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## Phylogeny, taxonomy, and evolution of Neotropical cichlids (Teleostei: Cichlidae: Cichlinae)

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### Abstract

Despite recent progress on the higher-level relationships of Cichlidae and its Indian, Malagasy, and Greater Antillean components, conflict and uncertainty remain within the species-rich African, South American, and Middle American assemblages. Herein, we combine morphological and nucleotide characters from the mitochondrial large ribosomal subunit, cytochrome *c* oxidase subunit I, NADH dehydrogenase four, and cytochrome *b* genes and from the nuclear histone H3, recombination activating gene two, Tmo-4C4, Tmo-M27, and ribosomal S7 loci to analyse relationships within the Neotropical cichlid subfamily Cichlinae. The simultaneous analysis of 6309 characters for 90 terminals, including representatives of all major cichlid lineages and all Neotropical genera, resulted in the first well-supported and resolved generic-level phylogeny for Neotropical cichlids. The Neotropical subfamily Cichlinae was recovered as monophyletic and partitioned into seven tribes: Astronotini, Chaetobranchini, Cichlasomatini, Cichlini, Geophagini, Heroini, and Retroculini. Chaetobranchini + Geophagini (including the “crenicichlines”) was resolved as the sister group of Heroini + Cichlasomatini (including *Acaronia*). The monogeneric Astronotini was recovered as the sister group of these four tribes. Finally, a clade composed of Cichlini + Retroculini was resolved as the sister group to all other cichlines. The analysis included the recently described †*Proterocara argentina*, the oldest known cichlid fossil (Eocene), which was placed in an apical position within Geophagini, further supporting a Gondwanan origin for Cichlidae. These phylogenetic results were used as the basis for generating a monophyletic cichline taxonomy.

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Cichlids are a species-rich clade of acanthomorph fishes that have captured the attention of ecologists, ethologists, and micro- and macroevolutionary biologists (Keenleyside, 1991; Barlow, 2000; Kornfield and Smith, 2000). They represent the largest clade of freshwater euteleosts (Nelson, 2006) and exhibit a Gondwanan distribution, with representatives found throughout Africa, South and Middle America (including Texas), Madagascar, India, Sri Lanka, Cuba, Hispaniola, Syria, Israel, and Iran (Stiassny, 1991; Chakrabarty, 2004; Sparks and Smith, 2004). Initial morphology-based phylogenetic work suggested that the Neotropical and African lineages were polyphyletic due to the separation of *Heterochromis* and/or *Cichla* from

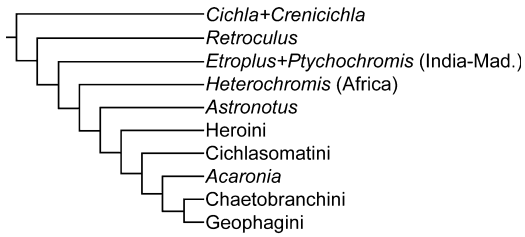
their continental allies (Oliver, 1984; Stiassny, 1987, 1991; Kullander, 1998), but recent family-level work has recovered all continental assemblages, with the exception of the Malagasy lineages, as monophyletic (Farias et al., 1999; Sparks, 2004; Sparks and Smith, 2004). Phylogeny of the Indian and Sri Lankan (Sparks and Smith, 2004; Sparks, 2008), Malagasy (Sparks and Smith, 2004; Stiassny and Sparks, 2006; Sparks, 2008), and Greater Antillean (Chakrabarty, 2006a, 2007) cichlids has been examined in detail, but conflict and uncertainty remain within the species-rich African and Neotropical clades (Kullander, 1998; Farias et al., 2000, 2001; Sparks and Smith, 2004; Fig. 1).

The Neotropical cichlids (Cichlinae *sensu* Sparks and Smith, 2004) or “cichlines” include the Middle American and Greater Antillean “heroines” (15 genera, approximately 110 species) as well as 41 extant genera

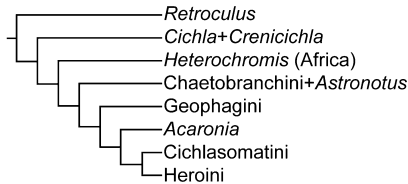
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\*Corresponding author:

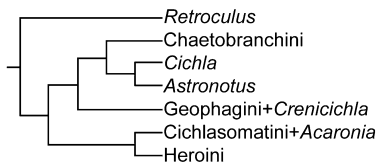
E-mail address: lsmith@fieldmuseum.org



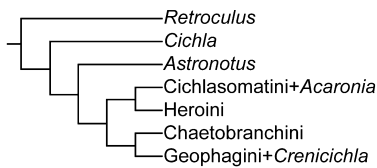
Kullander (1998) - equal weighting



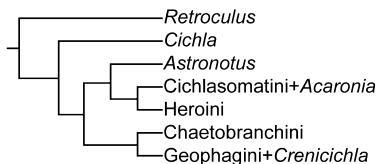
Kullander (1998) - successive weighting



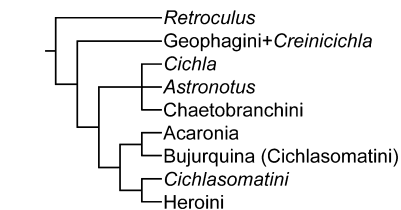
Farias et al. (1999)



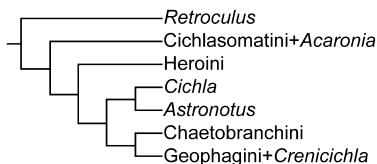
Farias et al. (2000) - total evidence



Farias et al. (2001) - total evidence



Sparks (2004) - 16S only



Sparks and Smith (2004)

and more than 350 species of South American cichlids (Kullander, 2003; Řičan and Kullander, 2006; Chakrabarty and Sparks, 2007; Schmitter-Soto, 2007). The Neotropics are also home to several important fossils (Casciotta and Arratia, 1993), including the recently described Eocene †*Proterocara* from Argentina (Malabarba et al., 2006), which represents the oldest known fossil cichlid.

For most of the 20th century, the taxonomy of cichlins followed Regan (1906). Knowledge of cichlid evolution changed significantly after the completion of Cichocki's (1976) dissertation, which provided the first real alternative to Regan's seminal work. Cichocki's (1976) study advanced our understanding of cichlid and cichline relationships, but the evidential significance and resulting hypothesis of relationships were hampered by their reliance on clique analysis (for discussion see Kullander, 1998). Shortly thereafter, a number of researchers built upon Cichocki's (1976) dataset to further our understanding of cichlid intrarelationships (Oliver, 1984; Stiassny, 1987, 1991); however, these studies emphasized the relationships of the family as a whole, providing little additional information on cichline intrarelationships beyond the placement of *Cichla*. Additionally, a number of influential revisionary and geographical studies were published that provided an incremental refinement of cichlid taxonomy, including the diagnosis and description of several South American genera (Kullander, 1983, 1986, 1988; Kullander and Nijssen, 1989; Kullander and Staeck, 1990). In 1998, our understanding of South American cichlid phylogeny improved dramatically when Kullander published the first generic-level phylogeny of this assemblage. His phylogenetic hypothesis was based on the analysis of a morphological dataset that critically evaluated and incorporated previous phylogenetic and taxonomic characters and included many new characters. Using the results of his successive weighting analysis, Kullander (1998) presented a revised taxonomy of South American cichlids. Subsequently, several molecular studies have provided insights into the relationships within Cichlinae (Farias et al., 1999, 2000, 2001; Sparks, 2004; Sparks and Smith, 2004; Fig. 1). These morphological, molecular, and combined studies have typically recovered monophyletic "chaetobranchines", "cichlasomatines", "geophagines" (often including *Crenicichla*), and "heroines" [including all Central American (except for a few cichlasomatins and geophagins) and Greater Antillean cichlids], but the interrelationships of these clades (all treated at the tribal level hereafter) and the interrelationships and inclusion/exclusion of the genera

Fig. 1. Prior higher-level hypotheses of Neotropical cichlid intrarelationships based on morphological (Kullander, 1998: fig. 9), molecular (Farias et al., 1999: fig. 2; Sparks, 2004: fig. 3; Sparks and Smith, 2004: fig. 1), or combined (Farias et al., 2000: fig. 3, 2001: fig. 7) evidence.

*Acaronia*, *Astronotus*, *Cichla*, *Crenicichla*, and *Retroculus* remain controversial (Fig. 1).

Despite several recurring phylogenetic patterns among cichlins, there are substantive differences between the various hypotheses at suprageneric levels (Fig. 1). However, this inconsistency represents indecisive rather than contradictory data, as evidenced by the limited branch lengths and support measures recovered for the majority of nodes in these analyses. These differences not only affect our phylogenetic understanding of the extant diversity, but the disagreement between morphological, molecular, and combined studies has ramifications for the placement of †*Proterocara*. Given that †*Proterocara* is the oldest known cichlid fossil, its phylogenetic placement is crucial for understanding the evolution and timing the diversification of both Cichlinae and Cichlidae.

