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Systematics, biogeography, and evolution of the Neotropical peacock basses *Cichla* (Perciformes: Cichlidae)

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Abstract

To investigate forces influencing diversification in Neotropical fishes, the phylogenetic relationships among species and populations of the cichlid genus *Cichla* were examined. Mitochondrial DNA was sequenced for 454 individuals of the 5 nominal *Cichla* species and several putative undescribed species. Phylogenetic analyses support the distinction of two major clades of *Cichla*. Clade A includes *C. temensis* and two undescribed species from the lower Amazonas and Xingu Rivers. Clade B includes *C. orinocensis*, *C. monoculus*, *C. ocellaris*. *C, intermedia*, and an undescribed species from the upper Madeira River. Species boundaries were relatively well-circumscribed for clade B, while incomplete lineage sorting was inferred for clade A. Three probable instances of introgression were observed, including a regional population of *C. orinocensis* from the Negro River that shows a history of introgression. Biogeographic patterns from *Cichla* are partially congruent with those seen in several other Neotropical fish clades, and the diversification of *Cichla* species is inferred to result from both vicariance and sympatric divergence.

Keywords: Neotropics; Cichlid; Peacock bass; *Cichla*; Tucunare; Biogeography; Species boundaries; Phylogeography; Speciation; Mitochondrial DNA; Introgression; Hybridization

1. Introduction

Molecular investigations of the higher-level systematics of tropical South American freshwater fishes are becoming more common (e.g., Orti and Meyer, 1997; Farias et al., 1999; Hrbek and Larson, 1999; López-Fernández et al., 2005), but relatively few molecular studies of species-level or intraspecific phylogeny have been published (Dergam et al., 1998; Hrbek and Larson, 1999; Lovejoy and de Araújo, 2000; Andrade et al., 2001; Sivasundar et al., 2001; Dergam et al., 2002; Montoya-Burgos, 2003; Moyer et al., 2004; Turner et al., 2004; Hrbek et al., 2005a,b; Říčan and Kullander, 2006; Renno et al., 2006). Species-level phylogenies are essential for reconstructing biogeographic events, and for understanding the geographic context of speciation (Harrison, 1998; Barraclough and Vogler, 2000). Studies of intraspecific genetic diversity, particularly with reference to geography (intraspecific phylogeography) can provide further insight into the role of geography in population structure, gene flow, and incipient speciation (Bermingham and Moritz, 1998). When combined, intraspecific and interspecific phylogenetic approaches can also identify instances of hybridization and introgression, clarify species boundaries, and uncover cryptic and polymorphic species (Doyle, 1992; Maddison, 1997; Baric and Sturmbauer, 1999; Mendlelson and Shaw, 2002; Weins and Penkrot, 2002). Furthermore, in light of the possibility of incomplete lineage sorting,

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correct inference of species phylogeny is more likely when many individuals from each putative species are included in the analysis (Maddison and Knowles, 2006). Thus, molecular phylogenetic investigations based on extensive species and population sampling could provide an exceptional window on the diversification of Neotropical fishes (Bermingham and Martin, 1998; Smith and Bermingham, 2005).

Although the tropics of South America exhibit the greatest diversity of freshwater fishes in the world (Reis et al., 2003), the major causes of this diversification have yet to be determined. A continuing debate exists over the relative importance of different modes of speciation (e.g., vicariance vs. adaptive or ecological speciation) in generating diversity in the tropics (Moritz et al., 2000). These alternatives can be investigated and tested using knowledge of ecology, phylogeny, and biogeography. For example, it is possible to distinguish between the vicariant effects of river drainage changes versus ecological shifts in promoting speciation. The hypothesis of vicariant speciation predicts that sister species will be distributed allopatrically, while the hypothesis of ecological speciation predicts that sister species will exhibit divergent ecologies (Lynch, 1989; Berlocher, 1998; Barraclough and Vogler, 2000). While there remains uncertainty about the reliability of estimating historical parameters, such as the geographical ranges of taxa at the time of speciation, or whether divergent ecologies between sister species are a cause or result of speciation, combining information from various sources may provide us with a robust estimate of patterns of speciation (Losos and Glor, 2003).

Biogeographic studies of freshwater fishes can also be used to understand historical changes in river drainage patterns. Freshwater fishes are physiologically restricted to aquatic habitats, and can be isolated by even minor terrestrial barriers (Vari, 1988). Thus, species and population phylogenies of freshwater fishes may closely record historical connections and isolation of river systems at a variety of geographic scales (Bermingham and Martin, 1998). A very simple expectation for river biogeography might be that closely related species and populations would be found in the same river system. In South American fishes, morphological and molecular phylogenies have revealed several violations of this simplistic idea, indicating instead that populations may often show close relationships between rather than within drainages (Weitzman and Weitzman, 1982; Vari, 1984, 1989a,b, 1991, 1992, 1995; Schaefer, 1997; Hrbek and Larson, 1999; Lovejoy and de Araújo, 2000; Sivasundar et al., 2001; Montoya-Burgos, 2003; Turner et al., 2004; Castro and Vari, 2004). For example, despite a direct connection between the Amazonas and Orinoco river drainages via the Casiquiare corridor (Fig. 1), molecular phylogenetic investigations of widespread lowland Neotropical fishes have emphasized closer relationships among taxa in the Amazonas, lower Orinoco, and coastal drainges of the Guyanas, to the exclusion of lineages in the upper Orinoco (Lovejoy and de Araújo, 2000; Sivasundar et al., 2001). This incongruence between biological pattern and contemporary hydrography highlights the complex nature of river drainage history in the Neotropics (Hoorn et al., 1995; Lundberg et al., 1998), and suggests that species phylogenies may play a useful role in untangling past paleogeographic events (Smith and Bermingham, 2005).

To explore patterns of speciation in Neotropical fishes and historical river geography of South America, we investigated the phylogeny, biogeography, and population structure of the endemic South American cichlid genus *Cichla*. Among tropical fishes, cichlids (Perciformes: Cichlidae) have been recognized as an excellent group for evolutionary study because of the diversity of ecological niches, life history strategies, and morphological and behavioral adaptation this group exhibits (Lowe-McConnell, 1969, 1991; Barlow, 2000). The best known examples of cichlid diversity are the species flocks of the great lakes of Africa, which are hypothesized by many to represent sympatric radiations based on sexual selection and adaptive divergence (Seehausen et al., 1997; Verheyen et al., 2003). However, systematists of Neotropical cichlids have hypothesized both adaptive radiation in ecomorphological form (López-Fernández et al., 2005) and vicariance resulting from large scale geologic forces such as drainage capture and division (Kullander, 1983) as driving forces generating Neotropical diversity. The genus *Cichla*, also known as peacock bass or peacock cichlid, is a particularly interesting and important group of cichlids. *Cichla* are large-bodied, diurnal piscivores and major determinants of community structure and ecosystem dynamics in many fluvial habitats of South America (Jepsen et al., 1997; Winemiller et al., 1997). Studies in their native range suggest that *Cichla* have a significant effect on species diversity. These fishes provide an intense size-selective predation pressure on a variety of prey fishes (Layman and Winemiller, 2004), potentially reducing competitive exclusion by dominant species and facilitating higher species density (Layman and Winemiller, 2004; *sensu* Paine, 1966). They have also been implicated in an allochthonous nutrient subsidy which may increase the productivity of nutrient poor blackwater systems by trapping energy from prey fishes migrating from the productive whitewater floodplains (Winemiller and Jepsen, 1998). Significant resource partitioning has been observed among sympatric species of *Cichla* (Jepsen et al., 1997; Winemiller et al., 1997), and in general *Cichla* species differ significantly in their color pattern and habitat preferences (Winemiller, 2001). *Cichla* are also important as subsistence and commercial food resources and recreational resources through sport fishing.

Phylogenetic analyses have shown *Cichla* to be among the earliest extant genera to diverge in the monophyletic Neotropical cichlid clade (Stiassny, 1987; Farias et al., 1999, 2000, 2001). *Cichla* species have natural distributions in rivers throughout most of northern South America, but have also been introduced to the Pananá-Paraguay drainage (the major drainage for southern South America), as well as Lake Gatun in the Canal Zone of Panama (Zaret and Paine, 1973), Florida, Texas, and Hawaii in the United States (Shafland, 1993), Puerto Rico, and Singapore. *Cichla*

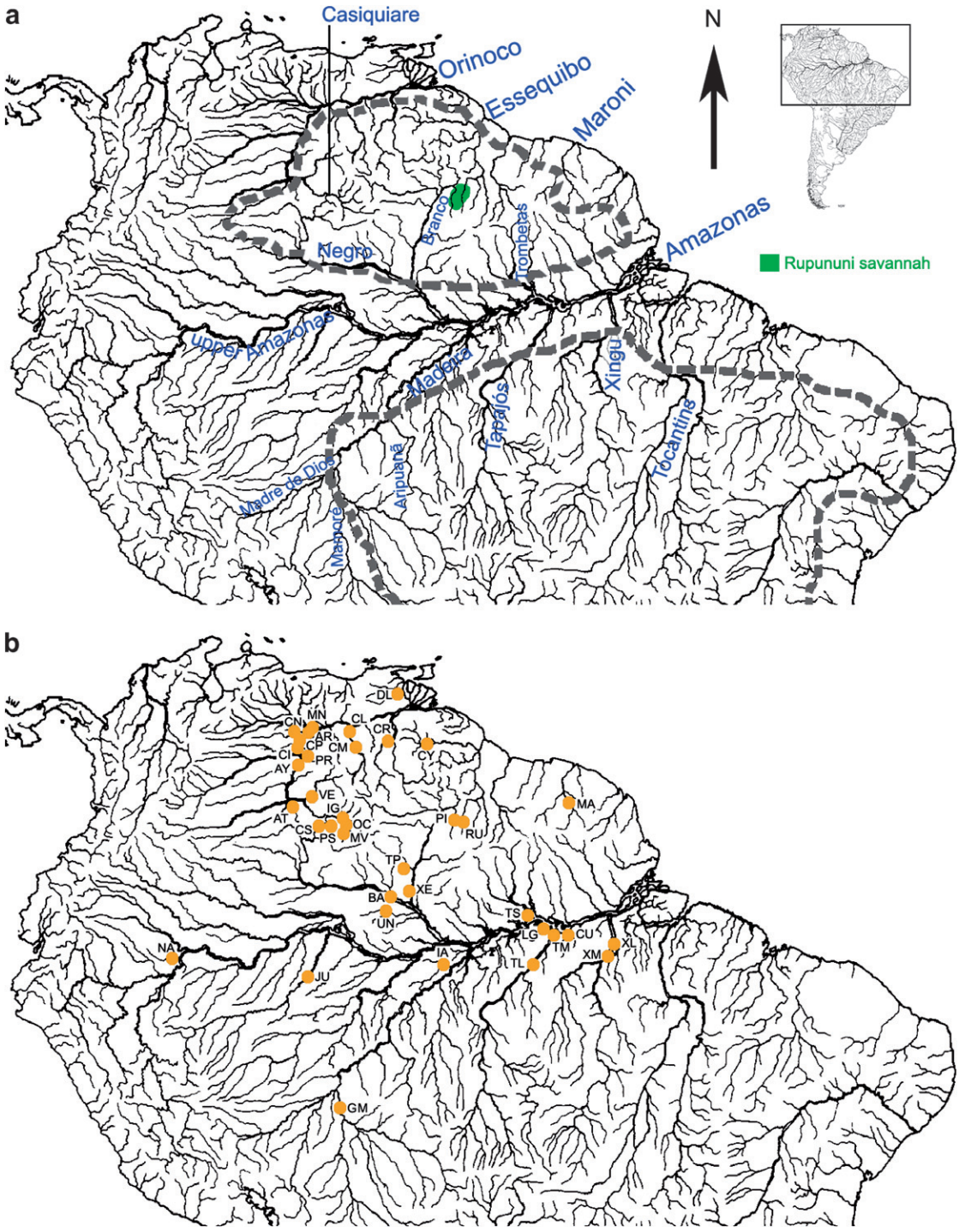

Fig. 1. (a) Major drainages and tributaries of northern South America. Dashed lines indicate the Guayana (north) and Brazilian (south) shield formations. (b) Distribution of sample localities. Locality abbreviations follow Table 1. Base map by Marilyn J. Weitzman and available from the Neotropical Ichthyological Association.

