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RESEARCH ARTICLE

Population genetics of the speckled peacock bass (*Cichla temensis*), South America's most important inland sport fishery

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Abstract The Neotropics harbor the world's most diverse freshwater fish fauna, with many of these species supporting major commercial, subsistence, or sport fisheries. Knowledge of population genetic structure is available for very few Neotropical fishes, thereby restricting management. To address this need, we examined population genetic variation in mtDNA control region sequences and twelve microsatellite loci in the speckled or barred peacock bass, *Cichla temensis*. Moderate and statistically significant genetic divergence among localities indicates that migration is low in this species, implying that populations inhabiting tributaries or even smaller spatial units should constitute management units. Analysis of molecular variance of mtDNA sequences identified six areas with largely exclusive haplotype clades, and a seventh area of high admixture, but major drainage basins harbored non-monophyletic haplotype groups. On the other hand, molecular variation in the microsatellite data was best explained by drainage basin and, subsequently, by the seven areas. Populations in these seven areas could be considered evolutionarily significant units (ESUs), and, therefore, we tested hypotheses explaining the discordant signal of mtDNA and microsatellite data using approximate Bayesian computation. This analysis indicated that the divergence of mtDNA clades preceded the divergence of contemporary ESUs across basins, with subsequent lineage sorting among ESUs due to reduced gene flow. Available genetic and ecological information indicates that *C. temensis* populations of major tributary rivers should be managed as separate stocks that likely are adapted to local environmental conditions.

Keywords Amazon · Fish stock · Evolutionary significant units · Lineage sorting · Management unit

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Introduction

The tropical regions of Central and South America (Neotropics) host the largest assemblage of freshwater fishes on earth, estimated at nearly 5000 species, approximately 10 % of vertebrate diversity (Reis et al. 2003). Despite the importance of fisheries in the Amazon and other inland regions of South America, there has been scant

Table 1 Sample sizes and diversity statistics for mitochondrial control region and twelve microsatellites for each sampling locality of *Cichla temensis*

		mtCR				Microsatellites		
		N	π	θ	$\hat{H}$	N	H_{obs}	H_{exp}
Sipao	SI	10	0.0093	0.0090	0.69	10	0.242	0.260
Cunavichito	CN	1	–	–	–	1	–	–
Capanaparo	CP	10	0.0016	0.0006	0.71	10	0.258	0.308
Cinaruco	CI	12	0.0013	0.0006	0.62	26	0.314	0.303
Parguaza	PZ	2	0	0	0	2	–	–
Atabapo	AT	10	0.0006	0.0006	0.68	10	0.350	0.381
Ventuari	VE	9	0	0	0	9	0.370	0.375
Iguapo	IG	1	–	–	–	1	–	–
Curamoni	CR	4	0.0109	0.0119	0.83	4	0.250	0.326
Pasiba	PS	10	0.0096	0.0099	0.73	10	0.333	0.362
Uaupés	UA	11	0	0	0	10	0.350	0.394
Marauiá	MR	9	0.0054	0.0035	0.42	10	0.207	0.657
Teá	TE	1	–	–	–	1	–	–
Uneiuxi	UE	10	0.0058	0.0076	0.64	7	0.619	0.608
Preto	PT	1	–	–	–	1	–	–
Barcelos	BC	7	0.0010	0.0015	0.52	7	0.619	0.590
Pirara	PI	5	0	0	0	5	0.183	0.292
Xeriuini	XE	9	0	0	0	9	0.481	0.501
Tapera	TP	13	0.0021	0.0023	0.78	10	0.458	0.480
Unini	UN	15	0.0005	0.0011	0.26	10	0.567	0.570
Novo Airão	NA	10	0.0006	0.0004	0.82	9	0.566	0.595
Preta da Eva	PE	4	0.0009	0.0010	0.50	4	0.431	0.431
Urubu	UR	3	0	0	0	3	0.542	0.586
Igapo-Açu	IA	10	0.0010	0.0006	0.53	10	0.383	0.482

Codes for localities and species names are used in Fig. 1. *N* sample size, π Nei and Li's (1979) nucleotide diversity, θ Watterson's (1975) nucleotide diversity, $\hat{H}$ haplotype diversity, H_{obs} observed heterozygosity, H_{exp} expected heterozygosity

research on population genetics of Neotropical fishes (Hrbek et al. 2014). Identification of population structure is critical for delineation of management units, or stocks, i.e., groups of individuals with independent demographic processes and/or unique adaptive genetic variation (Hillborn et al. 2003; Funk et al. 2012). Overexploitation of stocks erodes fishery sustainability and can threaten species persistence, especially when there are additional impacts. Lack of effective management has led to the decline of a number of Neotropical fish stocks, the most notable being the pirarucu, *Arapaima gigas*, one of the world's largest and most unique freshwater fishes (Hrbek et al. 2005). Although some progress has been made in elucidating the range-wide population structure of a few highly targeted Amazonian species (e.g. Batista and Alves-Gomes 2006; Pereira et al. 2009; Farias et al. 2010; Amado et al. 2011), almost nothing is known about the population structure of literally hundreds of exploited Neotropical fishes.

An ecologically and economically important group of Amazonian fishes are the *tucunaré* or peacock basses, genus *Cichla* (Bloch and Schneider 1801). These large, diurnal predators in the family Cichlidae superficially resemble "basses" of freshwater (Centrarchidae) and marine families (Serranidae). The largest of these is *Cichla temensis* (von Humboldt 1821), known as the 'speckled' (*tucunaré paca*, *pavón lapa*) or 'barred' (*tucunaré açu*, *pavón cinchado*) peacock bass. With anecdotal reports of individuals over one meter in length and more than 13 kg in weight, *C. temensis* is likely to be the largest member of the family Cichlidae (Winemiller 2001; Bailey 2011). The two sets of common names reflect color polymorphism that caused taxonomic confusion for fishery managers, but that is now recognized as being associated with maturation and reproductive and non-reproductive states (Winemiller 2001; Reiss et al. 2012). Following extensive molecular analysis of the genus (Willis et al. 2012), *C. temensis* was found to be a clearly morphologically and genetically circumscribed species. Tagging studies conducted on this species indicate relatively low vagility (Hoeinghaus et al. 2003). *Cichla temensis* spawns several thousand eggs once