## Materials and methods

### Taxon sampling

To provide a robust test of cichline monophyly, two non-cichlid families (Percidae and Embiotocidae), both etropline genera, all five ptychochromine genera, and 17 pseudocrenilabrine genera (including *Heterochromis*) were included as outgroups. The topology was rooted with a percid (*Perca*). The 64 cichline terminals analysed herein included representatives of all Central American, South American, and Greater Antillean cichlid genera, eight additional Neotropical species that have been occasionally or consistently separated from their congeners in phylogenetic analyses [“*Aequidens*” *diadema*, “*A.*” *hoehnei*, “*A.*” *pulcher*, “*A.*” *rivulatus*, “*Cichlasoma*” *festae*, *Geophagus brasiliensis*, *G. steindachneri*, and *Nannacara (Ivanacara) adoketa*], and one Argentine fossil (†*Proterocara*). Due to slight differences in the taxonomic sampling used in this and prior studies (Kullander, 1998; Farias et al., 2000, 2001), we have chosen, when necessary, to combine data from different congeneric species into single generic terminals (Table 1). The use of supraspecific taxa as terminals follows López-Fernández et al. (2005b) and was done with the goals of including data from all prior explicit higher-level cichline phylogenies (i.e. to test previous hypotheses) and increasing resolution at the generic level. To avoid confusion, we use the ending -ine(s) for subfamilies (e.g. “cichlins” for Cichlinae) and the ending -in(s) for tribes (e.g. “cichlins” for Cichlini).

### Molecular sequence data

A total of 6218 aligned nucleotides [based on the implied alignment (Wheeler, 2003a)] from four mitochondrial [large ribosomal subunit (16S), cytochrome

*c* oxidase subunit I (COI), NADH dehydrogenase four (ND4), and cytochrome *b* (Cyt-*b*)] and five nuclear [histone H3 (H3), recombination activating gene two (RAG2), intron one of the S7 ribosomal protein (S7), Tmo-4C4 (4C4), and Tmo-M27 (M27)] gene regions were analysed simultaneously with the morphological data coded in or from Kullander (1990, 1998) and Malabarba et al. (2006). The terminals analysed in the present study and GenBank accession numbers corresponding to the gene fragments sequenced are listed in Table 1. All previously published DNA sequence data analysed in this study were taken from the following studies: Zardoya et al. (1996), Lydeard and Roe (1997), Roe et al. (1997), Streelman and Karl (1997), Martin and Bermingham (1998), Mayer et al. (1998), Song et al. (1998), Bernardi and Bucciarelli (1999), Kumazawa et al. (1999), Seegers et al. (1999), Farias et al. (1999, 2000, 2001), Salzburger et al. (2002a,b), Schlieven and Klee (2004), Smith and Wheeler (2004), Sparks (2004), Sparks and Smith (2004), López-Fernández et al. (2005a,b), Westneat and Alfaro (2005), Chakrabarty (2006a,b), Hulsey et al. (2006), Řičan and Kullander (2006), Schelly et al. (2006), Concheiro-Perez et al. (2007), Higham et al. (2007), and Musilová et al. (2008). Note that the Cyt-*b* sequence of *Paratilapia* sp. from Farias et al. (2001) was excluded because it appears to be a misidentified etropline cichlid (unpublished data).

### Acquisition of nucleotide sequences

Fish tissues were preserved in 70–95% ethanol prior to extraction of DNA. Nuclear and mitochondrial DNA was extracted from muscle or fin clips using a DNeasy Tissue Extraction Kit (Qiagen, Valencia, CA, USA). PCR was used to amplify four gene fragments. Double-stranded amplifications were performed in a 25- $\mu$ L volume containing one Ready-To-Go PCR bead (GE Healthcare, Piscataway, NJ, USA), 1.25  $\mu$ L of each primer (10 pmol), and 2–5  $\mu$ L of undiluted DNA extract. Primers and PCR conditions for novel sequences from the 16S, COI, H3, and 4C4 genes follow Smith and Wheeler (2004) and Sparks et al. (2005).

The double-stranded amplification products were desalted and concentrated using AMPure (Agencourt Biosciences, Beverly, MA, USA). Both strands of the purified PCR fragments were used as templates and amplified for sequencing using the original amplification primers and a Prism Dye Terminator Reaction Kit Version 1.1 (Applied Biosystems, Foster City, CA, USA) with minor modifications to the manufacturer’s protocols. The sequencing reactions were cleaned and desalted using cleanSEQ (Agencourt Biosciences). The nucleotides were sequenced on a 3730XL automated DNA sequencer (Applied Biosystems). Contigs were built in



Table 1  
Continued

Terminal analysed	Molecular	Morphological	16S	COI	Cyt-b	ND4	4C4	H3	M27	S7	RAG2
<i>Cichlasoma (sensu stricto)</i>	<i>C. bimaculatum</i>	<i>C. amazonarum</i>	AY263836	AY263863	AF145128	AY566778	AF113075	AY662929	AF112613	EF432966	AY566747
<i>Cleithracara</i>	<i>C. maronii</i>	<i>C. maronii</i>	EU888045	EU888046	AY050614	N/A	N/A	EU888047	N/A	EF432993	N/A
<i>Krobia</i>	K. sp.	<i>K. sp.</i>	EU888048	EU888049	EF432931	N/A	N/A	EU888050	N/A	EF432961	N/A
<i>Laetacara</i>	<i>L. thayeri</i>	<i>L. jfavlabrasi</i>	EU888051	EU888052	AY050608	N/A	AF113079	EU888053	AF112617	EF433001	N/A
		<i>L. thayeri</i>									
		<i>L. sp.</i>									
<i>Nannacara (sensu stricto)</i>	<i>N. taenia</i>	<i>N. taenia</i>	EU888054	EU888055	EF432921	N/A	N/A	EU888056	N/A	EF432991	N/A
<i>Nannacara (Ivanacara)</i>	<i>N. adoketa</i>	<i>N. adoketa</i>	EF432903	N/A	EF432946	N/A	N/A	N/A	N/A	EF432995	N/A
<i>Tahuantinsuyoa</i>	<i>T. macantatzata</i>	<i>T. macantatzata</i>	EU888057	EU888058	EF432915	N/A	EU888059	EU888060	N/A	EF432983	N/A
Cichlini											
<i>Cichla</i>	<i>C. temensis</i>	<i>C. temensis</i>	AY662729	AY662781	AF370644	AY566793	AY662853	AY662928	U63666	N/A	AY566755
Geophagini											
ACARICHTHYINA											
<i>Acarichthys</i>	<i>A. heckelii</i>	<i>A. heckelii</i>	AY662726	AY662778	AF370653	AY566768	AY662848	AY662923	AF112621	N/A	AY566733
<i>Guianacara</i>	<i>G. sp.</i>	<i>G. sp.</i>	EU888061	EU888062	AF370654	AY566762	AF113084	EU888063	AF112622	N/A	AY566730
		<i>G. owroewéfi</i>									
CRENICARATINA											
<i>Biotoceus</i>	<i>B. dicentrarchus</i>	<i>B. dicentrarchus</i>	EU888064	EU888065	N/A	AY566792	N/A	EU888066	N/A	N/A	AY566754
<i>Crenicara</i>	<i>C. punctulatum</i>	<i>C. punctulatum</i>	EU888067	EU888068	AF370655	N/A	EU888069	EU888070	AF112628	N/A	AY566742
<i>Crenicichla</i>	<i>C. alta</i>	<i>C. lenticulata</i>	AY263837	AY263860	AF370646	AY566785	AY662854	AY662930	AF112625	N/A	AY566750
		<i>C. lepidota</i>									
		<i>C. proteus</i>									
<i>Dicrosuss</i>	<i>D. sp.</i>	<i>D. filamentosus</i>	AY662730	AY662782	N/A	AY566767	AY662855	AY662931	N/A	N/A	AY566731
		<i>D. maculatus</i>									
† <i>Proterocara</i>	N/A	† <i>P. argentina</i>	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>Teleocichla</i>	<i>T. sp.</i>	N/A	AY662734	AY662785	AF370647	N/A	AY662858	AY662936	AF112624	N/A	N/A
GEOPHAGINA											
<i>Apistogramma</i>	<i>A. sp.</i>	<i>A. borellii</i>	AY662727	AY662779	AF370656	AY566787	AY662850	AY662925	AF112633	DQ119272	AY566749
		<i>A. combracae</i>									
		<i>A. regani</i>									
<i>Apistogrammoides</i>	<i>A. pucallpaensis</i>	N/A	EU888071	EU888072	N/A	AY566770	N/A	EU888073	N/A	N/A	AY566735
<i>Biotodoma</i>	<i>B. warrini</i>	<i>B. cupido</i>	EU888074	EU888075	AF370657	AY566784	EU888076	EU888077	AF112620	N/A	AY566726
<i>Geophagus (sensu stricto)</i>	<i>G. megasema</i>	<i>G. altifrons</i>	EU888078	N/A	AF370658	AY566763	AF113093	EU888079	AF112631	N/A	AY566727
		<i>G. brachybranchus</i>									
		<i>G. grammepareus</i>									
		<i>G. harreri</i>									
		<i>G. taeniotoparcus</i>									
<i>Geophagus brasiliensis</i>	<i>G. brasiliensis</i>	<i>G. brasiliensis</i>	EU888080	EU888081	AF370659	AY566766	EU888082	EU888083	AF112626	N/A	AY566732
<i>Geophagus steindachneri</i>	<i>G. steindachneri</i>	<i>G. steindachneri</i>	DQ119188	DQ119217	AF370660	AY566765	DQ119246	EU888084	N/A	DQ119275	AY566730
<i>Gymnogeophagus</i>	<i>G. gymnogynys</i>	<i>G. gymnogynys</i>	EU888085	EU888086	AF370661	AY566775	EU888087	EU888088	AF112623	N/A	AY566738
		N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
<i>Mazarunia</i>	<i>M. mazarunii</i>	<i>M. mazarunii</i>	EU888089	EU888090	N/A	AY566764	AF113089	EU888091	AF112627	N/A	AY566729
<i>Mikrogeophagus</i>	<i>M. altispinosus</i>	<i>M. ramirzei</i>	AY263838	AY263861	AB018986	AY566783	AY662935	AY662935	N/A	N/A	AY566745
<i>Satanoperca</i>	<i>S. leucosticta</i>	<i>S. jurupari</i>	EU888092	EU888093	AF370665	AY566769	AF113094	EU888094	AF112632	N/A	AY566734
<i>Taeniacara</i>	<i>T. candidi</i>	N/A									
Heroini											
<i>Amatitlania</i>	<i>A. nigrofasciata</i>	N/A	DQ119167	DQ119196	DQ990698	N/A	DQ119225	N/A	N/A	DQ119254	N/A
<i>Amphilophus</i>	<i>A. citrinellus</i>	N/A	DQ119169	DQ119198	AY843348	N/A	DQ119227	N/A	U63669	DQ119256	N/A

