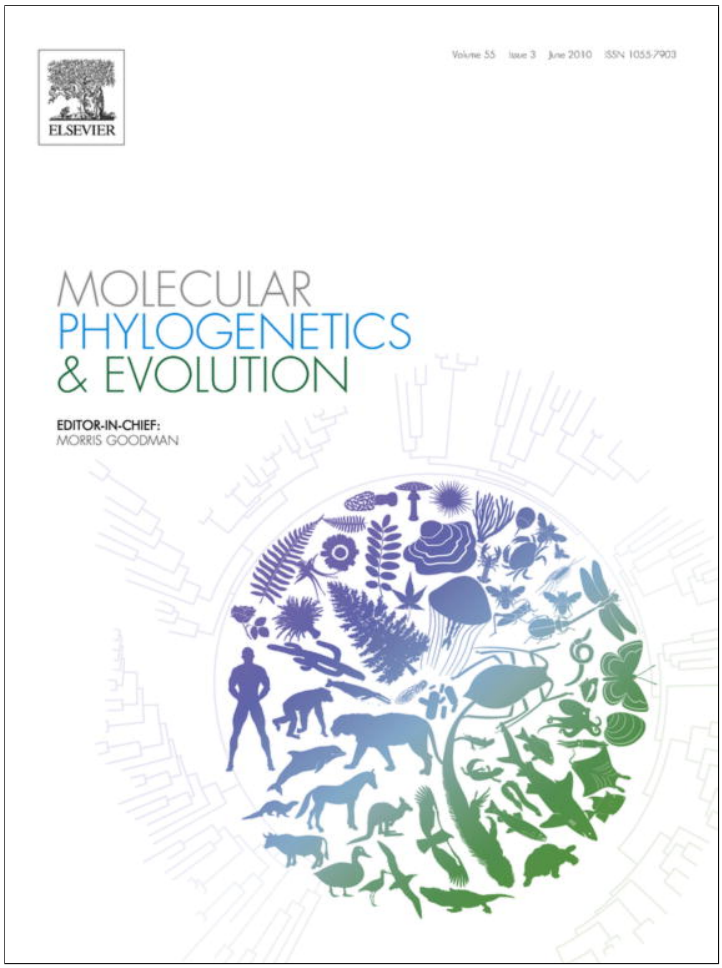

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Multilocus phylogeny and rapid radiations in Neotropical cichlid fishes (Perciformes: Cichlidae: Cichlinae)

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ABSTRACT

Neotropical cichlid fishes comprise approximately 60 genera and at least 600 species, but despite this diversity, their phylogeny is only partially understood, which limits taxonomic, ecological and evolutionary research. We report the largest molecular phylogeny of Neotropical cichlids produced to date, combining data from three mitochondrial and two nuclear markers for 57 named genera and 154 species from South and Central America. Neotropical cichlids (subfamily Cichlinae) were strongly monophyletic and were grouped into two main clades in which the genera *Retroculus* (Tribe Retroculini) and *Cichla* (Cichlini) were sister to a monophyletic group containing all other lineages. This group included the tribes Chaetobranchini, Astronotini, Geophagini, Cichlasomatini and Heroini. Topological comparisons with previously published hypotheses indicated that our results are congruent with recent analyses of the tribe Cichlasomatini, but significantly more likely than published hypotheses for Geophagini, Heroini and the entire Cichlinae. Improved resolution and support are attributed to increased taxon sampling and to the addition of taxa never before included in phylogenetic analyses. Geophagini included two major subclades congruent with our own previous findings but more strongly supported; we also found a new and strongly supported sister-group relationship between *Guianacara* and *Mazarunia*. Cichlasomatini relationships were similar to recently proposed topologies, but contrastingly, we found a monophyletic *Cichlasoma* and support for a monophyletic grouping of the *Aequidens diadema* and *A. tetramerus* groups. Three basal South American Heroini lineages were recovered: (*Hypselecara* + *Hoplarchus*), *Pterophyllum*, and a grouping we refer to as mesonautines. Three other South American clades, caquetaines, *Australoheros* and the '*Cichlasoma*' *festae* group, were nested within Central American clades. Most Heroini diversity was divided into two relatively well-supported large groups: the Southern Central American Clade, including clades herein referred to as nandopsines, caquetaines and amphilophines, and the Northern Central American Clade, including astatheroines, tomocichlines and herichthyines. Some of these groups have been previously identified, but often with different taxonomic compositions. Further resolution of Neotropical cichlid relationships, especially within the large amphilophine clade of Heroini, will require additional phylogenetic analysis. Nevertheless, the topology from this study provides a robust phylogenetic framework for studying evolutionary diversification in Neotropical cichlids. Significantly-short branches at the base of Geophagini and Heroini are compatible with early bursts of divergence that are characteristic of adaptive radiations. This pattern suggests diversification of Neotropical cichlid genera occurred rapidly, with subsequent convergent, adaptive ecomorphological diversification among and within South and Central American clades.

1. Introduction

The rivers of South and Central America harbor the most diverse freshwater fish fauna on Earth, with some estimates exceeding 6000 species, perhaps representing about 20% of all fishe and 10% of all species of vertebrates (Reis et al., 2003). Nearly 600 of these species are cichlids, making the family the third most diverse

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in the Neotropics after Characidae and Loricariidae (Reis et al., 2003). Neotropical cichlids (Cichlinae) are a monophyletic clade that is sister to a monophyletic African cichlid clade (Pseudocrenilabrinae, Stiassny, 1991; Farias et al., 2000; Sparks and Smith, 2004), and are found in nearly all rivers of South and Central America, with species extending from the edge of Patagonia to Texas. The Neotropical clade includes approximately 60 genera with many species still undescribed (Reis et al., 2003, HLF pers. obs.). The great majority of Neotropical cichlid diversity has been classified into three major tribes (Kullander, 1998; Smith et al., 2008): Geophagini, Cichlasomatini and Heroini, with a handful of additional species placed in the tribes Cichlini, Retroculini, Chaetobranchini and Astronotini. Geophagini is restricted to South America and southern Panama and includes approximately 18 genera and about 250 species (López-Fernández et al., 2005a,b). Cichlasomatini includes 11 described genera and more than 70 species distributed in South America and Panama. Heroini includes approximately 30 genera and about 150 spp. distributed in South and Central America, with one genus in Cuba and Hispaniola.

Remarkably, little is known about the origin of Neotropical cichlid diversity, as most studies of cichlid evolutionary diversification have focused on the unique adaptive radiations associated with the East African lakes Victoria, Tanganyika and Malawi (e.g. Meyer, 1993; Kornfield and Smith, 2000; Streelman and Danley, 2003; Kocher, 2004). In contrast to the Late Neogene-Quaternary origin of African-lake cichlids (<7 Ma, e.g. Verheyen et al., 2003; Joyce et al., 2005), Neotropical cichlids have a long history of diversification that appears to go back to the Late Cretaceous (>90 Ma, e.g. Chakrabarty, 2006; López-Fernández and Albert, in press; Lundberg et al., 2010). Consequently, Neotropical cichlid phylogenies should be more tractable than those of the African-lake radiations (e.g. Farias et al., 2000; López-Fernández et al., 2005a,b; Concheiro Pérez et al., 2006; Rican et al., 2008; Musilová et al., 2009). Despite increasing interest in their evolutionary history, a fairly large number of studies addressing aspects of their phylogeny, and a recent claim to have solved their inter-generic relationships (Smith et al., 2008), the Neotropical cichlid phylogeny remains only partially understood. This inability to obtain a clear phylogenetic hypothesis for Neotropical cichlids has multiple causes, including: (1) widespread homoplasy associated with convergent ecomorphological diversification; (2) high taxon diversity with some critical taxa unavailable for examination until recently; (3) rapid diversification at basal nodes (e.g. López-Fernández et al., 2005a); (4) rampant rate heterogeneity of sequence divergence associated with at least some of the major lineages (e.g. Farias et al., 1999; López-Fernández et al., 2005a); and (5) inadequate sampling of either taxa or taxonomic characters (both molecular and morphological) among studies performed to date. Several analyses of intergeneric relationships have recently become available for the major clades (Roe et al., 1997; Martin and Bermingham, 1998; Farias et al., 1999, 2000, 2001; Sides and Lydeard, 2000; Hulsey et al., 2004; López-Fernández et al., 2005a,b; Chakrabarty, 2006; Concheiro Pérez et al., 2006; Musilová et al., 2009; Rican et al., 2008) or even for the entire Neotropical assemblage (Kullander, 1998; Smith et al., 2008), but even in these studies, taxon sampling and character sets are inadequate for revealing relationships within the entire lineage. Although, in general, most of these studies have contributed greatly to clarifying the relationships among American cichlids, they have also revealed that this is a more difficult problem than many anticipated. A robust phylogeny is essential to achieve a stable taxonomy of Neotropical cichlids, especially for the Central American clade, which has been in a constant state of flux (e.g. Concheiro Pérez et al., 2006; Smith et al., 2008). In addition, a phylogeny provides an indispensable framework for evolutionary and ecological studies of Neotropical cichlids (e.g. López-Fernández et al., 2005a; Rican et al., 2008). For example, based on a multilocus phylogeny, López-Fernández et al. (2005a) proposed that the remarkable morphological, ecological and behavioral diversity of Geophagini genera was the outcome of an adaptive radiation, older than but similar to that seen for African lacustrine cichlids. Despite only partial success in resolving the geophagine phylogeny, López-Fernández et al.'s (2005a) work showed that detailed taxon sampling and multiple datasets are important for both phylogenetic resolution and in-depth investigation of speciation within the highly diverse Neotropical cichlid assemblage. Therefore, lack of a solid phylogeny limits taxonomic, ecological and evolutionary research.

A relatively stable taxonomy of South American cichlids has resulted largely from the work of Kullander, who over a period of approximately three decades has revised nearly every South American group and proposed the first classification of the Cichlinae (e.g. Kullander, 1980, 1983, 1986, 1988, 1989, 1990, 1996, 1998; Kullander and Nijssen, 1989; Kullander and Silfvergrip, 1991). Kullander's (1983) revision of the genus *Cichlasoma*, which restricted the genus to twelve South American species, "orphaned" many species that were left without formal generic assignment. This decision resulted in a series of studies describing a number of new genera of South American Cichlasomatini and Heroini and revisions within Geophagini (e.g. Kullander, 1986, 1988, 1990; Kullander and Nijssen, 1989), producing a more stable and tractable classification for South American cichlids. Unfortunately, the same has not been the case for the Central American Heroini, the taxonomy of which has remained in a constant state of confusion and flux.

In the absence of clear generic definitions for the Central American cichlids previously included in *Cichlasoma* sensu lato, Central American taxonomy requires major revision, but such revision has proven slow and difficult and is far from complete. Kullander (1983) and Stiassny (1991) suggested that, until a clear phylogenetic understanding and diagnostic characters for the genera were available, most orphaned species of the former *Cichlasoma* should be designated as '*Cichlasoma*' until their taxonomy is fully revised. Kullander (1983) also pointed out that some former *Cichlasoma* could be treated as full genera (e.g. *Thorichthys*) because they were well diagnosed in earlier work, especially by Regan (1905), as distinct "sections". However, while some of Regan's sections have proven useful (e.g. *Astatheros*, *Herichthys*), others are clearly polyphyletic groupings as originally defined (e.g. *Theraps*), as are some of his newly defined groupings (e.g. *Parapetenia*). Recent revision of types and analysis of new taxa, particularly the genus *Heroina*, have helped further clarify the identity of some Central American taxa such as *Parachromis*, and have provided descriptions of characters to diagnose the Heroini (Kullander, 1996; Kullander and Hartel, 1997). Kullander (1998) proposed the first phylogenetically-based classification of Neotropical cichlids and formally recognized a monophyletic Heroini, but his classification did not include the Central American taxa. Recently, several phylogenetic studies with dense taxon sampling of Central American cichlids have become available, and these have begun to clarify the generic composition of the Heroini (e.g. Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008). Collectively, these studies have helped reveal two major problems associated with the current classification of Central American cichlids. First, there is a lack of consensus as to the description and definition of monophyletic genera, especially as to which species actually belong to currently recognized genera. Second, phylogenetic relationships among genera are poorly resolved, making it difficult to both develop a stable classification and to use phylogenetic information for comparative evolutionary and ecological studies. Addressing these problems requires a combination of dense taxon sampling at the species-level and the inclusion of a large number of characters (see also Concheiro Pérez et al., 2006; Rican et al., 2008). Reduced taxon sampling

that focuses on allegedly well-defined lineages almost certainly fails to include members of lineages yet undetected (e.g. Smith et al., 2008).