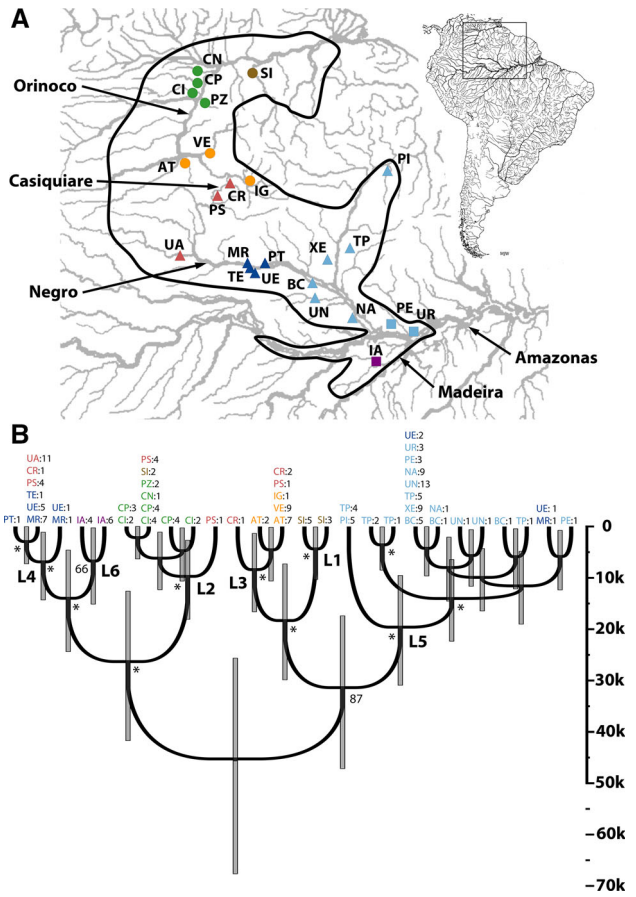

Fig. 1 Sampling Localities and MtCR Genealogy of *Cichla temensis* **a** Map of sampling localitiesin northern South America. Rivers referred to by the text are labeled. Codes for localities follow Table 1. Symbols refer to drainage sub-basin (Orinoco, Negro, Amazonas), while *colors* refer to putative ESUs (see text) **b** Time-calibrated genealogy of mtCR haplotypes from BEAST. Localities and sample sizes are plotted for each haplotype, and *colors* correspond to the putative ESUs in panel (**a**). Haplotype clades referenced in the text are labeled L1–L6. Asterisks indicated posterior clade probability >0.95, and additional nodes >0.5 are labeled with the corresponding value. *Bars* on each node correspond to the 95 % highest posterior density of divergence time

per year into nests followed by biparental care of eggs, larvae and juveniles (Winemiller 2001). *Cichla* in general, and *C. temensis* in particular, have been identified as playing key roles in food webs of freshwater ecosystems in the Neotropics (Zaret and Paine 1973; Winemiller and Jepsen 1998; Layman and Winemiller 2004). Because of their large size, abundance, and quality of their flesh, these fishes are strongly targeted by subsistence and commercial fishermen throughout their range. In addition, because peacock bass are diurnal predators that aggressively attack artificial baits, there are important recreational fisheries for these species throughout South America as well as other regions of the world where they have been introduced. Given its life history and other ecological attributes, *C. temensis* may be vulnerable to overexploitation (Winemiller 2001), with significant trophic cascades in local ecosystems (Layman and Winemiller 2004). Management strategies of peacock bass fisheries are highly variable and range from bans on "non-subsistence" or "non-indigenous" fishing, to seasonal closure during the reproductive season, and catch limits. Enforcement of fishing regulations varies by country and area, and fisheries near moderately populated areas are, with few exceptions, overexploited (Willis, pers. obs.; see also Winemiller 2001; Holley et al. 2008).

Cichla temensis is distributed within the Amazonas and Orinoco river basins, and these are connected by the Casiquiare River, a tributary of the upper Negro River (an Amazonas tributary) that captures water from the upper Orinoco (Fig. 1). Although the Casiquiare provides a corridor for fish migration between drainages, analyses of fish assemblages determined that this natural waterway functions more like a zoogeographic filter, with many species apparently unable to tolerate environmental conditions on one or the other side of the longitudinal gradient (Winemiller et al. 2008; Winemiller and Willis 2010). Part of the mitochondrial control region (mtCR) dataset used in the current study was previously used to ask whether the Casiquiare River facilitated recent migration and/or historical dispersal for three species of peacock bass, including *C. temensis* (Willis et al. 2010). Conspecifics within each drainage had a non-monophyletic assemblage of mtCR haplotypes, although subsets of localities did show largely exclusive groups of related haplotypes. Whereas coalescent analyses further implied a history of gene flow via the Casiquiare, strict interpretation of the mtCR haplotype distribution under an assumption of migration-drift equilibrium would suggest very complicated gene exchange dynamics for this species. However, it has been noted that while mtDNA has the advantage of having an accelerated rate of coalescence coupled with high mutation rates, at best it only provides a single sample of the distribution of coalescent patterns resulting from a species' demographic history. Moreover, due to its maternal inheritance mode, it may be subject to artifacts of sex-biased gene flow or selection (Ballard and Whitlock 2004; Galtier et al. 2009). Thus, inferences on population structure from mtDNA alone must be made with caution.

Here we combine intraspecific analyses of an expanded mtCR dataset with genotypes of twelve nuclear microsatellite loci generated for our previous analysis of species boundaries in this genus (Willis et al. 2012) to delineate population genetic structure of *C. temensis* throughout the native range. We find that although the patterns of population structuring provided by microsatellite and mtDNA data do not appear consistent upon first inspection, further analysis suggests that

implied contemporary and historical processes provide complementary interpretations. Using this combined dataset, we identify evolutionarily significant units and discuss their relevance for fisheries management.

