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ORIGINAL ARTICLE

Fish assemblages of the Casiquiare River, a corridor and zoogeographical filter for dispersal between the Orinoco and Amazon basins

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ABSTRACT

Aim The aim of this study was to determine whether the Casiquiare River functions as a free dispersal corridor or as a partial barrier (i.e. filter) for the interchange of fish species of the Orinoco and Negro/Amazon basins using species assemblage patterns according to geographical location and environmental features.

Location The Casiquiare, Upper Orinoco and Upper Negro rivers in southern Venezuela, South America.

Methods Our study was based on an analysis of species presence/absence data and environmental information (11 habitat characteristics) collected by the authors and colleagues between the years 1984 and 1999. The data set consisted of 269 sampled sites and 452 fish species (> 50,000 specimens). A wide range of habitat types was included in the samples, and the collection sites were located at various points along the entire length of the Casiquiare main channel, at multiple sites on its tributary streams, as well as at various nearby sites outside the Casiquiare drainage, within the Upper Orinoco and Upper Rio Negro river systems. Most specimens and field data used in this analysis are archived in the Museo de Ciencias Naturales in Guanare, Venezuela. We performed canonical correspondence analysis (CCA) based on species presence/absence using two versions of the data set: one that eliminated sites having < 5 species and species occurring at < 5 sites; and another that eliminated sites having < 10 species and species occurring at < 10 sites. Cluster analysis was performed on sites based on species assemblage similarity, and a separate analysis was performed on species based on CCA loadings.

Results The CCA results for the two versions of the data set were qualitatively the same. The dominant environmental axis contrasted assemblages and sites associated with blackwater vs. clearwater conditions. Longitudinal position on the Casiquiare River was correlated ($r^2 = 0.33$) with CCA axis-1 scores, reflecting clearwater conditions nearer to its origin (bifurcation of the Orinoco) and blackwater conditions nearer to its mouth (junction with the Rio Negro). The second CCA axis was most strongly associated with habitat size and structural complexity. Species associations derived from the unweighted pair-group average clustering method and pair-wise squared Euclidean distances calculated from species loadings on CCA axes 1 and 2 showed seven ecological groupings. Cluster analysis of species assemblages according to watershed revealed a stronger influence of local environmental conditions than of geographical proximity.

Main conclusions Fish assemblage composition is more consistently associated with local environmental conditions than with geographical position within the river drainages. Nonetheless, the results support the hypothesis that the mainstem

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Casiquiare represents a hydrochemical gradient between clearwaters at its origin and blackwaters at its mouth, and as such appears to function as a semi-permeable barrier (environmental filter) to dispersal and faunal exchanges between the partially vicariant fish faunas of the Upper Orinoco and Upper Negro rivers.

Keywords

Blackwater, clearwater, dispersal barrier, freshwater fish assemblages, hydrochemistry, multivariate analysis, pH, species dispersal, Venezuela, vicariance.

INTRODUCTION

The Neotropical region harbours the Earth's richest diversity of freshwater fishes, with at least 6000 species estimated to inhabit the great river basins of South America (Reis *et al.*, 2003). The integration of studies from various scientific disciplines (e.g. geology, palaeontology, climate history) has revealed that the Neotropical realm has had a long and complex history. In particular, there is substantial evidence that South America has undergone marked changes over the last 90 Myr, including major marine incursions, weathering of ancient shields, uplift of the Andean chain and palaeo-arches, significant reconfigurations in the size and drainage patterns of the region's principal river basins, tremendous accumulation of alluvial sediments, and periodic climatic shifts from very dry to very wet (Hoorn, 1994; Hoorn *et al.*, 1995; Lundberg *et al.*, 1998; Baker *et al.*, 2001). The complex and diverse evolution of the region's geomorphology and environmental conditions resulted in natural selection on fishes and other organisms that has promoted biological diversification (e.g. López-Fernández *et al.*, 2005; Sidlauskas, 2007). However, considerable uncertainty remains concerning the precise links between the region's physical history and the evolutionary origin, geographical distribution, ecology, and phenotypic characteristics of the modern Neotropical fish fauna.

The Amazon and Orinoco rivers are two of the world's major fluvial systems, covering 6,915,000 km^2 and 983,000 km^2, respectively, and draining much of central and northern South America. The two basins are connected by the Casiquiare River (also known as the Casiquiare Canal), a natural channel that is over 300 km long with a mean discharge of *c.* 2100 m^3 s^{-1} (Global Runoff Data Centre, 2007). The Casiquiare flows near the interface between the south-western edge of the Guayana (Guiana) Shield and the easternmost reach of the *Llanos* (savannas) of the Orinoco at their contact zone with western Amazonia. The largest natural waterway linking two major river basins, the Casiquiare offers biogeographers a unique opportunity to assess the manner in which ecology and historical geography have influenced fish distributions. The Casiquiare originates as a bifurcation of the main channel of the Upper Orinoco, downstream from several of the Orinoco's major tributaries (e.g. Mavaca, Ocamo and Padamo). At the point of diversion, the Casiquiare is *c.* 100 m wide and takes one-eighth to one-quarter (depending on water level) of the Upper Orinoco's main channel volume (Sternberg, 1975). The river has a low gradient and flows in a general south-west direction over a meandering course that cuts through a lowland plain of rain forests and contains several rapids. Downstream, the Casiquiare increases substantially in width and volume as it receives water from a number of large tributaries (e.g. Pasiba, Siapa, and Pasimoni). At its mouth, which is more than 500 m wide, the Casiquiare merges with the Guainía River to form the source of the Rio Negro, the largest tributary of the Amazon (Fig. 1). Hydrologically, the Casiquiare represents a stream capture in progress, but there is some debate concerning its formation (Sternberg, 1975). Nevertheless, if natural processes continue unabated, it is likely that the Casiquiare will capture a large portion of the Upper Orinoco drainage and deliver its waters to the Rio Negro (von Stern, 1970; Sternberg, 1975).