Table 1  
Continued

Terminal analysed	Molecular	Morphological	16S	COI	Cyt-b	ND4	4C4	H3	M27	S7	RA62
<i>Archocentrus</i>	<i>A. centrarchus</i>	N/A	DQ119162	DQ119163	AF009931	N/A	DQ119164	N/A	N/A	DQ119165	N/A
<i>Australoheros</i>	<i>A. facetum</i>	<i>A. facetum</i>	EU888095	EU888096	AY998666	N/A	N/A	EU888097	N/A	N/A	N/A
<i>Caquetaia</i>	<i>C. spectabilis</i>	<i>C. myersi</i>	EU888098	EU888099	AF370671	N/A	EU888100	EU888101	AF112609	DQ836805	N/A
" <i>Cichlasoma</i> "	<i>C. festae</i>	<i>C. atramaculatum</i>	DQ119187	DQ119216	AY050610	N/A	DQ119245	EU888102	N/A	DQ836812	N/A
<i>festae</i>											
<i>Herichthys</i>	<i>H. carpintis</i>	N/A	DQ119172	DQ119201	DQ990717	N/A	DQ119230	N/A	N/A	DQ119259	N/A
<i>Heroina</i>	<i>H. isonycterina</i>	<i>H. isonycterina</i>	N/A	N/A	AY998670	N/A	N/A	N/A	N/A	N/A	N/A
<i>Heros</i>	<i>H. appendiculatus</i>	<i>H. appendiculatus</i>	DQ119189	DQ119218	DQ010102	N/A	DQ119247	EU888103	AF112605	DQ119276	N/A
<i>Herotilapia</i>	<i>H. multispinosa</i>	N/A	DQ119166	DQ119195	AY843371	N/A	DQ119224	N/A	N/A	DQ119253	N/A
<i>Hoplarichus</i>	<i>H. psittacus</i>	<i>H. psittacus</i>	EU888104	EU888105	AF370673	AY566789	EU888106	EU888107	AF112612	N/A	AY566760
<i>Hypselecara</i>	<i>H. temporalis</i>	<i>H. temporalis</i>	DQ119190	DQ119219	AY050612	N/A	DQ119248	EU888108	AF112611	DQ119277	N/A
<i>Hypophrys</i>	<i>H. nicaraguensis</i>	N/A	DQ119173	DQ118202	AY843370	N/A	DQ119231	N/A	N/A	DQ119260	N/A
<i>Mesonauta</i>	<i>M. festivum</i>	<i>M. festivum</i>	EU888109	EU888110	DQ494392	AY566782	AF113066	EU888111	AF112604	DQ836809	AY566748
<i>Nandopsis</i>	<i>N. ramsdeni</i>	N/A	AY662731	AY662787	AY998668	N/A	DQ119182	AY662932	N/A	DQ119269	N/A
<i>Parachromis</i>	<i>P. mangwenis</i>	N/A	DQ119174	DQ119203	DQ990702	N/A	DQ119232	N/A	N/A	DQ119261	N/A
<i>Paraneotrophus</i>	<i>P. bulleri</i>	N/A	N/A	N/A	AY324004	N/A	N/A	N/A	N/A	N/A	N/A
<i>Petenia</i>	<i>P. splendida</i>	<i>P. splendida</i>	DQ119177	DQ119206	DQ990704	N/A	DQ119235	N/A	AF112608	DQ119264	N/A
<i>Pterophyllum</i>	<i>P. scalare</i>	<i>P. scalare</i>	AY662732	N/A	AF370676	N/A	AY662856	AY662933	AF112603	N/A	N/A
<i>Rocio</i>	<i>R. octofasciata</i>	N/A	DQ119168	DQ119197	AY843410	N/A	DQ119226	N/A	AF112610	DQ119255	N/A
<i>Symphysodon</i>	<i>S. discus</i>	<i>S. aequifasciatus</i>	EU888112	EU888113	AY840119	N/A	AF113069	N/A	AF112607	N/A	N/A
<i>Theraps</i>	<i>T. wesseli</i>	N/A	EU888114	EU888115	AY843384	N/A	N/A	N/A	N/A	N/A	N/A
<i>Thorichthys</i>	<i>T. aureus</i>	N/A	DQ119178	DQ119207	U88859	N/A	DQ119236	N/A	N/A	DQ119265	AY279875
<i>Tomocichla</i>	<i>T. asfraci</i>	N/A	AY662735	AY662786	AF009941	N/A	DQ119237	AY662937	N/A	DQ119266	N/A
<i>Uaru</i>	<i>U. amphiacanthoides</i>	<i>U. amphiacanthoides</i>	DQ119191	DQ119221	AF370678	N/A	DQ119249	EU888116	AF112606	DQ119278	N/A
<i>Vieja</i>	<i>V. synspila</i>	N/A	DQ119180	DQ119209	AY50625	N/A	DQ119238	N/A	N/A	DQ119267	N/A
<i>Retroculini</i>											
<i>Retroculus</i>	<i>R. xinguensis</i>	<i>R. lapidifer</i>	AY662733	AY662784	AF370641	AY566774	AY662857	AY662934	AF112600	N/A	AY566737

Underlined GenBank accession numbers represent sequences for which the analysed species was a different congener from the taxon listed under the molecular heading. All morphological data were taken from Kullander (1998) except †*Proterocara* (data taken from Malabarba et al., 2006) and *Mazarunia* (data coded by the current authors from Kullander, 1990).

N/A, not applicable.

Sequencher (Gene Codes, Ann Arbor, MI, USA) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Sequencher and Bioedit (Hall, 1999). All novel sequences were submitted to GenBank and assigned accession numbers EU888021–EU888116.