Methods

Dataset retrieval

New analyses were performed on a subset of a dataset previously generated to infer species boundaries across the genus *Cichla*; information on molecular techniques used to collect those data can be found in Willis et al. (2012). The mitochondrial control region or D-loop (mtCR) dataset is available from Genbank (DQ841909-DQ841929, GU295 739-GU295740, JQ926775-JQ926782), and the microsatellite matrix is stored with Dryad (http://dx.doi.org/10.5061/dryad.h4s73s5c). Sequences and genotypes of *C. temensis* from the Guri Reservoir in Venezuela, which were determined to be hybrids with *C. orinocensis*, were excluded from this dataset (although all mitochondrial haplotypes from these fishes matched the most common haplotype from the nearby Sipao locality). Sample sites (Fig. 1; Table 1) include localities throughout the distribution of *C. temensis* in the Orinoco Drainage, Negro Drainage, and several Amazonas sites peripheral to the mouth of the Negro River. *Cichla temensis* are also reported patchily moving west along the Amazonas (Solimões) River until approximately Tefé, although samples were unavailable for the current analysis. Our sampling strategy focused on geographic diversity at the expense of sample size, although, in aggregate, our population sample was adequate to resolve a robust pattern of population structure (Kalinowski 2002, 2005). Voucher information is available in our previous study (Willis et al. 2012).

MtDNA sequence analysis

The alignment of *C. temensis* mtCR sequences was made with the L-INS-i algorithm of MAFFT (Katoh et al. 2005). Unique haplotypes were confirmed from our previous study with DNAsp (Rozas et al. 2003) (considering gaps as a fifth state), which was also used to calculate molecular diversity at each locality. Tests of selective neutrality or historical changes in population size were estimated for each locality using Tajima's *D* (1989) and Fu's *Fs* (1997) statistics in ARLEQUIN (Excoffier and Lischer 2010). Significance was assessed using 10,000 permutations.

To visualize the phylogeographic pattern of genetic diversity, we inferred a genealogy of unique mtCR haplotypes using BEAST 1.7.5 (Drummond and Rambaut 2007) using 10 million generations and the coalescent (constant size) prior, with 3 replicate runs summarized together post-burn-in. The mutation model for the mtCR sequences, Tamura-Nei + gamma, was chosen using MrModeltest (Nylander 2004). This tree was time calibrated using mtCR mutation rates estimated from African cichlids (Genner et al. 2007). Because it has been recognized that substitution rates appear to decline with the age of the sequence divergences involved, a pattern ascribed to selection or saturation of mutational hotspots (Ho et al. 2007), time calibrations and conversions of demographic estimates (above) were made using the average of rates from Genner et al. (2007) up to ~0.1 million years ago. Localities and number of observations were mapped onto each haplotype on this phylogeny.

Using the mtCR sequences, we made a hierarchical analysis of molecular variance (AMOVA) to compare which division of localities described the greatest amount of genetic variation and, therefore, best described potential evolutionarily significant units of *C. temensis* (Excoffier et al. 1992). Groups of localities that were tested included (1) the two river drainages, Amazonas vs. Orinoco, (2) three groups that represented the two river drainages but with geographically intermediate upper Negro separated (CR, PS, UA in Fig. 1), and (3) seven groups corresponding to areas with largely exclusive sets of closely related haplotypes. Each test was made using ARLEQUIN with significance assessed with 10,000 permutations.

Microsatellite analyses

The dataset with genotypes of twelve microsatellite loci, developed by us for our previous study (Macrander et al. 2012), was reduced to a *C. temensis*-only dataset. Observed and expected heterozygosity for each locality was calculated with GENODIVE 2.0b25 (Meirmans and Van Tienderen 2004). Tests of consistency with Hardy–Weinberg equilibrium and linkage equilibrium in each locality were made with GENEPOP (Raymond and Rousset 1995). Tests for null alleles (excess homozygosity) were made with MICRO-CHECKER (van Oosterhout et al. 2004). To estimate population level differentiation, a multi-locus AMOVA-based F_{ST} (Excoffier et al. 1992) was calculated using GENODIVE. The significance of F_{ST} was assessed using 10,000 permutations. To test whether *C. temensis* is constrained by a pattern of isolation by distance (IBD) among localities, we performed a Mantel test (Mantel 1967) of F_{ST} versus approximate river distance between localities using GENODIVE, the significance of which was assessed with 1000 permutations. We subsequently analyzed subsets of the data to see which portion and scale of the geographic distribution contributed the most to the pattern of IBD.

In order to assess how well localities could be treated as independent units, or conversely how much demographic exchange (migration) occurs between localities, based on how well individuals conformed to the allele frequencies observed at their locality of capture, we performed population assignment using the program STRUCTURE (Pritchard et al. 2000) to determine the posterior probability of an individual's multi-locus genotype originating in its locality of capture under admixture. Unlike the all-or-nothing designation of traditional population assignment, STRUCTURE estimates what proportion of a multi-locus genotype is derived from any of the included localities. This analysis was run for 100,000 generations after 100,000 of burn-in, with a default migration prior of 0.05 K, the number of clusters, equaled the number of sampling localities. We also performed STRUCTURE analyses in order to estimate the number of evolutionary significant units (ESUs), groups of localities which show a higher degree of gene exchange among themselves than with localities in other such groups, and are thus expected to experience more independent migration-drift-selection evolutionary dynamics and harbor unique and important phenotypic and genetic variation (Waples 1991; Funk et al. 2012). First, we performed analyses without individuals pre-assigned to a population. STRUCTURE attempts to match individuals to clusters that best correspond to a model of Hardy–Weinberg and linkage equilibrium, a model that implies a high degree of gene flow within clusters but lower or no gene flow between clusters. With STRUCTURE we made 10 runs of the program with each K (number of clusters) from 1 to 10. We used the second order rate of change between runs of different K (ΔK) to estimate the optimal clusterings, K* (Evanno et al. 2005). We ran the program with the *r* (locality) prior implemented (Hubisz et al. 2009). Posterior values of this prior between 0 and 1 indicate that locality data are informative for clustering, whereas values above 1 indicate that they are not. We made these runs with an initial value of *r* at 1 and an upper limit of 100. We ran each replicate for 100,000 generations after the same number for burn-in. Evanno et al. (2005) found that their metric, ΔK, identified the optimal clusters at the highest hierarchical level in the data; inferring subsequent structure required dividing the original dataset. Thus after this initial series of runs, we divided the data according to the clusters and made another series of runs as above with K from 1 to $N + 1$, where *N* equaled the number of localities in that cluster. For each optimal K for each series of runs, we averaged the posterior probability of individual assignment across all runs using CLUMPP (Jakobsson and Rosenberg 2007). We assessed the percent of genetic variance explained by the groupings from the STRUCTURE analyses using AMOVAs in ARLEQUIN (see above).