are absent from the trans-Andean (western versant) drainages of South America, coastal drainages of southern Brazil, and the northern coastal drainages of Venezuela. Five species are currently considered valid. *Cichla ocellaris* Schneider, 1801 was described from Surinam and is reported from several coastal drainages of the Guyanas (Guyana, Surinam, French Guiana); *Cichla temensis* Humbolt and Valenciennes, 1821 was described from the upper Orinoco, and is widely distributed in the Orinoco (Colombia, Venezuela) and Negro (Brazil) basins; *Cichla orinocensis* Humbolt and Valenciennes, 1821 was originally reported from the Orinoco basin (Colombia, Venezuela) where it appears to be common, and is also found in the Negro basin (Brazil); *Cichla monoculus* Agassiz, 1831 (in Spix and Agassiz, 1831) was described from the Brazilian Amazonas and is also common in the Amazonas basin in

Peru, Colombia, and Ecuador, and allegedly occurs in the coastal drainages north of the Amazon including the Araguari in Brazil and Oyapock on the border of Brazil and French Guiana; *Cichla intermedia* Machado-Allison, 1971 is patchily distributed in tributaries of the Orinoco and Casiquiare rivers (Colombia and Venezuela) (Kullander, 1986, 2003; Kullander and Nijssen, 1989; Jégu and Keith, 1999; Winemiller, 2001). However, despite the commercial and ecological importance of *Cichla* the distributions of even the valid species remain uncertain, and putative undescribed species of *Cichla* have been reported from regions not densely sampled ichthyologically, such as the lower Amazonas river and several Amazonas tributaries such as Xingu, Tapajós, Madeira, and Tocantins (Fig. 1a).

To investigate forces influencing diversification in *Cichla*, we examined the phylogenetic relationships among species using DNA sequence data from the mitochondrial genome (mtDNA). We identified nominal and putative species of *Cichla* based on morphology from many localities in northern South America, and evaluated the congruence between morphogically defined species and molecular phylogeny to refine our inference of the morphological, genetic, and geographical boundaries of species. We tested the hypothesis that morphologically defined species would show monophyletic clades of mitochondrial haplotypes, and attempted to distinguish incidences of mismatch between haplotype lineage and morphotypes as morphological convergence, incomplete lineage sorting, or hybridization/introgression. We also assessed the biogeography of *Cichla*, in order to understand the relationship between geography and species diversification. We hypothesized that sister lineages would most frequently show allopatric distributions, implicating a null hypothesis of vicariance as the predominant cause of divergence. Upon finding vicariant patterns we considered the contemporary and paleogeographic barriers which might have resulted in lineage isolation. When sister clades were found to be partially or wholly overlapping, we were unlikely to reject the vicariant null hypothesis unless there was also corroborating evidence for ecological partitioning (see Coyne and Orr, 2004 for a review of criteria distinguishing alternative geographic scenarios). Finally, we briefly investigated the extent to which *Cichla* biogeographic patterns parallel those seen in other Neotropical aquatic taxa.

2. Methods

Tissue samples were collected from localities in Brazil, French Guiana, Guyana, Peru, and Venezuela, including the Amazonas, Essequibo, Maroni, and Orinoco river drainages and tributaries thereof (Fig. 1b, Table 1). An effort was made to obtain samples of every morphologically defined species present at a locality. For the purposes of investigating introgression and incomplete lineage sorting, morphologically defined species were identified based on characters, generally color characters and meristics, which have been used to distinguish the species of *Cichla* currently considered valid. To identify unrecognized but separate species, we used similar characters that consistently distinguished groups of individuals not strictly referable to the valid species (information available upon request from corresponding author). Samples collected by the authors were taken from fish caught with hook and line, fish spears, cast nets, gill nets, or purchased from local markets. Fin or muscle tissue was collected from each fish and preserved in DMSO–EDTA buffer (20% dimethylsulphoxide, 0.25 M EDTA, pH ~8.0, saturated with NaCl; Seutin et al., 1991) or 95% ethanol. Voucher specimens of geographical representatives were regularly taken (information available upon request from corresponding author). However, when possible the majority of fish were released alive and only a portion of the regenerable soft dorsal or anal fin and a photograph were taken. Distribution data for *Cichla* were collected by the authors in the field and supplemented by published reports (e.g., Kullander, 1986; Kullander and Nijssen, 1989) and specimens from known localities in natural history collections.

Total genomic DNA was extracted from tissues using proteinase K and Qiagen spin columns following the Qiagen DNeasy kit protocol. Portions of the mitochondrial control region (CR) and the cytochrome *b* gene (cyt *b*) were amplified via polymerase chain reaction (PCR). The cyt *b* region codes for a protein product that is involved in mitochondrial respiration and is constrained in its mutation pattern, while the CR provides the origin of replication of the mitochondrial heavy strand and is thought to be more free to vary than the cyt *b* region (Meyer, 1993; Lee et al., 1995). PCR primers were GLUDG-5′ (CGAAGCTTGAC TTGAARAACCAYCGTTG) and Cytb3-3′ (GCCAAAT AGGAARTATCATTC) for cyt *b* and tPro2-5′ (ACCCT AACTCCCAAAGC) and HN-20-3′ (GTGTTATGCTTT AGTTAAGC) for CR (Lee et al., 1995; Palumbi, 1996). Primers for CR were designed to amplify the entire control region (~900 bp), but accurate sequencing of the 3′ portion downstream of a poly-T region was problematic. Therefore the 5′ ~550 bp were used in the analyses here, a fragment that included both the most variable (5′) and conserved (central) portions of the control region. For cyt *b*, 25 μL reaction volumes contained 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.08 μM each primer, 1 U *Taq* polymerase, and 1 μL DNA extract (~50 μg). For CR, 25 μL reaction volumes contained 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 3 mM $MgCl_2$, 200 μM each dNTP, 0.16 μM each primer, 1 U *Taq* polymerase, and 1 μL DNA extract. Both amplifications were successful with the following thermocycling conditions: initial denaturation at 94 °C for 30 s, followed by 30 cycles of 30 s at 94 °C, 45 s at 52 °C, and 90 s at 72 °C, a final extension of 5 min at 72 °C, and held at 4 °C. PCR products were purified using Qiaquick spin columns (Qiagen) and sequenced using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Inc.) and an ABI 377 automated sequencer. External primers were used to sequence

Table 1
Localities and samples analyzed for each species

	Locality	Drainage	*temensis*	sp. "Amazonas"	sp. "Xingu"	*ocellaris*	*monoculus*	sp. "Madeira"	*orinocensis*	*intermedia*
DL	Orinoco delta	Orinoco							**1**/10	
CR	Caroní	Orinoco	**2**/11							
CL	Caura (lower)	Orinoco	10						**1**/10	
CM	Caura (middle)	Orinoco								**2**/12
MN	Manipicito	Orinoco							4	
AR	Arichuna	Orinoco							4	
CN	Cunavichito	Orinoco	1						3	
CP	Capanaparo	Orinoco	10						10	
CI	Cinaruco	Orinoco	**1**/12						**1**/9	**1**/10
PR	Parguaza	Orinoco	2						11	2
AY	Ayacucho	Orinoco					**2**/7			
AT	Atabapo	Orinoco	**1**/10						**1**/10	2
VE	Ventuari	Orinoco	**1**/9						**2**/11	**1**/12
IG	Iguapo	Orinoco	**1**/1							9
OC	Ocamo	Orinoco								**1**/9
MV	Mavaca	Orinoco					10			
CS	Casiquiare	Amazonas					**3**/10			**1**/10
PS	Pasiba	Amazonas	**1**/10						**1**/10	
TP	Tapera	Amazonas	**2**/11						**2**/3	
BR	Negro (Barcelos)	Amazonas	5				9			
XE	Xeruini	Amazonas	**3**/7						**3**/5	
UN	Unini	Amazonas	**5**/15				**3**/3		**3**/10	
PI	Pirara	Amazonas	1			**2**/2				
CY	Cuyuni	Essequibo				**1**/1				
RU	Rupununi	Essequibo				**2**/2				
MA	Maroni	Maroni				**2**/2				
NA	Nanay	Amazonas					**2**/2			
JU	Juruá	Amazonas					8			
IA	Igapo-Açu	Amazonas	10				10			
TS	Terra Santa	Amazonas					10			
LG	Lago Grande	Amazonas		3						
TM	Tapajós (mouth)	Amazonas		**2**/4						
TL	Tapajós (lower)	Amazonas		10			10			
CU	Curuá-Una	Amazonas		**1**/5						
XL	Xingu (lower)	Amazonas		**1**/4			10			
XM	Xingu (middle)	Amazonas			**2**/11					
MM	Mamoré	Amazonas						**5**/10		
Totals			**17**/125	**4**/26	**2**/11	**7**/7	**10**/89	**5**/10	**15**/110	**6**/66

For each locality, bold numbers preceding the slash indicate samples sequenced for cytochrome *b*, and numbers following the slash indicate samples sequenced for the control region.

cyt *b*, while CR was sequenced using internal primers CR(L) (AGTAAGAGCCCACCATCA) and CR(E) (CCT GAAGTAGGAACCAGATG), from Lee et al. (1995).

Sequences were imported into Sequencher (Gene Codes Corp.) and checked by eye against their chromatograms. Individuals showing identical sequences were recorded and eliminated from the alignment, leaving haplotypes that were at least one base-pair transformation event different from any other haplotype (transition, transversion, insertion/deletion). Additional alignment of cyt *b* haplotypes was not necessary, as is typically the case for protein-coding regions. The CR haplotypes were further aligned using ClustalX (Thompson et al., 1997). Because the choice of parameter values for alignment can affect recovered phylogenetic topology (Ogden and Rosenberg, 2006), we performed alignments using several different gap opening parameters (5, 15, and 50) and deleted positions that varied across these alignments following Gatesy et al. (1993) (gap extension cost held at 6.66; all other parameters default). All alignments are available from the first author.

Two datasets were arranged using MacClade 4.08 (Maddison and Maddison, 2000). The first dataset (hereafter referred to as the combined dataset) was used to infer the mtDNA phylogeny of *Cichla* species. It contained all cyt *b* haplotypes observed in representatives of the morphologically defined species collected from several localities throughout their respective ranges (Table 1), and was concatenated with corresponding CR haplotypes from each individual. The concatenation of cyt *b* and CR data is supported on theoretical grounds because the mitochondrial genome is a non-recombining unit with a single evolutionary history (Meyer, 1993; Avise, 1995). A total of 27 ingroup OTUs (haplotypes) were included. The cichlid genera *Retroculus* and *Astronotus*, which also consistently are recovered at deeper nodes in phylogeny of the Neotropical cichlid clade (Farias et al., 1999), were included in the matrix as outgroups. As previous studies were inconsistent

as to which outgroup genus shares a more recent common ancestor with *Cichla*, we performed each of the parsimony searches (below), with one, the other, or both outgroup sequences included in the matrix. This allowed us to examine the influence of including each outgroup on the topology of the *Cichla* tree. CR data for *Retroculus* and *Astronotus* were coded as "missing" in the combined data matrix because alignment of the CR regions between *Cichla* and the outgroups could not be made unambiguously (see below).