The Orinoco and Amazon rivers were closely associated in the distant past. For many millions of years prior to the late Miocene, they formed a single fluvio-lacustrine system that – for a period – drained northwards to the Caribbean (Wesselingh & Salo, 2006). During the late Miocene (8–10 Ma), the Orinoco and Amazon basins became separated by the rise of the Vaupés arch between the Sierra de La Macarena and Andes mountains in the west and the western edge of the Guayana Shield in the east. At this time, the final elevation of the Mérida Andes caused the Orinoco to flow to the north-east of South America, approximating its present course. Nearly simultaneously, the Amazon acquired its current easterly course as the previously isolated foreland basin of the Andes in western Amazonia filled with sediments, flooding the Purus arch and becoming a unified fluvial system running from the Andes to the Atlantic (Hoorn *et al.*, 1995; Lundberg *et al.*, 1998; Albert *et al.*, 2006). From the end of the Miocene to the present, the foreland basin of the Andes north of the Vaupés arch has continued to fill with Andean alluvium, forming the vast Orinoco floodplain known as the *Llanos*. As a result of a combination of erosion and sediment accumulation, the once separated Amazon and Orinoco basins are again connected by a continuous water channel, the Casiquiare River (von Stern, 1970). The Casiquiare has a very shallow gradient, with an elevation difference of only 20 m between its source at the Orinoco and its mouth at the Rio Negro (Mago-Leccia, 1971).

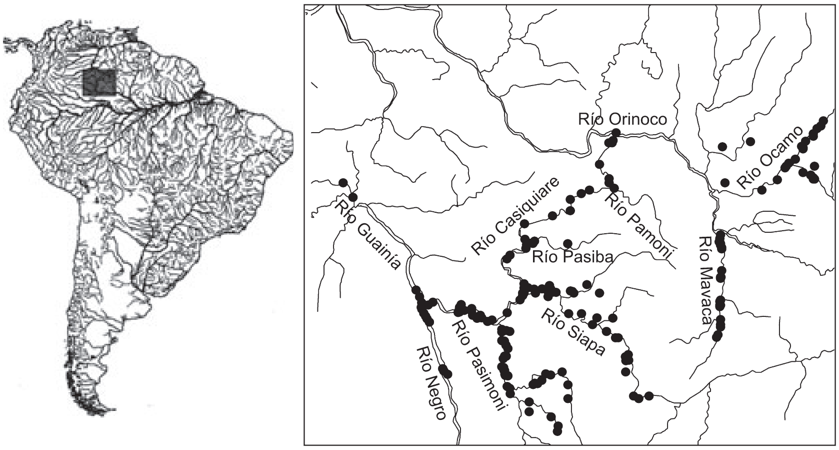

Figure 1 Map showing the location of the Casiquiare River between the Upper Orinoco River and the Upper Negro River of the Amazon Basin, with an inset map showing the locations of survey localities within the Casiquiare region.

Numerous and variable river systems drain the highly heterogeneous landscape of the Casiquiare region. The region's streams range considerably in colour, sediment load, and physical and chemical parameters – properties that are strongly influenced by the geology, vegetation cover and climatic regimes of local watersheds (Sioli, 1984; Huber, 1995). Traversing this diverse landscape, the Casiquiare links watersheds with markedly different physicochemical characteristics (Fig. 2). The Upper Orinoco Basin contains mostly clearwater streams with relatively high transparency, slightly acid pH, a moderate concentration of dissolved organic and inorganic substances, and clay-bearing sediments (Weibezahn *et al.*, 1990). In contrast, the Upper Rio Negro Basin is dominated by blackwater streams with high transparency but strong staining by tannins and other organic compounds leached from vegetation, extremely low pH, negligible amounts of solutes, and substrates of fine quartz sand (Sioli, 1984; Goulding *et al.*, 1988).

Figure 2 Photographs of (a) the Pasimoni River, a blackwater tributary of the lower Casiquiare River, and (b) the Siapa River, a clearwater tributary of the middle Casiquiare.

The Casiquiare and its many tributaries are a mosaic of different water types (Table 1). Consequently, over its course the Casiquiare main channel exhibits a marked hydrogeochemical gradient, ranging from clearwaters near its bifurcation with the Orinoco to black waters in its lower reaches. As a result of this gradient in water type, it is hypothesized that the Casiquiare influences the movement of aquatic organisms between the Orinoco and Amazon basins. Given its large size and shallow elevational gradient, the Casiquiare should serve as a major corridor for dispersal of aquatic biota between these two great river basins; however, several Amazonian fish taxa are absent from the Orinoco Basin. Included among these, for example, are three species of bonytongues (Osteoglossidae), the lungfish (*Lepidosiren paradoxa*), and discus cichlids (*Symphysodon* spp.). Moreover, there is evidence that a number of sister taxa demonstrate marked vicariant distribution patterns, with one species of the pair widely distributed but limited to the Amazon Basin and the other species wide ranging but limited to the Orinoco Basin (e.g. the redbelly piranhas *Pygocentrus nattereri* in the Amazon, and *P. cariba* in the Orinoco). Recent studies have also revealed large genetic differentiation between populations of fish species that occur across the Casiquiare, suggesting that the river is a barrier to gene flow (e.g. Lovejoy & de Araújo, 2000; Turner *et al.*, 2004; Willis *et al.*, 2007).

Knowledge of the role the Casiquiare has played in shaping (and reshuffling) the distributions of fishes in northern South America may be key to a better understanding of the region's fish diversity. To assess the potential for the Casiquiare River to function as a selective corridor for fishes of the Orinoco and Amazon basins, the current study had three objectives: (1) to

Table 1 Summary of physico-chemical characteristics of water within 12 drainages of the Casiquiare region based on measurements reported in Museo de Ciencias Naturales de Guanare field records.

Drainage	No. sites with data	Water type	pH average	pH range (no. sites)	Conductivity range (μS s^{-1}) (no. sites)	Dissolved oxygen range (mg L^{-1}) (no. sites)	Secchi depth range (m) (no. sites)
Ocamo	12	Clear	6.7	6.5–7.0 (9)	–	–	0.6–1.0 (4)
Padamo	3	Clear	6.3	6.0–6.5 (2)	–	–	0.9–1.0 (2)
Orinoco	5	Clear	6.2	5.0–7.0 (4)	–	–	0.9 (2)
Mavaca	18	Clear	5.9	5.5–6.5 (13)	–	–	0.3–0.9 (5)
Casiquiare	96	Black–Clear	5.5	4.1–6.2 (48)	15.5–62.0 (33)	3.6–4.0 (3)	0.4–2.0 (30)
Pasiba	1	Black	5.5	–	23.0	–	–
Siapa	35	Black–Clear	5.4	4.4–6.5 (9)	20.0–22.0 (7)	–	1.4 (5)
Pasimoni	23	Black	4.2	4.0–4.4 (9)	24.2–33.6 (9)	1.5–3.3 (9)	2.0 (5)
Baría	1	Black	4.2	–	26.7 (1)	1.3 (1)	1.5–2.5 (1)
Yatúa	9	Black	4.3	4.3 (2)	28.2 (2)	1.0 (2)	–
Guainía	3	Black	4.3	4.1–4.4 (2)	19.6–20.0 (2)	4.9–5.1 (2)	–
Negro	9	Black	4.8	4.7–5.0 (6)	12.6–13.8 (4)	4.9–5.0 (3)	0.8–1.0 (5)