We tested alternative demographic and biogeographic models for *C. temensis* to reconcile the mtCR and microsatellite data. In this analysis, data from localities within each ESU were pooled into a single population. We used only data from the nine loci that conformed to a stepwise mutation model. We used approximate Bayesian computation (ABC) (Beaumont et al. 2002), implemented in the program DIY-ABC (Cornuet et al. 2014), to test the topological pattern of divergences between ESUs distributed in the two drainage basins. ABC bypasses the difficulty of estimating model probabilities after calculating data likelihoods by instead calculating vectors of population genetic summary statistics from prior distributions of model parameters. These multiple summary statistics can then be compared to statistics from observed data by calculating the Euclidean distance (Beaumont et al. 2002). The superiority of alternative models can be determined by comparing the distance between simulated vectors from different priors to the observed statistic, because the closest *N* vectors are expected to approximate the posterior via a partial linear regression. DIY-ABC models population size, population divergence times, and mutation; however, it only accommodates gene exchange (migration) by designating individual populations as 'admixed' and modeling admixture proportions. Three models were constructed with identical, wide priors and nearly identical inequality constraints (e.g. $t0 < t1$) on divergence times (see Supplemental Information). Each ESU was specified as a terminal (sampled) unit connected by different population 'phylogenies', but in each construction, the upper Negro ESU was specified as derived from admixture of the upper Orinoco and middle Negro ESUs (see Results). No other populations were admixed. ESU population trees were arranged that corresponded to three models: (1) the mtCR genealogy, (2) chord distance calculated from microsatellite data, and (3) a hybrid model in which ESUs across basins were monophyletic following the microsatellite chord distances but within basins were related as in the mtCR genealogy (Supplemental Fig. 1). We made two separate analytical constructions where two of the candidate models were tested: model 1 versus model 2, and model 2 versus model 3. Additional information about analysis construction and run conditions for

Table 2 Percent variation in AMOVA of the mitochondrial control region and microsatellites of *Cichla temensis*

# Groups	mtCR			Micosatellites		
	2	3	7	2	3	7
Among groups	26.76	31.54	77.58	18.3	20.39	15.63
Within groups	57.12	52.07	5.98	12.96	9.09	10.63
Within localities	16.11	16.38	16.44	68.74	70.52	73.74

MIGRATE-N and DIYABC are available as Supplemental Information.

Results

Mitochondrial DNA

A 550 base pair alignment of mtCR was arranged for 179 individuals of *C. temensis*, in which there were 32 variable positions (21 parsimony informative; nucleotide diversity, π, on a per site basis, of 0.01334), and which exhibited 26 unique sequences among individuals (haplotypes, including gaps). Nei and Li's (1979) estimator of diversity (π,) at localities for which we had more than one sample ranged from 0 to 0.109, Watterson's (1975) estimator of diversity (θ) ranged from 0 to 0.0119, and haplotype diversity ranged from 0 to 0.83 (Table 1). Statistics of neutrality or demographic change (Tajima's *D* and Fu's *Fs*) were marginally significant for a few localities, but not after correction for multiple tests.

The average of recent mutation rates in some African cichlids was ~0.2178 changes/site/million years (Genner et al. 2007), which places the root age of the genealogy inferred with BEAST (Fig. 1) at ~45.2 thousand years ago (kya). The rooting of this tree between lineages L2–L4–L6 and L1–L3–L5 by relaxed molecular clock was consistent with previous outgroup-based roots using mtDNA from other species of *Cichla* (Willis et al. 2012). When the distribution of each locality was mapped onto the genealogy, it reflected previously observed patterns but with more detail. There were haplotype clades (labeled L1–L6 in Fig. 1) that were largely exclusive to (L1) the lower Orinoco (SI), (L2) the middle Orinoco (CN, CP, CI, PZ), (L3) upper Orinoco (AT, VE, IG), (L4) the middle Negro (MR, PT, UE, TE), (L5) the lower Negro and northern peripheral drainages (BC, UN, PI, TP, XE, NA, PE, UR), and (L6) the lower Madeira (IA). Importantly, the closest relationship between clades (L1–L6) was often not between clades in adjacent localities or even in the same drainage basin. There were also several sites with haplotypes from two or more clades, including SI (with haplotypes from L1 to L2) in the lower Orinoco, CR (with haplotypes from L3 to L4) and PS (with haplotypes from L2, L3 and L4) in the upper Negro, and UE (with haplotypes from L4 to L5) and MR (with haplotypes from L4 to L5) in the middle Negro. Notably, the localities with the highest number of haplotype groups (Fig. 1) and nucleotide diversity (Table 1) were the Pasiba (PS) and Curamoni (CR), localities that lie in the geographic corridor between the two drainages (the Casiquiare). In agreement with this distribution of genetic diversity, AMOVA analyses with localities grouped by drainage (2 groups: Orinoco vs. Amazonas) or with the upper Negro as a third group (3 groups: Orinoco, Amazonas, upper Negro), while significant (F_{CT} $p < 0.001$), explained less of the total genetic variance than a division into seven groups according to the distribution of the mtCR haplotype clades in six areas (lower Orinoco, middle Orinoco, upper Orinoco, middle Negro, lower Negro + northern peripherals, and lower Madeira) plus a zone of high diversity (upper Negro) (Table 2). This geographic structure was also apparent in a network of unique haplotypes (Supplemental Fig. 2). Within each of these seven areas, there was significant haplotype sharing among localities. These population groupings were designated as putative (ESUs) based on their geographical and genetic disjunction.