The second dataset contained all of the CR haplotypes observed in individuals collected throughout the range of each morphologically defined species, and was used to investigate population structure and haplotype lineage/morphotype mismatch in *Cichla* species. The matrix included 128 ingroup OTUs (haplotypes). High levels of divergence and correspondingly high numbers of insertion/deletion events characterized sequence comparisons between *Cichla* and the outgroups *Retroculus* and *Astronotus*. Alignments of CR that included the outgroups exhibited few clearly homologous positions and large numbers of indels. Those regions that were unambiguously alignable showed no nucleotide polymorphism or contained mutations unique to single ingroup haplotypes (autapomorphy). As these alignments were thus unusable for phylogenetic analyses, the CR dataset was aligned and analyzed using *Cichla* sequences only.

For the combined dataset, a heuristic search was made with 1000 random addition sequences (hereafter RAS) using the maximum likelihood (ML) criterion in PAUP* ver. 4.0b10 (Swofford, 2000). The appropriate model of evolution was chosen under the Akaike Information Criterion (AIC) (Akaike, 1974) using the program Modeltest 3.6 (Posada and Crandall, 1998). Additionally, a heuristic search with 10 000 RAS was performed in PAUP* using the parsimony criterion. In these analyses, gap positions were treated as missing data. All phylogenetic searches used tree bisection and reconnection (TBR) branch swapping. The ML tree was evaluated with 100 ML bootstrap pseudoreplicates, each with 100 RAS. Node support in the parsimony trees was evaluated using bootstrap analyses (Felsenstein, 1985) with 1000 pseudoreplicates with 100 RAS in PAUP*, as well as Bremer decay indices (Bremer, 1988, 1994) using TreeRot (Sorenson, 1996) and PAUP*.

For the CR dataset, the large number of haplotypes observed (128) combined with the minimal divergence among many of them made routine rigorous phylogenetic analyses computationally prohibitive. Therefore, we conducted tree searches for this dataset using the parsimony ratchet (Nixon, 1999). The ratchet weights a portion of randomly selected characters in sequential tree searches, making it easier to jump between tree islands, and increasing the possibility of finding the globally optimal tree island. Twenty runs of 200 iterations each, in which 15% of randomly chosen characters were doubled in weight (original weight = 1, alternative weight = 2) were performed using data files constructed using PAUPMacRat (Sikes and Lewis, 2001) and executed in PAUP*. Gaps were treated as missing data. Bootstrap analysis of node support consisted of 1000 parsimony pseudo-replicates each with 100 RAS in which the MULTREES option in PAUP was off (so that only one tree per RAS was saved). Bremer decay indices were calculated using the parsimony ratchet using batch files created using PRAP (Müller, 2004) and executed in PAUP*. As described above, CR data for *Astronotus* or *Retroculus* were not useful for rooting CR trees, due to alignment difficulties. Thus, the CR trees were rooted in the position indicated by the combined analysis tree. This is the same rooting indicated by midpoint-rooting using minimum *f*-value optimization (Farris, 1972) for each of the observed equally parsimonious trees.

3. Results

For cyt *b*, 664 bases were sequenced for 66 *Cichla* specimens representing eight morphologically defined species. Twenty-seven ingroup haplotypes were determined (plus one from each outgroup). In general these haplotypes were unique to particular drainages or broad geographic areas (e.g., upper Orinoco, lower Amazonas). Uncorrected sequence divergence between *Cichla* cyt *b* haplotypes ranged from a single base difference to over 7%, while sequence divergence between ingroup and outgroups for cyt *b* was between 11% and 18%. These sequences are available from Genbank (Accession Nos. DQ841790–DQ841818) (Table 2).

Modeltest suggested the "transversion" model of evolution for the combined data (aligned 1115 bp, 664 bp of cytb and 451 bp of CR, 301 variable sites), a model that includes (2) equal transition rates but (4) unequal transversion rates, with a proportion of nucleotide sites invariant and inclusion of the gamma shape parameter (TVM + I + Γ; Posada and Crandall, 1998). Parameter values were: $I = 0.4630$, $\alpha = 0.4927$, fA = 0.2926, fC = 0.2910, fG = 0.1319, fT = 0.2846; the rate matrix is available upon request. The ML heuristic search in PAUP* with 29 taxa resulted in a single most-likely tree (−ln likelihood = 4377.1148). The topology was not affected by the inclusion of either or both outgroup sequences. This ML tree (Fig. 2) supports the monophyly of the morphologically defined species, with the exception of polyphyly for *C. orinocensis* and the paraphyly of *Cichla* sp. "Amazonas" and *Cichla* sp. "Xingu". The tree also shows two main clades of *Cichla*. The first clade contains *C. temensis* and the two putative undescribed species from the lower Amazonas and Xingu rivers, and is hereafter referred to as "clade A." The second clade contains the remaining described species, *C. orinocensis*, *C. intermedia*, *C. monoculus*, and *C. ocellaris*, and the putative undescribed species from the upper Madeira (Guajará-Mirim), and is hereafter referred to as "clade B."

In clade A, the haplotypes from *C. temensis* form a monophyletic group of haplotypes nested within the lineages of *Cichla* sp. "Amazonas." *Cichla* sp. "Xingu" is similarly nested among lineages of *Cichla* sp. "Amazonas," but

Table 2
Average uncorrected sequence divergence among haplotypes in species and clades of *Cichla*

	Clade A	*temensis*	sp. X ingu	sp. Amazonas	Clade B	Clade B1	*monoculus* N.	*ocellaris*	*orinocensis*	sp. Madeira	Clade B2	*orinocensis s.s.*	*intermedia*
Clade A	—	—	—	—	**5.96**	**5.70**	—	—	—	—	**6.69**	—	—
temensis	—	—	2.56	2.46	—	—	5.60	5.75	5.20	6.05	—	6.75	6.55
sp. Xingu	—	8.48	—	2.73	—	—	5.45	5.95	5.40	6.25	—	6.68	6.55
sp. Amazonas	—	8.20	7.21	—	—	—	5.62	5.84	5.45	6.15	—	6.75	6.55
Clade B	**11.28**	—	—	—	—	—	—	—	—	—	—	—	—
Clade B1	**11.56**	—	—	—	—	—	—	—	—	—	**5.30**	—	—
monoculus	—	11.45	11.89	11.30	—	—	—	1.86	1.41	2.51	—	5.52	5.47
ocellaris	—	11.13	11.62	11.32	—	—	5.87	—	1.56	2.56	—	5.35	5.05
orinocensis N.	—	11.68	11.33	10.74	—	—	5.74	5.04	—	2.21	—	4.92	4.77
sp. Madeira	—	11.83	11.69	11.56	—	—	4.99	5.05	4.60	—	—	5.54	5.42
Clade B2	**10.90**	—	—	—	—	**9.35**	—	—	—	—	—	—	—
orinocensis s.s.	—	11.47	8.82	10.17	—	—	8.84	8.65	9.14	9.66	—	—	4.07
intermedia	—	12.19	9.75	10.93	—	—	9.98	9.77	9.29	10.50	—	5.35	—

No correction has been made for variation within species/clades. Above diagonal are divergences in cytochrome *b*, and below diagonal are divergences in the control region. Bold values are those calculated by averaging across species.

its two haplotypes do not form a monophyletic group without the inclusion of a *Cichla* sp. "Amazonas" haplotype from the lower Xingu river. Clade A shows a primarily Amazonian distribution, with the exception of *C. temensis'* presence in the Orinoco drainage.

Clade B is composed of two sub-clades, one containing *C. monoculus*, *C. ocellaris*, *Cichla* sp. "Madeira," and a portion of *C. orinocensis* haplotypes (clade B1 in Fig. 2), and the other containing *C. intermedia* and another portion of *C. orinocensis* haplotypes (clade B2 in Fig. 2). The non-monophyly of *C. orinocensis* is associated with geography: all *C. orinocensis* in clade B1 are fishes from the lower and middle Negro river (Amazonas drainage), while all the *C. orinocensis* in clade B2 are from various localities in the Orinoco and Casiquiare drainages where *C. intermedia* also occurs. The latter *C. orinocensis* haplotypes (from B2) will hereafter be referred to *C. orinocensis sensu stricto*, because the type locality of *C. orinocensis* is the Orinoco basin (Humboldt, 1821), and the representatives in the Negro basin will hereafter be referred to as *C. orinocensis* Negro. In clade B1, *C. monoculus* is sister to the two *C. ocellaris* lineages. *Cichla* sp. "Madeira" is sister to the remaining lineages of clade B1. Lineages in clade B1 are distributed primarily in the Amazonas drainage and coastal drainages of the Guyanas, while lineages in clade B2 are distributed in the Orinoco and Casiquiare. Thus, the species of clade B roughly encircle the Guyana Shield region.

The parsimony heuristic search of the combined matrix (197 parsimony-informative sites) recovered different numbers of most-parsimonious trees depending on the outgroup(s) used. With either *Astronotus* or *Retroculus* included in the search, PAUP found 12 trees (with the same topologies found in both searches) of length 506 steps and C.I. 0.615 or length 528 and C.I. 0.636 for *Astronotus* or *Retroculus* respectively. The topology of these trees (not shown) agreed with the ML tree except for disagreement in the internal topology within the species *C. temensis*, *C.* sp. "Madeira," and *C. monoculus*. When both outgroup sequences were included in parsimony searches, 24 trees were recovered, each of length 582 and C.I. 0.625 (not shown). A strict consensus of these trees revealed ambiguity in the deeper nodes of the tree, with the 2 major clades previously recovered (clades A and B) collapsing to 3 clades (A, B1, and B2).

Sequencing the 5′ portion of the CR from 454 individuals of *Cichla* from 37 different localities provided 128 different CR haplotypes. These sequences had lengths ranging from 501 to 505 nucleotides from near the Proline-tRNA (5′) to shortly following the central conserved displacement loop region (3′). In almost all cases, haplotypes were not shared across species (see below). Also, haplotypes were almost always exclusive to a geographic region, if not a single locality. These haplotypes (*Cichla* only) differed by a single transition to over 14% uncorrected sequence divergence (after alignment variable positions were deleted). The original haplotype sequences are available from Genbank (accession nos. DQ841819-DQ841946).