This paper reports a molecular phylogeny of Neotropical cichlids that includes a combination of data from five different molecular markers and 57 named genera and 154 species. All known major lineages of Neotropical cichlids are represented, including a large number of undescribed taxa and the first molecular analysis of the South American genus *Mazarunia*. Here we provide (1) the most comprehensively sampled phylogeny of Neotropical cichlids to date, including taxa never before available for molecular analysis, and (2) a preliminary evaluation of the patterns of diversification in Neotropical cichlids.

2. Materials and methods

2.1. Taxon sampling

Our ultimate goal was to produce a phylogenetic hypothesis for all lineages of Neotropical cichlids. Taxon sampling was maximized by both targeting taxa examined in previous studies and by combining our new sequence data with previously published data. With few exceptions, vouchers for all specimens examined were deposited in ichthyology collections in a variety of museums (see Supplement Table 1 for details). Outgroup taxa included the Indian species *Etroplus maculatus* and the Malagasy *Paretroplus polyactis* and *Paratilapia polleni*, which are recognized as sister to both African and Neotropical cichlids in several recent analyses (e.g. Stiassny, 1991; Kullander, 1998; Farias et al., 2000; Sparks and Smith, 2004). The riverine African cichlids *Hemichromis fasciatus*, *Chromidotilapia guntheri* and *Heterochromis multidens* were included to represent the sister-group to all Neotropical cichlids. The ingroup included at least one species of every described genus and representative taxa whose generic assignments remain uncertain. We included eight species of the three putative basal Neotropical genera *Cichla*, *Retroculus* and *Astronotus* and one species each of *Chaetobranchus* and *Chaetobranchopsis* [Kullander's (1998) Chaetobranchinae]. The tribe Geophagini (López-Fernández et al., 2005a,b) was represented by 48 species (14.8% of the estimated total) in all 18 putative genera. Following results from previous studies (e.g. Kullander, 1998; López-Fernández et al., 2005a,b), we treated the clades '*Geophagus*' *brasiliensis* and '*Geophagus*' *steindachneri* as genera in need of description (contra Smith et al., 2008). We also included, for the first time in any molecular phylogenetic analyses, specimens of the Guyanese genus *Mazarunia*, that included *M. mazarunii* and two undescribed species. Cichlasomatini (Kullander, 1998; Musilová et al., 2008, 2009) was represented by 20 species (17.5% of the estimated total species) in all described genera. Samples of Heroini included 83 species (51.2% of the estimated total), representing all South American genera and at least one representative of every described Central American genus. Unnamed Central American lineages are highlighted throughout the paper as '*Cichlasoma*' as proposed by Kullander (1983) and Stiassny (1991) and as used in the standard Checklist of Freshwater Fishes of South and Central America (CLOFFSCA, Reis et al., 2003). We attempted to include at least one species of each putative Middle American lineage still in need of description (e.g. '*Cichlasoma*' *wesseli*, '*C.*' *urophthalmus*). We also attempted to test the monophyly of described putative genera by including, whenever possible, at least two species of each. Overall, we maximized taxon sampling, with the goal of increasing resolution at the genus and above-genus levels of the phylogeny in accordance with a variety of studies showing that increased taxon sampling is often more critical in recovering accurate phylogenies than a greater amount of data (e.g. Graybeal, 1998; Zwickl and Hillis, 2002; Hillis et al., 2003; Rican et al., 2008, and see Section 4.1).

2.2. Data collection

DNA sequences of three mitochondrial genes (Cytochrome *b*, NADH dehydrogenase subunit 4 [ND4], and ribosomal 16S), and two nuclear genes (Recombination Activating Gene subunit 2 [RAG2], and ribosomal protein S7 intron 1) were obtained from 166 terminals. Total genomic DNA from muscle or fin-clip samples preserved in either 95% ethanol or DMSO was isolated using the DNeasy kit (Qiagen) or standard phenol–chloroform extractions (Sambrook et al., 1989). Whenever possible, two specimens per terminal were sequenced to confirm sequence identity. Primers and annealing temperatures for all loci sequenced in this study are listed in Table 1. The mitochondrial gene ND4 was amplified and sequenced using conditions described in López-Fernández et al. (2005a) and included an additional amplification primer designed specifically for this study (Table 1). Cytochrome *b* and ribosomal 16S fragments followed amplification conditions described in Farias et al. (2000, 2001). For some species, an additional primer was used for both amplification and sequencing of a smaller fragment of Cytochrome *b* following Willis (Pers. Comm, see Table 1). Conditions for S7 are those described in Chow and Hazama (1998). RAG2 fragments were generally amplified using primers developed specifically for Neotropical cichlids (Table 1). Some taxa were amplified and sequenced using primers from Lovejoy and Collette (2001) under the same amplification conditions and gel-extraction protocols described by López-Fernández et al. (2005a). Forward and reverse automated sequencing of all specimens was performed with either an ABI 3100 or 3130 (Applied Biosystems) genetic analyzer and followed protocols recommended by the manufacturer. Sequence editing and consensus contigs of the forward and reverse sequences for each specimen were built and exported for analysis using Sequencher 4.8 (Genecodes).

Preliminary multiple sequence alignment was performed in Clustal X (Thompson et al., 1994) for all fragments. Alignments of coding sequences (ND4, Cytochrome *b*, RAG2) were visually evaluated by aligning their amino acid sequences in McClade 4.0 (Maddison and Maddison, 2000) to ensure that no stop codons were present. Sequences of the mitochondrial ribosomal 16S were aligned using the secondary structural model for *Xaenopus leavis* (GenBank sequence M10217) proposed by the Guttell laboratory at The University of Texas at Austin. Further alignment of 16S followed the structural alignment employed by López-Fernández et al. (2005a), in which 29 base pairs were removed because positional homology could not be unambiguously established. The alignment for S7 from Clustal X was slightly modified by eye, and 229 bp on the 3′ end of the sequences for *Etroplus*, *Paretroplus* and *Paratilapia* were removed from the final alignment because positional homology with the rest of the dataset could not be established. Aligned lengths for the sequences from each locus are given in Table 1 and GenBank accession numbers, names and voucher information of all sequences are given in Supplement Table 1.

2.3. Phylogenetic analyses

Aligned sequences from all five markers were concatenated and kept as five independent partitions for all analyses. Maximum Parsimony (MP) tree searches were performed using 100 random addition sequences and the Tree Bisection and Reconnection (TBR) algorithm in PAUP* 4.0b10 (Swofford, 2002). The best score from each search was used to instruct PAUP* to save only trees of that length or shorter in further searches. Under this constraint we performed additional 100 TBR search replicates until no shorter

Table 1
Primers, annealing temperature for PCR amplification, alignment lengths and models of nucleotide substitution estimated and implemented for loci used in phylogenetic analyses. See Methods for details of each phylogenetic analysis.

Locus	Primers (5′–3′ direction)	Original reference	Ta (°C)	Aligned length	Nucleotide composition homogeneity test (X^2, df, p-value)	Substitution model estimated	Substitution model implemented
16S	F: 16Sa-L2510 CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)	50	543	63.6, 495, 1.0	GTR + I + Γ	GTR + I + Γ
	R: 16Sb-H3080 CCGGTCTGAACTCAGATCACGT	Palumbi et al. (1991)					
ND4	F1: ND4LB CAAAACCTTAATCTYCTACTACAATGCT	Bielawsky et al. (2002)	48	677	323.3, 495, 1.0	GTR + I + Γ	GTR + I + Γ
	F2: ND4-Hist CTGTCTTTAGAATCACAATC	This study					
	R: Nap2 TGGAGCTTCTACGTGRGCTTT	Arévalo et al. (1994)					
Cyt *b*	F: GluDG.L TGACTTGAARAACCAYCGTTG	Martin and Bermingham (1998)	48	1128	364.8, 495, >0.9	GTR + I + Γ	GTR + I + Γ
	R: Cb6b.H GGAATTCACCTCTCCGCTTTACAAGAC	Martin and Bermingham (1998)					
	R: CytbIntR GGTGAAGTTGTCTGGGTC	S. Willis Pers. Comm.					
S7	F: S7RPEX1F TGGCCTCTTCCTTGGCCGTC	Chow and Hazama (1998)	55	527	96.2, 495, 1.0	TVM + I + Γ	GTR + I + Γ
	R: S7RPEX2R AACTCGTCTTTCTTCCTTGGCCGTC	Chow and Hazama (1998)					
RAG2	F1: NeoRAG2F AAACTGAGGGCCATTTCCTT	This study	53	993	53.0, 495, 1.0	K80 + I + Γ	GTR + I + Γ
	R1: NeoRAG2R GGGTTCTTTCTTCCTCTTTGG	This study	53				
	F2: RAG2-F2 ARACGCTCMTGTCMACTGG	Lovejoy and Collette (2001)	56–62[a]				
	R2: RAG2-R7 AAGTAGAGCTCCTCAGAGTC	Lovejoy and Collette (2001)	56–62[a]				

[a] Touchdown amplification protocol from López-Fernández et al. (2005a).

trees were found. A final search with 1000 TBR replicates was performed under the constraint of not keeping any more than one tree one-step longer than the shortest tree. From this last round, a final tree was produced using a 50% majority rule consensus. This tree was used to create a constraints file in MacClade 4.0 (Maddison and Maddison, 2000) to calculate Bremer Support values in PAUP* based on 100 replicates of random addition sequence and TBR for each constrained node.

Model selection for Bayesian analysis was performed in ModelTest (Posada and Crandall, 1998, see Table 1). Bayesian Inference (BI) analysis of all unlinked partitions was run in MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003) under a GTR + I + Γ model allowing for independent parameters of molecular evolution to be calculated for each partition. The combined dataset was analyzed in four independent runs of MC^3, each with two parallel searches using six Markov chains. To facilitate tree-space sampling, five of the chains were manipulated to more easily accept proposals to change topological state by increasing the "heating" parameter of MrBayes from 0.2 to 0.3 and keeping 1 cold chain to record sampling of tree space. The search was stopped at 12×10^6 generations after the split variance parameter, comparing the two parallel searches in each run arrived at $\leqslant 0.01$ (Ronquist and Huelsenbeck, 2003). Convergence of the independent searches was further explored by both evaluating likelihood vs. generation plots with the use of the sump command in MrBayes and importing the parameter output files of MrBayes into Tracer 1.4 (Drummond et al., 2006). The latter procedure was used to confirm that both an unimodal distribution of the estimated parameters and a minimum of 100 independent samples from the Markov chains had been attained (ESS parameter in Tracer). With tree sampling set every 100 generations, a combined total of 180,000 trees per run were analyzed after discarding a burn-in of 60,000 trees. Support for Bayesian topologies was estimated using node posterior probabilities from the posterior distribution of topologies as estimated by the sumt command of MrBayes.

Maximum Likelihood (ML) analysis was performed with independent GTR + I + Γ models for each partition in RAxML version 7.0.3 for Windows (Stamatakis, 2006). Bootstrap support estimates were based on 1000 independent searches with random starting trees and the rapid bootstrapping algorithm implemented in the program (Stamatakis et al., unpublished).

2.4. Hypothesis testing

The resultant topology was used to test several hypotheses pertaining to: (1) congruence among different data partitions, (2) previously proposed phylogenies, and (3) the evidence for rapid radiations based on internal branch lengths. In a previous analysis of the geophagine clade of Neotropical cichlids, López-Fernández et al. (2005b) found evidence of significant topological incongruence between Cytochrome *b* and other molecular and morphological data. At that time, the reasons for this incongruence were beyond the scope of the study and the authors opted for the removal of the Cytochrome *b* dataset until further exploration of the potential reasons for incongruence could be performed. In the context of this paper, we used TreeRot version 2 (Sorenson, 1999), with 100 heuristic replicates per clade, to calculate Partitioned Bremer Support (PBS, Baker et al., 1998, 2001) as a measure of a posteriori congruence among partitions for both the MP and BI trees. We did not perform this analysis on the ML topology because it was virtually identical to the BI tree. PBS results were summarized as the contribution of each partition to the combined topology. We followed the method of Sota and Vogler (2001), which uses Spearman correlations to perform node-to-node pairwise comparisons among PBS values for each partition. Positive correlations indicate congruent phylogenetic signal between partitions

and negative correlations indicate conflict (Damgaard and Cognato, 2003; López-Fernández et al., 2005b).