### Phylogenetic analyses

For the phylogenetic analysis, the nucleotide characters from the nine gene fragments were combined with the 91 morphological characters identified by Kullander (1998). Two taxa were added to Kullander's (1998) morphological dataset: †*Proterocara*, which was coded by Malabarba et al. (2006), and *Mazarunia*, which was coded herein for all characters that could be scored from its original description (Kullander, 1990). The characters and state (in parentheses) that we were able to code for *Mazarunia* are as follows: 5(0), 7(1), 12(0), 13(1), 15(2), 39(1), 40(0), 41(2), 42(0), 45–46(0), 62(1), 65(0), 66–67(1), 78(3), 79(0), 81–84(0), 85–86(1), 87–91(0). These morphological and molecular data were simultaneously analysed under the optimality criterion of parsimony with equal weights (i.e. morphological transformations, insertions, deletions, transitions, and transversions all given a weight of 1). The parsimony analysis was conducted using direct optimization (Wheeler, 1996) and iterative pass (Wheeler, 2003b) as implemented in the program POY (Wheeler et al., 2003, 2006). Unlike traditional multiple sequence alignment, which is divorced from the search for optimal tree topologies, direct optimization combines alignment and tree-search into a single procedure to produce globally optimal trees.

The analysis began by generating 250 random addition sequences (RAS), followed by tree fusing (Goloboff, 1999), SPR, and TBR branch swapping. The best trees resulting from these analyses were submitted to 100 TBR-ratchet replicates (Nixon, 1999), tree fusing (Goloboff, 1999), and TBR branch swapping. Following the methods in previous studies (Smith and Wheeler, 2006; Smith and Craig, 2007), all equally optimal trees resulting from this analysis were submitted to POY for more exhaustive tree searching using the commands iterative pass (Wheeler, 2003b) and exact (Wheeler et al., 2006). This second step of the analysis consisted of 100 rounds of TBR ratcheting followed by tree fusing and TBR branch swapping.

The length of the resulting implied alignment (Wheeler, 2003a) was verified in PAUP\* (Swofford, 2002). To estimate the “robustness” of the clades recovered in the phylogenetic hypotheses, jackknife percentages (200 replications, five RAS per replicate, using the “emulate jac” option) and Bremer supports (Bremer, 1994; Sorenson, 1999) were calculated in PAUP\* based on the resulting implied alignment with †*Proterocara* and

*Mazarunia* removed. †*Proterocara* and *Mazarunia* were removed for the support calculations because they were coded for only 17 and 28 of the 6309 characters, respectively; the addition of taxa with extensive missing data has been shown to improve phylogenetic estimates, but their addition necessarily reduces support measures (Norell and Wheeler, 2003). Branch length calculations represent unambiguous parsimony transformations only, and they were calculated using the program WinClada (Nixon, 2002).

### Results

The combined analysis of the nine gene fragments and the morphological dataset (6309 characters) for 90 terminals resulted in a single most parsimonious tree that had a length of 19 921 steps. The optimal phylogenetic hypothesis had a consistency index (CI; Kluge and Farris, 1969) of 0.28 and a retention index (Farris, 1989) of 0.43 when uninformative characters were retained and is presented in Fig. 2. The only lack of resolution in the topology involved rearrangements of the clade composed of *Crenicichla*, †*Proterocara*, and *Teleocichla* due to the lack of morphological data for *Teleocichla* and the lack of molecular data for †*Proterocara*. In the 88-taxon support dataset (excluding †*Proterocara* and *Mazarunia*), a single optimal tree was recovered; this tree was identical to the tree in Fig. 2 if †*Proterocara* and *Mazarunia* were removed (see dashed lines in Fig. 2 to note placement of †*Proterocara* and *Mazarunia*). This tree had a length of 19 916 steps. Most of the 85 nodes represented in the 88-taxon support analysis were well supported, with 71 nodes (84%) having a Bremer support  $\geq 5$  and 43 nodes (51%) having a Bremer support  $\geq 10$ . Additionally, 68 nodes (80%) were supported by a jackknife value  $\geq 70$  and 47 nodes (55%) had a jackknife value  $\geq 90$ .

The family Cichlidae was recovered as monophyletic with strong support (jackknife resampling of 100% and Bremer support of 41). Furthermore, the limits and interrelationships of all four subfamilies (Ectoplineae, Ptychochrominae, Cichlinae, and Pseudocrenilabrinae) were well supported and match Sparks and Smith (2004). The higher-level relationships within Cichlinae differed from all previous explicit hypotheses (compare Figs 1 and 2).

We recovered a monophyletic Cichlasomatini (with the inclusion of *Acaronia*), Heroini, Chaetobranchini, and Geophagini (with the inclusion of *Crenicichla*, †*Proterocara*, and *Teleocichla*). Geophagini + Chaetobranchini formed a clade that was recovered as the sister-group of Cichlasomatini + Heroini. *Astronotus* (Astronotini) was resolved as the sister-group to the clade composed of these four cichline tribes



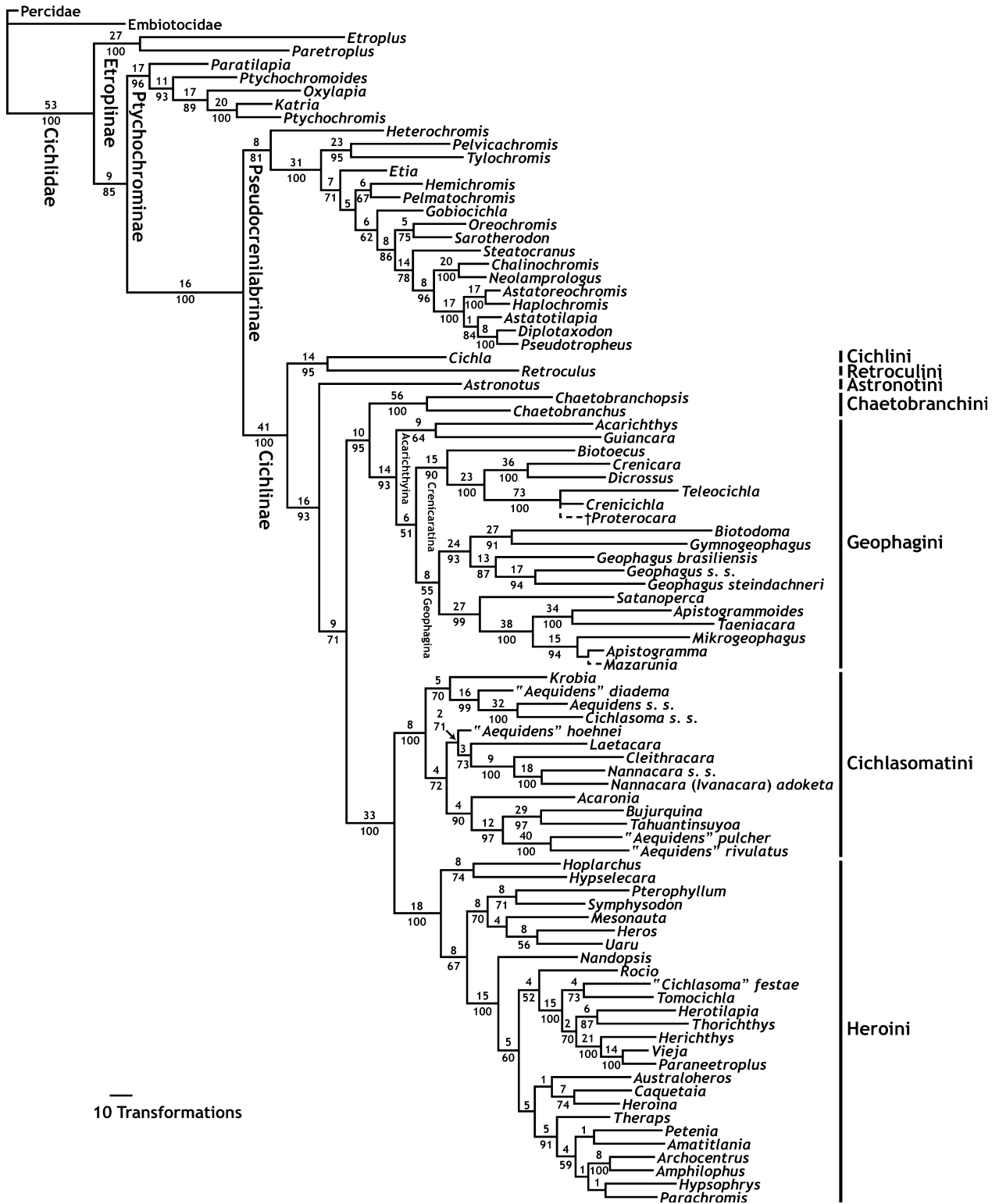


Fig. 2. Single most parsimonious tree with familial, subfamilial, tribal, and subtribal clades identified. Numbers above branches represent Bremer supports and numbers below branches represent jackknife resampling percentages (> 50%) for each resolved node in the 88-taxon support analysis with †*Proterocara* and *Mazarunia* (dashed branches) removed. The abbreviation *s.s.* following *Aequidens*, *Cichlasoma*, *Geophagus*, and *Nannacara* represents *sensu stricto*. Branch lengths represent unambiguous parsimony transformations.