For the complete set of *Cichla* CR haplotypes, 476 base positions remained after alignment-variable positions were deleted. Of these, 144 positions were variable and 133 were parsimony informative. Twenty tree search replicates using the parsimony ratchet each with 200 iterations resulted in 3304 trees of length 422 and CI of 0.455, the strict consensus of which is shown in Fig. 3. This tree was rooted in the position indicated by the combined analysis (Fig. 2), which is the same root indicated by mid-point rooting (Farris, 1972). The many equally parsimonious trees mainly differed in topological arrangements of haplotypes within species. The trees showed that most morphologically defined species were characterized by well-differentiated haplotype clades. However, some species exhibited non-monophyletic haplotype clades (e.g., *C.* sp. "Amazonas"). The topology of the CR strict consensus tree agreed with the combined data trees from ML and MP analyses with a few notable exceptions. In the CR consenus, *C. orinocensis* Negro was sister to *C. monoculus*, rather than *C. monoculus* + *C. ocellaris*. Also, a clade including haplotypes of *Cichla* sp. "Xingu" and *Cichla* sp. "Amazonas" from the lower Xingu was

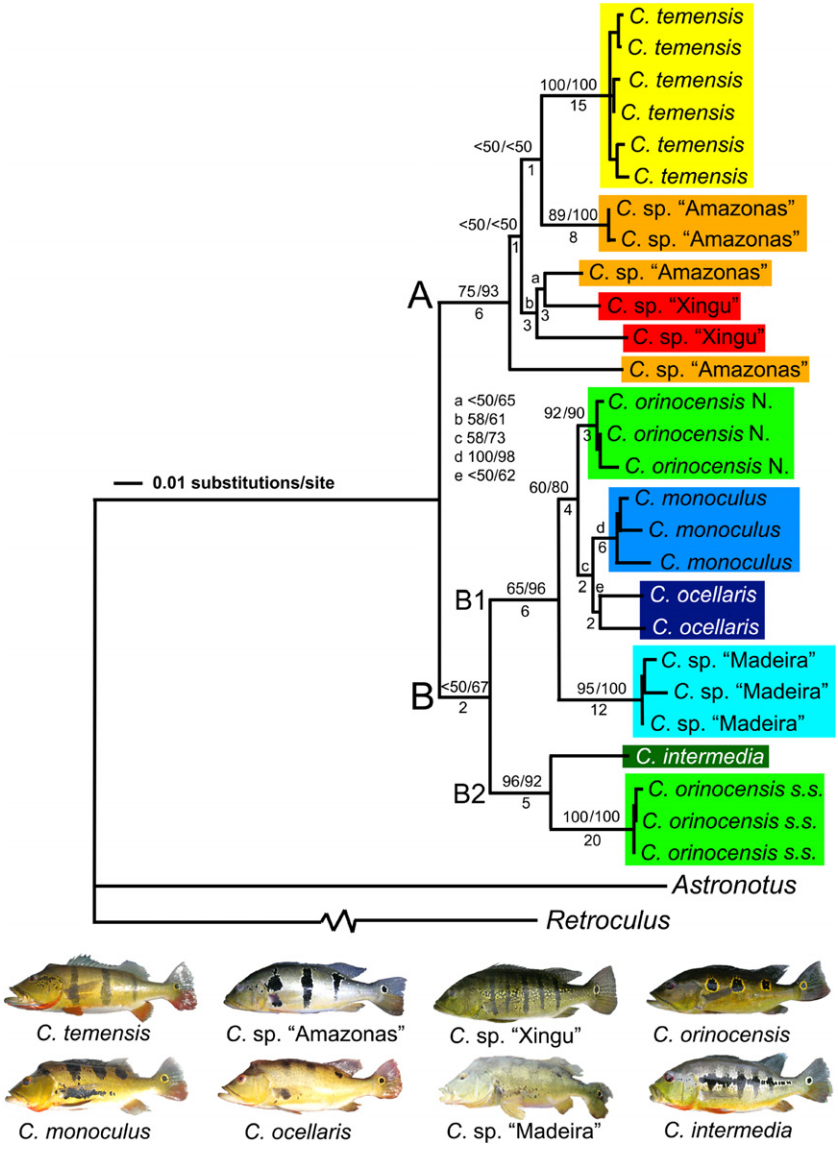

Fig. 2. Maximum likelihood phylogeny for *Cichla*, based on combined cytochrome *b* and control region data. Values above branches are bootstrap proportions for likelihood/parsimony, and values below branches are decay indices (parsimony and decay values are derived from the analysis including only *Astronotus*). Color blocks show species identity determined by morphology. A, B, B1, and B2 identify clades discussed in the text. Fish profiled at the bottom show morphologies typical for the morphologically defined species. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

sister to the remaining clade A haplotypes (*C. temensis* and *Cichla* sp. "Amazonas" from other localities).

The CR analysis revealed four cases that we consider to represent mismatches between morphotype and haplotype lineages (Fig. 3): (1) the single haplotype from *C. intermedia* in the Parguaza river clustered with *C. orinocensis sensu stricto*; (2) the two haplotypes from *C. monoculus* in the Mavaca river clustered with *C. temensis*; (3) all four haplotypes from *Cichla* sp. "Amazonas" from the lower Xingu nested within *Cichla* sp. "Xingu" from the middle Xingu; and (4) *C. orinocensis* showed a bi-phyletic division of haplotypes corresponding to geography, with one group sister to *C. intermedia* and another sister to *C. monoculus*. This mismatch was confirmed by cyt *b* data as well for all cases (see Fig. 2; not shown for cases 1 and 2).

4. Discussion

4.1. Phylogeny of Cichla

Few studies have reconstructed the phylogeny of *Cichla*, and none has incorporated molecular data from all valid species and multiple undescribed species. Farias et al. (1999, 2000, 2001) included three species of *Cichla* (*C. temensis*, *C. orinocensis*, and *C. monoculus*) in their phylogeny of Neotropical cichlids. They found, in agreement with our phylogeny, that *C. orinocensis* and *C. monoculus* grouped together to the exclusion of *C. temensis*. Similarly, López-Fernández et al. (2005) included *C. temensis*, *C. orinocensis*, and *C. intermedia* in their Neoptropical cichlid phylogeny, and found that *C.*

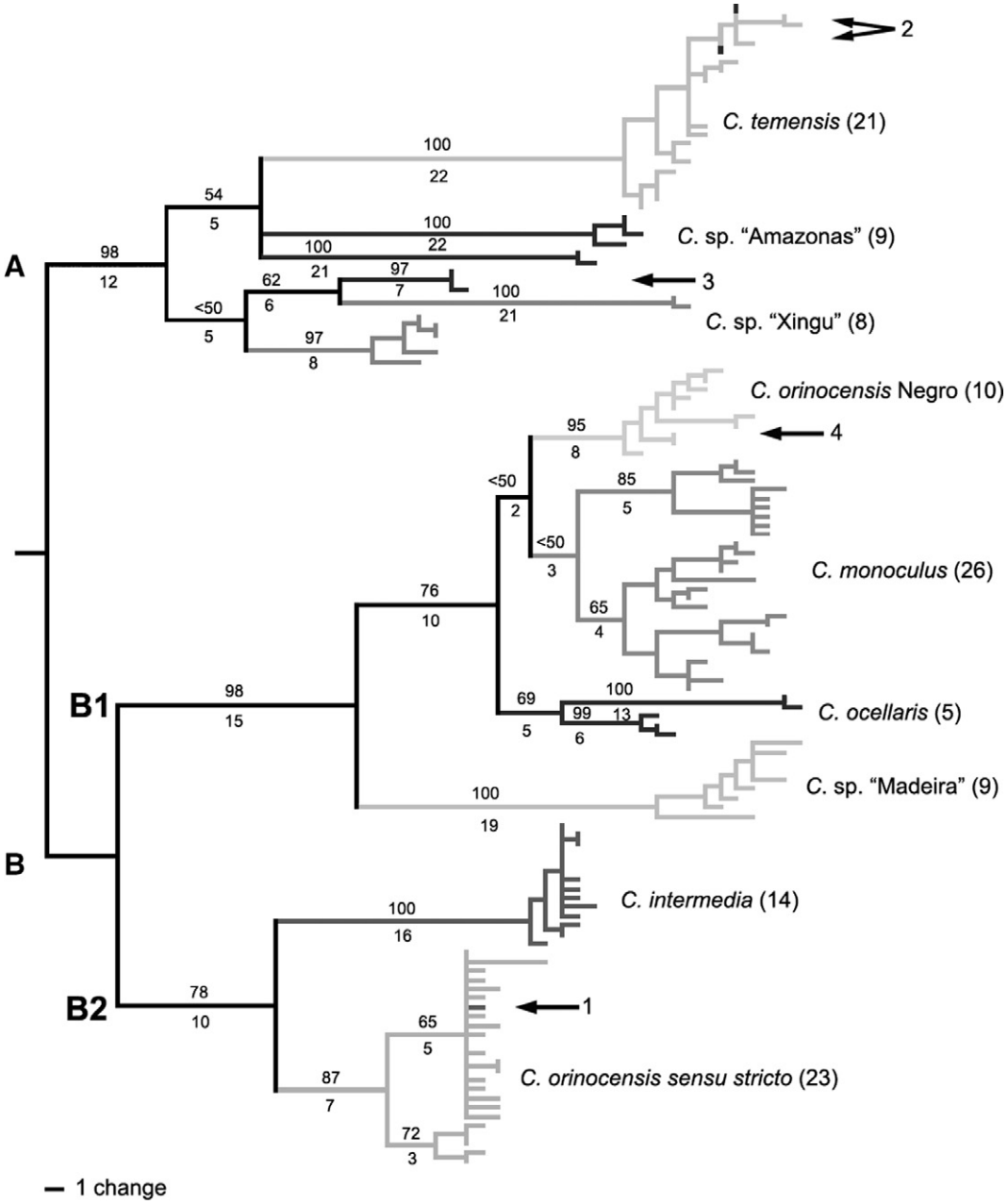

Fig. 3. Strict consensus phylogram of 3304 equally parsimonious trees for *Cichla*, based on the mitochondrial control region data. Tree is rooted at the position indicated by combined data analysis (Fig. 2). Values above branches are bootstrap proportions, and values below branches are decay indices. Branch color corresponds to species identity determined by morphology, as in Fig. 2. A, B, B1, and B2 identify clades discussed in the text. Values next to species name indicate the number of haplotypes observed. Arrows identify four morphotype-lineage mismatches (see text). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

intermedia and *C. orinocensis* grouped to the exclusion of *C. temensis*. More recently, Renno et al. (2006) investigated the nature of *Cichla* from the upper Madeira in Bolivia using a phylogeny including *C. monoculus*, *C. ocellaris*, *Cichla* sp. "Madeira," and *C. temensis*. They found, as in our phylogeny, that *C. monoculus* and *C. ocellaris* were sister species, to the exclusion of *Cichla* sp. "Madeira," while *C. temensis* diverged at the base of the tree. Thus, our phylogenetic results confirm previous limited investigations of *Cichla* relationships, but add new species and resolve a number of novel species-groups.

The phylogeny provides some insight into morphological evolution in *Cichla*. All the species of *Cichla* show a mouth and head morphology appropriate for piscivory (Stiassny, 1987), traits that match the observed ecology of all species as pursuit predators of fishes (Winemiller, 2001). Within this constraint, however, the two clades of *Cichla* seem to have diverged. Species in clade A are generally more elongate and achieve larger average body sizes than species in clade B, which are more deep-bodied and smaller (K. Winemiller, pers. comm.; C. Montaña, unpublished data). This may be associated with ecology:

Jepsen et al. (1997) reported that *C. orinocensis* (clade B) prefer lagoon and backwater types of habitats with shallow water and slow current velocity, while *C. temensis* (clade A) prefer deeper water habitats in both lagoons and the main channel. In addition, *Cichla* color patterns show phylogenetic conservatism (not withstanding intraspecific variation and polymorphism). Most *Cichla* exhibit variation on a central theme: three dark patches along the flank. In some species these spots are more vertically elongate (*C. temensis*) or reduced (*C. ocellaris*), and they may be ocellated (bordered by scales of a lighter color, as in *C. orinocensis*) or borderless (*C. temensis*). However, an exception to this color theme is seen once in both clade A and clade B. *Cichla intermedia* and *C.* sp. "Xingu" both exhibit six or more vertical stripes along the flank, double the number of dark patches in other *Cichla*. The phylogenetic independence of this pattern indicates either convergence or parallelism. Interestingly, this pattern may also correlate with ecology. Jepsen et al. (1997) reported that *Cichla intermedia* prefers habitats with available structural cover (rocks or logs) in areas of high current velocity, usually in the main river channel. *Cichla* sp. "Xingu" also seems to predominantly inhabit these types of areas in the Xingu. An intriguing line of inquiry would be to investigate the underlying genetic and developmental basis of these novelties to determine whether replicated patterns in independent branches result from the same (parallelism) or different phenomena (convergence).