document regional taxonomic diversity of fishes throughout the region of the Upper Orinoco, Casiquiare, and Upper Rio Negro in southern Venezuela; (2) to examine geographical patterns of species distributions and assemblage structure within the region; and (3) to examine ecological correlates of species assemblage structure, particularly in the context of environmental gradients on the landscape. This paper includes the first analysis of correlations between freshwater fish diversity, distribution and environmental conditions in the Casiquiare region. We demonstrate that environmental factors associated with the longitudinal fluvial gradient of the Casiquiare River are significantly correlated with species assemblage structure, with some species associated with blackwater habitats, some with clearwater habitats, and others distributed across a wide range of water conditions. Our findings support the idea that a combination of phylogeographical and ecological studies are highly useful for understanding the factors that influence geographical patterns of biological diversity in the world's richest freshwater fish fauna.

METHODS

Field collections and data sets

We compiled a data base of fish samples taken during ichthyofaunal surveys conducted by the authors and colleagues in southern Venezuela between 1984 and 1999. Fish samples were collected using small and large seines, castnets, dipnets, gillnets of various lengths and mesh sizes, fish traps and hook and line. A total of 269 survey sites were distributed among 12 drainages of the Upper Rio Negro, Casiquiare and Upper Orinoco rivers (Fig. 1). Field records describing habitat features and fish data (> 50,000 preserved fish specimens) from these collections are archived in the Ichthyological Collection of the Museo de Ciencias Naturales de Guanare (MCNG), Venezuela.

The composition of fish assemblages from survey sites was analysed based on species presence or absence. The diverse nature of the shallow-water habitats, species, and methods used to obtain these samples prevented us from performing abundance-based analyses, which would require standardized collections over time and across sites. Similarly, records of habitat characteristics at each site varied in terms of the level of detail and the sets of variables recorded across collecting events performed by different individuals. To overcome this deficiency, we created a standard set of coarse-scale habitat variables. The first step was to compile a matrix that included all survey sites and every type of habitat variable recorded during the surveys. From this data base we derived four coarse-scale habitat variables that reliably characterized aspects of habitat at each site in comparable terms. Two categorical variables (channel and water type) included non-overlapping categories (Table 2) that could be assigned unambiguously to each site from field records at the MCNG. The categories blackwater and clearwater were based on water colour and transparency characteristics that were associated with pH in particular, with blackwater having pH values < 5.0 (Table 1).

Table 2 Description of categorical habitat variables.

Categorical variable	Category name	Category attribute
Channel type	Small channel	0–25 m maximum width
	Medium river	25–100 m maximum width
	Large river	> 100 m maximum width
	Isolated pools	Interrupted main channel of any of the above
	Lagoon	Oxbow or permanent side channel with lentic water
	Lagoon mouth	Channels linking lagoons to a main channel
Water type	Black	Categories follow descriptions in Sioli (1984)
	Clear	
	White	

Table 3 Continuous habitat variables: substrate heterogeneity and habitat structural complexity with their individual components. See text for a description of calculations of complexity indices.

Continuous variable	Components
Substrate heterogeneity	Bedrock
	Rock
	Gravel
	Sand
	Clay, mud
	Silt
	Leaf litter
	Flooded grass
	Flooded forest
Structural complexity	Emergent vegetation
	Submerged macrophytes
	Floating macrophytes
	Filamentous algae
	Overhanging vegetation
	Flooded grass
	Flooded bushes
	Flooded forest
	Submerged individual trees
	Rocks
	Submerged logs
	Leaf litter

In addition, two continuous metrics (substrate heterogeneity and structural complexity) were derived from descriptions of substrate and habitat structure in the field records. For each survey site, the presence/absence of individual components of these two habitat features (Table 3) was recorded in a matrix. For example, overall habitat structural complexity was derived by summing across elements such as rocks, submerged logs, submersed aquatic vegetation, etc. Standardized indices for habitat structural complexity and substrate composition were derived using the following formula:

$$S = \frac{\sum x_i}{N},$$

where S is an index of substrate heterogeneity or habitat structural complexity, x_i represents each of the individual components of substrate or habitat structure present at a given site (Table 3), and N represents the number of combined elements of substrate or structure observed at the site having maximum substrate or structural complexity. By standardizing the number of observed complexity elements at a given site by the number of elements at the site with maximum complexity, we obtained an index that ranged from 0 (minimum complexity) to 1 (maximum complexity) and thus allowed us to provide coarse-scale but comparable values for substrate heterogeneity and habitat complexity for each collecting site. Other habitat variables could have been included, but this would have reduced the number of survey sites in our analysis of the ecological gradient associated with the Casiquiare over a regional scale. Thus, we elected to include as many localities as possible, accepting a reduced resolution of environmental variables. Because a smaller set of habitat variables reduces the probability of finding an environmental gradient, our approach provides a conservative test of the hypothesis that the Casiquiare represents an environmental filter for fish dispersal.

Analyses of faunal and environmental gradients

Canonical correspondence analysis

Relationships between fish assemblage and habitat attributes at each site were explored using canonical correspondence analysis (CCA). This multivariate method is a direct gradient analysis technique that ordinates a set of observations by directly relating them to a series of associated environmental variables (ter Braak, 1986). In our case, the set of observations consisted of a matrix of 269 survey sites with species recorded as present (coded as 1) or absent (coded as 0). The associated environmental variables consisted of a matrix including the four habitat variables derived for each site where fishes were collected. Categorical variables were coded as dummy variables (coded as 1 when a given category applied to a site, with all other categories coded as 0). CCA simultaneously associates the fish assemblage with the habitat variables at each site, and uses multiple regression to constrain the ordination to represent the maximum correlation between fish and habitat. Orthogonal canonical axes are derived based on habitat variables, and these in turn model a proportion of the variation in fish assemblages (ter Braak, 1986; ter Braak & Šmilauer, 1998). The null hypothesis that habitat variables do not explain any of the observed variation in fish assemblage was tested with an *F*-ratio of the eigenvalues of the canonical axes and the residual sum of squares. Two hundred permutations of Monte Carlo simulations were used to generate a 95% confidence interval for all canonical axes. CCA and Monte Carlo simulations were run in CANOCO ver. 4.0 (ter Braak & Šmilauer, 1998).