(Chaetobranchini, Cichlasomatini, Geophagini, and Heroini). Finally, we recovered a clade composed of *Cichla* + *Retroculus* as the sister-group to all other cichlins. Our placement of †*Proterocara* within Geophagini differs from Malabarba et al. (2006) who recovered this taxon outside of the Chaetobranchini + Cichlasomatini + Geophagini + Heroini clade.

## Discussion

This study was designed to look specifically at the intergeneric relationships of Neotropical cichlids. In particular, we focused on the limits and relationships of, and within, the cichline tribes and the placement of †*Proterocara*. Furthermore, this study provided an opportunity to refine Kullander's (1998) taxonomy in light of additional taxa and novel morphological and molecular sequence data. Beyond Cichlinae, our results provided an opportunity further to test and re-examine some of the higher-level relationships within Cichlidae. Our results corroborate Sparks and Smith's (2004) phylogeny regarding the limits and relationships of Cichlidae and its four subfamilies. This hypothesis posits that the African Pseudocrenilabrinae and Neotropical Cichlinae are reciprocally monophyletic and sister taxa. The results also support the Malagasy Ptychochrominae as this African–Neotropical clade's sister group and the Malagasy–Indian Etroplinae as the sister-group of all other cichlids.

The current study provides an opportunity to test some of the more contentious problems in cichlid phylogenetics (e.g. placement of *Cichla* and *Heterochromis* within the cichlid radiation). Morphological studies (Oliver, 1984; Stiassny, 1991; Kullander, 1998) have excluded *Heterochromis* from Pseudocrenilabrinae. Furthermore, Oliver (1984) excluded *Cichla* from the Neotropical assemblage, placing the genus in an unresolved trichotomy with *Heterochromis* and the combined Neotropical–African lineage (excluding *Cichla* and *Heterochromis*). Stiassny (1987) refuted Oliver's placement of *Cichla* and provided morphological evidence for the placement of *Cichla* in the Neotropical clade. Molecular studies have corroborated Stiassny's (1987) placement of *Cichla* within the Neotropical assemblage and have generally recovered *Heterochromis* within Pseudocrenilabrinae (Farias et al., 1999, 2000; Sparks, 2004; Sparks and Smith, 2004; but see Farias et al., 2001: figs 3–6). As with recent molecular and combined analyses, we recovered the continental lineages, except Madagascar, as monophyletic. Additionally, this study recovered anatomical support for the monophyly of the African cichlids, which has been elusive (Sparks and Smith, 2004). Kullander (1998) suggested that the loss of ceratobranchial four toothplates and the presence of two posterodorsal palatine

wing-lateral ethmoid ligaments would support a monophyletic Pseudocrenilabrinae, a supposition corroborated by the present analysis.

## Cichlinae

Casciotta and Arratia (1993) were the first researchers explicitly to examine relationships within Neotropical cichlids in a cladistic framework; their work included approximately half of the South American genera and focused on the placement of several Tertiary fossils. Kullander (1998) built upon the work of Cichocki (1976), Casciotta and Arratia (1993), and Stiassny (1991). Kullander (1998) noted problems with the prior studies [e.g. the clique analysis of Cichocki (1976), the overemphasis of piscivory-related characters in Casciotta and Arratia (1993)], and he augmented the existing datasets with many new characters and included most South American genera. Kullander (1998) did not include Central American and Greater Antillean heroins in his analysis; this was justified on the view that Central American cichlids essentially represent a single lineage within Heroini (see also Chakrabarty, 2006b). More recent phylogenetic work (Chakrabarty, 2006a; Concheiro-Perez et al., 2007; present study) has continued to support this idea, with the caveat that several taxa (e.g. *Heroina*, *Caquetaia*) represent South American exemplars of this largely Middle American heroin lineage (Kullander, 1998).

In agreement with other molecular or combined morphological and molecular studies (Farias et al., 1999, 2000, 2001; Sparks, 2004; Sparks and Smith, 2004), we have recovered the Neotropical cichlids as monophyletic. This is in contrast to Kullander (1998) whose optimal trees included both Ptychochrominae and *Heterochromis* in the least inclusive clade that included all cichlins (Fig. 1). Our relationships within Cichlinae differ from those of all previous phylogenetic studies. In contrast to previous studies that have typically reported strong re-sampling support (> 70%) for approximately one-quarter of the suprageneric cichline nodes, our results recovered strong support for approximately 80% of the suprageneric nodes.

## *Retroculini*, *Cichlini*, and *Astronotini*

We recovered the genera *Astronotus*, *Cichla*, and *Retroculus* outside of the larger Chaetobranchini + Cichlasomatini + Geophagini + Heroini clade. The separation of these genera from the remainder of the cichlins was also found in the nuclear gene and combined analyses of Farias et al. (2000), whereas all other multi-gene and combined analyses failed to separate these three genera from the remainder of the cichlins. Kullander's (1998) successive weighting

analysis, but not his most parsimonious trees, recovered *Retroculus* sister to a clade composed of *Heterochromis* and the non-*Retroculus* cichlins (Fig. 1). This placement was unorthodox because previous studies (Regan, 1906; Cichocki, 1976) had consistently treated *Retroculus* as a geophagin. Kullander's unexpected placement of *Retroculus* as the sister group of all other cichlins was supported in essentially all subsequent molecular and combined family-level analyses. However, this study recovered a clade comprised of *Cichla* and *Retroculus* sister to the remainder of the cichlins (as did López-Fernández et al., 2005a). Kullander's Cichlinae (*sensu stricto*) also included *Crenicichla*, but the present study, like all molecular (Farias et al., 1999; Sparks, 2004; Sparks and Smith, 2004) and combined (Farias et al., 2000, 2001) analyses, recovered *Crenicichla* and *Teleocichla* within Geophagini (Figs 1 and 2).

Our analyses recovered *Astronotus* sister to all other cichlins (less *Cichla* and *Retroculus*). This placement separates *Astronotus* from Chaetobranchini (namely *Chaetobranchius* and *Chaetobranchopsis*) with which it has historically been allied on the basis of morphological evidence (Regan, 1906; Cichocki, 1976; Stiassny, 1991; Casciotta and Arratia, 1993; Kullander, 1998). However, molecular studies [except the 16S analyses of Farias et al. (1999) and Sparks (2004)] and Kullander's (1998) optimal topology have separated *Astronotus* from Chaetobranchini.

Kullander (1998) treated *Cichla* and *Retroculus* as name-bearing types for cichlid subfamilies. In concordance with Sparks and Smith's (2004) biogeographically informative subfamilial taxonomy, we herein propose that *Cichla* and *Retroculus* be treated as the monogeneric cichline tribes Cichlini and Retroculini, respectively (see Appendix for subfamilial, tribal, and subtribal diagnoses and composition). Also, we propose that *Astronotus* be treated as the monogeneric cichline tribe Astronotini (see Appendix).

#### *Chaetobranchini*

As in all previous multi-gene or combined analyses that included chaetobranchins and geophagins (Farias et al., 2000, 2001; Sparks and Smith, 2004), we recovered Chaetobranchini as monophyletic and sister to Geophagini. Similarly, Kullander's (1998) equally weighted analysis recovered *Chaetobranchius* sister to his included geophagins (minus *Crenicichla*), a placement opposed to the hypothesis recovered in previous studies (Stiassny, 1991; Casciotta and Arratia, 1993) that recovered Chaetobranchini sister to *Astronotus* on the basis of several features, most notably similar microbranchiospine morphology. Herein, we propose that *Chaetobranchopsis* and *Chaetobranchius* be formally treated as the cichline tribe Chaetobranchini (Appendix).

#### *Geophagini (including †Proterocara)*

Kullander (1998), Farias et al. (1999, 2000, 2001), and Sparks and Smith (2004) all examined geophagin relationships, but López-Fernández et al. (2005a,b) provided the most comprehensive phylogeny of geophagin cichlids to date. In addition to the taxa included in "Geophaginae" (our Geophagini) by Kullander (1998), molecular and combined analyses have recovered *Teleocichla* and *Crenicichla* within this assemblage (Farias et al., 1999, 2000; Sparks, 2004; Sparks and Smith, 2004; present study), in contrast to their placement with *Cichla* in previous morphological studies (Stiassny, 1987, 1991; Kullander, 1998). Furthermore, we herein recovered †*Proterocara* deeply nested within Geophagini; this is in contrast to its placement in Malabarba et al. (2006), which suggested that this extinct taxon belongs outside the Chaetobranchini + Cichlasomatini + Geophagini + Heroini clade.