4.2. Genetic diversity and species status

For this study, we diagnosed species as geographically circumscribed and morphologically differentiated groups of individuals with distinct haplotypes or haplotype clades, indicating cohesive species lineages which are united by the processes of gene flow and recombination with minimal or no gene flow among cohesive groups (Templeton, 1989; de Queiroz, 1998). The monophyly of haplotypes within species was not required, in accord with the possibility of incomplete lineage sorting or minor but ongoing gene flow between otherwise cohesive species (Harrison, 1998; de Queiroz, 1998; Weins and Penkrot, 2002; Sites and Marshall, 2003, 2004). According to these criteria, we confirm the distinctiveness of the five currently recognized *Cichla* species. These include: *C. intermedia*, restricted to the upper Orinoco, tributaries of the middle Orinoco, and the Caura river; *C. orinocensis sensu stricto*, distributed throughout the Orinoco and upper Negro (Casiquiare) rivers; *C. monoculus*, widely distributed in the Amazonas drainage and patchily distributed in the upper Orinoco; *C. ocellaris*, from the coastal tributaries of the Guyanas (Essequibo, Maroni, and others) and upper Branco in the Negro/Amazonas drainage; and *C. temensis*, from the Orinoco river, Negro river, and Igapo-Açu region of the lower Madeira (Fig. 4).

Our criteria also suggest the existence of at least three more species in need of description. These include, from clade B, *Cichla* sp. "Madeira," restricted to the upper Madeira drainages of Mamoré, Guaporé, and Madre de Dios, above the rapids at Porto Velho (Brazil) (Fig. 4). Although commonly referred to *C. monoculus* due to only relatively minor morphological differences, these fish clearly form a distinct genetic group separate from either of the two described species of subclade B1. Recent additional sampling in the upper Madeira discussed by Renno et al. (2006) have confirmed this is the only *Cichla* known from this region. From clade A, putative species are *Cichla* sp. "Xingu", found only above the rapids near Belo Monte (Brazil) in the middle and upper Xingu river; and *Cichla* sp. "Amazonas," seemingly widely distributed in the mainstream Amazon downstream of the Negro river, and in the lower courses of Amazonas tributaries (i.e., Tapajós, Xingu, Tocantins). Although these two species did not each form reciprocally monophyletic haplotype lineages, they both exhibited well-differentiated groups of haplotypes that were private to those morphologically distinct species. Additional putative undescribed species of *Cichla* have been reported from the upper courses of other lower Amazonas tributaries such as the Tapajós, Tocantins-Araguaia, Trombetas, and Brazilian shield tributaries of the lower Madeira (see Fig. 1a), but we were unable to obtain tissue samples from these regions. Finally, as discussed below, the *C. orinocensis* population in the middle/lower Negro river may also warrant species status, but current data are insufficient to confirm this. We have not provided descriptions or diagnoses of these species here, as these have been provided elsewhere (Kullander and Ferreira, 2006).

4.3. Morphotype/mitochondrial lineage mismatch

In this study we initially assigned individual fishes to species using morphology, particularly diagnostic color patterns. We surveyed numerous individuals from multiple populations throughout the range of the most widespread species, with the vast majority of individuals being collected personally by the authors or supplied to us with individual color photographs. This allowed us to assign individuals to morphologically defined species with a high degree of accuracy, while taking intraspecific variation into account. Several species of *Cichla* are quite variable in color, exhibiting intraspecific morphological variation possibly related to local environmental influences, population genetic structure, age and reproductive status (Winemiller, 2001; P. Reiss, unpublished data). This has caused species identification in smaller scale studies to be difficult, complicating previous investigations of molecular patterns in *Cichla* and inferences of hybridization from morphological and molecular data (e.g., Andrade et al., 2001; Teixeira and de Oliveira, 2005). Isolated examples of hybridization, while intriguing, do not permit robust conclusions about the long-term efficacy of reproductive barriers between species or the importance of introgression in the evolution of the species involved. Our study provides the most comprehensive investigation of natural patterns of introgressive hybridization in *Cichla* to date.

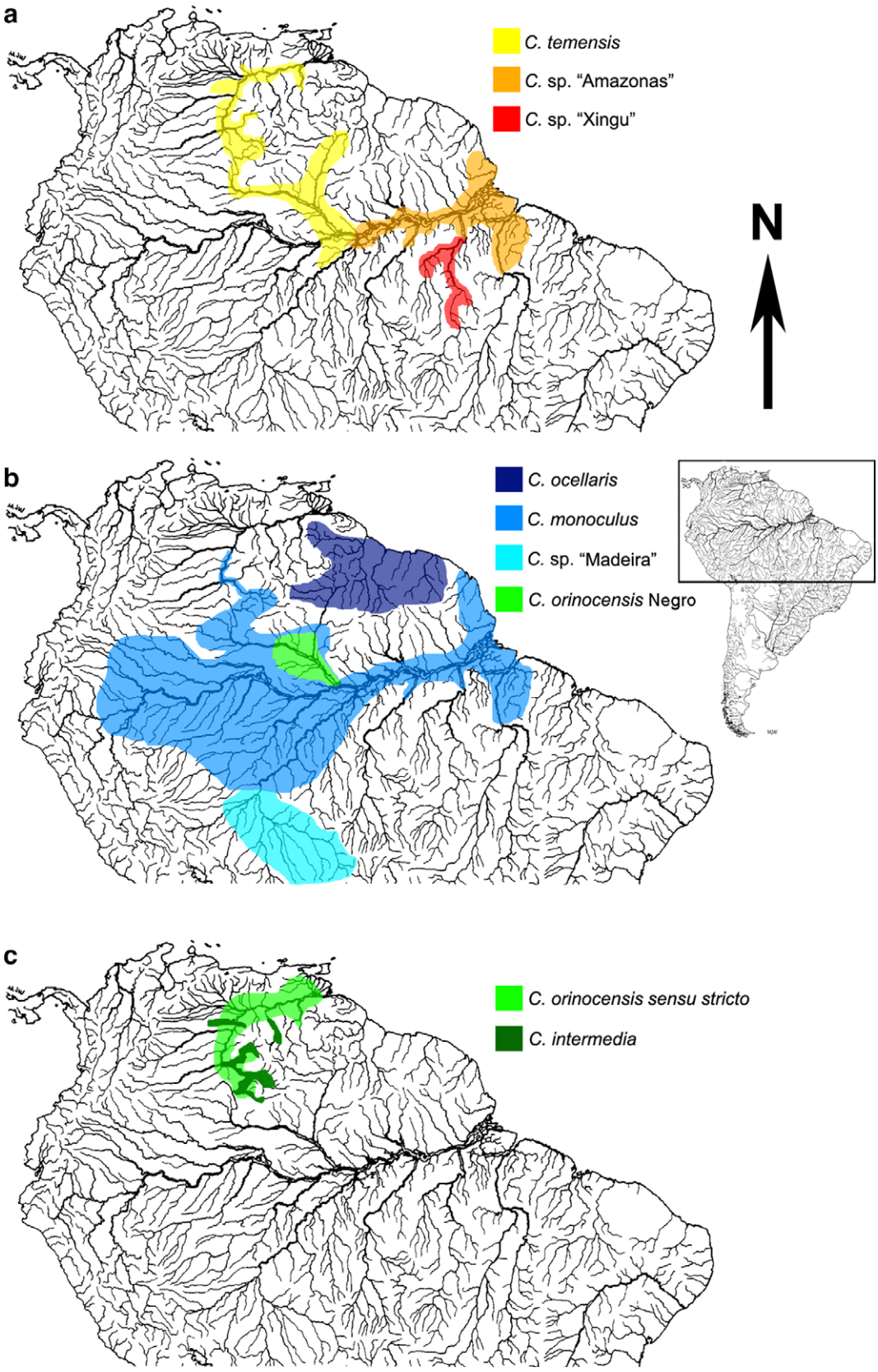

Fig. 4. Estimated distributions of the morphologically defined *Cichla* species, by clade. (a) Clade A: *C. temensis* (yellow), *C.* sp. "Amazonas" (orange), *C.* sp. "Xingu" (red); (b) Clade B1: *C. ocellaris* (darkest blue), *C. monoculus* (middle blue), *C.* sp. "Madeira" (lightest blue), *C. orinocensis* Negro (light green); (c) Clade B2: *C. orinocensis sensu stricto* (lighter green), *C. intermedia* (darker green). Distribution data were collected by the first author, extracted from the literature, and compiled from museum specimens. Base map by Marilyn J. Weitzman. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

We found that most *Cichla* species are represented by monophyletic haplotype clades. However, in a few cases we observed that CR haplotypes were not restricted to a single morphologically defined species, or did not conform to the morphology-based predictions of placement in the phylogenetic tree (Fig. 3). Specifically, this was the case for: (1) the haplotype from the two *C. intermedia* from the Parguaza river that grouped among *C. orinocensis sensu stricto*; (2) both haplotypes from the 13 *C. monoculus* from the Mavaca river that grouped most closely with *C. temensis*; (3) all three haplotypes from four *C.* sp. "Amazonas" from the lower Xingu that grouped with C. sp. "Xingu"; and (4) all 10 haplotypes from 18 *C. orinocensis* from the middle and lower Negro river which grouped most closely with *C. monoculus* and *C. ocellaris* rather than with *C. orinocensis sensu stricto*. These mismatches were confirmed with cyt *b* sequences as well, and thus most likely constitute the mitochondrial haplotype rather than a nuclear paralog (see Fig. 2a; not shown for case 1 or 2 above). As all tissue samples were collected personally by the authors and carefully rechecked for sequence identity, we do not believe these instances result from sample misidentification, but rather from actual morphotype/lineage mismatch.

Assuming that morphological convergence is an unlikely explanation, these patterns may be the result of two separate phenomena: incomplete sorting of ancestral polymorphism, or introgression via hybridization (Doyle, 1992; Smith, 1992). Incomplete lineage sorting may occur when speciation is recent and ancestral allelic polymorphism has not yet evolved into species-specific monophyletic lineages (Nei, 1986; Neigel and Avise, 1986; Avise, 2000). Thus, closely related species might be composed of paraphyletic or polyphyletic groups of haplotypes. Introgressive hybridization, on the other hand, occurs when alleles from one species are transferred to another due to breakdown of reproductive isolating barriers (Arnold, 1997; Dowling and Secor, 1997; Avise, 2000). While both incomplete lineage sorting and introgression can cause morphotype/haplotype mismatches, distinguishing between the two may be difficult. Introgression may be implicated when an individual's haplotype is identical or very closely related to haplotypes from a sympatric, but not necessarily closely related, species from the same locality (Sullivan, 2002). Patterns of haplotype paraphyly or polyphyly between recently diverged and not necessarily sympatric species may signal incomplete lineage sorting (Moran and Kornfield, 1993). However, intermediate or more complex situations may be common in nature, such as ancient introgression followed by subsequent evolution of the introgressed alleles.

In the case of (1) *C. intermedia* from the Parguaza River and (2) *C. monoculus* from the Mavaca River, mismatches are most likely the result of relatively recent hybridization resulting in the introgression of mitochondria from one *Cichla* species to another. Two lines of evidence support this idea. First, the sampled individuals of the putative "recipient" species all possessed mitochondrial haplotypes that were identical to or very closely related to haplotypes found in individuals of the "donor" species from the same geographic areas. Second, individuals of the recipient species were uncommon in each of the locations where putative-introgression was observed (in comparison to the donor species) suggesting the populations were relatively small and potentially isolated from the main populations of the recipient species. These have been suggested to be prime conditions for genetic introgression from a more common species (Hubbs, 1955; Arnold, 1997; Dowling and Secor, 1997). This hypothesis probably requires actual pairing between individuals of different species. Although *Cichla* exhibit external fertilization and sperm could hypothetically drift to fertilize the eggs of another species, nest proximity would have to be above average and spawning almost completely synchronous (K. Winemiller, pers. comm.). Perhaps more likely, heterospecifics form pairs and spawn in rare instances. All *Cichla* species for which cytogenetic studies have been conducted have equal chromosome numbers (48 non-degenerate chromosomes; Thompson, 1979; Brinn et al., 2004), reducing the likelihood of reproductive isolation due to aneuploidy (Coyne and Orr, 2004). Additionally, it should be noted that all sampled individuals in the putatively introgressed populations possessed the mismatched haplotypes, suggesting that hybridization may have been followed by a founder effect or fixation of these foreign mitochondria either by genetic drift or selective sweep.

