullander, 1998, fig. 7). Stiassny (1991) proposed a feature of the vomer-parasphenoid articulation to unite the Neotropical cichlids (also shared with *Heterochromis*), but a monophyletic Neotropical assemblage was not recovered in that study (Stiassny, 1991, fig. 1.20). Likewise, Sparks

(2001) was unable to establish monophyly of the Neotropical cichlids based solely on the analysis of morphological evidence.

In general, relationships within Cichlinae are in agreement with other recent studies that are based on molecular evidence (e.g., Farias et al., 1999, 2000; Sparks, 2004a). *Retroculus* is recovered as the sister taxon to the remaining Neotropical lineages, as it has been in all recent molecular studies. In contrast to results from recent molecular studies (Farias et al., 1999; Sparks, 2004a), the cichlasomine cichlids [Cichlasomatinae of Kullander (1998), i.e., Acaronini, Heroini, and Cichlasomatini] are not monophyletic. In our reconstruction, these lineages comprise two non-sister clades whose composition is congruent with the heroine and cichlasomine (including Acaronini and Cichlasomatini) classifications proposed by Farias et al. (1999), but which correspond neither to the traditional group A and B classifications of Stiassny (1991), nor to the pattern of relationships recovered by Kullander (1998, figs 7, 9). Whereas Kullander's (1998) Cichlasomatinae is not monophyletic in any of the six shortest trees he recovered, his Heroini and Cichlasomatini are monophyletic in all of these reconstructions (Kullander, 1998, fig. 7); a strict consensus was not presented. In the current study, a clade comprising *Astronotus* and *Cichla* is recovered as the sister taxon to a clade including the chaetobranchine, geophagine, and crenicichline lineages. In addition, *Chaetobranchopsis* is recovered as the sister taxon to the (geophagine + crenicichline) clade, a result that is congruent with the total evidence phylogeny of Farias et al. (2000). Also, congruent with results of recent molecular studies (Farias et al., 1999, 2000; Sparks, 2004a), but in contrast to morphology-based analyses (Stiassny, 1982, 1987, 1991; Kullander, 1998) that recover a sister-group relationship between *Cichla* and the crenicichline cichlids (*Crenicichla* and *Teleocichla*), crenicichlines are herein recovered within a clade that also includes the geophagine lineages, whose relationships remain unresolved.

Subfamily Pseudocrenilabrinae (Africa)

A clade (= Pseudocrenilabrinae) comprising the African cichlid lineages, including *Heterochromis*, is recovered and receives strong support (Fig. 1). To date, morphology-based family level studies have not recovered a monophyletic African lineage that included the monotypic Central African cichlid genus *Heterochromis* (e.g., Oliver, 1984; Stiassny, 1991; Kullander, 1998; Sparks, 2001). Nevertheless, a number of apomorphic features have been advanced to unite the African cichlids, less *Heterochromis* (Cichocki, 1976; Oliver, 1984; Stiassny, 1990, 1991; Sparks, 2001), including modified ligaments and muscle attachments involving elements of the oral and pharyngeal jaws, a reduced entopterygoid, and an elongate and uniquely oriented uncinate process of the first epibranchial bone.

Relationships within Pseudocrenilabrinae are for the most part congruent with other recent studies based on the analysis of nucleotide characters (e.g., Farias et al., 1999, 2000; Schliewen and Stiassny, 2003; Sparks, 2004a). *Heterochromis* is recovered, with strong support, as the sister taxon to the remaining African lineages. *Hemichromis* is recovered as the sister taxon to the remaining African lineages, less *Heterochromis*, and *Etia* is the sister taxon to the remaining African lineages, less *Heterochromis* and *Hemichromis*. In contrast, prior molecular studies based solely on mitochondrial nucleotide characters have recovered *Heterochromis* and *Hemichromis* as sister taxa (e.g., Farias et al., 1999; Sparks, 2004a). Like Schliewen and Stiassny (2003), our results indicate that *Pelmatochromis*, *Pelvicachromis*, and *Tylochromis* are closely related, however, based on our evidence, *Hemichromis* is not a member of this clade. This *Pelmatochromis*, *Pelvicachromis*, and *Tylochromis* clade is recovered as the sister taxon to a clade comprising various riverine and tilapiine lineages, the lamprologine cichlids, and the haplochromine lineages. The lamprologine lineages are recovered as the sister taxon to the haplochromine lineages from lakes Malawi and Victoria that were included in this study.