Although our phylogeny shares many similarities with previous hypotheses, there are also many differences, particularly at higher levels within Geophagini. As for cichline relationships generally, this study was the first to recover strong re-sampling support for the majority of suprageneric geophagin clades. Despite various differences between the included and previous phylogenies, there are a number of geophagin clades that are consistently recovered across the diversity of published phylogenies. These include the sister-group pairing of *Crenicichla* and *Teleocichla* (Farias et al., 1999, 2000; Sparks, 2004; Sparks and Smith, 2004), the pairing of *Acarichthys* and *Guianacara* [Kullander, 1998; Farias et al., 1999, 2000; López-Fernández et al., 2005a,b (RAG2 only)], the pairing of *Crenicara* and *Dicrossus* (Kullander, 1998; López-Fernández et al., 2005a,b), and the pairing of *Biotodoma* and *Gymnogeophagus* (Farias et al., 2000, 2001).

Kullander (1998) recognized three tribes within his "Geophaginae". The monophyly of two of these tribes (our Crenicaratina and Geophagina) was not supported in the analyses of López-Fernández et al. (2005a,b). However, the current study largely recovered Kullander's (1998) tribal structure, particularly for the genera analysed by Kullander (1998). The limited changes to Kullander's tribal composition include the incorporation of *Crenicichla*, †*Proterocara*, and *Teleocichla* into the Crenicaratina and the movement of *Mazarunia* from the Crenicaratina to the Geophagina. Of these four genera, only *Crenicichla* was explicitly analysed in Kullander (1998). Herein, the three geophagin clades, first identified and treated as tribes by Kullander (1998), are recognized as the subtribes Acarichthyina, Crenicaratina, and Geophagina (Appendix). Despite essentially recovering the same geophagin clades as Kullander (1998), the current study's subtribal interrelationships differed in that Acarichthyina was recovered as the

sister-group of Geophagina + Crenicaratina, albeit with limited support.

Acarichthyina is the least species-rich geophagin subtribe. *Acarichthys* is monotypic and *Guianacara* has five species (Kullander, 2003; López-Fernández et al., 2006). The monophyly of this subtribe has been corroborated in several molecular and combined studies (Farias et al., 1999, 2000; López-Fernández et al., 2005a,b), and there is ample evidence for the monophyly of *Guianacara* and its separation from its historical ally *Aequidens* (Kullander and Nijssen, 1989; Kullander, 1998; López-Fernández et al., 2006).

The composition of Crenicaratina, as recognized in the present study, differs from Kullander (1998) in that it includes *Crenicichla*, *Teleocichla*, and †*Proterocara* and it excludes *Mazarunia*. *Crenicara* and *Dicrossus* are well diagnosed and each has two species (Kullander and Staek, 1990). Molecular and morphological data have consistently supported a close relationship between *Crenicara* and *Dicrossus* (López-Fernández et al., 2005a, b; present study). Similarly, molecular data have supported a close relationship between *Teleocichla* and *Crenicichla*, but the placement of these genera within Geophagini has varied among studies (Farias et al., 1999, 2000, 2001; Sparks and Smith, 2004; present study). Kullander (1998) intimated a close relationship between *Crenicichla* and *Teleocichla* and suggested that it was likely that *Teleocichla* was nested within *Crenicichla*, so additional phylogenetic research on the species-rich genus *Crenicichla* is required to address the taxonomy of these genera despite the clear support for their monophyly. Finally, there is the placement of the two species in the well-diagnosed genus *Biotoecus* (Kullander, 1989). Following Kullander's (1998) placement of *Biotoecus* with *Crenicara* and *Dicrossus*, only López-Fernández et al. (2005a,b) had tested this hypothesis. The analysis of morphological data in López-Fernández et al. (2005a) supported a close relationship between *Biotoecus* and *Crenicara* + *Dicrossus*, but their molecular and combined analyses (Farias et al., 2000; López-Fernández et al., 2005a,b) have generally suggested a close relationship between *Biotoecus* and *Crenicichla*. Finally, the current study and Malabarba et al. (2006) have suggested a close relationship between †*Proterocara* and *Crenicichla*. Our more apical placement of the Eocene †*Proterocara* within Cichlinae lends support to the idea that cichlids are of Cretaceous age and are considerably older than previously suggested (see also Sparks and Smith, 2004, 2005).

Geophagina is the largest of the geophagin subtribes with nine genera and approximately 121 species. Prior to the current study, none of the published molecular or combined studies has recovered a Geophagina with a composition approximating that of Kullander (1998; his Geophaginae). Additionally, the genus *Geophagus*,

which has been consistently recovered as para- or polyphyletic when *G. brasiliensis* and *G. steindachneri* have been included (Kullander, 1998; Farias et al., 2001; López-Fernández et al., 2005a,b) was recovered as monophyletic and sister to *Biotodoma* + *Gymnogeophagus* in the present study. This assemblage was also recovered in Farias et al. (2000, 2001), but refuted in López-Fernández et al. (2005a,b) who frequently recovered *Crenicara*, *Dicrossus*, and/or *Mikrogeophagus* within this clade. The evidence for the monophyly and diagnoses of *Gymnogeophagus* and *Biotodoma* were discussed by Gosse (1976), Reis and Malabarba (1988), and Reis et al. (1992). In the current study, this *Biotodoma* + *Geophagus* + *Gymnogeophagus* clade was recovered as the sister group of a clade composed of *Satanoperca* and the “dwarf cichlids” (*Apistogramma*, *Apistogrammoides*, *Mazarunia*, *Mikrogeophagus*, and *Taeniacara*). Although there is evidence for the monophyly of the “dwarf cichlids” as a whole, Kullander (1998) suggested that *Taeniacara* and *Apistogrammoides* might be nested within the species-rich genus *Apistogramma*, and he separated *Mazarunia* and *Mikrogeophagus* from the other “dwarf cichlid” genera. Clearly, significant phylogenetic and revisionary work is needed within the “dwarf cichlids” to clarify generic limits. The sister-group pairing of *Satanoperca* and the “dwarf cichlids” has generally been recovered in molecular and combined studies (Farias et al., 1999, 2000, 2001; López-Fernández et al., 2005a,b), except that *Mikrogeophagus* has been typically separated from the other “dwarf cichlids”. López-Fernández et al. (2005b) formally referred to this assemblage (minus *Mazarunia* and *Mikrogeophagus*) as the “*Satanoperca* clade”. Our placement of *Mazarunia* in Geophagina contradicts the findings of Kullander (1990) who suggested that *Mazarunia*, *Crenicara*, and *Dicrossus* formed a clade. Kullander (1990) noted character conflict with his hypothesized relationships and highlighted that *Mazarunia*, *Apistogramma*, *Taeniacara*, *Biotoecus*, *Hemichromis*, *Anomalochromis*, and *Dicrossus* had lost their anguloarticular canal, but that it was present in *Crenicara* and other South American cichlids.

#### *Cichlasomatini*

Following Kullander's (1998) formal recognition of Cichlasomatini, all studies, except Sparks (2004), that have examined the relevant genera have recovered this clade (with the inclusion of *Acaronia*). Although the relationships recovered in the current study differ from Kullander (1998), Marescalchi (2005), and Musilová et al. (2008), there are many similarities including the separation of *Aequidens* (*sensu stricto*) from “*Aequidens*” *hoehnei*, “*A.*” *pulcher*, and “*A.*” *rivulatus* and the sister-group relationship between *Cleithracara* and *Nannacara* (including *Ivanacara*). Similarly, the relationships are

not identical to other molecular or combined analyses (Farias et al., 1999, 2000, 2001; Musilová et al., 2008), but there are more similarities than differences in the recovered relationships. The most comprehensive study of Cichlasomatini to date is Musilová et al. (2008) whose relationships were quite similar to ours. The only major difference is our placement of *Laetacara* sister to the *Cleithracara*–*Nannacara* clade (NIC clade of Musilová et al., 2008). Furthermore, the current study, like previous molecular and combined studies, found comparatively high support for subclades within Cichlasomatini. Herein, we follow Kullander (1998) in recognizing this clade (now including *Acaronia*) as the cichline tribe Cichlasomatini (Appendix).

Cichlasomatini (less *Acaronia*) was separated by Kullander (1983, 1986) and Stiassny (1991) from all other South American cichlids, and it is frequently referred to as the “cichlasomine group b” radiation. Historically, members of this tribe were classified in *Aequidens*, *Cichlasoma*, and *Nannacara*. The genera *Nannacara* and *Cichlasoma* (following its tremendous restriction in Kullander, 1983) have evidence for their monophyly (Kullander, 1983, 1988; Kullander and Prada-Pederos, 1993; but see Musilová et al., 2008). *Aequidens*, however, has consistently been recovered as polyphyletic (Kullander, 1998; Farias et al., 2000; Marescalchi, 2005; Musilová et al., 2008; present study), so additional taxonomic work is clearly warranted. The remainder of the genera (*Bujurquina*, *Cleithracara*, *Krobia*, *Laetacara*, and *Tahuantinsuyoa*) were recently described and diagnosed by Kullander (1986) and Kullander and Nijssen (1989) as part of the ongoing refinement of the generic limits within this tribe. *Ivanacara* was recently described (Römer and Hahn, 2007) for *Nannacara adoketa* and *N. bimaculata*, but phylogenetic evidence supporting the reciprocal monophyly of *Nannacara* and *Ivanacara* remains to be demonstrated. The only phylogenetic studies to include both putative genera (Kullander, 1998; Musilová et al., 2008; present study) recovered the included species as a clade; thus, the evidence necessary to support their separation into two distinct genera is lacking.