The case of (3) *Cichla* sp. "Amazonas" in the Xingu is more suggestive of incomplete sorting of ancestral polymorphism. Indeed, the CR topology suggests that incomplete lineage sorting may be common in clade A. Fishes from the Xingu above the rapids near Belo Monte, Brazil, were unequivocally assigned using morphology to *Cichla* sp. "Xingu", while all those from below the falls were unequivocally assigned to *Cichla* sp. "Amazonas". Thus these two morphologically defined species are allopatrically distributed, and these two regions of the Xingu are separated by a geographic barrier (waterfalls and rapids) that has likely precluded at least recent opportunities for interbreeding. Additionally, two other deeply divergent lineages of *C.* sp. "Amazonas" were found sympatrically in the lower Tapajós, lower Amazonas, and Curuá-Una rivers (the two lineages which group with of *C. temensis* in Fig. 3). This evidence may suggest that *Cichla* sp. "Amazonas" exhibits polymorphism ancestral to the divergences of the more spatially bounded *Cichla* sp. "Xingu" and *C. temensis* (making it a persistent ancestor; see Graybeal, 1995; Olmstead, 1995; Weins and Penkrot, 2002). Additional sampling of both *Cichla* sp. "Amazonas" and *Cichla* sp. "Xingu" is necessary to establish the frequency of non-monophyletic lineages and their geographic distribution in the Xingu, lower Amazonas, and lower courses of other lower Amazonas tributaries.

The case of (4) *C. orinocensis* is quite complex. All 10 haplotypes of the *C. orinocensis* morphotype from the middle and lower Negro river were closely related to *C. mono-*

culus and *C. ocellaris* (Fig. 3). *Cichla orinocensis sensu stricto*, is the sister species *C. intermedia*. This makes the two haplotype clades of *C. orinocensis* deeply divergent and separated considerably on the phylogenetic tree. Nevertheless, *C. orinocensis sensu stricto* and *C. orinocensis* Negro morphotypes are essentially indistinguishable: both share three distinct ocellated round spots along the flank with no other spots or marks apparent on the body against a light or dark green background (see Fig. 2). Indeed, more morphological variation was observed within either population than was consistently apparent between the two.

One explanation for this pattern is a history of incomplete lineage sorting. Under this scenario, ancestral polymorphism from the clade B ancestor is sorted among descendants in such a way that (1) *C. orinocensis* Negro and *C. monoculus* haplotypes are most closely related and (2) *C. orinocensis sensu stricto* and *C. intermedia* haplotypes are most closely related. However, we find this scenario unlikely. First, the observation that haplotype clades are otherwise tightly matched to specific morphologically defined species suggests that the CR is evolving quickly enough in clade B (via mutation and genetic drift) to accurately record cladogenetic events. If incomplete lineage sorting was a problem we might expect many more mismatches between morphologically defined species and haplotype lineages. Second, the placement of *C. orinocensis* Negro among the crown species of clade B1 makes incomplete or differential lineage sorting unlikely. If *C. orinocensis* suffers problems of incomplete lineage sorting, why do other species such as *Cichla* sp. "Madeira" not exhibit similar phenomena? Together, *C. monoculus*, *C. ocellaris*, and *Cichla* sp. "Madeira" cover a much larger geographic range than *C. orinocensis*; thus if geographic range translates into larger effective population size, it is surprising that ancestral polymorphism would have been completely sorted in these species but not in *C. orinocensis*.

Instead, we hypothesize an ancient introgression event between *C. orinocensis* and an early *C. monoculus*/*C. ocellaris* lineage, possibly as one or the other species colonized the lower/middle Negro River. In this scenario, the hybrid fishes received the characteristic morphology of *C. orinocensis*, but acquired mtDNA from the *C. monoculus*/*C. ocellaris* lineage. Subsequently, the hybrid population evolved as a distinct lineage, explaining the sequence divergence between its mtDNA and that of *C. monoculus*/*C. ocellaris*. Also, the genetic diversity and haplotype monophyly of *C. orinocensis* Negro suggests that the hybrid lineage has remained relatively stable since its origination. An intriguing possibility is that the initial hybridization event may have resulted in a novel lineage that was reproductively isolated and has evolved independently from both parental species (hybrid speciation) (Dowling and Secor, 1997). Hybrid speciation has been reported in fishes, specifically in cichlids, and indeed was hypothesized to be the basis for an entire radiation of cichlids in Lake Victoria in Africa (Seehausen, 2004). Clearly, confirmation of the hybridization hypothesis will require evaluation of nuclear DNA and additional sampling of *C. orinocensis* populations from across the species range.

It appears that hybridization is not uncommon in *Cichla*, and may provide a significant path to genetic diversification. Studies have shown that introgression has the potential to transfer adaptive alleles from one species to another, potentially opening habitats previously unsuitable for colonization (e.g., Lewontin and Birch, 1966). It is interesting that where introgression was inferred to occur, all the individuals in the sampled localities exhibited the foreign haplotypes. The sedentary nature of some fish species, such as *Cichla* (Hoeinghaus et al., 2003), may reduce the effective population size of the species by limiting gene flow (Wright, 1931). The result may be that stochastic events such as introgression have a greater impact than might otherwise be expected.

4.4. Diversification and biogeography of Cichla

Species-level phylogenies allow the identification of biogeographic events that have affected the distributions and origins of extant species. Accurate phylogenies can also be used to infer geographic mode of speciation (Lynch, 1989; Barraclough and Vogler, 2000). Moreover, comparisons of biogeographic patterns from multiple taxa can potentially shed light on the paleogeographic history of a region (Bermingham and Moritz, 1998; see also Hunn and Upchurch, 2001). Such investigations may be particularly valuable in South America, where tropical rivers contain the world's most speciose assemblage of vertebrates (Vari, 1988; Bermingham and Martin, 1998; Bermingham and Moritz, 1998; Moritz et al., 2000; Reis et al., 2003). Here, we synthesize phylogenetic and distributional data for *Cichla*, and attempt to infer geographical mode of speciation for lineage divergences. We also briefly compare *Cichla* biogeographic patterns to those of other Neotropical aquatic taxa.

The earliest divergence within *Cichla* was between clade A, and clade B (Fig. 2). The distribution of these two clades overlaps significantly (Fig. 4), and a geographic explanation for their divergence is not readily obvious. One possible scenario is that an initial allopatric separation occurred between clade A (once distributed exclusively in the Amazonas basin) and clade B (once distributed exclusively in rivers draining the northern and eastern Guyana shield), followed by considerable dispersal and range expansion by both clades. However, there are complications with this theory. For example, if clade A originated in the Amazonas, and clade B1 colonized the Amazonas from previous allopatry in the Guyanas, why is only the latter clade present in the upper Maderia? Alternatively, the initial divergence between the two *Cichla* clades may have been adaptive. Fishes in clade A tend to be larger and more elongate, while species in clade B tend to be smaller and deeper bodied. Additionally, species in clade B (with the possible exception of *C. intermedia*) are more tolerant of the higher conductivities and higher sediment content (lower visibility) of whitewater conditions than species of clade A (Winemiller, 2001;

see also Shafland, 1993). Thus, ecologically driven divergence in sympatry or parapatry is another possibility. However, it may be impossible to determine whether these morphological and ecological differences were the primary cause, or a secondary result of the divergence (Coyne and Orr, 2004).

We hypothesize that the divergence between clades B1 and B2 was vicariant, as these clades are largely allopatric (Fig. 4). Areas of overlap between the two clades in the upper Orinoco and Negro rivers may be due to recent range expansions. Sequences from *Cichla monoculus* in the upper Orinoco and Casiquiare are relatively derived within *C. monoculus* and show no nucleotide diversity (the same haplotype was found in all individuals from both locations; Willis et al., in preparation), suggesting recent range expansion (Templeton, 2001). Similarly, the putative hybridization event for *C. orinocensis* in the Negro River may suggest that the species colonized the area recently in comparison to its divergence from *C. intermedia*. The limited hybridization of closely related lineages that diverged in allopatry where there was no selection for reproductive isolation *per se* is not uncommon (Endler, 1982; Hewitt, 2001; Templeton, 2001). The biogeography of *Cichla* clades B1 and B2 is repeated in several other Neotropical fish taxa. For example, the genera *Prochilodus* and *Semaprochilodus* also have sister clades distributed in the Orinoco versus the Amazonas or Amazonas + Guyanas drainages (Sivasundar et al., 2001; Castro and Vari, 2004).

At least two cladogenetic events must have occurred during the diversification of clade A *C. temensis*, *Cichla* sp. "Amazonas," *Cichla.* sp. "Xingu," and *C. temensis*. Both *Cichla* sp. "Xingu" and *C. temensis* appear to be allopatric but contiguous with *C.* sp. "Amazonas" (Fig. 4a). *Cichla* sp. "Xingu" is isolated by a series of rapids and water falls between the lower and middle Xingu rivers near Belo Monte, Brazil, corresponding to the northern edge of the Brazilian shield (see also Fig. 1a). These rapids may have isolated an ancestral clade A population which eventually became the new species *Cichla* sp. "Xingu". The evolution of endemic taxa in the Brazilian shield tributaries of the Amazonas is a particularly common pattern in Neotropical fishes. For instance, *Prochilodus* (*P. britskii*; Castro and Vari, 2004), *Semaprochilodus* (*S. brama*; Castro and Vari, 2004), *Potamotrygon* (*P. henlei*, *P. leopoldi*; Carvalho et al., 2003), and *Retroculus* (*R. lapidifer*, *R. xinguensis*; Kullander, 2003) all exhibit species restricted to these southern tributaries. In the lower Negro and lower Madeira rivers where the distribution of *C. temensis* is contiguous but non-overlapping with that of *Cichla* sp. "Amazonas" there are no obvious geological barriers to separate these two species. However, the rivers inhabited by *C. temensis* have different water chemistries (acidic blackwater; Sioli, 1984) compared to the habitats of *Cichla* sp. "Amazonas" in the lower portions of the lower Amazonas tributaries (clearwater, with neutral pH and high transparency; Sioli, 1984). Thus, adaptation to different water chemistries may have contributed to reduced gene flow and eventual speciation between *C. temensis* and *Cichla* sp. "Amazonas."

Within clade B1, *Cichla* sp. "Madeira" diverged from the lineage that gave rise to *C. monoculus* and *C. ocellaris*. A series of rapids separates the Mamoré and Madre de Dios rivers from the middle Madeira and remainder of the Amazonas, and may define a barrier to gene flow that caused the divergence of *Cichla* sp. "Madeira" (Fig. 4b). Other Neotropical taxa, including freshwater needlefishes (*Potamorrhaphis eigenmanni*; Collette, 1982; Lovejoy and de Araújo, 2000), and river dolphins + (*Inia boliviensis*; Banguera-Hinestroza et al., 2002) exhibit similar endemicity in the Mamoré and Madre de Dios rivers. A second divergence in clade B1 resulted in the separation of *C. monoculus* and *C. ocellaris*. The cause of divergence was probably vicariant, since these species are allopatric. The two coastal Guyanan drainages from which *C. ocellaris* was collected, the Essequibo (Cuyuni and Rupununi) and Maroni rivers, each showed a relatively well-differentiated monophyletic clade of haplotypes (Figs. 2 and 3). As these coastal drainages are separated by a terrestrial barrier, this divergence may suggest the initial evolution of distinct species. Interestingly, haplotypes of *C. ocellaris* in the Essequibo (Rupununi) were shared by *C. ocellaris* in the upper Branco (Pirara), a tributary of the Negro river in the Amazonas drainage. Seasonal inundation of the low-lying Rupununi savannah which separates these two drainages has been hypothesized to provide an ephemeral aquatic connection between two rivers (e.g., Lowe-McConnell, 1964; Lovejoy and de Araújo, 2000), and the distribution of genetic diversity in *Cichla* supports this hypothesis. A close connection between the coastal Guyanan drainages and the Amazonas has also been observed in other Neotropical fishes, such as *Potamorrhaphis* (*P. guianensis*; Lovejoy and de Araújo, 2000) and *Prochilodus* (*P. nigricans* and *P. rubrotaeniatus*; Castro and Vari, 2004; Turner et al., 2004).