Congruence between our topology and those obtained by previous authors was also evaluated. We performed topological comparisons using the Shimodaira–Hasegawa test as implemented in PAUP* 4.0b10. In order to match previously published datasets, we used Mesquite (Maddison and Maddison, 2009) to prepare pruned versions of our dataset to match the taxon sampling in the published topologies for Geophagini (Smith et al., 2008), Cichlasomatini (Musilová et al., 2009; Smith et al., 2008), Heroini (Concheiro Pérez et al., 2006; Rican et al., 2008; Smith et al., 2008) and Cichlinae (Smith et al., 2008). An effort was made to maximize the similarity between datasets, so species removed from our analyses were, whenever possible, those that were not present in the other studies. For example, in the comparison with Smith et al.'s (2008) topology, we used *Cichla temensis* sequences because that is the species they used in their study. Removal of species from our dataset for comparisons also minimized missing data. Comparisons were limited to Neotropical taxa and rooted with one species from the same set of taxa used in the published versions of previous topologies. Only one outgroup taxon was used in order to focus the topological comparisons on the ingroup by avoiding increasing similarity through the use of multiple outgroups whose relationships were not questioned and may have artificially increased topological similarity.

Finally, a bootstrap-based internal branch test (IBT, Dopazo, 1994; Sitnikova, 1996) was used to determine whether internal branches in the topology were either significantly different from zero or compatible with a polytomy (Nei and Kumar, 2000). IBTs were implemented in MEGA 4 (Tamura et al., 2007, 2008) and evaluations were based on both Neighbor-Joining and Minimum Evolution distance-based topologies, derived with and without gamma-corrections for among-site rate heterogeneity and estimated proportion of invariant sites (and see López-Fernández et al., 2005a).

2.5. Taxonomic conventions and classification of the Cichlinae

Given the historical instability of Neotropical cichlid nomenclature and the absence of a detailed phylogenetic hypothesis, especially for the Central American Heroini, it is not always clear what to name a given clade. Whenever possible, we followed and expanded the approach used by Rican et al. (2008) that used available generic names (sensu the International Code of Zoological Nomenclature, ICZN 1999) for clades containing the type species to which a generic name was first assigned. The decision of which generic name to apply to monophyletic clades found in this paper was strictly driven by the conventions of priority and common usage, in which the oldest name available is the one that should be utilized unless a different name dominates common usage in which case the latter may be preferred. Thus, for example, we have applied the names *Paraneetroplus* Regan (1905) and *Theraps* Günther (1862) instead of *Vieja* Fernández-Yépez (1969) for clades that include the type species *Paraneetroplus bulleri* and *Theraps irregularis*, respectively. Although our generic assignments often coincide with those of Rican et al. (2008), differences in the topology and the strict use of taxonomic priority in this study resulted in some changes. Decisions on available names were based on the original literature, largely guided by the detailed historical analysis of nomenclature performed by Kullander (1983, 1996) and Kullander and Hartel (1997). References to type species for genera follow Kullander (2003) and Eschmeyer and Fricke (2009). For suprageneric nomenclature we follow Smith et al. (2008) at the tribe- level, and Concheiro Pérez et al. (2006) and Rican et al. (2008) in the use of informal names for categories below tribe. When possible, both diagnostic and total molecular apomorphies as well as nucleotide and amino acid transformations per locus for both suprageneric clades and genera are given in Supplement Tables 2 to 5. Diagnostic molecular characters were listed for each clade by parsimony mapping of nucleotide and amino acid substitutions (in the case of coding sequences) on the topology in Fig. 1 and counting the number of unambiguous changes (Consistency Index = 1) at each node using PAUP* 4.0b10 (Swofford, 2002) and Mesquite version 2.6 (Maddison and Maddison, 2009). Additionally, uniquely shared gaps in the alignments of non-coding genes were tallied and given as diagnostic for certain clades when available (Supplement Tables 2 and 3, and see Musilová et al., 2009).

3. Results

3.1. Patterns of divergence in different partitions

Chi-square tests implemented in PAUP* 4.0b10 (Swofford, 2002) failed to reject the hypothesis of homogeneity of nucleotide composition (Table 1) for all positions combined in any of the genes. However, third positions in ND4 and Cytochrome *b* had a significant anti-guanine bias (G = 6.3%, $X^2 = 695.80$, df = 495, $p < 0.01$; G = 3.5%, $X^2 = 986.48$, df = 495, $p < 0.01$, respectively). Mean values of base composition in ND4 presented an additional deficiency of guanine in first (16%) and second (12%) positions, but these were not enough to reject homogeneity. Cytochrome *b* had a reduced proportion of guanine in second positions (12.9%), but again not enough to reject homogeneity. Results for Cytochrome *b* are similar to those obtained by Farias et al. (2001) who found 4% guanine in third positions and an overall mean of 14% in an analysis of 78 cichlid sequences. The aligned length of the combined dataset was 3868 base pairs; aligned lengths for each of the five loci are given in Table 1. Both the 16S and S7 alignments showed some variation in length due to numerous insertions and deletions. Among the coding genes, only ND4 was found to have 1-codon deletions at position 130 for *Retroculus* and *Heterochromis multidens* and at position 136 for *Cichla* and *Biotodoma cupido*. Saturation plots (not shown) revealed saturation in third codon positions for ND4 and Cytochrome *b* above approximately 20% uncorrected divergence, but no saturation was detected in RAG2 at any position. These results coincide with previous analyses of all three genes (Farias et al., 2001 for Cytochrome *b*, López-Fernández et al., 2005a for ND4 and RAG2). The minimum amount of uncorrected genetic divergence in any fragment was observed in RAG2 between some species of the genera *Geophagus*, *Guianacara*, *Heros*, *Amphilophus*, *Cryptoheros*, *Herichthys*, and *Paraneetroplus*, all of which had identical sequences within each genus. The minimum non-zero divergence in RAG2 was 0.1% between *Herichthys cyanoguttatus* and *H. carpintis* and *H. tamasopoensis*, and the maximum was 7.5% between *Apistogramma pucallpaensis* and the African *Heterochromis multidens*. The maximum divergence observed in the dataset was 42% in ND4 between *Andinoacara coeruleopunctatus* and *Crenicichla* sp. 'Orinoco wallacii'; the minimum divergence in ND4 was 0% between *Amphilophus citrinellus* and *A. labiatus*. Divergence in 16S varied between 0.19% in the pairs *Cichla intermedia* × *C. orinocense* and *Guianacara stergiosi* × *G.* sp. 'Takutu' and 16.3% in *Dicrossus* sp. × *Heterochromis multidens*. Divergence in Cytochrome *b* varied between 1.8% in *Paretroplus polyactys* × *Paratilapia polleni* and 28.9% in *Nannacara taenia* × *Taeniacara candidi*. Distances in S7 ranged between 0.2% in *Paraneetroplus maculicauda* × *P. bifasciatus* and *P. melanurus* and 25.8% between *Gymnogeophagus rhabdotus* × *Etroplus maculatus*.

3.2. Phylogenetic analyses

Maximum parsimony analysis (MP) of the five-gene dataset with equal weights produced 12 MP trees of 21647 steps (CI = 0.18, RI = 0.57, RC = 0.10) that were very similar to the ML

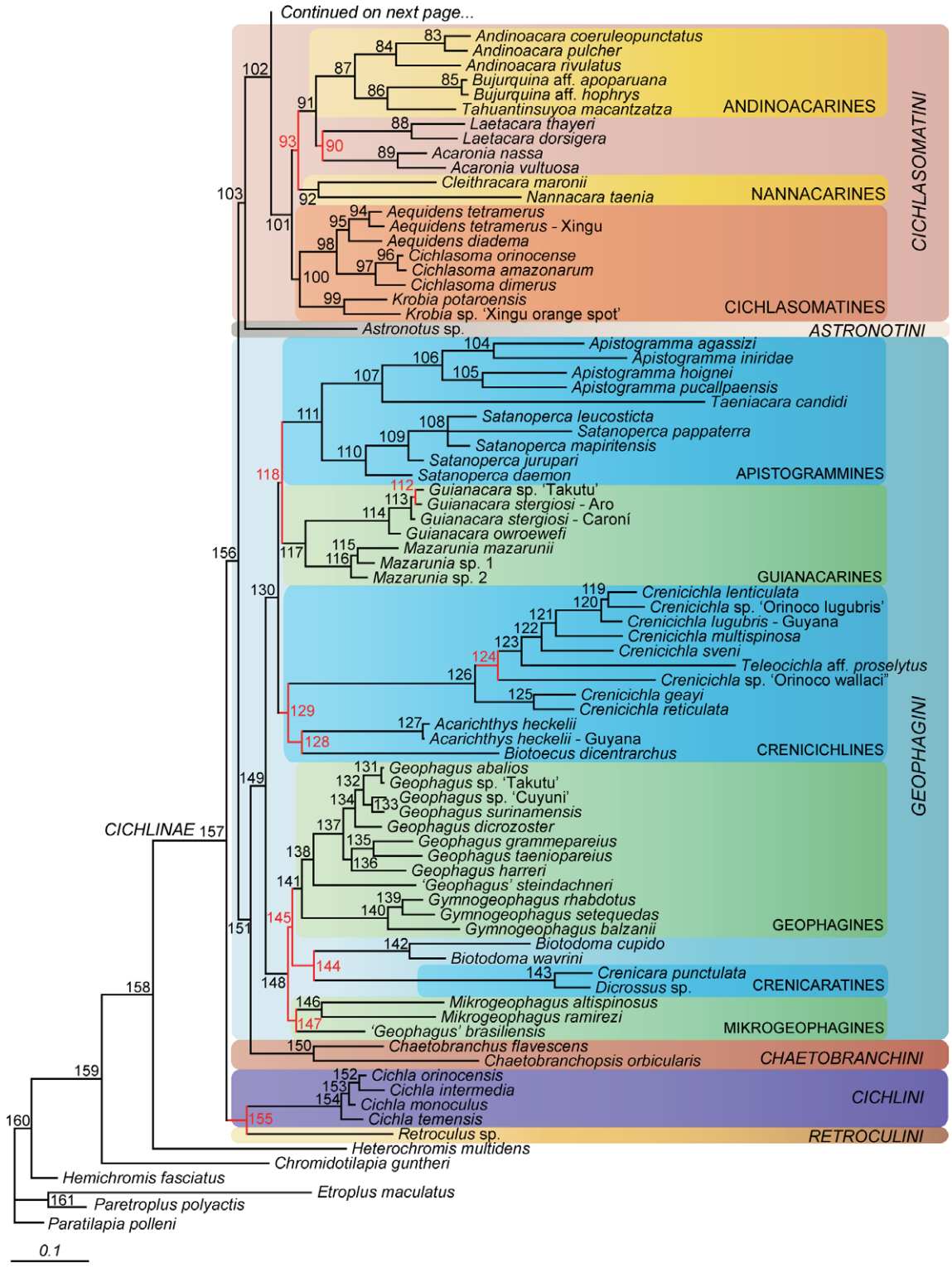

Fig. 1. Phylogenetic relationships of Neotropical cichlid fishes proposed in this paper based on 3868 base pairs from five loci. The tree represents Bayesian relationships recovered in a partitioned, unlinked analysis with 12×10^6 replications using three mitochondrial (16S, Cytochrome *b*, ND4) and two nuclear DNA fragments (S7 Intron 1, RAG2). Node numbers correspond to those given in Table 2 providing support for the topology under Bayesian, Maximum Likelihood and Maximum Parsimony optimality criteria. Red numbers and branches depict branches whose length is not statistically different from zero according to the Internal Branch Test (see Section 2). Taxa accompanied by an asterisk have South American distribution but show phylogenetic affinity with the Central American Heroini. Colored boxes illustrate the composition of each clade as per the nomenclature used throughout the paper. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(−ln = −94480.1212) and BI topologies (Fig. 1, node support from all three methods is given in Table 2). Topologies obtained with BI and ML analyses were essentially identical. We performed an additional MP analysis with third positions of ND4 and Cytochrome *b* removed to explore the effects of observed saturation and base composition biases. This search revealed an identical pat-