The inclusion of small samples and rare species introduces error and skew into multivariate analyses designed to reveal gradients of species composition (ter Braak & Šmilauer, 1998). With this in mind, we performed CCA after eliminating sampling sites that contained fewer than five species and species that occurred at fewer than five sites. We also performed the CCA by eliminating samples with fewer than 10 species and species occurring at fewer than 10 sites. As the overall results did not differ, here we report only the results of the first analysis.

Environmental groupings of species were examined with a neighbour-joining cluster analysis (centroid method) based on pair-wise species Euclidean distances for CCA species assemblage axis-1 and axis-2 scores using SPSS software (SPSS Inc., 2002).

Association between fluvial and ecological gradients

Even if habitat attributes are significantly associated with fish assemblage composition, this association does not need to follow the longitudinal fluvial axis of the Casiquiare River. In other words, an ecological gradient affecting community assemblage may not coincide with the physical waterway connecting the Upper Orinoco and Upper Negro rivers. Thus, we were interested in determining if the ecological gradient revealed by the CCA overlapped with the longitudinal fluvial gradient represented by the Casiquiare. Using the geographical coordinates of each sampling locality along the main channel of the Casiquiare, and following the meandering of the channel on a 1 : 100,000 scale map of the region, we measured the river channel distance between each sampled locality and the blackwater endpoint of the fluvial gradient at the juncture of the Casiquiare with the Río Negro (the mouth of the Casiquiare). We then performed a linear regression of the CCA axis-1 score of each locality vs. its fluvial distance from the Río Negro. We used axis-1 scores because this axis represented the strongest ecological gradient (essentially clearwater to blackwater) between the Upper Orinoco and Upper Rio Negro. Because we were interested in the potential role of the mainstem Casiquiare as a dispersal corridor, the analysis excluded tributaries of the Casiquiare as well as watersheds outside its direct drainage basin. To reduce error from undersampling of fishes, sites with fewer than seven recorded species were excluded from this analysis. Whenever the same site was surveyed in more than 2 years, the collection with the greater number of recorded species was used.

Inter-basin faunal similarity

We examined the potential association between fish assemblage composition and position within the Casiquiare Basin using the site × species presence/absence matrix to calculate pair-wise assemblage similarity values among all watersheds. We estimated inter-basin faunal similarity using the 'tripartite similarity calculator' (Tulloss & Tulloss, 2007), which implements the non-biased similarity index described by Tulloss (1997). These similarity values were used to calculate inter-basin faunal distances (1 – similarity) for use in a neighbour-joining cluster analysis using MEGA ver. 3.0 (Kumar *et al.*, 2004).

RESULTS

Taxonomic richness

Our complete survey data matrix for the Casiquiare region documents 41 fish families, 216 genera and 452 species, including some previously undescribed taxa (see Appendix S1 in Supplementary Material). Several species were morphologically divergent from species described from other regions of South America, and these putative undescribed species were recorded as *Genus* sp., followed by a description or letter when there were multiple undescribed forms. A few fish species were common and collected at 30 or more survey sites, but most species were collected at fewer than 10 sampling sites (Fig. 3).

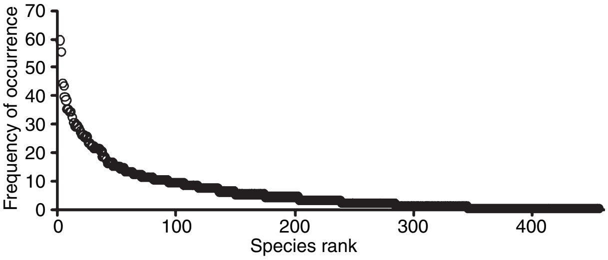

Figure 3 Frequency of occurrence of fish species in surveys plotted according to ranks.

The relationship between the number of sites sampled and the number of species documented within a given watershed was linear and did not reach an asymptote (Fig. 4), which indicates that surveys did not completely document species assemblages within sites and watersheds, even in those watersheds with the greatest numbers of samples (e.g. Siapa). Although some rivers, such as the Padamo and Pamoni, were probably grossly undersampled, this does not prevent achievement of the objective of our analysis, namely, to demonstrate faunal patterns on the landscape that correlate with broad geographical and habitat features. Incomplete species documentation would pose a serious problem for demonstration or interpretation of faunal patterns if species were systematically omitted from collections according to habitat or region, but this was not the case. Thus, our analysis should be conservative with regard to type-I statistical error (i.e. the likelihood of discerning a pattern when one is not actually present).

Assemblage composition–environment relationships

The first and second set of axes from CCA modelled 26% and 17% of the species–environment variance in the data set comprising 269 survey sites, 452 species, and four environmental variables (two continuous variables and two categorical variables, the latter implemented as nine dummy variables). The first CCA environmental axis described a general gradient

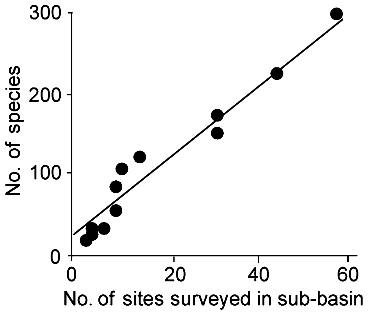

Figure 4 Relationship between the number of sites surveyed within a sub-basin and the total number of species collected from the sub-basin (no. species = 4.29(no. sites) + 19.2; $r^2 = 0.97$; $P < 0.01$).

Table 4 Canonical correspondence analysis (CCA) results for habitat variables. Only the six variables with the highest loadings on axis 1 and the six species occurring at the most sites are listed.