Within clade B2, *C. orinocensis sensu stricto* diverged from *C. intermedia* in the Orinoco basin (Fig. 4c). These species are sympatric throughout the entire range of *C. intermedia*. However, they do not occur symtopically (in the same habitat). *C. orinocensis* prefers slow-moving, backwater habitats and is rather tolerant of low visibility, while *C. intermedia* is most common in rocky habitats with high current velocity and relatively high visibility. Thus, we consider an ecologically based divergence between these two species to be a possibility.

In most cases, sister lineages in *Cichla* are allopatrically distributed, and in a number of instances specific geographic isolating barriers can be identified. Thus, vicariance seems to have played a predominant role in the evolution of species diversity in this genus. However, we documented at least one sympatric, ecologically divergent sister species pair (*C. orinocensis* and *C. intermedia*), suggesting that ecological speciation cannot be discounted. Recent work has suggested that other Neotropical cichlids (geophagines) have radiated based on ecology (López-Fernández et al., 2005). It is likely that adaptive phenomena are not isolated to cichlid fishes, but represent a widespread contributing

factor in the accumulation of biological diversity in the Neotropics (Moritz et al., 2000).

5. Conclusion

We have presented the first detailed and relatively complete molecular phylogenetic analysis of the genus *Cichla*. Our broad intraspecific sampling made possible the identification of several putative cases of mitochondrial introgression. Based on this finding, we suspect that introgression is a significant source of genetic mixing in *Cichla*, and may play an important role in the molecular evolution of this genus. By extension, hybridization may play an unrecognized role in the origin of biological diversity of fishes in the Neotropics, as has been emphasized recently in more well known regional faunas (Dowling and Secor, 1997; Hewitt, 2001). Biogeographic analysis of *Cichla* indicates that most sister lineages have allopatric distributions, suggesting the predominance of vicariance. However, at least one *Cichla* divergence may be more parsimoniously explained by ecological speciation. Peacock bass (*Cichla* spp.) are a conspicuous and important part of the Neotropical aquatic fauna. Their ease of collection, widespread distribution, and manageable diversity make them an excellent group for further investigation. The completion of a robust phylogenetic hypothesis further enhances the value of *Cichla* as model system for understanding Neotropical biodiversity.

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References

Akaike, H., 1974. A new look at statistical model identification. IEEE Transactions of Automatic Control 19, 716–723.

Andrade, F., Schneider, H., Farias, I., Feldberg, E., Sampaio, I., 2001. Análise filogenética de duas espécies simpátricas de tucunaré (*Cichla*, Perciformes), com registro de hibridização em diferentes pontos da bacia Amazônica. Revista Virtual de Iniciação Acadêmica de Universidade Federal do Para 1, 1–11.

Arnold, M.L., 1997. Natural Hybridization and Evolution. Oxford Univ. Press, Oxford.

Avise, J.C., 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conserv. Biol. 9, 686–690.

Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Banguera-Hinestroza, E., Cárdenas, H., Ruiz-García, M., Marmontel, M., Gaitán, E., Vázquez, R., García-Vallejo, F., 2002. Molecular identification of evolutionary significant units in the Amazon river dolphin *Inia* sp. (Cetacea: Iniidae). J. Heredity 93, 312–322.

Baric, S., Sturmbauer, C., 1999. Ecological parallelism and cryptic species in the genus *Ophiothrix* derived from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 11, 157–162.

Barlow, G., 2000. The Cichlid Fishes: Nature's Grand Experiment in Evolution. Perseus Publishing, Cambridge, MA.

Barraclough, T.G., Vogler, A.P., 2000. Detecting the geographical pattern of speciation from species-level phylogenies. Am. Nat. 155, 419–434.

Berlocher, S.H., 1998. Can sympatric speciation via host or habitat shift be proven from phylogenetic and biogeographic evidence. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York, pp. 99–113.

Bermingham, E., Martin, A.P., 1998. Comparative mtDNA phylogeography of Neotropical freshwater fishes: Testing shared history to infer the evolutionary landscape of lower Central America. Mol. Ecol. 7, 499–517.

Bermingham, E., Moritz, C., 1998. Comparative phylogeography: concepts and applications. Mol. Ecol. 7, 367–369.

Bremer, K., 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution 42, 795–803.

Bremer, K., 1994. Branch support and tree stability. Cladistics 10, 295–304.

Brinn, M.N.A., Porto, J.I.R., Feldberg, E., 2004. Karyological evidence for interspecific hybridization between *Cichla monoculus* and *C. temensis* (Perciformes, Cichlidae) in the Amazons. Hereditas 141, 252–257.

Carvalho, M.R., Lovejoy, N.R., Rosa, R.S., 2003. Family Potamotrygonidae. In: Reis, R.E., Kullander, S.O., Ferraris, C.J. (Eds.), Checklist of the Freshwater Fishes of South America. EDIPUCRS, Porto Alegre, Brazil, pp. 22–28.

Castro, R.M., Vari, R.P., 2004. Detritivores of the South American fish family Prochilodontidae (Teleostei: Ostariophysi: Characiformes): a phylogenetic and revisionary study. Smit. Contr. Zool. 622, 1–189.

Collette, B.B., 1982. South American freshwater needlefishes of the genus *Potamorrhaphis* (Beloniformes: Belonidae). Proc. Biol. Soc. Wash. 95, 714–747.

Coyne, J.A., Orr, H.A., 2004. Speciation. Sinauer Associates, Sunderland, MA.

de Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: Howard, D.J., Berlocher, S.J. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York, pp. 57–75.

Dergam, J.A., Suzuki, H.I., Shibatta, O.A., Duboc, L.F., Júlio Jr., H.F., Guiliano-Caetano, L., Black IV, W.C., 1998. Molecular biogeography of the Neotropical fishes *Hoplias malabaricus* (Erythrynidae: Characiformes) in the Iguaçu, Tibagi, and Paraná rivers. Genet. Mol. Biol. 21, 493–496.

Dergam, J.A., Paiva, S.R., Schaeffer, C.E., Godinho, A.L., Vieira, F., 2002. Phylogeography and RAPD-PCR variation in *Hoplias malabaricus* (Bloch, 1794) (Pisces: Teleostei) in southeastern Brazil. Genet. Mol. Biol. 25, 379–387.

Dowling, T.E., Secor, C.L., 1997. The role of hybridization and introgression in the diversification of animals. Annu. Rev. Ecol. Syst. 28, 593–619.

Doyle, J.J., 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Syst. Bot. 17, 144–163.

Endler, J.A., 1982. Problems in distinguishing historical from ecological factors in biogeography. Am. Zool. 22, 441–452.

Farias, I.P., Orti, G., Sampaio, I., Schneider, H., Meyer, A., 1999. Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and fast molecular evolution of the Neotropical assemblage. J. Mol. Evol. 48, 703–711.

Farias, I.P., Ortí, G., Meyer, A., 2000. Total evidence: molecules, morphology and the phylogenetics of cichlid fishes. Mol. Develop. Evol. 288, 76–92.

Farias, I.P., Ortí, G., Sampaio, I., Schneider, H., Meyer, A., 2001. The cytochrome *b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. J. Mol. Evol. 53, 89–103.

Farris, J.S., 1972. Estimating phylogenetic trees from distance matrices. Am. Nat. 106, 645–668.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Gatesy, J., DeSalle, R., Wheeler, W., 1993. Alignment ambiguous nucleotides and the exclusion of systematic data. Mol. Phylogenet. Evol. 2, 152–157.

Graybeal, A., 1995. Naming Species. Syst. Biol. 44, 237–250.

Harrison, R.G., 1998. Linking evolutionary pattern and process: the relevance of species concepts for the study of speciation. In: Howard, D.J., Berlocher, S.J. (Eds.), Endless Forms: Species and Speciation. Oxford University Press, New York, pp. 19–31.

Hewitt, G.M., 2001. Speciation, hybrid zones and phylogeography – or seeing genes in space and time. Mol. Ecol. 10, 537–549.

Hoeinghaus, D.J., Layman, C.A., Arrington, D.A., Winemiller, K.O., 2003. Movement of *Cichla* species in a Venezuelan floodplain river. Neotrop. Ich. 1, 121–126.

Hoorn, C., Gurrero, J., Sarmiento, G.A., Lorente, M.A., 1995. Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. Geology 23, 237–240.

Hrbek, T., Larson, A., 1999. The evolution of diapause in the killifish family Rivulidae: a molecular phylogenetic and biogeographic perspective. Evolution 53, 1200–1216.

Hrbek, T., Farias, I.P., Crossa, M., Sampaio, I., Porto, J.I.R., Meyer, A., 2005a. Population genetic analysis of *Arapaima gigas*, one of the largest freshwater fishes of the Amazon basin: implications for its conservation. Anim. Conserv. 8, 297–308.

Hrbek, T., Taphorn, D.C., Thomerson, J.E., 2005b. Molecular phylogeny of *Austrofundulus* Myers (Cyprinodontiformes: Rivulidae), with revision of the genus and the description of four new species. Zootaxa 825, 1–39.

Hubbs, C.L., 1955. Hybridization between fish species in nature. Syst. Zool. 4, 1–20.

Hunn, C.A., Upchurch, P., 2001. The importance of time/space in diagnosing the causality of phylogenetic events: towards a "chronobiogeographical" paradigm? Syst. Biol. 50, 391–407.

Jégu, M., Keith, P., 1999. Le bas Oyapock limite septentrionale ou simple étape dans la progression de la faune des poissons d'Amazonie occidentale. C.R. Acad. Sci. Paris, Sciences de la vie 322, 1133–1143.

Jepsen, D.B., Winemiller, K.O., Taphorn, D.C., 1997. Temporal patterns of resource partitioning among *Cichla* species in a Venezuelan blackwater river. J. Fish Biol. 51, 1085–1108.

Kullander, S.O., 1983. Review of the South American cichlids. Ph.D. thesis, Univ. Stockholm, Stockholm, Sweden.

Kullander, S.O., 1986. Cichlid Fishes of the Amazon River Drainage of Peru. Swedish Museum of Natural History. Springer, Berlin.

Kullander, S.O., 2003. Family Cichlidae. In: Reis, R.E., Kullander, S.O., Ferraris, C.J. (Eds.), Checklist of the Freshwater Fishes of South America. EDIPUCRS. Porto Alegre, Brazil, pp. 605–654.

Kullander, S.O., Ferreira, E.J.G., 2006. A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). Ichthyol. Explor. Freshwaters 17, 289–398.

Kullander, S.O., Nijssen, H., 1989. The Cichlids of Suriname. Brill, Leiden, Netherlands.

Layman, C.A., Winemiller, K.O., 2004. Size-based responses of prey to piscivore exclusion in a species-rich Neotropical river. Ecology 85, 1311–1320.

Lee, W.J., Conroy, J., Howell, W.H., Kocher, T.D., 1995. Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. 41, 54–66.

Lewontin, R.C., Birch, L.C., 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20, 315–336.

López-Fernández, H., Honeycutt, R.L., Winemiller, K.O., 2005. Molecular phylogeny and evidence for an adaptive radiation of geophagine cichlids from South America. Mol. Phylogenet. Evol. 34, 227–244.