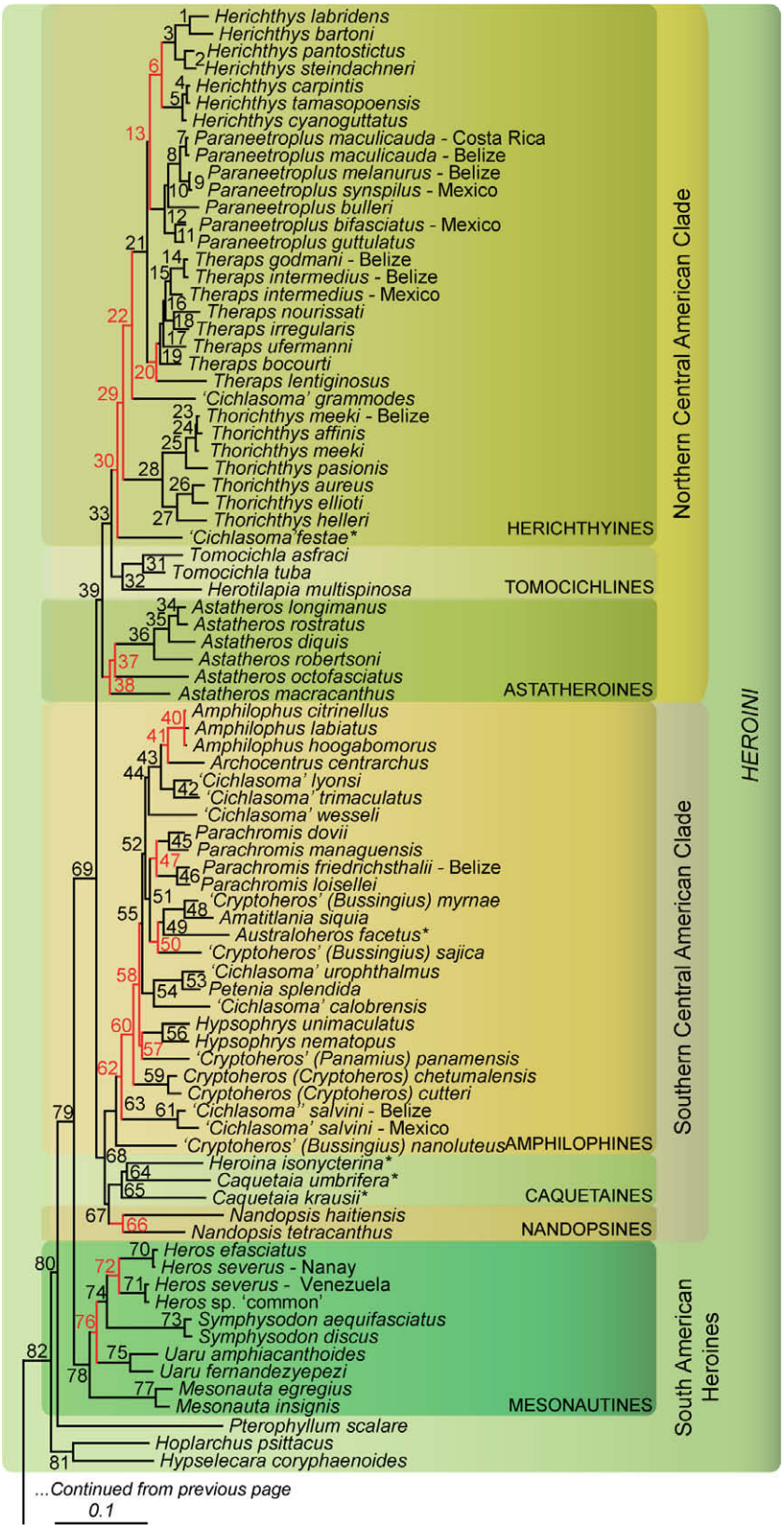

Fig. 1 (*continued*)

tern of higher level divergence to that shown by the entire dataset, but resolution was lower near the tips of the tree, i.e. within genera and among genera inside major clades (not shown). These results suggest that third positions contained important phylogenetic information and that use of the entire dataset, even under parsimony, provides better resolution than an analysis that excludes third positions. Topological disagreement among trees obtained with different methods was mainly observed among clades with

significantly-short basal branches and low support in all analyses (branches highlighted in red in Fig. 1, and see Table 2 and Section 4). All searches strongly supported the monophyly of the Neotropical Cichlidae. Relationships among outgroup taxa were consistent with previous analyses of African and Indo-Malagasy cichlids (subfamilies Pseudocrenilabrinae and (Ptychochrominae + Etroplinae), respectively, see e.g. Stiassny, 1991; Farias et al., 2000; Sparks and Smith, 2004). Neotropical cichlids (subfamily Cichlinae) were grouped into two main clades in which the genera *Retroculus* (Tribe Retroculini) and *Cichla* (Cichlini) are sister to a monophyletic group containing all other lineages. This group is subdivided into five clades: the tribes Chaetobranchini, Geophagini, Astronotini, Cichlasomatini and Heroini (Fig. 1). Within this major group, the Chaetobranchini (genera *Chaetobranchus* and *Chaetobranchopsis*) and Geophagini and the Astronotini (genus *Astronotus*) and (Cichlasomatini + Heroini) form respective sister groups.

All analyses supported monophyly of the tribe Geophagini, containing six groups in two major subclades (see Table 2). The first major subclade of Geophagini (node 148) includes geophagines, mikrogeophagines and crenicaratines. While well-supported, branches at the base of the first two groups are significantly short according to IBT tests, and relationships among the three clades remain poorly supported (Node 145, Fig. 1 and Table 2). Geophagines include the genera *Geophagus* sensu stricto, '*Geophagus*' *steindachneri* and *Gymnogeophagus*; mikrogeophagines include *Mikrogeophagus* and the undescribed '*Geophagus*' *brasiliensis* group; and crenicaratines include *Crenicara* and *Dicrossus*, which are weakly grouped with *Biotodoma* (node 144). The second large clade of Geophagini (node 130) is also strongly supported and includes three subclades: (1) a moderately supported crenicichlines that groups *Crenicichla* and the putative genus *Teleocichla* with an also fairly well-supported sister-group relationship between *Acarichthys* and *Biotoecus*; (2) the previously undetected and strongly supported guianacarines containing the genera *Guianacara* and *Mazarunia*, both endemic to the Guiana Shield of northern South America (and see Section 4); and (3) the strongly supported apistogrammines, grouping *Apistogramma*, *Taeniacara* and *Satanoperca*. A sister-group relationship between guianacarines and apistogrammines is suggested but poorly supported by a significantly-short branch (Fig. 1 and Table 2, node 118). Despite strongly supported monophyly, basal relationships among the two major clades and six groups of Geophagini remain poorly supported and/or with significantly-short basal branches (Fig. 1, nodes 118, 128, 129, 144, 145 and 147).

The second large clade of Neotropical cichlids is the Tribe Cichlasomatini, which contains three well-supported subclades (Fig. 1, Table 2): cichlasomatines include *Cichlasoma* and *Aequidens* sensu stricto as sister to *Krobia*, which in our study includes '*Aequidens*' *potaroensis* (see Section 4 and Musilová et al., 2008). Andinoacarines contain the genera *Bujurquina* and *Tahuantinsuyoa*, which are sister to the mostly north-western South American species in the genus *Andinoacara* (and see Musilová et al., 2009); the genera *Acaronia* and *Laetacara* are weakly supported as a clade sister to andinoacarines (Fig. 1 and Table 2, node 90). Finally, andinoacarines and the *Acaronia* + *Laetacara* clade are weakly united to nannacarines, which is a fairly well-supported grouping of *Nannacara* and *Cleithracara* (Fig. 1, Table 2, node 92). Despite well-supported relationships, some basal groupings among clades of Cichlasomatini remain tentative, as evidenced by low support and basal branches that are significantly short in at least two nodes at or near the base of the clade (Fig. 1, Table 2, nodes 90 and 93).

The third large clade is the tribe Heroini, a well-supported sister clade to Cichlasomatini in which the South American genera *Hoplarchus* and *Hypselecara* are sister to each other and to a large clade in which *Pterophyllum* is sister to all other heroine cichlids (Fig. 1). Within this larger clade, mesonautines (Fig. 1, node 78) are strongly supported and include the South American *Mesonauta*, *Uaru*, *Symphysodon* and *Heros*, all of which are sister to a large clade of mostly Central American affiliation representing the bulk of heroine diversity. This large heroine group is divided into two major clades, which we informally refer to as the Southern and Northern Central American Clades (SCAC and NCAC, respectively) on the basis of their roughly geographic composition of taxa (Fig. 1). Both of these groups have good Bayesian support, but little or no bootstrap and Bremer support (Fig. 1, Table 2, nodes 39 and 68). The SCAC includes the sister nandopsines and caquetaines (Fig. 1, node 67) as sisters to amphilophines (Fig. 1, node 63, *sensu* this paper). Caquetaines include the South American *Heroina* nested within *Caquetaia* (but see Section 4) and nandopsines contain the Greater Antilles genus *Nandopsis*. Amphilophines include a fairly well-supported monophyletic group of Central American genera plus the South American *Australoheros* weakly placed in a small clade with *Amatitlania* and 2 '*Cryptoheros*' species. Monophyly for some amphilophine genera (e.g. *Parachromis*) is strongly supported as are some suprageneric groupings (e.g. *Amphilophus* + *Archocentrus*, '*Cichlasoma*' *lyonsi* clade, Fig. 1, nodes 41 and 42); however, the genus *Cryptoheros* and its subgenera (sensu Schmitter-Soto, 2007a) was recovered as polyphyletic, and most basal relationships within amphilophines remain unresolved or are weakly supported by extremely short branches (Fig. 1, Table 2, nodes 41, 50, 57, 58, 60 and 62). The NCAC clade includes three well-defined clades: the moderately supported astatheroines and herichthyines and the strongly supported tomocichlines. Astatheroines include a monophyletic genus *Astatheros* that includes *Rocio* (see Schmitter-Soto, 2007a, and see below); tomocichlines include *Tomocichla* and *Herotilapia* (contra Schmitter-Soto, 2007a, who had synonymised *Herotilapia* with *Archocentrus*); and herichthyines include the South American '*Cichlasoma*' *festae* at its base and the Central American *Thorichthys*, '*Cichlasoma*' *grammodes*, *Theraps* (*sensu* this paper), *Paraneetroplus* (*sensu* this paper) and *Herichthys* in a pectinate genus-level arrangement. Generic monophyly among herichthyines is strongly supported in all cases, but suprageneric relationships are often moderately supported and/or based on significantly-short branches (Fig. 1 and Table 2, nodes 13, 22, 29 and 30).

3.3. Phylogenetic congruence and branch length tests

Pearson's pairwise correlation analysis of PBS values among partitions for each node of the Bayesian and Parsimony trees revealed that support from Cytochrome *b* for the combined topology was generally negative and at odds with Bremer support from other partitions. Because these results suggested strong incongruence of Cytochrome *b* with the rest of the data, we repeated the phylogenetic analyses described above after removing the Cytochrome *b* partition (see also López-Fernández et al., 2005b). However, topologies obtained from this reduced dataset (not shown) only differed from the ones including Cytochrome *b* in the relative position of certain clades with extremely short basal branches (as revealed by the IBTs, see Section 2.4 and Fig. 1). Additionally, posterior probabilities and bootstrap support for most nodes were higher in the dataset including Cytochrome *b*.

Pseudogenes of mitochondrial loci are not uncommonly found in fishes and other organisms (e.g. Dubey et al., 2009; Mabuchi et al., 2004; Triant and DeWoody, 2007, 2008). We explored the possibility that topological incongruence may be caused by paralogous sequences of Cytochrome *b* introgressed into the nuclear genome of some taxa. Because selective constraints are relaxed on non-functional copies of coding genes, a larger ratio of non-synonymous (dN) to synonymous (dS) nucleotide substitutions is expected in the pseudogene of a functional sequence. Likewise, the

Table 2
Statistical support for nodes in the tree presented in Fig. 1 according to Bayesian posterior probabilities (BPP), Boostrap values for the RAxML Maximum Likelihood topology (MLBS), and Decay Index values for the Maximum Parsimony tree (MPDI). See Section 2 for details on the calculation of support for each clade and Section 3 for explanations on the taxonomic composition of each supragenerical grouping.