	Axis 1	Axis 2
Eigenvalue	0.344	0.222
Percentage species–environment variance	26	17
Correlations of environmental variables with axes		
Blackwater	0.60	−0.10
Clearwater	−0.54	< 0.01
Lagoon	−0.35	0.02
Structural complexity	−0.35	0.58
Small channel	0.27	0.31
Large river	0.13	−0.46
Correlations of fish species with strongest negative loadings on axis 1		
Ochmacanthus orinoco	−2.17	0.86
Characidium zebra	−2.17	0.86
Steindachnerina argentea	−1.84	0.74
Potamorhina altamazonica	−1.83	0.18
Serrasalmus altuvei	−1.75	0.02
Psectrogaster ciliata	−1.72	−0.05
Correlations of fish species with strongest positive loadings on axis 1		
Crenicichla lenticulata	1.93	−0.31
Pseudopimelodus sp.	1.78	2.19
Liosomadoras oncinus	1.38	1.32
Trachelyopterichthys taeniatus	1.27	0.12
Amblydoras sp.	1.21	0.58
Microphilypnus ternetzi	1.18	1.16
Correlations of fish species with highest frequency of occurrence at sites		
Hemigrammus analis (60 sites)	0.17	−0.10
Hemigrammus vorderwinkleri (56)	0.43	−0.06
Mesonauta insignis (45)	0.39	−0.08
Moenkhausia copei (44)	−0.17	0.28
Hemigrammus schmardae (40)	−0.32	−0.14
Copella nattereri (39)	0.59	−0.06

spanning blackwater (large positive correlation) to clearwater (large negative correlation) conditions (Table 4). This gradient was influenced to a lesser extent by lagoons, habitat structural complexity, small channels, and large rivers. According to this gradient, lagoons and habitats with high structural complexity, such as flooded riparian zones, tended to be associated with blackwater conditions. Fish species with large negative loadings on the first species assemblage axis, such as *Steindachnerina argentea* and *Catoprion mento*, tended to be associated with clearwater conditions, whereas those with large positive loadings, such as *Liosomadoras oncinus* and *Crenicichla lenticulata*, were essentially restricted to blackwater habitats (Table 4). The species captured at the greatest number of sites tended to have intermediate correlations with the first two CCA axes (Table 4). The second CCA environmental axis was influenced by structural complexity and small channels (large positive correlations) and large rivers (large negative correlations). Thus, the second axis describes an environmental gradient spanning small bodies of water with high structural complexity to large rivers.

Ordination of survey sites on the first two CCA species assemblage axes reveals variable degrees of overlap among the Orinoco, Negro, Casiquiare, Pasimoni and Siapa rivers (the latter two being the largest blackwater and clearwater watersheds of the middle Casiquiare, respectively) (Fig. 5). Upper Orinoco sites tended to have lowest scores on axis 1 and not overlap with sampling sites from the Negro, Pasimoni, and Casiquiare. Siapa and Casiquiare sites occupied intermediate space in the ordination plot. Sites within the Upper Orinoco and Siapa watersheds had the greatest spread of site scores on axis 2, suggesting a greater range of habitat types surveyed in those river systems.

Cluster analysis based on CCA assemblage axis-1 and axis-2 scores for fish species yielded seven groupings (Fig. 6). An undescribed catfish *Pseudopimelodus* sp. (shallow blackwater), the piranha *Serrasalmus manueli* (deep blackwater), and the frugivorous characid *Brycon bicolor* (deep clearwater) were outliers that did not cluster with any species. The other four clusters were diverse fishes from shallow blackwater (17 species), shallow blackwater and clearwater (66 species), deep clearwater (12 species) and shallow to deep clearwater (nine species). Some species were restricted to clearwater habitats (e.g. *Cichla intermedia*, *Pimelodus blochii*), some were restricted to blackwater habitats (e.g. *Paracheirodon axelrodi*, *Pterophyllum altum*), and others were common in both water types (e.g. *Hoplias malabaricus*, *Serrasalmus rhombeus*).

Cluster analysis of river drainages based on dissimilarities of fish species presence/absence grouped species according to water type and habitat location within the regional drainage network (Fig. 7). Three relatively small blackwater tributaries

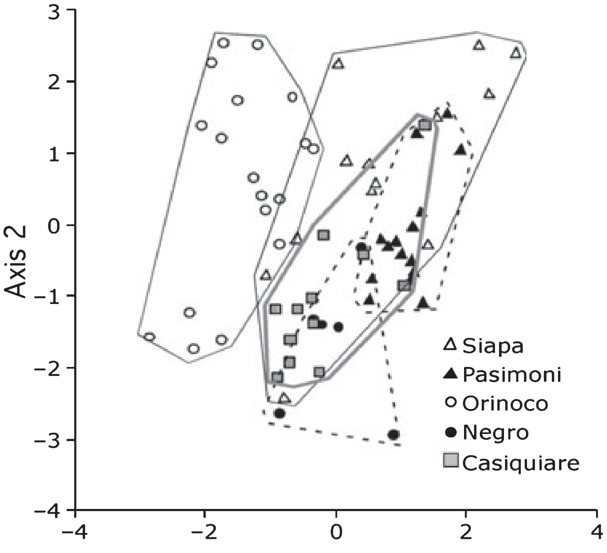

Figure 5 Plot of sample canonical correspondence analysis (CCA) scores on axis 1 and axis 2 based on the matrix of species assemblage composition and the matrix of habitat variables. Symbols for sub-basins are: open circles, Upper Orinoco; open triangles, Siapa; black circles, Upper Negro; black triangles, Pasimoni; squares, Casiquiare.

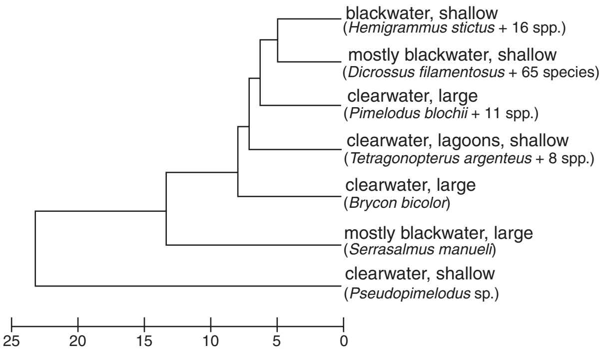

Figure 6 Dendrogram of species habitat groupings derived from cluster analysis (unweighted pair group method with arithmetic mean using pair-wise species Euclidean distances for canonical correspondence analysis axis-1 (CCA) and axis-2 scores).