Table 3 Microsatelliteallelic diversity and size range for *Cichla temensis*

Locus	Alleles	Size range	H_{obs}	H_{exp}
A6	3	267–271	0.198	0.177
B3	17	201–241	0.512	0.578
D12	5	152–162	0.289	0.277
E3	2	276–278	0.132	0.176
Cin22	10	147–169	0.207	0.267
D2	20	285–323	0.617	0.702
G4	8	292–316	0.175	0.213
F12	20	260–304	0.747	0.814
B6.2	13	286–314	0.372	0.485
SM2	17	238–278	0.591	0.578
C11	11	219–243	0.486	0.569
C1	3	225–229	0.268	0.316

H_{obs}: observed heterozygosity, H_{exp} expected heterozygosity. For additional information, see Macrander et al. (2012)

Microsatellites

Genotypes from 12 microsatellites were arranged for 174 individuals of *C. temensis*, with 0.57 % missing data. The number of alleles per locus ranged from 2 (E3) to 20 (D2) (Table 3). There was no evidence of linkage disequilibrium or deviation from Hardy–Weinberg expectations in individual localities after correction for multiple tests (Benjamini and Hochberg 1995). Heterozygosity was moderate at most sites for which there were 3 or more samples, and generally similar to expectations (Tables 1, 3). However, MICROCHECKER suggested there was significant excess homozygosity at Igapo-Acu for locus G4 and at Pirara and Uaupes for locus F12, and that null alleles may be present. At each of these localities, samples were pooled from several sub-sites (e.g. creeks or small tributaries), indicating that local sub-structure and small sample size may have contributed to this pattern. As there was no other evidence

Table 4 Pairwise F_{ST} (AMOVA-based) among localities of *Cichla temensis*

	SI	CN	CP	CN	PZ	AT	VE	IG	CR	PS	UA	MR	TE	UE	PT	BC	PI	XE	TP	UN	NA	PE	UR	IA
N	10	1	10	26	2	10	9	1	4	10	10	7	1	5	1	7	5	9	10	10	9	4	3	10
CN	na	–																						
CP	**0.101**	na	–																					
CI	**0.100**	na	**0.037**	–																				
PZ	0.141	na	0.059	0.046	–																			
AT	**0.100**	na	**0.111**	**0.120**	0.033	–																		
VE	**0.193**	na	**0.159**	**0.191**	**0.182**	**0.115**	–																	
IG	na	na	na	na	na	na	na	–																
CR	**0.377**	na	**0.357**	**0.339**	0.360	**0.256**	**0.208**	na	–															
PS	**0.289**	na	**0.287**	**0.276**	**0.273**	**0.205**	**0.172**	na	−0.006	–														
UA	**0.367**	na	**0.385**	**0.387**	**0.333**	**0.264**	**0.296**	na	**0.197**	**0.172**	–													
MR	**0.322**	na	**0.300**	**0.322**	**0.215**	**0.225**	**0.236**	na	**0.140**	**0.152**	**0.130**	–												
TE	na	na	na	na	na	na	na	na	na	na	na	na	–											
UE	**0.381**	na	**0.361**	**0.390**	0.261	**0.275**	**0.258**	na	**0.156**	**0.182**	**0.153**	**0.070**	na	–										
PT	na	na	na	na	na	na	na	na	na	na	na	na	na	na	–									
BC	**0.339**	na	**0.328**	**0.353**	**0.238**	**0.237**	**0.267**	na	**0.188**	**0.181**	**0.133**	**0.037**	na	**0.090**	na	–								
PI	**0.503**	na	**0.503**	**0.479**	**0.507**	**0.421**	**0.459**	na	**0.452**	**0.397**	**0.390**	**0.261**	na	**0.279**	na	**0.235**	–							
XE	**0.346**	na	**0.354**	**0.367**	**0.289**	**0.283**	**0.323**	na	**0.291**	**0.249**	**0.239**	**0.093**	na	**0.162**	na	**0.064**	**0.166**	–						
TP	**0.355**	na	**0.377**	**0.381**	**0.310**	**0.311**	**0.351**	na	**0.299**	**0.273**	**0.264**	**0.113**	na	**0.175**	na	**0.096**	**0.123**	**0.042**	–					
UN	**0.290**	na	**0.293**	**0.319**	**0.220**	**0.209**	**0.220**	na	**0.197**	**0.198**	**0.180**	**0.061**	na	**0.109**	na	**0.071**	**0.266**	**0.104**	**0.130**	–				
NA	**0.289**	na	**0.281**	**0.313**	**0.200**	**0.210**	**0.216**	na	**0.159**	**0.145**	**0.154**	**0.043**	na	**0.066**	na	0.016	**0.193**	**0.058**	**0.078**	**0.043**	–			
PE	**0.450**	na	**0.398**	**0.407**	0.369	**0.312**	**0.304**	na	**0.179**	**0.198**	**0.186**	**0.083**	na	**0.106**	na	**0.140**	**0.382**	**0.198**	**0.236**	**0.140**	**0.100**	–		
UR	**0.379**	na	**0.329**	**0.356**	0.249	**0.254**	**0.296**	na	**0.270**	**0.249**	**0.261**	**0.086**	na	**0.097**	na	**0.078**	**0.280**	**0.107**	**0.106**	**0.056**	0.045	**0.130**	–	
IA	**0.346**	na	**0.342**	**0.357**	**0.255**	**0.252**	**0.257**	na	**0.199**	**0.203**	**0.120**	**0.095**	na	**0.113**	na	**0.121**	**0.202**	**0.188**	**0.151**	**0.140**	**0.095**	**0.270**	**0.260**	–

Statistically significant permutations are indicated by bold font. Locality codes follow Table 1