Node	Clade	BPP	MLBS	MPDI	Node	Clade	BPP	MLBS	MPDI	Node	Clade	BPP	MLBS	MPDI
1		0.54	55	9	55		1.00	52	–	109		1.00	100	12
2		1.00	100	16	56	*Hypsophrys*	1.00	100	24	110	*Satanoperca*	1.00	100	21
3		0.54	54	9	57		0.78	<50	–	111	Apistogrammines	1.00	100	5
4		1.00	100	10	58		0.86	62	–	112		0.98	70	–
5		1.00	100	36	59		1.00	100	22	113		0.98	85	2
6	*Herichthys*	1.00	100	10	60		0.57	–	–	114	*Guianacara*	1.00	100	7
7		0.55	100	10	61		1.00	100	22	115		1.00	95	2
8		1.00	100	12	62		0.81	–	–	116	*Mazarunia*	1.00	100	16
9		1.00	100	19	63	Amphilophines	1.00	84	9	117	Guianacarines	1.00	100	13
10		0.55	–	2	64		0.69	62	5	118		0.64	<50	–
11		1.00	100	10	65	Caquetaines	1.00	97	8	119		0.99	69	–
12	*Paraneetroplus*	1.00	100	6	66	nandopsines	1.00	99	6	120		1.00	100	24
13		0.55	51	2	67		0.97	<50	–	121		0.93	71	3
14		1.00	100	7	68	SCAC	1.00	<50	1	122		0.57	52	2
15		0.98	88	5	69	SCAC + NCAC	1.00	100	17	123		0.83	–	–
16		0.81	66	2	70		1.00	100	66	124		0.99	64	6
17		0.88	<50	1	71		1.00	100	36	125		1.00	100	59
18		0.99	92	5	72	*Heros*	1.00	100	11	126	*Crenicichla*[a]	1.00	100	39
19		0.99	69	–	73	*Symphysodon*	1.00	100	90	127	*Acarichthys*	1.00	100	61
20	*Theraps*	1.00	94	5	74		1.00	91	4	128		0.96	76	–
21		1.00	100	15	75	*Uaru*	1.00	100	40	129	Crenicichlines	0.99	80	–
22		1.00	95	3	76		0.81	–	2	130		1.00	99	6
23		0.87	55	1	77	*Mesonauta*	1.00	100	81	131		1.00	100	38
24		1.00	100	19	78	Mesonautines	1.00	100	7	132		1.00	100	11
25		1.00	100	29	79		1.00	100	7	133		1.00	100	14
26		1.00	100	25	80		1.00	81	–	134		1.00	100	16
27		1.00	95	14	81		1.00	100	–	135		1.00	100	16
28	*Thorichthys*	1.00	100	35	82	Heroini	0.99	100	18	136		0.95	70	1
29		0.99	69	0	83		1.00	100	58	137	*Geophagus*	1.00	100	32
30	Herichthyines	0.99	86	0	84	*Andinoacara*	1.00	100	29	138		1.00	98	4
31	*Tomocichla*	1.00	100	20	85	*Bujurquina*	1.00	100	76	139		1.00	95	13
32	Tomocichlines	1.00	94	0	86		1.00	100	17	140	*Gymnogeophagus*	1.00	100	42
33		0.99	66	0	87	Andinoacarines	1.00	100	19	141	Geophagines	1.00	100	4
34		1.00	100	17	88	*Laetacara*	1.00	100	36	142	*Biotodoma*	1.00	100	60
35		1.00	100	20	89	*Acaronia*	1.00	100	56	143	Crenicaratines	1.00	100	26
36		1.00	100	31	90		0.77	68	–	144		0.72	50	6
37		0.75	54	4	91		1.00	95	4	145		0.60	<50	4
38	Astatheroines	1.00	79	5	92	Nannacarines	1.00	81	11	146	*Mikrogeophagus*	1.00	100	21
39	NCAC	0.97	62	0	93		0.56	–	3	147	Mikrogeophagines	0.96	76	4
40	*Amphilophus*	0.93	100	38	94		1.00	100	37	148		1.00	100	6
41		1.00	98	11	95	*Aequidens*	1.00	83	15	149	Geophagini	1.00	100	6
42	'*Heros*' *lyonsi*	1.00	100	15	96		1.00	100	51	150	Chaetobranchini	1.00	100	6
43		1.00	97	7	97	*Cichlasoma*	1.00	100	46	151		1.00	97	3
44		0.89	<50	–	98		1.00	100	32	152		0.96	72	5
45		1.00	100	9	99	*Krobia*	1.00	100	26	153		0.66	–	4
46		1.00	100	21	100	Cichlasomatines	1.00	88	–	154	Cichlini	1.00	100	77
47	*Parachromis*	1.00	85	–	101	Cichlasomatini	1.00	100	15	155		1.00	97	2
48		1.00	100	22	102		1.00	100	15	156		0.96	80	3
49		0.76	<50	–	103		0.96	<50	8	157	Cichlinae	1.00	100	23
50		0.99	62	–	104		0.99	83	1	158		1.00	99	–
51		0.69	<50	–	105		1.00	100	7	159		1.00	96	–
52		0.93	<50	2	106	*Apistogramma*	1.00	100	12	160		1.00	96	–
53		1.00	100	43	107		1.00	100	17	161		1.00	96	–
54		1.00	60	11	108		1.00	80	–					

[a] Including *Teleocichla* (and see Section 4).

overall number of amino acid substitutions in a functioning coding gene should be smaller than in a non-functional copy of the same gene. We compared patterns of dN to dS nucleotide substitutions and overall amino acid substitutions between Cytochrome *b* and ND4 because they are both coding mitochondrial genes, but ND4 was congruent with the combined topology. We used MEGA 4 (Tamura et al., 2007) to calculate dN, dS, genetic distance and amino acid differences for all clades in the phylogeny. ANCOVA analysis of the mean number of amino acid differences against the amount of nucleotide divergence in different clades of the phylogeny revealed non-significant interactions between genetic divergence and loci ($p > 0.05$), suggesting that Cytochrome *b* and ND4 have similar rates of amino acid change. Likewise, ANCOVA revealed non-significant interactions between dN/dS and loci ($p > 0.05$), indicating that the ratios of non-synonymous to synonymous amino acid substitutions in both genes are also similar. Based on these results we decided to include Cytochrome *b* in all further analyses for three reasons. First, topological differences are restricted to short branches. Second, support is higher when Cytochrome *b* is included. Finally, Cytochrome *b* showed no indicators of changes expected for a pseudogene.

Topological comparisons of the phylogeny presented in this paper (Fig. 1) along with those previously published indicate that although congruent with some published analyses (Musilová et al., 2009, Shimodaira–Hasegawa test $p > 0.05$ for the tribe Cichlasomatini), our tree in general is significantly more likely than

those previously proposed. In particular, the recent analysis of American cichlids by Smith et al. (2008) is qualitatively the most incongruent of all previous topologies, and is quantitatively incongruent when either the entire Cichlinae (S–H test, $p < 0.01$ −ln L Smith et al's tree = 68519.58, −ln L this study = 67745.39) or each major subclade is compared with our results (S–H test, −ln L Smith et al's Geophagini = 30318.34, −ln L this study = 29675.98, $p < 0.001$; −ln L Smith et al.'s Cichlasomatini = 18283.01, −ln L this study = 18244.30, $p < 0.05$; −ln L Smith et al's Heroini = 26188.60, −ln L this study = 26088.04, $p < 0.05$). Although congruent in several qualitative aspects, our topology for the Heroini is also significantly more likely than those proposed by Concheiro Pérez et al. (2006) (S–H test, −ln L Concheiro Pérez et al. = 55153.71, −ln L this study 54832.94, $p < 0.01$) and Rican et al. (2008) (S–H test, −ln L Rican et al. 33566.51, −ln L this study 33313.15, $p < 0.01$).

Tests of internal branch lengths revealed a number of significantly-short branches at the base of all three major clades of Cichlinae (Fig. 1, branches highlighted in red). These short branches generally coincide with poorly supported nodes reinforcing the notion that some nodes may not be distinguishable from polytomies (e.g. nodes 60, 145). However, some nodes with short branches had high statistical support (e.g. nodes 22, 41, 128, 155, Fig. 1 and Table 2), suggesting that short basal branches do not necessarily translate into weak phylogenetic resolution.

4. Discussion

4.1. Taxon sampling and diagnosis of genera of Neotropical cichlids

The first attempt to classify the Neotropical Cichlidae within a modern, explicit phylogenetic context was that of Kullander (1998), who used a matrix of 91 morphological characters to build a phylogeny of most South American cichlid genera. Since then, a number of studies have addressed the phylogeny of either specific clades (e.g. Geophagini, López-Fernández et al., 2005a,b; Cichlasomatini, Musilová et al., 2008, 2009; Heroini, Concheiro Pérez et al., 2006; Rican et al., 2008) or the entire subfamily (Smith et al., 2008). The majority of these studies found large incongruences with Kullander's early phylogeny (e.g. see López-Fernández et al., 2005a,b), and much of his original classification has been challenged in light of newly proposed topologies. The most recent classification of the Cichlinae was proposed by Smith et al. (2008) on the basis of parsimony analysis of a super matrix of seven loci combined with Kullander's (1998) morphological dataset. Unfortunately, Smith et al.'s (2008) topology has proven to be remarkably incongruent both quantitatively and qualitatively (see Section 3.3) with all other studies, and we consider the classification derived from their analysis inadequate in light of recent studies based on much larger taxon sampling and more widely accepted methods of phylogenetic analysis. A number of observations seem appropriate regarding Smith et al.'s newly proposed classification.

Considering that all of the morphological characters (or modifications of them) and DNA loci used by Smith et al. (2008) were analyzed in previous studies (Kullander, 1998; Farias et al., 1999, 2000, 2001; Hulsey et al., 2004; Sparks and Smith, 2004; López-Fernández et al., 2005a,b; Chakrabarty, 2006b; Concheiro Pérez et al., 2006; Rican and Kullander, 2006; Rican et al., 2008; Musilová et al., 2008, 2009) and that in the case of Geophagini their study and ours include the same genera, it is puzzling that their topology is so strongly incongruent with other hypotheses (see Section 3.3). The original morphological dataset they used was modified in other studies to address geophagine relationships (López-Fernández et al., 2005b) and was found to be inadequate to resolve relationships among Central American cichlids (Rican et al., 2008). Smith et al. (2008) used a method of simultaneous alignment and topology search (direct optimization) for their phylogenetic analysis that, although interesting in principle and the subject of ongoing research (e.g. Liu et al., 2009), has been widely criticized in its specific implementation both on methodological and epistemological grounds (e.g. Rieppel, 2007, and references therein). Besides these criticisms, it seems clear that the major problem with Smith et al.'s (2008) tree and derived classification stems from insufficient taxon sampling. Despite their claim to have produced the "first well-supported and resolved generic-level phylogeny for Neotropical cichlids" (Smith et al. 2008, p. 625 in Abstract), their study lacks many taxa essential to do that. Particularly, their representation of the deeply problematic Central American taxa, a group requiring dense sampling of lineages because generic assignment is not clear or is weakly supported by previous hypotheses (e.g. the former genus *Archocentrus* sensu lato, see Schmitter-Soto, 2007a), is severely limited. For example, their use of '*Cichlasoma*' *wesseli* (within the complicated amphilophines and probably part of an undescribed clade including '*C.*' *istlanus*, among others, Concheiro Pérez et al., 2006 and see Fig. 1, this paper) as a representative of the genus *Theraps*, effectively excluded *Theraps* from their analysis. Furthermore, their analysis lacks, at a minimum, representatives of the problematic *Cryptoheros* sensu lato, '*Cichlasoma*' *urophthalmus*, '*C.*' *calobrensis*, '*C.*' *salvini*, '*C.*' *lyonsi*, and '*C.*' *grammodes* lineages. All of these taxa were recovered in ours and in other studies as belonging to yet undescribed lineages of Central American Heroini (see Fig. 1, and Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008). Consequently, no test of genus-level monophyly or clarification of the taxonomic status of problematic lineages is possible with Smith et al.'s (2008) dataset. Clearly, much broader taxon sampling is necessary to resolve Neotropical cichlid generic relationships. In light of these shortcomings, we limit our discussion of Smith et al.'s (2008) paper to cases in which their findings are compatible with other analyses or in which their interpretation of the results is judged to require clarification.