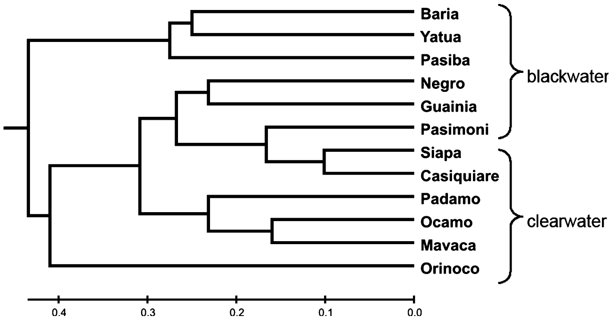

Figure 7 Dendrogram of river sub-basin species assemblage relationships derived from cluster analysis (unweighted pair group method with arithmetic mean using pair-wise sub-basin dissimilarities based on fish species presence/absence).

of the Pasimoni and Casquiare clustered together (Baria, Yatua, Pasiba), as did clearwater tributaries of the Upper Orinoco (Padamo, Ocamo, Mavaca). The clearwater Orinoco was an outlier, and the blackwater Negro and Guainía (uppermost Rio Negro) clustered together. The remaining cluster was the Casiquiare, Pasimoni and Siapa, the latter two being the largest tributaries entering the mid-reaches of the Casiquiare. The Casiquiare clustered closer to the Siapa (clearwater) than it did to the Pasimoni (extreme blackwater).

Regression of site scores for species assemblage CCA axis 1 as a function of position along the longitudinal fluvial gradient of the mainstem Casiquiare River (as indicated by fluvial distance to the Casiquiare mouth) yielded a coefficient of determination of 0.33 (Fig. 8). Although this relationship would be considered weak–moderate, it reveals a significant ($P < 0.05$) association between species assemblage composition constrained by environmental conditions and geographical location along the river corridor.

DISCUSSION

Does the Casiquiare River represent a corridor permitting free movement of fishes between the Orinoco and Amazon basins,

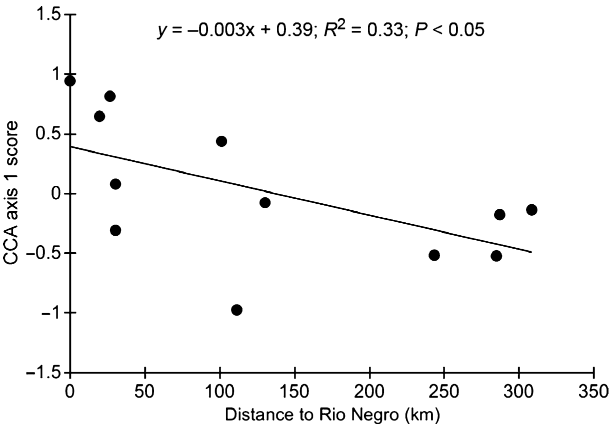

Figure 8 Relationship between sample location along the longitudinal river gradient of the Casiquiare and fish assemblage sample canonical correspondence analysis (CCA) score on axis 1, where the latter is interpreted as indicating where the sample falls along a general blackwater–clearwater environmental gradient.

or does it function as a zoogeographical filter? At present, the river is a large permanent aquatic connection between the Upper Orinoco River near the village of Tama Tama, Venezuela, and the Upper Rio Negro just upstream from the town of San Carlos, Venezuela. The connection between the Casiquiare and Orinoco is a stream capture (anastomosis) that is believed to be fairly recent in terms of geological time (von Stern, 1970), following a long period of isolation between the two basins after the uplift of the Vaupes Arch in the foreland of the Andes around 8–10 Ma (Hoorn, 1993, 1994; Hoorn *et al.*, 1995). The precise age of this connection, in which the Casiquiare, a tributary of the Rio Negro, partially captured waters draining the Upper Orinoco, is unknown. von Stern (1970) cited evidence that the connection is growing larger in response to the natural forces of erosion and deposition. If this progression continues, the Casiquiare will completely capture the uppermost drainage of the Orinoco Basin, which would result in designation of the headwaters of the Orinoco as the Cunucunuma River drainage (von Stern, 1970; Sternberg, 1975). von Stern (1970) also described old channels that formerly connected the Casiquiare and Orinoco rivers at two locations located several kilometres downstream from the current bifurcation of the Orinoco channel, and that continue to receive over-bank floodwaters during extreme wet periods.

We found a significant trend in fish assemblage structure and environmental conditions that is correlated with the fluvial gradient of the Casiquiare. A great many fish species are distributed throughout the Orinoco, Casiquiare, and Rio Negro drainages. This observation is based on current taxonomy, and Neotropical ichthyology remains in a dynamic state, with many new species described each year. In many cases, vicariant species with similar appearances have been identified based on morphology or genetics. For example, two species of bagre rayado (pimelodid catfishes in the genus *Pseudoplatystoma*) formerly considered widespread throughout the Amazon and Orinoco basins have been split into eight vicariant species (Buitrago-Suárez & Burr, 2007). Nonetheless, other extensively and recently studied species seem to be distributed within both basins (e.g. Crampton & Albert, 2003; Freeman *et al.*, 2007; Littmann, 2007; Willis *et al.*, 2007). Our surveys obtained many species that were widely distributed throughout the region, with conspecifics captured from both clearwater habitats of the Upper Orinoco and Upper Casiquiare and blackwater habitats of the lower Casiquiare and Rio Negro. Examples of these widespread fishes include the piranha *Serrasalmus rhombeus*, all known species of the ctenoluciid genus *Boulengerella*, the cichlids *Cichla temensis* and *Mesonauta insignis*, and the catfishes *Pimelodus blochii* and *Scorpiodoras heckelii*. Even very small species with restricted ranges have been found at locations within both the Upper Orinoco and Upper Rio Negro drainages (e.g. Ferraris & Mago-Leccia, 1989). Thus, if the Casiquiare is a zoogeographical filter, it appears to be a porous one that permits certain species to move freely between the two basins.

Our study also revealed that, for other fish species, the gradient of environmental conditions along the course of the Casiquiare constitutes an ecological barrier for dispersal. The river's longitudinal fluvial gradient transitions between clearwater conditions having upland hydrological influences, and blackwater conditions associated with the drainage of the sandy, oligotrophic alluvial plains of the Cuchivero geological province (Vegas-Vilarrúbia *et al.*, 1988; Fig. 9). Although conductivity and pH are low for both of these water types (Table 1), the blackwater conditions of the Upper Negro/Lower Casiquiare are extremely acidic and probably pose physiological constraints upon aquatic organisms. Low pH influences ionic balance (Wilson *et al.*, 1999; Matsuo & Val, 2002) and a host of other physiological processes in fishes, including oxygen affinity of haemoglobin, digestion, and osmotic balance (reviewed by Val & Almeida-Val, 1995). Black waters also tend to have low dissolved oxygen content (Table 1), apparently because of the combined effects of photochemical and biological oxygen demand associated with the processing of dissolved humic and fulvic substances (Amon & Benner, 1996; Mayorga & Aufdenkampe, 2002). Thus, blackwater fishes appear to be specifically adapted to the extreme physicochemical conditions of their environment (Val & Almeida-Val, 1995).