### *Heroini*

Among Neotropical cichlid tribes, no group has been explicitly examined as often as the Heroini; however, much of this phylogenetic work has focused on the less species-rich Middle American (vs. South American) component of this assemblage using only the mitochondrial *Cyt-b* gene (Lydeard and Roe, 1997; Roe et al., 1997; Martin and Bermingham, 1998; Farias et al., 2001; Hulsey et al., 2006; Řičan and Kullander, 2006; Concheiro-Perez et al., 2007). Heroins have also been examined using morphological data (Kullander, 1998;

Chakrabarty, 2007), partial mitochondrial sequences of the large ribosomal subunit (Farias et al., 1999; Sparks, 2004), multiple mitochondrial and nuclear gene sequences (Sparks and Smith, 2004; Chakrabarty, 2006a), and combined morphological and multi-gene molecular datasets (Farias et al., 2000, 2001; Chakrabarty, 2006b). Herein, we follow Kullander (1998) in recognizing this clade as the cichline tribe Heroini (Appendix).

With respect to prior studies (Kullander, 1983; Chakrabarty, 2006a,b; Řičan and Kullander, 2006; Concheiro-Perez et al., 2007), our phylogeny is in general agreement with results suggesting that the South American taxa *Australoheros*, *Caquetaia*, “*Cichlasoma*” *festae*, and *Heroina* are nested within a “Middle American” clade. Concheiro-Perez et al. (2007) and Hulsey et al. (2006) recovered the Greater Antillean genus *Nandopsis* as the sister group to all other members of the “Middle American” clade. Within the “Middle American” clade, our results are largely in agreement with Concheiro-Perez et al. (2007) who generally broke this assemblage into the informal “herichthyines” and “amphilophines”, except that we recovered *Australoheros* and *Theraps* within the “amphilophines”. Our placement of *Australoheros* is supported by the results of Řičan and Kullander (2006), and although our placement of *Theraps* is unorthodox, this is more a reflection of the poor state of heroin taxonomy than a major phylogenetic change. Concheiro-Perez et al. (2007) recovered our included species, *Theraps wesseli*, among their “amphilophines”, but they found the type species, *T. irregularis*, in its more traditional placement within their “herichthyines”. Clearly, the demonstrable polyphyly of the Middle American genera *Amphilophus*, *Archocentrus*, “*Cichlasoma*”, *Theraps*, *Tomocichla*, and *Vieja* [as recognized by Kullander (2003) and tested by Chakrabarty (2006a,b), Řičan and Kullander (2006), and Concheiro-Perez et al. (2007)] renders our phylogeny within the “Middle American” clade largely preliminary. The combination of dense sampling of the “Middle American” heroins, as was analysed by Concheiro-Perez et al. (2007) using only *Cyt-b* sequences, for multiple genes and morphology, as well as traditional revisionary work, will be required to resolve the complicated phylogeny and taxonomy of this species-rich assemblage.

As in prior studies, we recovered a “deep-bodied” clade sister to the “Middle American” clade. Previous phylogenies have generally recovered a “deep-bodied” clade composed of *Heros*, *Mesonauta*, *Symphysodon*, and *Uaru* (Farias et al., 2000, 2001; Concheiro-Perez et al., 2007), but the placement of *Pterophyllum* has been more elusive. In previous studies, the placement of *Pterophyllum* has ranged from within this “deep-bodied” clade (Farias et al., 2000), to the sister-group of all other heroins (Farias et al., 1999, 2001), to the

sister-group of a combined Cichlasomatini + Heroini (Concheiro-Perez et al., 2007). We recovered *Pterophyllum* in a more traditional placement (Regan, 1906), sister to *Symphysodon*, within the “deep-bodied” clade. Finally, our analysis recovered a clade composed of *Hoplarchus* + *Hypselecara* that was sister to all other heroins. Typically, these genera have fallen out near each other (often sister), but their placement has also varied from a close relationship with *Symphysodon* (Farias et al., 1999) to their current placement as the sister-group of all other heroins (Farias et al., 2000).

The taxonomy of Heroini, or what has often been referred to as the “cichlasomine group a” radiation (Stiassny, 1991), is complicated, but most of this controversy revolves around the limits of the former *Cichlasoma* and the Middle American species. As noted above, the demonstrable polyphyly of the Middle American genera *Amphilophus*, *Archocentrus*, “*Cichlasoma*”, *Theraps*, *Tomocichla*, and *Vieja* makes any discussion of Middle American taxonomy futile in the absence of a species-level analysis. Among the South American heroins, monophyly of the genera within the “deep-bodied” clade (*Heros*, *Mesonauta*, *Pterophyllum*, *Symphysodon*, and *Uaru*) has been previously discussed (Kullander, 1986; Kullander and Silfvergrip, 1991; Bleher et al., 2007) or are indisputable because of their extreme modifications (e.g. the popular aquarium angel-fishes in *Pterophyllum*). Monophyly of the other South American heroin genera was discussed in their descriptions [Kullander, 1986 (*Hypselecara*); Kullander, 1998 (*Heroina*); Řičan and Kullander, 2006 (*Australoheros*)] and in subsequent phylogenetic revisions (Schmitter-Soto, 2007). Finally, monophyly of the small genus *Caquetaia* and the monotypic genus *Hoplarchus* has not been explicitly discussed, but Řičan and Kullander (2006) did recover *Heroina* nested within *Caquetaia*.

#### *Evolution of Cichlinae*

In the first family-level molecular phylogeny for Cichlidae, Farias et al. (1999) noted that Neotropical cichlins harbour significantly higher levels of genetic variation than their African pseudocrenilabrine sister group. They argued that cichlins had experienced accelerated rates of molecular evolution, and they highlighted their finding that a particularly high evolutionary rate was found within Geophagini. In agreement with Farias et al. (2000, 2001), the present study found support for these comparatively longer geophagin branches. Despite the significantly longer branches found within Geophagini (Farias et al., 1999), López-Fernández et al. (2005b) suggested that geophagins represented an adaptive radiation that was characterized, in part, by “short basal branches”. López-Fernández et al. (2005b, p. 242) argued that it was

“improbable that (their) lack of resolution and support at the base of the geophagine tree is due to either inadequate or insufficient data. Instead short branches at the base of the tree suggest that the different geophagine genera may have originated rapidly and/or over a short period” of time. Short basal branches are used by these authors as evidence to satisfy Schluter’s (2000) phylogenetic requirements for an adaptive radiation.

In contrast to the findings of López-Fernández et al. (2005a,b), the current study, which included additional geophagin genera, outgroups, and data, does not reveal “short basal branches” with limited support (Fig. 2). In contrast to all previous studies, we recovered strong support and ample branch lengths for the majority of the suprageneric nodes within Geophagini. These findings minimally question, if not outright reject, the phylogenetic evidence for geophagins representing a rapid radiation.

In this study, we have included complete generic sampling of Neotropical cichlids and have incorporated substantial novel genetic data in a simultaneous analysis of available morphological and molecular data. This significant increase in data has resulted in the first well-supported tribal, sub-tribal, and generic phylogeny for Cichlinae, and we have updated Kullander’s (1998, 2003) taxonomy accordingly. Despite this phylogenetic progress, many taxonomic problems remain, including the limits of many of the species-rich genera (e.g. *Apistogramma*, *Aequidens*, *Crenicichla*) and the classification of the “Middle American” heroins. Clearly, detailed species-level phylogenies and type-based revisionary studies for most of these problematic clades are the next step in further resolving cichline relationships.

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## Appendix

Proposed classification, morphological diagnoses, and composition of Cichlinae and its included tribes and subtribes.

### *Cichlinae* Bonaparte 1840

**Type genus:** *Cichla* Bloch and Schneider 1801.

**Sister taxon:** Pseudocrenilabrinae.

**Concept and content:** Approximately 480 species classified in seven tribes: Astronotini, Chaetobranchini, Cichlasomatini, Cichlini, Geophagini, Heroini, and Retroculini.

**Diagnostic feature:** Stiassny (1991) diagnosed Cichlinae with one morphological feature: strongly interdigitating suture between vomerine shaft and parasphenoid bar.