The above situation illustrates a more general problem and provides further empirical evidence supporting the use of extensive taxon sampling in phylogenetic analysis (e.g. Graybeal, 1998; Zwickl and Hillis, 2002; Hillis et al., 2003; Rican et al., 2008). This point is further strengthened by our finding of the previously unknown Geophagini clade, referred to herein as guianacarines, containing the South American genera *Guianacara* and *Mazarunia* (Fig. 1). Until this study, a clade uniting *Guianacara* and *Acarichthys* [Kullander (1998) tribe Acarichthyini] was generally recovered based on morphological characters (Kullander, 1998, but see López-Fernández et al., 2005b) and molecular data, albeit with moderate support (e.g. Farias et al., 2000; López-Fernández et al., 2005a,b). Our addition of all known species of the previously unavailable genus *Mazarunia* has recovered the strongly supported Guianacarines and a well-supported grouping of *Acarichthys* with *Biotoecus* as part of the crenicichlines (Fig. 1, Table 2, node 128). Therefore, incorporating additional taxa had the effect of revealing an entirely new phylogenetic arrangement in which the position of two previously problematic genera is more clearly resolved.

Finally, results from this study strongly suggest that further resolution of clades that remain poorly supported should benefit from incorporating more taxa. More conclusive characterization of Central American genera, particularly within amphilophines, will require more detailed phylogenetic analysis and morphological descriptions beyond those used to date because they clearly are insufficient to diagnose the majority of genera. In combination, our results and those of Concheiro Pérez et al. (2006) and Rican et al. (2008) indicate that traditional morphological characters used to define either the Central American cichlid genera (e.g. Günther, 1862; Meek, 1904; Schmitter-Soto, 2007a) or sections of the

former *Cichlasoma* (Regan, 1905) are generally subject to pervasive homoplasy and are thus misleading when used as the sole information to define evolutionary lineages. Characters such as tooth or body shape seem to vary extensively within clades as starkly exemplified by *Theraps* (sensu this study), in which extreme reophilic forms with elongate bodies (e.g. *T. irregularis*) are part of the same clade as deep-bodied species such as *T. intermedius*. In another case, the reophilic adaptations of *Paraneetroplus bulleri* repeatedly led to treating this species as a monotypic genus (e.g. Miller et al., 2005), while our molecular phylogenetic analysis found it as part of a much larger clade with at least one unique molecular diagnostic character and as much ecomorphological variation as that encompassed by the *Theraps* clade.

Our phylogenetic results include a sufficiently large taxon sampling to allow a first revision of the nomenclature of these genera with the concomitant elimination of superfluous names (e.g. *Vieja* Fernández-Yépez) in preference of available names with taxonomic priority (e.g. *Theraps*, *Paraneetroplus*). Despite the advances provided by the latest molecular phylogenetic studies, including this one, resolution of Central American cichlid taxonomy requires further research. Molecular diagnosis of genera and the emerging image of their relationships leaves us in the unsatisfactory state that many genera are not clearly diagnosable morphologically, which will surely hinder the efforts of evolutionary biologists, ecologists, and conservationists. Thus, we acknowledge that further morphological analysis is necessary, especially for the Central American Heroini. Nevertheless, the tree provided in this study provides the most complete phylogenetic framework available for studying the tempo and processes of evolutionary diversification within ecologically diverse assemblages of Neotropical cichlids.

4.2. Higher-level relationships of the American Cichlidae

In general, the higher-level relationships among Neotropical cichlids found in this study are consistent with previous results (Kullander, 1998; Farias et al., 2000; Sparks and Smith, 2004). Most of the diversity of Cichlinae is concentrated in the three clades Geophagini, Cichlasomatini and Heroini. We found the genus *Astronotus* (Astronotini) as sister to the (Cichlasomatini + Heroini) clade (and see Concheiro Pérez et al., 2006), whereas other studies have found it closer to the clade of (Cichlini + Retroculini) (Farias et al., 2000; López-Fernández et al., 2005b) or to Chaetobranchini (Kullander, 1998). The moderate support for this position (node 103, Table 2) suggests that additional taxon sampling may still determine further changes in the position of Astronotini. As in several previous studies, the tribe Chaetobranchini (*Chaetobranchus* + *Chaetobranchopsis*) was confirmed as the sister group to Geophagini (e.g. Farias et al., 2000), but with stronger support than previously recovered (node 150, Table 2). A well-supported but significantly-short branch united Cichlini and Retroculini (node 155, Fig. 1, Table 2). Interestingly, this relationship was strongly recovered by López-Fernández et al. (2005b), using their combined molecular and morphological data, but has not been recovered in either other molecular (Farias et al., 2000; Sparks and Smith, 2004) or combined analyses that included Kullander's (1998) morphological dataset (Farias et al., 2000, 2001). In combination with recently published studies (e.g. López-Fernández et al., 2005b; Concheiro Pérez et al., 2006; Rican et al., 2008; Musilová et al., 2009), our results further clarify the increasingly resolved higher-level framework of seven tribes and confirm some recently proposed intra-clade relationships (e.g. Musilová et al., 2009). More significantly, our study's expanded taxon sampling of Geophagini and Heroini, together with the inclusion of a larger amount of molecular data provides evidence for several previously unknown groupings. These additions clearly improve our understanding of the taxonomy, phylogenetic history, and evolutionary processes underlying the diversification of Neotropical cichlids. Below we discuss the most relevant relationships in light of previous findings, and examine some general implications of the phylogeny for the evolutionary origin of Cichlinae.

4.3. Relationships among Geophagini

This study expands López-Fernández et al.'s (2005a,b) taxon sampling of Geophagini by both adding the genera *Teleocichla* (1 species) and *Mazarunia* (3 spp.) and increasing the number of species in several genera (e.g. *Guianacara*, *Crenicichla*). When compared with previous analyses, resolution and support of Geophagini did increase for many of the relationships within the major clades (see Table 2 versus López-Fernández et al., 2005a,b). For instance, our study provides strong support for a clade grouping geophagines, crenicaratines, mikrogeophagines and the genus *Biotodoma*, and this grouping is identical to the "B" clade identified by López-Fernández et al. (2005b) (see node 148, Table 2, this study) in their combined molecular and morphological analysis. Additionally, increased taxon sampling in our study and the inclusion of formerly missing molecular data (see López-Fernández et al., 2005a) provide stronger support for clades such as geophagines and mikrogeophagines (nodes 141 and 147, respectively, Table 2). Despite these improvements, however, short basal branches still prevent us from clarifying the basal relationships between geophagines, mikrogeophagines, crenicaratines, and *Biotodoma*. Although both this study and the previous analyses suggest that *Biotodoma* is sister to crenicaratines, support for that grouping remains low (node 144, and see López-Fernández et al., 2005b), and further study will be necessary to confirm that relationship.

The rest of Geophagini grouped into a second strongly supported clade that includes three groupings (node 130, Table 2). Apistogrammines are identical to the "*Satanoperca* clade" of López-Fernández et al. (2005a,b), and our data provide increased support for this grouping (node 111, Table 2). *Apistogramma pucallpaensis*, previously placed in the monotypic genus *Apistogrammoides* Meinken 1965, is nested within a clade containing other species of *Apistogramma* (Fig. 1, node 106). Therefore, to keep *Apistogramma* as a monophyletic genus, we consider *Apistogrammoides* Meinken 1965 a junior synonym of *Apistogramma* Regan 1913. The clade crenicichlines provides further confirmation that the genus *Teleocichla* is related to *Crenicichla* (e.g. Stiassny, 1987; Kullander, 1988; Farias et al., 2000; Smith et al., 2008), but its deeply nested placement within a clade containing species of *Crenicichla* challenges the recognition of *Teleocichla* as a distinct genus (see also Farias et al., 2000). Nevertheless, we hesitate to recommend synonymy of *Teleocichla* with *Crenicichla* until more data are available, because in comparison to other Neotropical cichlids, crenicichlines reveal some of the most rapid and heterogeneous rates of molecular evolution (Farias et al., 1999; López-Fernández et al., 2005a), and we only present data for one species of *Teleocichla*. Also within crenicichlines, *Acarichthys* groups with *Biotoecus*, to the exclusion of *Guianacara*, a result conflicting with most previously published analyses (see above, Section 4.1). Since our present analysis strongly places *Guianacara* as sister to the poorly known genus *Mazarunia*, we have referred to both genera as guianacarines (node 117, Table 2). Originally thought by Kullander (1990), on the basis of some morphological evidence, to be part of a clade with *Dicrossus* and *Crenicara* (Kullander, 1998), *Mazarunia* has not been well studied because neither specimens nor tissue samples were available until very recently. With the finding of guianacarines, this paper provides the first evidence for a geophagine clade with a history of isolated evolution and specialization for life in clear and often fast waters on the slopes and foothills of the Guiana Shield of northern South America (Kullander, 1990; Kullander

and Nijssen, 1989; López-Fernández et al., 2006). Whereas the addition of *Mazarunia* revealed that the moderately supported grouping of *Guianacara* and *Acarichthys* was an artifactual result of incomplete taxon sampling (and see Section 4.1), addition of taxa and improvement of support still does not allow resolving the basal relationships among guianacarines, crenicichlines, and apistogrammines. These results are congruent with those of López-Fernández et al. (2005a,b) in that basal relationships within Geophagini are difficult to resolve with certainty due to short basal branches, even though some of them are strongly supported (e.g. nodes 130, 148, Table 2).

4.4. Relationships among Cichlasomatini

In terms of taxon sampling, the most detailed studies of the Cichlasomatini are those of Musilová et al. (2008, 2009). Although our study includes a smaller number of cichlasomatine taxa, the overall amount of sequence data is larger. Both our study and those of Musilová et al. (2008, 2009) share three loci (16S, Cytochrome *b*, S7 intron 1) in common, yet differ in that they included RAG1 while we present data from ND4 and RAG2.

A Shimodaira–Hasegawa test (see Section 3: Hypotheses testing), comparing relationships among the Cichlasomatini for our topology and that proposed by Musilová et al. (2009) showed that the two topologies do not differ significantly. Nonetheless, differences among the studies remain, particularly in regards to the composition of *Cichlasoma* and *Aequidens*.

Musilová et al. (2009, Fig. 2) indentified a clade in which the *Aequidens tetramerus* group and *Cichlasoma* are sister taxa, and in turn are sister to a group that includes *A. diadema*, rendering *Aequidens* paraphyletic. On the other hand, we recovered a putatively monophyletic and well-supported *Aequidens* sensu stricto (i.e. *A. tetramerus* + *A. diadema* groups, node 95, Table 2), which is in turn sister to *Cichlasoma* (node 98, Table 2). Musilová et al. (2009) further questioned the monophyly of *Aequidens*, as they found *A. patricki* grouping with *Cichlasoma* rather than with other species of *Aequidens*, a relationship we could not test because *A. patricki* was not included in our study. As pointed out by Musilová et al. (2009), further phylogenetic analysis of *Aequidens* sensu lato is required to unequivocally diagnose *Aequidens* and to determine which taxa currently included in that genus may belong to different lineages. Based on our results, however, we disagree with Musilová et al.'s (2009, p. 13) proposal that *Aequidens* should be synonymized with *Cichlasoma*. Although some species like *A. patricki* may belong in *Cichlasoma*, species within the clade we tentatively call *Aequidens* sensu stricto are recovered as a well-supported sister-group to *Cichlasoma*.

Both our study and those of Musilová et al. (2008, 2009) found strong support for a monophyletic *Andinoacara* as sister to *Bujurquina* and *Tahuantinsuyoa*, a grouping we informally refer to as andinoacarines (BTA clade of Musilová et al., 2008, 2009). Our results differ, however, in that they find andinoacarines to be sister to *Acaronia* and both as sister to *Laetacara* (e.g. Musilová et al. 2009, Fig. 2). In contrast, we found *Laetacara* and *Acaronia* united by a poorly supported node and a significantly-short branch (node 90, Fig. 1, Table 2) as sister to andinoacarines. Regardless of these relatively minor differences, it is interesting that both topologies imply a close relationship between the mostly *trans*-Andean genera *Andinoacara*, *Bujurquina*, and *Tahuantinsuyoa* and the widespread *cis*-Andean lowland forms *Laetacara* and *Acaronia* (Kullander, 1986, 1991; Casciotta, 1998; Staeck and Schindler, 2007).