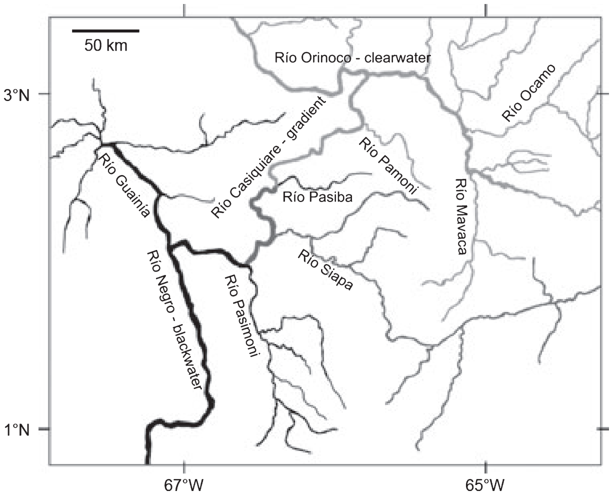

Figure 9 Map revealing the general habitat mosaic of the Casiquiare region, which functions as an environmental filter for fish dispersal between the Upper Orinoco River (clearwater rivers shown in light grey) and Negro River (blackwater rivers shown in black). The middle reach of the mainstem Casiquiare (between the mouths of the Pasiba and Pasimoni rivers) has waters that are a mixture (shown in dark grey) of clearwaters delivered by the Upper Orinoco, Pamoni and Siapa rivers and the blackwater Pasiba River. Below the mouth of the Pasimoni River, the Casiquiare acquires strong blackwater characteristics.

Clearwaters of the Upper Orinoco, Upper Casiquiare, and Upper Siapa (a major Casiquiare tributary draining the mountain ranges that form Venezuela's southernmost international boundary) are associated with more moderate pH and dissolved oxygen concentrations, but also with higher levels of suspended particulate matter, including clay, particularly during periods of high flow. Most of the clearwater rivers of our study region have high water transparency during periods of base-flow conditions, but many of these rivers may become slightly to moderately turbid during periods of high flow. In clearwater rivers, such as the Mavaca, sustained low-flow conditions result in lower water transparency owing to higher concentrations of phytoplankton (Lewis, 1988; Cotner *et al.*, 2006). Our findings indicate that some species are largely restricted to blackwater habitats, and others occur entirely or mostly in clearwater habitats of the region. Examples of blackwater-adapted fishes include the characid *Paracheirodon axelrodi*, the 'cachorro' *Acestrorhynchus grandoculis*, the piranha *Pristobrycon maculipinnis*, the leaffish *Monocirrhus polyacanthus*, and the cichlids *Laetacara fulvipinnis* and *Satanoperca daemon*. Clearwater- or whitewater-adapted species include *Steindachnerina argentea* (Curimatidae), *Catoprion mento* (Serrasalmidae), *Cichla intermedia* (Cichlidae), the characids *Aphyocharax alburnus* and *Bryconamericus orinocense*, and the anostomids *Laemolyta taeniatus* and *Schizodon scotorhabdotus*. Other investigators have noted water-type affiliations of Neotropical fishes (Goulding *et al.*, 1988; Vari, 1988). Several recently described taxa appear to be endemic to the Upper Orinoco and upstream reaches of the Casiquiare, for example *Pseudancistrus sidereus* (Armbruster, 2004) and *P. pectegenitor* (Lujan *et al.*, 2007). In contrast, other species have geographical ranges restricted to the Rio Negro and the lowermost reaches and tributaries of the Casiquiare, where extreme blackwater conditions exist, for example *Serrasalmus gouldingi* (Freeman *et al.*, 2007), *Hypophthalmus fimbriatus* (López-Fernández & Winemiller, 2000), and *Geophagus winemilleri* (López-Fernández & Taphorn, 2004).

The main channel of the Casiquiare appears to be a gradient between clear waters received at its origin from the Upper Orinoco, and black waters as it approaches the Rio Negro. Along its upper course, the Casiquiare first receives clear waters from several relatively small tributaries (Caripo, Pamoni), and then is joined by black waters delivered by a succession of tributary streams and rivers. The Siapa River is the first large tributary to join the Casiquiare. The upper tributaries of the Siapa have clear waters drained from the mountain ranges on the southern border of Venezuela. The Siapa fluvial gradient is associated with a transition in water quality, and the water exiting at the Siapa mouth is dominated by inflow from blackwater streams and lagoons along the lower reaches. The other major Casiquiare tributary is the blackwater Pasimoni, a river that drains sandy alluvial plains lying north of the Neblina formation. From the mouth of the Pasimoni to the junction with the Rio Negro, the Casiquiare has extreme blackwater characteristics (Fig. 9). Thornes (1969) demonstrated a gradual decline in pH values (6.5–4.6) along the longitudinal gradient of the Casiquiare channel (see also Table 1), with small spikes in pH downstream from the mouths of the Pamoni and Siapa, and sharp declines below the mouths of the Pasiba and Pasimoni. Conductance also declined gradually along the river course, with a small spike below the Siapa outflow.

In addition to water quality, other habitat features vary along the fluvial gradient of the Casiquiare River, and these potentially could inhibit dispersal by fishes with narrow habitat requirements. As mentioned previously, the Casiquiare has a very shallow elevational gradient and a meandering course with many broad sandbanks on the inside of bends. Its main channel also includes several shoals, these characterized by fast water passing over granite outcrops and boulders. During the low-water season, these shoals could constitute hydraulic barriers to upstream dispersal by certain kinds of fishes; however, during high-water conditions, the shoals are completely submerged, and river water floods into the riparian forest. Even for the most lentic-adapted fishes, there appear to be no effective hydraulic barriers to upstream or downstream dispersal. Fishes with specialized habitat requirements might have limited dispersal as a result of the patchy nature of the river mosaic, but this seems unlikely. For example, species that seemed restricted mostly to small forest streams during the dry season (e.g. the dwarf cichlids *Laetacara fulvipinnis*, *Dicrossus filamentosus*, *Apistogramma* species) were sometimes captured from the flooded riparian forest during the wet season. Thus, species requirements for structural habitat features are probably less restrictive for dispersal than are those for water physicochemistry.