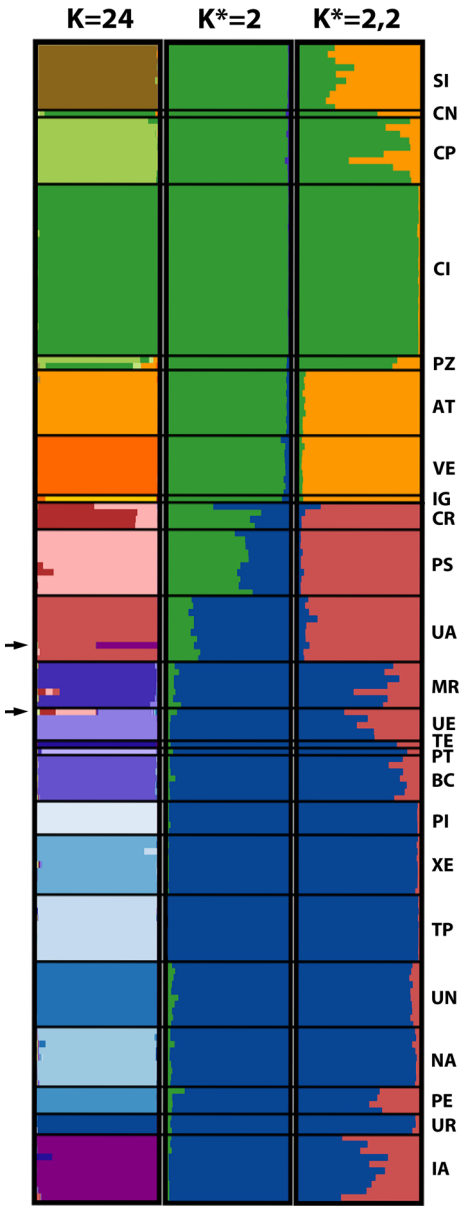

Fig. 2 Results from STRUCTURE analysis and population assignment of microsatellite data from *Cichla temensis*. Locality codes follow Table 1. The first column represents individual posterior probability/admixture proportions of individuals to their sampled localities (USEPOPINFO = 1). To the left of this column, two putative migrants identified by population assignment tests are indicated with arrows. The second column shows an analysis with only locality data (r prior). The third column represents analyses of the Orinoco (SI through IG) and Amazonas (CR through IA) data separately. Above each column are the K values (no. clusters) corresponding to those results

of null-alleles at other localities, and it did not affect overall results (not shown), we proceeded without correcting for potential null alleles.

Pairwise F_{ST} values among localities indicated moderate genetic divergence (mean pairwise $F_{ST} = 0.22$ excluding sites $N = 1$), and the majority of localities with $N > 2$ were significant based on permutation and after correction for multiple tests (Table 4). Alternatives indices of population divergence, which capture complementary aspects of population divergence (Bird et al. 2011), showed similar patterns (Supplemental Information).Mantel tests in GENODIVE confirmed that there was a significant pattern of IBD across the range of *C. temensis* ($p = 0.001$). However, IBD tests were not significant in the Orinoco alone, or in the Negro when the upper Negro localities and Igapo-Açu were not included, indicating that IBD only occurs at the largest spatial scales in this species.

Population assignment with STRUCTURE identified two fish as having significantly admixed genetic profiles (Fig. 2). These same two fish were identified by traditional population assignment methods as being likely migrants (Supplemental Information). Otherwise, fish from localities from which more than 2 individuals were sampled were always assigned to their locality of sampling with high probability, consistent with the moderate F_{ST} values among localities (Table 4). This suggests that demographic exchange between localities on a per-generation basis is very low.

When we ran STRUCTURE without population of origin, the optimal number of clusters was 2, with these two clusters corresponding to the Orinoco versus the Amazonas, with a zone of admixture in the upper Negro (CR, PS, UA) (Fig. 2). Within the Orinoco cluster, there was an additional two optimal clusters, and these corresponded to the upper Orinoco (AT, VE, IG) versus middle Orinoco (CN, CP, CI, PZ), with the lower Orinoco (SI,) admixed between them. Within the Amazonas, two additional clusters corresponded to the upper Negro (CR, PS, UA) versus lower Negro and northern peripheral drainages (UR, PE), with the middle Negro (MR, TE, UE, PT) and Igapo-açu (IA) admixed between these two. In all of the STRUCTURE runs, the posterior value for r (locality prior) was less than one, indicating that locality information contributed to the posterior. A principal components analysis exhibited very similar structure (Supplemental Fig. 3).These divisions in the microsatellite data thus correspond well to the seven putative ESUs identified in the mtDNA data. However, in contrast to the mtCR data, the microsatellite data was better explained in AMOVA analyses by the drainage division (2 or 3 groups) than the 7 groups that best fit the mtDNA data (Table 2), though all divisions were significant (F_{CT} $p < 0.001$). We hypothesized that this reflected the phylogeographic structure of the nuclear data, that is, that ESUs within drainages are monophyletic, in contrast to the genealogy of the mtDNA.

To test whether the microsatellite data could be significantly better described by a model wherein the ESUs in each drainage are monophyletic, versus a model based on the mtDNA genealogy wherein ESUs are polyphyletic

between drainages, we used ABC. We used wide priors on model parameters (ancestral population sizes and divergence times), and as a result, vectors of population statistics from most parameter draws for each model were not close to the observed statistic (Supplemental Figs. 4a, 5a). However, posterior predictive analysis indicated that an adequate number of simulations (out of 20 million) were close enough to the observed statistic to reflect the posterior of each model (Supplemental Figs. 4b, c, 5b, c). In comparing the fit of the microsatellite data to a model where the ESUs in each drainage were monophyletic (Scenario 2) versus a model where they were polyphyletic (Scenario 1), the monophyletic model was clearly superior. The direct posterior probability from the closest 50–500 vectors ranged from 0.94 to 0.82, and the logistic regression approach also overwhelmingly supported the monophyletic model (PP: 1.0; Supplemental Fig. 6). The type 1 and type 2 error were estimated by simulation to both be $\sim$0.02. The superiority of this model suggests that the divergence of the two oldest clades in the mtCR tree precedes the divergence of the ESUs in each basin, and that subsequent lineage sorting resulted in differential retention of these clades in the respective ESUs.