A striking result in both studies is the weak support for the suprageneric relationships of nannacarines (NIC clade in Musilová et al. 2008, 2009). Although all studies recovered a monophyletic nannacarines, its relationship to the rest of the Cichlasomatini differs among all three studies. Musilová et al. (2008) found nannacarines as sister to (andinoacarines + (*Acaronia* + *Laetacara*)), whereas the "all molecular tree" of Musilová et al. (2009) (see their Fig. 2) placed it as sister to Cichlasomatini. In contrast, the combined analysis of both morphological and molecular data by Musilová et al. (2009) placed nannacarines as sister to either andinoacarines + *Krobia* (their Fig. 4A) or all Cichlasomatini minus *Acaronia* (their Fig. 4B). Our BI and MP analyses grouped nannacarines with (andinoacarines + *Acaronia* + *Laetacara*), but with extremely low support and a significantly-short basal branch (node 93, Fig. 1, Table 2). Although our results and Musilová et al.'s (2009) combined morphological and molecular dataset suggest that nannacarines are probably related to andinoacarines, it is remarkable that large datasets with extensive taxon sampling do not clearly support this relationship. Much like in Geophagini, short and poorly supported branches at the base of Cichlasomatini prevent unequivocal resolution of some suprageneric relationships (nodes 90 and 93, Fig. 1, Table 2).

4.5. Basal South American Heroini

South American genera of heroines do not form a monophyletic assemblage within the tribe Heroini. Three basal South American lineages are recovered including: (1) (*Hypselecara* + *Hoplarchus*), (2) *Pterophyllum*, and (3) mesonautines (Fig. 1). In addition, three groups with South American distributions are nested well within the Central American clades: caquetaines, *Australoheros* and the 'Cichlasoma' *festae* group. The only published studies with comparable taxon sampling of South American heroines are those of Hulsey et al. (2004) and Concheiro Pérez et al. (2006). Nevertheless, both studies are based solely on Cytochrome *b*, and the relationships they recovered are incongruent with our results. Most notably, both studies placed *Pterophyllum* as a basal taxon placed between Geophagini and Cichlasomatini instead of being part of Heroini. In contrast, our study (Fig. 1) places *Pterophyllum* as a more basal lineage within Heroini and nested between a clade containing *Hypselecara* + *Hoplarchus* and the rest of Heroini.

Most South American heroine diversity is included within mesonautines (see also Concheiro Pérez et al., 2006). The remaining diversity of Heroini can be partitioned into two large groups, the Southern (SCAC) and Northern Central American Clades (NCAC, Fig. 1). Geographically, the Southern and Northern clades are approximately located in areas south and north of the Motagua fault, respectively (and see Concheiro Pérez et al., 2006). This grouping of the Middle American, Caribbean and some north-western South American Heroini is broadly compatible with clades found in other studies, but not necessarily identical (Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008). Both clades include considerable morphological and ecological diversity and neither has been extensively studied morphologically. Together, the two clades form the so-called Circum-Amazonian (CAM) Heroini as treated by Concheiro Pérez et al. (2006) and Rican et al. (2008).

4.6. The Southern Central American Clade (SCAC)

Monophyly of the SCAC is moderately supported (node 68, Fig. 1, Table 2), and contains the clades referred to as nandopsines, caquetaines and amphilophines. Although most studies agree with the general recognition of these three groups, their contents and relationships to one another vary. For instance, our study provides moderate support for the sister-group relationship between nandopsines and caquetaines (Fig. 1), whereas the topologies of Rican et al. (2008) varied depending on how the data were analyzed. Their separate analyses of morphology and Cytochrome *b* sequences (their Fig. 5) were congruent with our topology (Fig. 1), but their analysis with additional molecular data and morphology

(their Fig. 6) placed *Nandopsis* between *Australoheros* and the rest of the Middle American cichlids. In contrast to our study, the topology by Rican et al. (2008) also placed caquetaines between amphilophines and the Northern Central American Clade. The topology of Concheiro Pérez et al. (2006) placed *Nandopsis* at the base of their amphilophines, which included a basal *Caquetaia* (their Figs. 1 and 2). Other studies have not found nandopsines and caquetaines to be closely related (e.g. Hulsey et al., 2004; Smith et al., 2008), probably due to reduced taxon sampling or a small amount of data. Unlike Chakrabarty (2006), who recovered nandopsines nested between the two main Middle American clades and suggested a Central American origin for *Nandopsis*, our topology suggests the ancestor of the Caribbean genus may have originated in either continent. These results imply a biogeographic history different from previously proposed hypotheses and warrants further study of the possible scenarios of cichlid movement between South and Central America and the Caribbean (HLF, In prep.). A curious result across several studies is the frequent nesting of *Heroina* within *Caquetaia* [e.g. our Fig. 1, some of Rican et al.'s (2008) analyses], but Rican et al.'s (2008) combined analysis of molecular and morphological data recovers reciprocally monophyletic genera.

The most taxonomically problematic, species-rich and ecomorphologically diverse group of Central American cichlids is the heroine clade we refer to as amphilophines (and see Concheiro Pérez et al., 2006; Rican et al., 2008), which in our study is sister to the clade containing nandopsines and caquetaines (node 63, Fig. 1, Table 5). Few inter-generic relationships within amphilophines are well-supported, and at least one genus, *Cryptoheros*, clearly is not monophyletic. Some suprageneric relationships are congruent with those found in other studies including the grouping of *Amphilophus* sensu stricto (i.e. without *Astatheros*) with *Archocentrus* and both with the '*Cichlasoma*' *lyonsi* group (see also Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican and Kullander, 2006; Rican et al., 2008). On the other hand, Schmitter-Soto's (2007a) decision to place of *Herotilapia* in synonymy with *Archocentrus* has been unanimously rejected by all molecular evidence as the two genera have never been found together (see references above) and we recovered a strong relationship of *Herotilapia* with *Tomocichla* (Tomocichlines, node 32, Table 2, see below, and see also Hulsey et al., 2004). Unfortunately, the only species of *Archocentrus* (sensu Schmitter-Soto, 2007a) included in all molecular analyses is *A. centrarchus*, thus the monophyly of that genus has not been formally tested with the other putative species, *A. spinosissimus*.

No other putative genus in the Neotropical cichlid phylogeny is nearly as problematic as *Cryptoheros*. Originally separated from *Archocentrus* by Allgayer (2001), it was revised by Schmitter-Soto (2007a,b) who divided it into the subgenera *Cryptoheros*, *Panamius* and *Bussingius*. He further divided *Archocentrus* to distinguish the genus *Amatitlania*, synonymyzed *Herotilapia* as a third species of *Archocentrus*, and erected the genus *Rocio* for the group of species in the '*Cichlasoma*' *octofasciatus* group (Schmitter-Soto, 2007a, and see discussion about *Astatheros* in next section). However, no molecular dataset with sufficient taxon sampling has retrieved a monophyletic assemblage for any of these groups (e.g. Concheiro Pérez et al., 2006; Rican et al., 2008, this study). In our study, *Cryptoheros* (*Cryptoheros*) *chetumalensis* and *C.* (*C.*) *cutteri* form the only strongly supported grouping of species in the putative genus (node 59, Table 2). As Schmitter-Soto (2007a) restricted the subgenus *Cryptoheros* to include the type species (*C.* (*C.*) *spilurus*) along with *C.* (*C.*) *chetumalensis* and *C.* (*C.*) *cutteri*, herein we have used *Cryptoheros* as the genus for the three species, although this assumes that *C.* (*C.*) *spilurus* is part of a monophyletic clade consistent with Schmitter-Soto's (2007a) grouping; unfortunately we did not include *C.* (*C.*) *spilurus* in our analyses and the relationship remains to be tested. All other species originally included in *Cryptoheros* sensu lato are herein referred to as '*Cryptoheros*' until further taxonomic and phylogenetic revision provides a clearer picture of the group. We also consider the subgeneric classification of Schmitter-Soto's (2007a) in need of revision given the lack of monophyly for, at least, the subgenus *Bussingius*. '*Cryptoheros*' (*Bussingius*) *myrnae* groups with *Amatitlania siquia* with extremely high support (node 48, Fig. 1, Table 2), whereas '*Cryptoheros*' (*Bussingius*) *sajica* and '*C.*' (*B.*) *nanoluteus* are dispersed in other parts of the clade and not close to each other. '*Cryptoheros*' (*Panamius*) *panamensis* groups with *Hypsophrys*, but support is only moderate (node 57, Table 2), suggesting that further analysis is needed to place this southern species. Lack of monophyly for *Cryptoheros* sensu lato is not limited to our analysis, as at least three other studies found extensive paraphyly among their samples of the former genus *Archocentrus* (Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008).

The grouping of *Petenia splendida*, '*Cichlasoma*' *urophthalmus* and '*C.*' *calobrensis* and the inclusion of *Hypsophrys* within amphilophines is a result common to this and previous studies, but despite this congruence, the relationship of these clades to other groups of amphilophines remains poorly supported in all instances (e.g. node 51, Fig. 1 and Table 2, this study, and see Concheiro Pérez et al., 2006; Rican et al., 2008). The inclusion of '*C.*' *salvini* and *Australoheros* within amphilophines is rather controversial, as these taxa have been recovered in different parts of the phylogeny in different studies. Chakrabarty (2006) found an interesting grouping of '*Cichlasoma*' *salvini* with '*C.*' *sieboldii* and '*C.*' *tuyrense*. In contrast, Concheiro Pérez et al. (2006) found '*C.*' *sieboldii* and '*C.*' *tuyrense* to group with '*Cichlasoma*' *punctatus* and near '*Cichlasoma*' *istlanus* and '*C.*' *wesseli* at the base of the amphilophines, while '*C.*' *salvini* was at the base of *Thorichthys* within herichthyines (and see also Hulsey et al., 2004). Because we did not include '*C.*' *sieboldii* and '*C.*' *tuyrense*, it is difficult for us to address this disagreement. The more comparable study to ours is Rican et al.'s (2008) because they have similar taxon sampling and a larger dataset than either Concheiro Pérez et al. (2006) or Hulsey et al. (2004). They also found '*Cichlasoma*' *salvini* near *Thorichthys* and within the herichthyines, but support was not robust. Rican et al. (2008) recovered '*C.*' *sieboldii* as sister to ('*C.*' *istlanus* + '*C.*' *beani*) in the same position that we found '*C.*' *wesseli*, and Concheiro Pérez et al. (2006) found '*C.*' *wesseli* related to '*C.*' *istlanus*. It would be interesting to test whether all these species without generic assignment form a clade placed between *Parachromis* and the (*Amphilophus* + *Archocentrus* + '*Cichlasoma*' *lyonsi*) clade, where our analysis placed '*C.*' *wesseli*.

The genus *Australoheros*, although restricted in its distribution to southern South America's Paraná-La Plata basins, has been shown to be deeply nested among Central American taxa by all molecular studies. But, the position of *Australoheros* is far from congruent among these studies. While we recover the genus nested within amphilophines and weakly related to *Amatitlania* and part of '*Cryptoheros*', Hulsey et al. (2004) found it as sister to '*Cichlasoma*' *festae* and at the base of a clade including *Caquetaia umbrifera*, tomocichlines and herichthyines (sensu this paper). Similarly to Hulsey, Concheiro Pérez et al. (2006), found *Australoheros* at the base of *Astatheros* and the herichthyines, whereas Rican and Kullander (2006) found it as sister to a clade of amphilophines and our caquetaines, and Rican et al. (2008) placed it at the base of all Central American cichlids in their combined molecular trees and MP analysis of genes and morphology (Rican et al., 2008, Figs. 1–3, 6). Interestingly, Rican et al.'s (2008) morphological analysis weakly grouped *Australoheros* with '*Cryptoheros*' *panamensis* and placed it close to *Cryptoheros* sensu lato, *Archocentrus* and *Herotilapia*, further suggesting extensive morphological homoplasy within amphilophines (see above). Given the variable position of *Australoheros* and the fact that Rican et al. (2008) included the largest number of species from the genus, we interpret our results with

caution. This is especially true from a biogeographic point of view, since interpretation of the origin of *Australoheros* and its current geographic distribution seems more difficult on the basis of our topology (i.e. nested within an otherwise Central American clade) than that of Rican et al. (i.e. from a basal position with respect to all Central American taxa). Overall, all studies coincide in placing *Australoheros* within the Central American assemblage, and most studies place the genus in a position near to or inside amphilophines. Further study is needed to establish the position of *Australoheros* and to infer its biogeographic history.