Cichlid sister group

The sister group to Cichlidae is not known. Monophyly of Labroidei, a species-rich assemblage comprising cichlids, pomacentrids (damselfishes), embiotocids (surfperches), labrids (wrasses), odacids, and scarids (parrotfishes), has been hypothesized on the basis of several features of the pharyngeal jaw apparatus (e.g., Kaufman and Liem, 1982; Stiassny and Jensen, 1987); however, the naturalness of the group has been questioned (e.g., Johnson, 1993; Streelman and Karl, 1997). Apart from modifications of the pharyngeal jaws (i.e., pharyngeal jaws capable of manipulating and crushing prey, also called pharyngognathy), Johnson (1993) and Streelman and Karl (1997) contend that there is a lack of corroborative morphological evidence to support labroid monophyly.

Our results, admittedly based on a limited taxonomic sampling ($n = 9$) of labroid lineages, but including members of all labroid families, further indicate that Labroidei is not monophyletic and corroborate the results of Streelman and Karl (1997) (Fig. 1). A clade comprising the labroid families Labridae, Odacidae, and Scaridae is recovered as the sister taxon to Sparidae, and is not the sister group to any of the other putative labroid lineages (i.e., Cichlidae, Embiotocidae, and Pomacentridae). Embiotocids are recovered as the sister group to pomacentrids, and this clade is in turn

recovered, with strong support, as the sister group to a clade comprising plesiopids (roundheads) and grammatids (basslets).

A number of apomorphic features have been advanced to diagnose cichlids (e.g., Cichocki, 1976; Stiassny, 1981; Zihler, 1982; Gaemers, 1984; Oliver, 1984; Kullander, 1998; Sparks, 2001), including several modifications of the branchial arches and associated musculature. When compared broadly within Perciformes, members of a number of families, including pomacentrids, embiotocids, and haemulids, are found to share some of these putatively derived features with cichlids (Stiassny, 1981; Sparks, 2001), which has made identifying the cichlid sister group problematic. Our results indicate, somewhat unexpectedly, that the sister group to cichlids might comprise a large assemblage of diverse perciform lineages (Fig. 1), including but presumably not limited to the other "labroid" lineages, sparids, anabantids-nandids, haemulids, percids, moronids, and kyphosids. We caution that these results should be viewed as tentative, but also stress that they may at least partly explain why recovering the cichlid sister group has proven to be problematic in prior studies.

Biogeography

In our phylogenetic reconstruction, the Malagasy-South Asian cichlids are not monophyletic (Fig. 1).

Sparks (2004a) presented a hypothesis of relationships for cichlid fishes and, based on geological evidence and the recovered phylogenetic pattern, argued that the current distribution of Cichlidae was congruent with Gondwanan vicariance. Although Sparks (2004a) recovered a different pattern of higher-level relationships, in which the Malagasy-South Asian cichlids were monophyletic (albeit weakly supported), the topology presented in Fig. 1 is also congruent with the traditional hypothesis of Gondwanan break up in the Mesozoic (Smith et al., 1994; Storey, 1995; Reeves and de Wit, 2000) (Fig. 2).

This hypothesis posits that Madagascar-India rifted from Africa 165 Ma (Fig. 2A), with motion between Madagascar and Africa terminating ~120 Ma (Rabinowitz et al., 1983; Reeves and de Wit, 2000), at about the same time that India and Antarctica separated (Rabinowitz et al., 1983; Storey et al., 1995; Storey, 1995) (Fig. 2B). South America and Africa began to rift ~130 Ma and were subaerially isolated by the Mid-Cretaceous (~120–100 Ma) (Smith et al., 1994; Storey et al., 1995; Storey, 1995; Hay et al., 1999) (Fig. 2C), whereas Madagascar and India remained close until ~92–84 Ma (Storey et al., 1995; Hay et al., 1999; Torsvik et al., 2000) (Fig. 2D).