However, the ABC analysis was less clear as to whether relationships among ESUs within basins were better reflected by the microsatellite chord distance (Scenario 2) or mtCR genealogy (Scenario 3). In contrast to the previous comparison, the posterior probabilities of these two models was roughly equivalent, with Scenario 2 only slightly preferred (direct 500 PP: 0.53; logistic 100,000 PP: 0.76; Supplemental Fig. 6). However, the error rates were also larger with these models, estimated as $\sim$0.15. The equivalency of these models means that divergence among ESUs within drainages was within a short time period relative to population size, that gene flow has obscured the historical signal in the nuclear dataset, or that coalescence estimated among microsatellite alleles is insufficient to discriminate these models.

Discussion

Phylogeographic patterns

Cichla temensis is spatially structured both among and within drainage basins. A previous investigation of historical biogeography analyzed an mtCR dataset for *C. temensis* and congeners to test for vicariance associated with Andean orogeny and the break-up of the proto-Amazonas River that once flowed from Bolivia to the Maracaibo lagoon, as well as more recent inter-basin dispersal via the Casiquiare River (Willis et al. 2010). Based on the non-monophyletic haplotype clades in each basin, it was inferred that *C. temensis* had dispersed through the Casiquiare, most likely from the Amazonas to the Orinoco, rather than experiencing ancient vicariance dating to the proto-Amazonas, geologically dated to 8–10 million years ago (we note that our previous dispersal date estimates may in fact have been overestimated based on inappropriate mutation rates; see Genner et al. 2007; Ho et al. 2007). With the addition of data from a recent review of species boundaries in this genus (Willis et al. 2012), this pattern has not changed (Fig. 1). In contrast, confirmation of the patterns exhibited by mtCR with nuclear data from microsatellites is not straightforward. While the mtCR data are best explained by the distribution of 6 haplotype clades that are largely exclusive to subsets of localities (plus a high diversity zone in the upper Negro), the microsatellite data appear best explained by the division of populations between basins, plus the same mixing zone (Table 2). Upon, further analysis, however, it appears that the same seven areas are also present in the microsatellite data, as indicated by different degrees of admixture among localities (Fig. 2). This lack of congruence is likely related to our use of analyses that do not adequately account for phylogenetic (phylogeographic) history or coalescent variance in the multi-locus microsatellite data. In particular, Evanno et al. (2005) related that while their method for determining optimal clustering using STRUCTURE was successful in identifying the deepest division of clusters, identification of nested clusters (i.e. phylogeographic structure) required division and re-analysis of the data. As a result, we have identified these seven areas as likely ESUs of *C. temensis*, areas that experience more gene exchange within rather than between groups, that inhabit more similar hydrological and topographic regions, and as a result are expected to harbor unique genetic diversity adapted for the different natural selection regimes these populations experience. However, we note that there is some ambiguity in drawing the borders of each unit, and that the grouping of specific localities into each group should be seen as a guide for further analysis. For example, while we grouped the Uaupes (UA) with the upper Negro ESU, and Barcelos (UR) and Preta da Eva (PE) with the lower Negro ESU, each of these localities exhibited some admixture with localities in adjacent ESUs (Fig. 2), indicating 'fuzzy' boundaries between *C. temensis* ESUs. That this pattern was less pronounced in the Orinoco may be a result of our sample distribution and gaps between locality sets. To some extent, our sampling reflects the patchy distribution of *C. temensis* and its preference for blackwater habitats (Winemiller 2001), however, it also is possible that additional sampling could indicate fuzzy boundaries for these ESUs as well. The fuzzy nature of these ESUs (and the lack of overt phenotypic differences) reinforces the interpretation that these

units are sub-populations within an extended meta-population, rather than separate species.

Our analyses of demes and phylogeographic structure in this species were constrained by the challenge of sampling in the Neotropics and the sample sizes we garnered. Given the observed levels of genetic divergence, we expect most of our results to be robust to the relatively low sample sizes for several of our sites (Kalinowski 2005; Hale et al. 2012). However, as a result of our sample sizes versus the moderate genetic divergence between localities, we chose not to attempt to estimate actual rates of genetic migration and divergence times between localities or ESUs using fully Bayesian coalescent programs (e.g. IMa). These programs rely on a combination of genealogical information among alleles, which is reduced in fragment-size based data, and allele frequencies among populations, which suffers higher variance with small sample sizes. Nevertheless, we were able to further explore hypotheses explaining the non-monophyly between basins of mtCR clades versus the closer relationships within basins depicted by microsatellite loci using an ABC analysis that relies on population statistics that are more robust to small samples sizes and fragment size-based data (Beaumont et al. 2002; Kalinowski 2005). We tested two major hypotheses. First, we hypothesized that the mtCR genealogy reflected a degree of random lineage sorting among ESUs in each basin following dispersal from one basin to the other, rather than a *prima facie* interpretation that implies closer relationships between non-adjacent ESUs in each basin that must have resulted from dispersal across intervening ESUs. Second, we sought to test whether there was confidence in the relationships among ESUs within each basin as depicted by the microsatellite distance tree, which conflicted with mtCR relationships, even within basins (Supplemental Fig. 1). A lack of confidence in this population tree would suggest that gene exchange following initial population divergence had obscured the original relationships, which may in fact be depicted accurately by mtCR at this level. While these ABC analyses overwhelmingly supported the monophyly of ESUs within each basin, implying that lineage sorting had occurred, they were more ambivalent with regard to within-basin relationships. This could mean that ongoing migration between ESUs has obscured historical relationships, and/or that coalescent variance among loci was too great to discriminate among hypotheses with these microsatellite data. Moreover, consideration of coalescent variance introduces another requirement of the hypothesis of lineage sorting: that without gene flow, allele coalescence must precede or be coincident with the divergence of the populations from which they are sampled (Edwards and Beerli 2000). Specifically, without non-adjacent dispersal, we expect that the most recent divergence between mtCR haplotype clades among basins must precede the divergence of ESUs within each basin as depicted by microsatellites. Considering our estimates are based on external mutation rates (a 'fish standard' 1×10^{-4} mutations/generation for microsatellites; e.g. Yue et al. 2002) and estimated generation times (5 years/gen; Willis et al. 2010), it is interesting that the ABC modal posterior estimate for divergence time of the most recent common ancestor of all *C. temensis* (5640 generations, or ~28,000 years; data not shown) would correspond so well to the estimated nodal dates for the MRCA of mtCR lineages L1–3–5 (mean ~31,000, 95 %HPD 17,449–47,296) or L2–4–6 (mean ~26,000, 95 % HPD 12,398–41,786) (Fig. 1). We also note that faster microsatellite mutation rates, shorter generation times, or slower mtCR mutation rates, as may be the case, while causing less concordance between these dates, would also be consistent with the mtCR divergences preceding the population divergences estimated from microsatellites.