In addition to the Casiquiare, there are several other sites in the region that are likely to function as portals for fish dispersal between the Orinoco and Amazon basins. One such location is in the vicinity of the towns of Maroa and San Antonio de Yavita, where a tributary of the upper Atabapo River (a north-flowing blackwater tributary of the Orinoco) originates in flat terrain in close proximity to Caño Pimichin, a tributary of the Guainía River (upper Rio Negro system above the Casiquiare junction). Wet-season flooding could permit fishes to move between the two drainages, although it should be noted that the Atabapo has some endemic blackwater species never collected from the Rio Negro (e.g. *Geophagus gottwaldi*, *Uaru fernandezyepezi*). Similarly, within the flat terrain of the Colombian *Llanos*, the Inírida River (Orinoco Basin) is a few kilometres from the headwaters of the Guainía River, and the headwaters of the Guaviare River (Orinoco Basin) are near those of the Upper Vaupes River (Rio Negro Basin).

Inter-basin dispersal of fishes via seasonal portals occurs in other regions of South America. The waters of the Madeira River (Amazon tributary) and Paraguay River (Paraná tributary) unite during the rainy season within the northern Pantanal wetlands of eastern Bolivia and Brazil's Matto Grosso state. In a similar manner, the Rupununi River (Essequibo Basin) of Guyana has a seasonal connection with the Branco River (Negro-Amazon Basin) via the wetlands associated with the headwaters of the Takutu, a Branco tributary, and those of the Rupununi (Lowe-McConnell, 1964). Because much of the landscape of the Casiquiare region is low and flat, it is likely that – for some extended period prior to the stream capture event – there was periodic surface-water contact between the Orinoco and Amazon basins, if not seasonally then during extreme high-water periods. These flood events would have permitted at least a minimal exchange of fishes, perhaps similar to that in the current Rupununi Savannah situation.

Recent studies of population genetics are yielding important new insights into the phylogeography of South American fishes. Comparisons of mitochondrial haplotypes of *Prochilodus rubrotaeniatus* (Turner *et al.*, 2004; Moyer *et al.*, 2005) showed that specimens from the Caroní River, a major blackwater tributary of the lower Orinoco, were sister to *P. mariae*, a species widespread in Orinoco Basin whitewaters. Specimens from the Upper Rio Negro and Casiquiare region were sister to conspecifics from the Essequibo, suggesting inter-basin dispersal via the Rupununi region, and no dispersal to the Upper Orinoco via the Casiquiare. Based on mitochondrial genetic variation, Lovejoy & de Araújo (2000) similarly found that populations of *Potamorrhaphis guianensis* from the Apure River (a major whitewater tributary of the lower Orinoco) were more similar to main-stem Amazon and Lower Rio Negro specimens than they were to specimens from the Upper Orinoco. These authors proposed that an ancient connection between the Orinoco and Amazon through the Rupununi region of present-day Guyana was closed more recently than was the ancient connection through the Casiquiare/Vaupes Arch region.

Willis *et al.* (2007) examined mitochondrial haplotypes of *Cichla* species inhabiting northern South America. *Cichla temensis*, a species common in blackwater tributaries throughout the Casiquiare, Orinoco and Negro rivers, revealed relatively low genetic divergence, which suggests very recent and possibly extensive migration of individuals via the Casiquiare. In contrast, *Cichla orinocensis* specimens from the Orinoco were not closely related to specimens from the Lower Rio Negro (paraphyletic taxon). Specimens from the Lower Rio Negro identified, based on morphology, as *Cichla orinocensis* were haplotypes that were more closely related to *C. ocellaris* (a species distributed in the Essequibo and Upper Branco/Essequibo) and *C. monoculus* (distributed in the

Amazon, Casiquiare, and Upper Orinoco). *Cichla orinocensis* specimens from the Orinoco were sister to *C. intermedia*, a species restricted to the Orinoco Basin. Although inconclusive, these findings indicate a complicated history of isolation, dispersal, and introgression that differs from that of *Cichla temensis*. The two species differ in maximum size and habitat affinities (Jepsen *et al.*, 1997, 1999; Winemiller *et al.*, 1997), and these ecological differences may explain different patterns of phylogeography for species that inhabit the same landscapes. These findings, together with the results of our study on fish assemblage structure throughout the Casiquiare corridor, support Tuomisto's (2007) contention that historical biogeographical reconstructions must consider not only vicariance and dispersal based on physical barriers (e.g. Hubert & Renno, 2006), but also past and present environmental factors influencing species distributions.

The challenge of conserving tropical biodiversity in a world of rapidly growing human populations and economies requires accurate inventories of species and habitats, but also an understanding of the mechanisms that create and maintain biodiversity patterns. The Casiquiare Basin is one of the few remaining tropical wilderness areas, and many watersheds in the region are virtually untouched by modern human development. The species richness of the basin's fluvial freshwater ichthyofauna ranks among the highest on Earth, and protection is currently afforded by the Serrania La Neblina, Parima Tapirapecó, Sierra of Parima and Duida-Marahuaca national parks in Venezuela and by the Pico da Neblina National Park in Brazil, and by federal protection for additional areas occupied by indigenous people. Despite the region's remoteness (at present there are no roads from the outside that extend close to the Casiquiare, or to many areas in the Upper Orinoco and Upper Rio Negro drainages in Venezuela or Brazil), environmental impacts have been observed in recent years. During our expeditions to the region, we noted evidence of overharvesting of large-bodied fishes, such as pimelodid catfish and peacock cichlids (*Cichla* species), by subsistence fishers along the main-stem Casiquiare and the lower reaches of its tributaries. Sport-fishing and ecotourism ventures in the region provide employment for local people without apparent damage to habitats or biodiversity (Winemiller *et al.*, 1997), but these activities have been curtailed in recent years. Gold mining in southern Venezuela has had negative effects on aquatic habitats and fishes (Nico & Taphorn, 1994). In the Siapa River drainage such activities have harmed riparian habitats and increased sedimentation in stream channels, and hunting by the miners apparently reduced local populations of wildlife and fishes (O. Santaella, personal communication).

The Casiquiare River Basin is a truly unique region with respect to the biogeography of a hyperdiverse freshwater fish fauna, and for this reason alone it warrants environmental protection. Similar to the African rift lakes with their remarkable adaptive radiations of cichlid fishes, the Casiquiare's diverse fishes and rich mosaic of aquatic habitats provide a remarkable natural laboratory for the study of ecology and evolution.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available for this article:

Appendix S1 List of fish species in each sub-basin of the Casiquiare region.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2699.2008. 01917.x (this link will take you to the article abstract).

BIOSKETCH

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