Although a pattern of "duplicated geography" (or redundancy) is evident for Madagascar, this does not imply a Malagasy center of origin for Cichlidae and
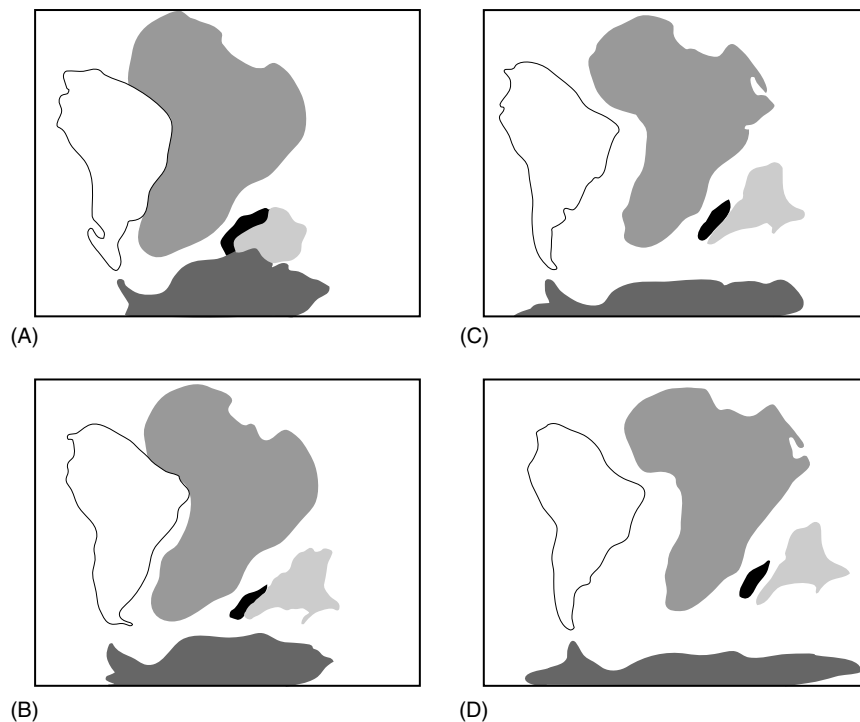
Fig. 2. Temporal sequence depicting major Gondwanan vicariant events occurring during the Cretaceous (after Rabinowitz et al., 1983; Smith et al., 1994; Storey et al., 1995; Storey, 1995; Reeves and de Wit, 2000; Torsvik et al., 2000). (A) at ~130 Ma; (B) at ~120 Ma; (C) at ~100 Ma; and (D) at ~85–80 Ma.

subsequent dispersal (Fig. 1). As Nelson and Ladiges (1996, 2001, p. 395) and Ebach (1999) stress, to assume as much relies on a geographically paralogous (basal) comparison, which is uninformative. Following the reasoning offered by Nelson and Ladiges (2001, p. 395, Fig. 10), for cichlid fishes, the implied vicariant history ("without a center of origin and dispersal therefrom", i.e., minimizing the number of implied dispersal events) can be explained by an initial split of a widespread ancestor, which isolated a population (Etroplinae) in the Madagascar-India block of Gondwana prior to break up of the southern supercontinent. This event was followed by a subsequent split between a (more restricted) population spanning parts of the rift between Madagascar and the Africa + South America landmass. In this scenario, the initial population (Etroplinae) that was isolated in Madagascar was subsequently split when Madagascar and India rifted in the Late Cretaceous. These two clades, Etroplinae (Madagascar + India/Sri Lanka) and (Ptychochrominae (Madagascar) + Africa + South America), exhibit patterns of relationship consistent or congruent with the conventional and well corroborated hypothesis of Gondwanan fragmentation (i.e., sister group relationships are consistent or congruent with an independently derived geological area cladogram; Fig. 3).