Our ABC models of population history only included a single parameter modeling gene exchange between ESUs in each basin, specified as the upper Negro being a hybrid population derived from admixture between the upper Orinoco and middle Negro. While this may have caused us to underestimate divergence dates for ESUs within basins, the inclusion of admixture between the basins should preclude the underestimate of divergence times between basins as a result of gene exchange. Moreover, there is ample evidence both in the mtCR (Fig. 1) and microsatellite data (Fig. 2) that there is recent admixture between basins. However, the scale and extent of this gene exchange has yet to be fully resolved. While the microsatellite data are consistent with exchange limited to the upper Orinoco and upper Negro, it is intriguing that the Casiquiare localities (CR, PS) exhibit haplotypes identical or very similar to others in three of the six mtCR lineages. While several of these may be retained ancestral alleles (see Supplemental Fig. 1), this also raises the possibility of ancient dispersal routes around the upper Orinoco. Given that dispersal corridors might have previously existed, or ephemerally still exist, between rivers farther west than the persistent Casiquiare connection (reviewed by Winemiller and Willis 2010), it is unfortunate that we did not have access to samples in the Llanos or Amazonas regions of Colombia. Future phylogeographic studies of Neotropical freshwater fishes should include samples from these regions.

Population genetics studies have been conducted for relatively few Neotropical fishes for comparison with findings for *C. temensis*. One, in particular, is the recent range-wide review of species boundaries and population structure in discus fish, genus *Symphysodon* (Amado et al. 2011). *Cichla* and *Symphysodon* are both cichlids that inhabit floodplain rivers and exhibit seasonal reproduction and extended parental care, but *Symphysodon* are smaller

and omnivorous. Like *C. temensis*, *Symphysodon* exhibit ESUs (in this case, equated partially but not fully with morphological species) with greater gene flow within than among, and their ESUs also exhibit fuzzy boundaries. Mean F_{ST} values in *Symphysodon* were generally similar to those in *C. temensis* (within ESU: 0.122 vs. 0.126, among ESU 0.242 vs. 0.186, respectively), and similar amounts of hierarchical molecular variance were explained by variation among ESUs (~20 %), among localities within ESUs (~10 %), and within localities and individuals (~70 %). This contrasts dramatically with Amazonian populations of *Colosomma macropomum*, a large (up to 1 meter), frugivorous serrasalmid (Characiformes) that has much higher fecundity than these cichlids (0.5–1 million eggs) and exhibits seasonal migrations to the flooded forests but returns to the trunk rivers during the dry season (Goulding 1980). Although this study was based exclusively on analysis of mtDNA, *C. macropomum* exhibited small and non-significant divergences among localities within either the Amazonas or the upper Madeira ($\Phi_{ST} < 0.016$) but moderate divergence between them ($\Phi_{CT} = 0.121$), which was ascribed to rapids along the Madeira that reduce dispersal (Farias et al. 2010). Perhaps not surprisingly, these results reinforce the idea that there are fundamental differences in the population biology of fishes that have high fecundity, limited or no parental care, and seasonal migration versus those with low fecundity, extended parental care, and sedentary natures (Winemiller and Rose 1993). Nevertheless, the lack of basic population data on the majority of exploited Neotropical fishes is a major hindrance to effective and sustainable management of these resources.

Population structure and conservation

Cichla temensis supports important commercial fisheries in the Amazon and recreational fisheries in rivers and reservoirs throughout its native range. Conservation of fishery resources requires determining sustainable rates of exploitation given stock characteristics and environmental conditions. Thus, fisheries management requires stock identification, and increasingly this is accomplished via analysis of genetic data. Stock dynamics may depend on both local adaptation and meta-population dynamics involving dispersal (Funk et al. 2012). We found that *C. temensis* is spatially structured, with moderate and highly significant F_{ST} values that imply relatively little effective gene exchange among localities over short time scales. Individual *C. temensis* were assigned to their respective sampling localities with high posterior probabilities, and few specimens exhibited admixture values reflecting recent ancestry derived from migration (Fig. 2). Our low estimates of gene exchange among *C. temensis* at different localities is consistent with results from tagging studies conducted in the Orinoco and Negro basins, where some fish moved several kilometers over the course of several years, but the majority stayed within a few hundred meters of where they were originally captured and released (Hoeinghaus et al. 2003; Holley et al. 2008). Only a single individual tagged by Hoeinghaus et al. had migrated from the Cinaruco to another, adjacent tributary of the Orinoco, the Capanaparo, apparently having dispersed across the flooded savanna during the annual flood pulse (Winemiller, pers. obs.). Thus, even though this species is highly sedentary, some individuals may opportunistically disperse over longer distances under suitable conditions.

Impacts from fishing seem to be strongly localized in this species. For example, Jepsen et al. (1999), in studying differences between the population size and age structures of *Cichla* populations in different parts of Venezuela, described how more than 20 years of fishing pressure in the Aguaro River resulted in the *de facto* extirpation of *C. temensis* and a reduction of the *C. orinocensis* stock (a smaller species) to one comprised almost entirely by age-1 fish. Winemiller (2001), citing data from Jepsen et al. (1999), related how initiation of recreational and subsistence fishing in Venezuela's remote Pasimoni River, after just a few years, radically changed the size structure of the *C. temensis* stock. The low vagility of *C. temensis* implies that management needs to be based on local stocks with different physiological and ecological response to local environmental conditions. Based on our findings, and until additional studies are performed at finer spatial scales, tributaries of major rivers probably provide the best basis for delineating stocks. Population genetic research and population modeling could help to determine whether or not dispersal is sufficient to affect dynamics of exploited stocks.

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