### *Astronotini* Hoedeman 1947

**Type genus:** *Astronotus* Swainson 1839.

**Sister taxon:** Chaetobranchini + Cichlasomatini + Geophagini + Heroini.

**Concept and content:** Two species classified in the genus *Astronotus* (Kullander, 2003).

**Diagnostic features:** The current study recovered five morphological apomorphies that diagnose Astronotini: microbranchiospines enlarged, with numerous teeth on exposed face (Kullander, 1998: character 13); insertion of pharyngocleithralis internus onto lower jaw complex with several tendons (Kullander, 1998: character 24); narrow insertion of Baudelot's ligament onto bilateral minor process on basioccipital (Kullander, 1998: character 29); no caudal opening of posterior myodome (Kullander, 1998: character 30); 17–20 anal-fin elements (Kullander, 1998: character 81).

### *Chaetobranchini* Fernández-Yépez 1951

**Type genus:** *Chaetobranchius* Heckel 1840.

**Sister taxon:** Geophagini.

**Concept and content:** Four species classified in two genera: *Chaetobranchius* and *Chaetobranchopsis* with two species each (Kullander, 2003).

**Diagnostic feature:** Casciotta and Arratia (1991) identified the following diagnostic morphological feature for Chaetobranchini: uncinat process of first epibranchial much longer than anterior arm.

### *Cichlasomatini* Kullander, 1998

**Type genus:** *Cichlasoma* Swainson 1839.

**Sister taxon:** Heroini.

**Concept and content:** Approximately 107 species classified in ten genera: *Acaronia*, *Aequidens*, *Bujurquina*, *Cichlasoma*, *Cleithracara*, *Krobia*, *Laetacara*, *Nannacara*, and *Tahuantinsuyoa* (Kullander, 2003).

**Diagnostic features:** The current study recovered four morphological synapomorphies that diagnose Cichlasomatini: suturing of mesethmoid and vomer absent (Kullander, 1998: character 31); anterior two post-lachrymal infraorbitals variously ossified with laminar ventral expansion (Kullander, 1998: character 43); no posterior expansion of gas bladder into caudal region (Kullander, 1998: character 74); predorsal scales triserial (Kullander, 1998: character 80).

### *Cichlini* Bonaparte 1840

**Type genus:** *Cichla* Bloch and Schneider 1801.

**Sister taxon:** Retroculini.

**Concept and content:** Approximately 15 species classified in the genus *Cichla* (Kullander and Ferreira, 2006).

**Diagnostic features:** The current study recovered 13 morphological apomorphies that diagnose Cichlini [see Kullander and Ferreira (2006) for additional characters and discussion]: uncinat process of epibranchial 1 wider than anterior arm (Kullander, 1998: character 4); epibranchial 1 with posterodorsal laminar expansion with sharp angle (Kullander, 1998: character 8); central ligament inserts on ceratobranchial 4 (Kullander, 1998: character 11); microbranchiospines with teeth on exposed face (Kullander, 1998: character 13); origin of pharyngocleithralis internus on the lateral face of cleithrum (Kullander, 1998: character 23); urohyal spine rostrally directed (Kullander, 1998: character 25); Baudelot's ligament with narrow insertion on bilateral minor process of basioccipital (Kullander, 1998: character 29); anterior notch on vomer (Kullander, 1998: character 32); anteriorly directed process on distal postcleithrum long and pointed (Kullander, 1998: character 49); anterodorsal palatomaxillary ligament well defined and originating from distinct dorsolateral fossa found approximately (at midpoint) on maxillary process of palatine (Kullander, 1998: character 53); ascending arm of premaxilla shorter than dentigerous arm (Kullander, 1998: character 64); abdominal vertebrae 13 or fewer (Kullander, 1998: character 67); final basapophysis on last abdominal vertebra (Kullander, 1998: character 68).

### *Geophagini* Haseman 1911

**Type genus:** *Geophagus* Heckel 1840.

**Sister taxon:** Chaetobranchini.

**Concept and content:** Approximately 217 species (Kullander, 2003; López-Fernández et al., 2005a, 2006) classified into three subtribes: *Acarichthyina*, *Crenicaratina*, and *Geophagina*.

**Diagnostic features:** The current study recovered three morphological synapomorphies that diagnose Geophagini [see López-Fernández et al. (2005b) for additional characters and discussion]: uncinat process of epibranchial 1 wider than anterior process (Kullander, 1998: character 4); one supraneural (Kullander, 1998: character 66); more than three procurrent caudal-fin rays (Kullander, 1998: character 84).

### *Acarichthyina* Kullander, 1998

**Type genus:** *Acarichthys* Eigenmann 1912.

**Sister taxon:** *Crenicaratina* + *Geophagina*.

**Concept and content:** Approximately seven species (Kullander, 2003; López-Fernández et al., 2006) classified in the genera *Acarichthys* and *Guianacara*.

**Diagnostic features:** The current study recovered four morphological synapomorphies that diagnose *Acarichthyina*: expanded basisphenoid and dorsal parasphenoid wing (Kullander, 1998: character 36); extensive overlap of lachrymal and first infraorbital (Kullander, 1998: character 44); anteroventral articulation of palatine slightly displaced from vomer, but maintaining ligamentous connection (Kullander, 1998: character 54); pelvic-fin shape pointed (Kullander, 1998: character 86).

### *Crenicaratina* Kullander, 1998

**Type genus:** *Crenicara* Steindachner 1875.

**Sister taxon:** *Geophagina*.

**Concept and content:** Approximately 89 species (Kullander, 2003) classified in six extant genera: *Biotoecus*, *Crenicara*, *Crenicichla*, *Dicrosuss*, and *Teleocichla*.

**Diagnostic features:** The current study recovered one morphological synapomorphy that diagnoses the Crenicaratina: caudal-fin lateral line absent (Kullander, 1998: character 78).

*Geophagina* Haseman 1911

**Type genus:** *Geophagus* Heckel 1840.

**Sister taxon:** Crenicaratina.

**Concept and content:** Approximately 121 species (Kullander, 2003) classified in nine genera: *Apistogramma*, *Apistogrammoides*, *Biotodoma*, *Geophagus*, *Gymnogeophagus*, *Mazarunia*, *Mikrogeophagus*, *Satanoperca*, and *Taeniacara*.

**Diagnostic features:** The current study recovered four morphological synapomorphies that diagnose *Geophagina*: uncinat process of epibranchial 1 relative to main axis of bone parallel or at very slight angle (Kullander, 1998: character 3); epibranchial lobe present (Kullander, 1998: character 5); interarcual cartilage long (Kullander, 1998: character 22); single palatoethmoid articulation (Kullander, 1998: character 59).

*Heroini* Kullander, 1998

**Type genus:** *Heros* Heckel 1840.

**Sister taxon:** Cichlasomatini.

**Concept and content:** Approximately 142 species classified in 26 genera (Kullander, 2003; Chakrabarty, 2006a; Řičan and Kullander, 2006; Chakrabarty and Sparks, 2007; Schmitter-Soto, 2007):

*Amatitlania*, *Amphilophus*, *Archocentrus*, *Australoheros*, *Caquetaia*, “*Cichlasoma*”, *Herichthys*, *Heroina*, *Heros*, *Herotilapia*, *Hoplarchus*, *Hypselecara*, *Hypsophrys*, *Mesonauta*, *Nandopsis*, *Parachromis*, *Paraneotroplus*, *Petenia*, *Pterophyllum*, *Rocio*, *Symphysodon*, *Theraps*, *Thorichthys*, *Tomocichla*, *Uaru*, and *Vieja*.

**Diagnostic features:** The current study recovered three morphological synapomorphies that diagnose *Heroini*: palatine displaced, lacking contact with vomer (Kullander, 1998: character 54); single (posterior) palatoethmoid articulation (Kullander, 1998: character 59); five or more anal-fin spines (Kullander, 1998: character 82).

*Retroculini* Kullander, 1998

**Type genus:** *Retroculus* Eigenmann and Bray 1894.

**Sister taxon:** Cichlini.

**Concept and content:** Approximately three species classified in the genus *Retroculus* (Gosse, 1971; Kullander, 2003).

**Diagnostic features:** The current study recovered six morphological apomorphies that diagnose *Retroculini*: uncinat process of epibranchial 1 considerably longer than anterior arm (Kullander, 1998: character 1); both uncinat process and anterior arm of epibranchial 1 posterodorsally angled without deep indentation in dorsal bone margin (Kullander, 1998: character 3); gill rakers on ceratobranchial 5 (Kullander, 1998: character 18); articulating process of premaxilla indistinct (Kullander, 1998: character 63); dorsal caudal-fin lateral line absent (Kullander, 1998: character 78); “*Tilapia* spot” present (Kullander, 1998: character 91).