Surprisingly, no modern phylogenetic evidence congruent with the conventional hypothesis of Gondwanan fragmentation (Smith et al., 1994; Storey, 1995; Reeves and de Wit, 2000) has been reported for any of Madagascar's extant terrestrial vertebrates (e.g., Yoder et al., 1996; Caccone et al., 1999; Jansa et al., 1999; Mausfeld et al., 2000; Raxworthy et al., 2002; Nagy et al., 2003; Yoder et al., 2003; Yoder and Yang, 2004). Paleontologists (e.g., Krause et al., 1997; Gottfried and Krause, 1998; Murray, 2001), molecular biologists (e.g., Vences et al., 2001), and biogeographers (e.g., Briggs, 2003) contend that Madagascar's freshwater fishes owe their origin to Cenozoic *trans*-oceanic dispersal, well after the Mesozoic break-up of Gondwana. For the most part, these claims have been advanced due to a lack of Cretaceous-age fossils for Madagascar's extant freshwater fish groups [Patterson, 1993a,b; but see Murray (2000) and Sparks (2004a) for discussion regarding placement of the morphologically advanced Eocene-age cichlid fossils, the oldest recovered to date], due to less than expected divergence time estimates based on a molecular "clock" [Vences et al., 2001; see Sparks and Smith (2005) for a critique and alternative interpretation], and due to inferred dispersal capability based on an alleged tolerance to salinity (Myers, 1938; Briggs, 2003).

Interestingly, phylogenetic patterns of relationship that are congruent with the conventional hypothesis of Gondwanan fragmentation (Smith et al., 1994; Storey, 1995; Reeves and de Wit, 2000) are also recovered for Madagascar's two other major clades of freshwater
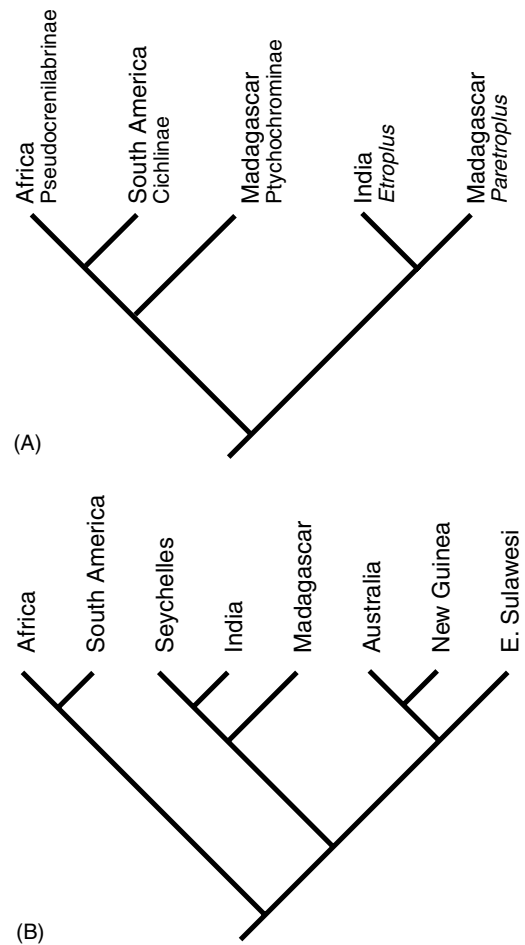
Fig. 3. (A) Taxon-area cladogram of cichlid intrarelationships generated from the strict consensus phylogeny. (B) Geological area cladogram of Gondwanan break up during the Mesozoic (after Rabinowitz et al., 1983; Smith et al., 1994; Storey et al., 1995; Storey, 1995; Reeves and de Wit, 2000; Torsvik et al., 2000).

fishes, aplocheiloid killifishes (Murphy and Collier, 1997) and rainbowfishes (Sparks and Smith, 2004). Along with cichlids, these two clades represent the only freshwater fishes with members present in Madagascar that exhibit a broad Gondwanan distribution (Sparks and Stiassny, 2003), and consequently, that can be used to test a Gondwanan vicariance hypothesis.

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