There is support for a monophyletic amphilophines, yet with few exceptions, relationships within the clade are far from settled. A number of very short and poorly supported basal branches limit the resolution of inter-generic relationships within the group, leaving amphilophines as the group of Central American cichlids in greatest need of revision. Both expansion of taxon sampling in future phylogenetic analyses and taxonomic reassessment, especially of lineages currently assigned to *Cryptoheros* sensu lato, are urgently needed. Likely, Schmitter-Soto's (2007b) detailed morphological analyses combined with molecular data, could be informative when more species of amphilophines are included. The amphilophines might present a similar situation to that of Geophagini (López-Fernández et al., 2005b) in which extensive homoplasy nonetheless was associated with morphological synapomorphies allowing diagnosis of at least some of the lineages.

4.7. The Northern Central American Clade (NCAC)

The NCAC contains three clades: astatheroines, tomocichlines and herichthyines. Our results coincide with those of Hulsey et al. (2004) in recovering a monophyletic *Astatheros* that includes the putative genus *Rocio* nested between *A. macracanthus* and the rest of *Astatheros*. Concheiro Pérez et al. (2006) also found *Astatheros* and *Rocio* (their '*Heros*' *octofasciatus*) at the base of their herichthyines, but the two genera did not form a clade. In Rican et al.'s (2008) study, *Astatheros* is sister to a clade of *Rocio* and *Herotilapia*, whereas we find *Herotilapia* strongly grouping with *Tomocichla* (node 32, Table 2, tomocichlines). Given that *Rocio* is nested within *Astatheros* and that none of the previously found alternative relationships for the genus are supported by our expanded dataset, we herein treat *Rocio* as part of *Astatheros* and use the combination *Astatheros octofasciatus* for the species included in our study. Hulsey et al. (2004) also found a clade of *Tomocichla* and *Herotilapia*, but in their study this clade was nested between '*Cichlasoma*' *festae* and *Thorichthys* rather than being basal to the herichthyines as in our tree. Whereas monophyly of tomocichlines in our analysis is well-supported, its position within the NCAC is supported by the Bayesian analysis, but only moderately or not at all by the ML and MP analyses node 33, Table 2).

With few exceptions, monophyly of genera within herichthyines is supported as well as their internal relationships within the clade. Nevertheless, relationships within herichthyines are far from resolved. Although the grouping of '*Cichlasoma*' *festae* and *Thorichthys* is well-supported, branch lengths are extremely short (Fig. 1, nodes 29 and 30, Table 2). Similarly, despite a significantly-short branch, support for the grouping of '*C.*' *grammodes* with the clade formed by *Theraps*, *Paraneetroplus* and *Herichthys* is very high (node 22, Table 2). Although the monophyly of *Thorichthys* has not been questioned in any molecular or morphological study, its closest relative is unclear. We found *Thorichthys* at the base of Herichthyines between '*Cichlasoma*' *festae* and '*C.*' *grammodes*, but other studies have found it as sister to '*C.*' *salvini* (Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008). In Rican et al.'s (2008) study, '*Cichlasoma*' *bocourti* was placed at the base of *Theraps* and these authors identified it as a different genus. In contrast, our study placed *Theraps lentiginosus* as the most basal species and recovered '*C.*' *bocourti* nested within a strongly supported *Theraps*. Therefore, we refer to that species as *Theraps bocourti*. (Rican et al., 2008, Fig. 6) also divide *Paraneetroplus* (sensu this paper) into three genera: *Paraneetroplus*, *Vieja* and *Paratheraps*. We prefer to keep all species as *Paraneetroplus* because taxon sampling is not complete in either study, relationships within the genus are not always unequivocally supported (e.g. node 10, Table 2) and morphological diagnostic characters have not been explored. Finally, monophyly of *Herichthys* is strongly supported by all molecular analyses with adequate taxon sampling (e.g. Hulsey et al., 2004; Concheiro Pérez et al., 2006; Rican et al., 2008, this study). Its close relationship with both *Theraps* and *Paraneetroplus* also seems unquestionable, but it is not entirely clear which of these genera is sister to *Herichthys*. We retrieved a weakly-supported short branch uniting *Herichthys* with *Paraneetroplus* (node 13, Fig. 1, Table 2), that coincides with the Cytochrome *b* results of Hulsey et al. (2004) and Concheiro Pérez et al. (2006), but Rican et al. (2008) recovered *Herichthys* as sister to a clade of *Paraneetroplus* and *Theraps*. Whatever the actual relationship among the three genera, support for a clade including them is extremely strong in all our analyses (node 21, Table 2) as well as in previous studies.

In summary, the NCAC is yet another clade within the Neotropical cichlids that is fairly well-supported with at least some short basal branches. Some of these short branches are evidence of low support for certain groupings, whereas others are extremely well-supported (e.g. node 22, Fig. 1, Table 2). Although this study used a larger sample of Central American cichlid genera than any previous study, relationships among these cichlids still need further analysis. Particularly, it is imperative to perform detailed anatomical studies that, in combination with molecular data, allow for more practical diagnosis of the various Central American genera, as well as improved taxonomic description of a number of lineages and a workable classification. It would be beneficial to combine the most recently analyzed molecular datasets with morphological matrices proposed by López-Fernández et al. (2005b) for Geophagini, by Rican et al. (2008) for Heroini, Schmitter-Soto (2007b) for some amphilophines and by Musilová et al. (2009) for Cichlasomatini. Such analysis would allow detection of morphological synapomorphies for different clades in a much broader context. Thorough anatomical analysis of all Neotropical cichlids incorporating such large numbers of characters can only come from a large effort, possibly involving collaboration from different teams working on the group.

4.8. Phylogenetic evidence for multiple adaptive radiations and convergent evolution in Neotropical cichlids

López-Fernández et al. (2005a) hypothesized that genera of geophagine cichlids diversified through adaptive radiation. They proposed this mechanism following Schluter's (2000) four criteria for a group of organisms to be considered an adaptive radiation: (a) monophyly, (b) rapid diversification, (c) phenotype-environment correlation, and (d) trait utility. They showed that the first two requirements were fulfilled by a strongly monophyletic tribe Geophagini (their Geophaginae) in which basal branches were not significantly different from zero, thus suggesting rapid diversification at the genus level. This interpretation was challenged by Smith et al. (2008), who claimed to have found a completely resolved Geophagini (but see Section 4.1) with "ample branch lengths". However, their branch lengths were based on a parsimony analysis, representing the minimum number of character state transformations optimized along each branch (Swofford et al., 1996; Felsenstein, 2004). In other words, these branch lengths are informative regarding the amount of change along a branch, but provide no information on the rate at which that change occurred. In contrast, the branch lengths used by López-

Fernández et al. (2005a) to hypothesize rapid diversification were based on models of sequence evolution and represent expected numbers of substitutions per site. These branch lengths are estimates of the relative rate at which change occurs along a branch (e.g. Yang, 2006), and short basal branches are subsequently interpretable as indication of fast divergence.

The present study not only reinforces López-Fernández et al.'s (2005a) proposal of rapid diversification at the base of Geophagini, but allows expanding that interpretation to include the Heroini and possibly Cichlasomatini. We found multiple significantly-short basal branches in the first two clades and one in the latter (Fig. 1, Section 3.3), suggesting that lineages associated with these branches diversified rapidly from their common ancestor. Interestingly, nodes associated with some of these significantly-short branches were very strongly supported under all phylogenetic optimization algorithms (e.g. nodes 22 and 41, Fig. 1, Table 2). This finding suggests that short branches need not necessarily hamper phylogenetic reconstruction. Enough phylogenetic signal is available in the characters associated with the base of these clades to recover a strongly supported phylogeny. In the case of Geophagini, it is interesting that even using fewer loci in this study than in previous analyses (López-Fernández et al., 2005a,b), we recovered very similar relationships and increased resolution and support for some short basal branches.

Short branches at the base of Geophagini and Heroini are compatible with a so-called "early burst of evolutionary divergence" seen as a typical pattern of an adaptive radiation (Gavrilets and Losos, 2009). Early speciation with significant morphological diversification in adaptive radiations has been documented in a number of phylogenetic studies (e.g. Verheyen et al., 2003; Poe and Chubb, 2004; Lukoschek and Keogh, 2006), analyses of the fossil record (e.g. Foote et al., 1999; Luo, 2007) and theoretical models of ecomorphological diversification (Pie and Weitz, 2005). Ecomorphological variation in Neotropical cichlids tends to be highest among genera, with intra-generic variation being generally subtle and often limited to cases in which congeneric species are syntopic (e.g. *Geophagus*, López-Fernández et al., 2005a). The pattern displayed by our phylogeny of Cichlinae suggests that phyletic and ecomorphological diversification of Neotropical cichlids associated with the origin of genera occurred rapidly near the beginning of the group's divergence, with subsequent convergent, adaptive ecomorphological diversification among and within South and Central American clades (Winemiller et al., 1995). Further phylogeny-based analysis of the timing, environmental circumstances and patterns of these divergence events is beyond the scope of this paper and these elements of Neotropical cichlid evolution are being discussed elsewhere (López-Fernández, Unpubl.).

Morphology of genera in Geophagini and Heroini is strongly associated with ecological function. The adaptive nature of morphological specialization is highlighted by repeated cases of convergent evolution associated with both feeding and habitat use. Convergent ecomorphological specialization among genera is amply documented (e.g. Winemiller et al., 1995) and includes elongate-bodied piscivores (e.g. *Cichla* and *Crenicichla* from South America, *Parachromis* and *Petenia* from Central America); rapids-dwelling, invertebrate feeders (e.g. *Teleocichla* from the South American Geophagini, *Theraps* from the Central American Heroini); lentic invertivores with disc-like bodies inhabiting highly complex habitats (e.g. *Mesonauta*, *Pterophyllum* and *Symphysodon*, in the South American basal Heroini, *Archocentrus centrarchus* from Central American Heroini); algae scrapers with specialized teeth (e.g. *Hypsophrys*, *Tomocichla*); and a variety of benthic sifters that extract invertebrates from the substrate by taking mouthfuls of sand or mud and winnowing out inedible particles using their pharynx (e.g. certain Geophagini from South America, *Thorichthys* and *Astatheros* of the Central American Heroini). In a few cases specialization is restricted to one group as in the case of detritivory, which is present in some Central American cichlids but not in South American taxa presumably because that niche is densely occupied by the detritivorous South American characiform families Curimatidae and Prochilodontidae (Winemiller et al., 1995). Although apparently less morphologically specialized, genera in the tribe Cichlasomatini can have significant body size differences, including several "dwarf" lineages (e.g. *Nannacara*, *Laetacara*). The most notably specialized Cichlasomatini is *Acaronia*, a predator with unusually protrusible oral jaws convergent with those of the heroine genera *Caquetaia* and *Petenia*.

Understanding the timing of ecomorphological diversification, and the forces driving the remarkably diverse functional specialization of Neotropical cichlid evolution requires an interdisciplinary research effort. Studies combining ecology, functional morphology and phylogenetics should begin to reveal patterns of ecomorphological diversification and convergence described above (e.g. López-Fernández et al., Unpubl.), as well as many other aspects of the evolution of Neotropical cichlid fishes. This paper contributes a framework on which to build this program by providing the most broadly sampled phylogeny of Neotropical cichlids available to date. We also have interpreted the patterns of molecular divergence within Neotropical cichlids as phylogenetic evidence of repeated instances of adaptive radiation. Further testing of this multiple-radiation hypothesis should be possible as the phylogeny provides the appropriate historical context for comparative analysis of Neotropical cichlid evolution. Within a clearer phylogenetic context, further studies should greatly enhance our understanding of cichlid evolution by providing much needed studies of the Neotropical fluvial taxa, which have lagged far behind those of the lacustrine adaptive radiations of East African cichlids.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.02.020.

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