Losos, J.B., Glor, R.E., 2003. Phylogenetic comparative methods and the geography of speciation. Trends Ecol. Evol. 18, 200–227.

Lovejoy, N.R., de Araújo, L.G., 2000. Molecular systematics, biogeography, and poplation structure of Neotropical freshwater needlefishes of the genus *Potamorrhaphis*. Mol. Ecol. 9, 259–268.

Lowe-McConnell, R.H., 1964. The fishes of the Rupununi savanna districts of British Guiana, Part 1. Ecological groupings of fish species and effects of the seasonal cycle on the fish. Zool. J. Lin. Soc. 45, 103–144.

Lowe-McConnell, R.H., 1969. The cichlid fishes of Guyana, South America, with notes on their ecology and breeding behavior. Zool. J. Lin. Soc. 48, 255–302.

Lowe-McConnell, R.H., 1991. Ecology of cichlids in South American and African waters, excluding the African Great Lakes. In: Keenleyside, M.H.A. (Ed.), Cichlid Fishes – Behavior, Ecology and Evolution. Chapman and Hall, London, pp. 60–85.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C., Wesselingh, F., 1998. The Stage for Neotropical Fish Diversification: A History of Tropical South American Rivers. In: Malabarba, L.R., Reis, R.E., Vari, R.P., Lucena, C.A.S., Lucena, Z.M.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. Museu de Ciências e Tecnologia, PUCRS, Porto Alegre, Brazil (Chapter 1).

Lynch, J.D., 1989. The gauge of speciation: on the frequencies of modes of speciation. In: Otte, D., Endler, J.A. (Eds.), Speciation and Its Consequences. Sinauer Associates, Sunderland, MA, pp. 527–553.

Maddison, W.P., 1997. Gene trees in species trees. Syst. Biol. 46, 523–536.

Maddison, W.P., Knowles, L., 2006. Inferring phylogeny despite incomplete lineage sorting. Syst. Biol. 55, 21–30.

Maddison, W.P., Maddison, D.R., 2000. MacClade: analysis of phylogeny and character evolution, version 3.0. Sinauer, Sunderland, Massachusetts.

Mendlelson, T.C., Shaw, K.L., 2002. Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis*. Genetica 116, 301–310.

Meyer, A., 1993. Evolution of mitochondrial DNA in fishes. In: Hachachka, P.W., Mommsen, T.P. (Eds.), Molecular Biology Frontiers: Biochecmistry and Molecular Biology of Fishes, vol. 2. Elsevier Science Publishers, Amsterdam, Netherlands. Chapter 1.

Montoya-Burgos, J.I., 2003. Historical biogeography of the catfish genus *Hypostomus*, with implications on the diversification of Neotropical ichthyofauna. Mol. Ecol. 12, 1855–1867.

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

Moritz, C., Patton, J.L., Schneider, C.J., Smith, T.B., 2000. Diversification of rainforest faunas: an integrated molecular approach. Annu. Rev. Ecol. Syst. 31, 533–563.

Moyer, G.R., Burr, B.M., Krajewski, C., 2004. Phylogenetic relationships of thorny catfishes (Siluriformes: Doradidae) inferred from molecular and morphological data. Zool. J. Lin. Soc. 140, 551–575.

Müller, K., 2004. PRAP - computation of Bremer support for large data sets. Mol. Phylogenet. Evol. 31, 780–782.

Nei, M., 1986. Stochastic errors in DNA evolution and molecular phylogeny. In: Gershowitz, H., Rucknagel, D.L., Tashian, R.E. (Eds.), Evolutionary Perspectives and the New Genetics. Allan R. Liss, New York, pp. 133–147.

Neigel, J.E., Avise, J.C., 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In: Nevo, E., Karlin, S. (Eds.), Evolutionary Processes and Theory. Academic Press, New York, pp. 515–534.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.
Ogden, T., Rosenberg, M., 2006. Multiple sequence alignment accuracy and phylogenetic inference. Syst. Biol. 55, 314–328.
Olmstead, R.G., 1995. Species concepts and plesiomorphic species. Syst. Bot. 20, 623–630.
Orti, G., Meyer, A., 1997. The radiation of characiform fishes and the limits of resolution of mitochondrial ribosomal DNA sequences. Syst. Biol. 46, 75–100.
Paine, R.T., 1966. Food web complexity and species diversity. Am. Nat. 100, 65–75.
Palumbi, S.R., 1996. Nucleic acid II: the polymerase chain reaction. In: Hillis, D.M., Moritz, G., Mable, B.K. (Eds.), Molecular Systematics. Sinauer Associates, Sunderland, MA, pp. 205–247.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Reis, R.E., Kullander, S.O., Ferraris, C.J., 2003. Checklist of the freshwater fishes of South America. EDIPUCRS, Porto Alegre, Brazil.
Renno, J.-F., Hubert, N., Torrico, J.P., Duponchelle, F., Nunez Robriguez, J., Garcia Davila, C., Willis, S.C., Desmarais, E., 2006. Phylogeography of peacock bass *Cichla monoculus* in the upper Madeira (Amazon, Bolivia): evidence of incipient speciation. Mol. Phylogenet. Evol. 41, 503–510.
Říčan, O., Kullander, S.O., 2006. Character- and tree-based delimination of species in the *'Cichlasoma' facetum* group (Teleostei, Cichlidae) with the description of a new genus. J. Zool. Syst. 44, 136–152.
Schaefer, S.A., 1997. The Neotropical cascudinhos: systematics and biogeography of the *Otocinclus* catfishes (Siluriformes: Loricariidae). Proc. Acad. Nat. Sci., Philadelphia 148, 1–120.
Seehausen, O., 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19, 198–207.
Seehausen, O., Van Alphen, J.J.M., Witte, F., 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science 277, 1808–1811.
Seutin, G., White, B.N., Boag, P.T., 1991. Preservation of avian blood and tissue samples for DNA analyses. Can. J. Zool. 69, 82–90.
Shafland, P.L., 1993. An overview of Florida's introduced butterfly peacock bass (*Cichla ocellaris*) sportfishery. Natura 96, 26–29.
Sikes, D.S., Lewis, P.O., 2001. PAUPRat: A tool to implement parsimony and likelihood ratchet searches using PAUP*. Univ. Calgary, Calgary, Canada.
Sioli, H., 1984. The Amazon and its main affluents: hydrography, morphology of the river courses, and river types. In: Sioli, H. (Ed.), The Amazon. In: Monographie Biologicae, vol. 56. Dr. W. Junk Publishers, The Hague, Netherlands.
Sites Jr., S.W., Marshall, J.C., 2003. Delimiting species: a renaissance issue in systematic biology. Trends Ecol. Evol. 18, 462–470.
Sites Jr., S.W., Marshall, J.C., 2004. Operational criteria for delimiting species. Ann. Rev. Ecol. Evol. Syst. 35, 199–227.
Sivasundar, A., Bermingham, E., Orti, G., 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*) in major South American rivers. Mol. Ecol. 10, 407–417.
Smith, G.R., 1992. Introgression in fishes: Significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41, 41–57.
Smith, S.A., Bermingham, E., 2005. The biogeography of lower Mesoamerican freshwater fishes. J. Biogeography 32, 1835–1854.
Sorenson, M.D., 1996. TreeRot. Univ. Michigan, Ann Arbor, MI.
Stiassny, M.L.J., 1987. Cichlid familial intrarelationships and the placement of the Neotropical genus *Cichla* (Perciformes: Labroidei). J. Nat. Hist. 21, 1311–1331.
Sullivan, J.P., 2002. Discovery and phylogenetic analysis of a riverine species flock of African electric fishes (Mormyridae: Teleostei). Evolution 56, 597–616.
Swofford, D.L., 2000. PAUP* Phylogenetic Analysis Using Parsimony (* and Other Methods). Sinauer, Sunnderland, MA.
Teixeira, A.S., de Oliveira, S.D., 2005. Evidence for a natural hybrid of peacock bass (*Cichla monoculus* vs *Cichla temensis*) based on esterase electrophoretic patterns. Genet. Mol. Res. 4, 74–83.
Templeton, A.R., 1989. The meaning of species and speciation: a genetic perspective. In: Otte, D., Endler, J.A. (Eds.), Speciation and Its Consequences. Sinauer Associates, Sunderland, MA, pp. 3–27.
Templeton, A.R., 2001. Using phylogeographic analyses of gene trees to test species status and processes. Mol. Ecol. 10, 779–791.
Thompson, K.W., 1979. Cytotaxonomy of 41 species of Neotropical Cichlidae. Copeia 1979, 679–691.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876.
Turner, T.F., McPhee, M.V., Campbell, P., Winemiller, K.O., 2004. Phylogeography and intraspecific genetic variation of prochilodontid fishes endemic to rivers of northern South America. J. Fish Biol. 64, 186–201.
Vari, R.P., 1984. Systematics of the Neotropical characiform genus *Potamorhina* (Pisces: Characiformes). Smit. Contr. Zool. 400, 1–36.
Vari, R.P., 1988. The Curimatidae, a lowland Neotropical fish family (Pisces: Characiformes): distribution, endemism, and phylogenetic biogeography. In: Heyer, W.R., Vanzolini, P.E. (Eds.), Proceedings of a Workshop on Neotropical Distribution Patterns. Academia Brasileira de Ciências, Rio de Janeiro, Brazil, pp. 343–377.
Vari, R.P., 1989a. Systematics of the Neotropical characiform genus *Curimata* Bosc (Pisces, Characiformes). Smit. Contr. Zool. 474, 1–63.
Vari, R.P., 1989b. Systematics of the Neotropical characiform genus *Psectrogaster* Eigenmann and Eigenmann (Pisces, Characiformes). Smit. Contr. Zool. 481, 1–43.
Vari, R.P., 1991. Systematics of the Neotropical characiform genus *Steindachnerina* Fowler (Pisces, Characiformes). Smit. Contr. Zool. 507, 1–118.
Vari, R.P., 1992. Systematics of the Neotropical characiform genus *Cyphocharax* Fowler (Pisces, Characiformes). Smit. Contr. Zool. 529, 1–137.
Vari, R.P., 1995. The Neotropical characiform fish family Ctenoluciidae (Teleostei: Ostariophysi): supra and intrafamilial phylogenetic relationships, with a revisionary study. Smit. Contr. Zool. 564, 1–97.
Verheyen, E., Salzburger, W., Snoeks, J., Meyer, A., 2003. Origin of the superflock of cichlid fishes from Lake Victoria, east Africa. Science 300, 325–329.
Weins, J.J., Penkrot, T.A., 2002. Delimiting species using DNA and morphological variation and discordant limits in spiny lizards (*Sceloporus*). Syst. Biol. 51, 69–91.
Weitzman, S.H., Weitzman, M.J., 1982. Biogeography and evolutiona7ry diversification in Neotropical freshwater fishes, with comments on the refuge theory. In: Prance, G.T. (Ed.), Biological Diversification in the Tropics. Columbia University Press, New York, pp. 403–422.
Winemiller, K.O., 2001. Ecology of peacock cichlids (*Cichla* spp.) in Venezuela. J. Aquaric. Aquat. Sci.: Cichlid Research: State of the Art IX, 93–112.
Winemiller, K.O., Jepsen, D.B., 1998. Effects of seasonality and fish movement on tropical river food webs. J. Fish Biol. 53, 1–30.
Winemiller, K.O., Taphorn, D.C., Barbarino-Duque, A., 1997. The ecology of *Cichla* (Cichlidae) in two blackwater rivers of southern Venezuela. Copeia 1997, 690–696.
Wright, S., 1931. Evolution in Mendelian populations. Genetics 16, 97–159.
Zaret, T.M., Paine, R.T., 1973. Species introduction in a tropical lake. Science 182, 449–455.