Cichlids Model the Role of Riverine Connectivity in Shaping the Biogeography, Diversification, and Population Structure of Fishes in the Guiana Shield, South America
by

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Abstract

Riverine fishes are confined by the hydrology of their river systems and their populations and communities are consequently modeled based on a dendritic network architecture. However, distinct river basins are known to become connected to one another through network-independent erosional processes and through flooding. The connections between adjacent river basins have been shown to allow out-of-network fish dispersal both contemporarily and over evolutionary time, and this is hypothesized to be an important mechanism shaping the diversity of riverine species. However, out-of-network fish dispersal violates the assumed longitudinal within-network dispersal of models, and remains poorly studied, making it a priority in better characterizing intra-network fish population structure and inter-network biogeographic patterns. The Guiana Shield of South America, as a region dominated by river systems, rich fish biodiversity, and a deep history of drainage rearrangements, is an ideal region in which to better understand the influences of inter-basin connections and out-of-network fish dispersal at several scales.

In Chapter II of this dissertation, I analyzed the biogeography of *Geophagus sensu stricto* (Subfamily: Cichlinae) across the Guiana Shield to detect the influences of largescale drainage rearrangements in shaping its evolution. Using a reduced-representation genomic dataset, I produced a resolved phylogeny of *Geophagus*, prioritizing thorough representation from within the Guianas. Using the *Geophagus* phylogeny, I estimated ancestral ranges, and evaluated the importance of hypothesized inter-basin corridors in shaping the diversity of *Geophagus.* Since

the mid-Miocene, drainage rearrangements have allowed *Geophagus* to move (i) into the lower Amazon, (ii) from western tributaries of the Amazon into the Guianas, and (iii) from the Rio Negro to the Orinoco River basin. Inter-basin corridors shaped the relationships of 31 lineages of *Geophagus* identified in this study. Chapter II showcases a spectrum of diversification outcomes for *Geophagus,* including various range expansion events across the Guiana Shield that highlight the importance of drainage rearrangement in shaping biogeography and diversification processes.

In Chapter III, I further investigated out-of-network dispersal in structuring the populations of an upland fish species, *Krobia potaroensis*, in the Pakaraima Mountains of Guyana. Using the same genomic approach as in Chapter II, I determined the genetic relationships of *K. potaroensis* between several interdigitating river systems. The results highlight admixture in the uppermost riverine tributaries, suggesting a recent history connection. Dispersals between the river systems in the Pakaraima Mountains are discussed in the context of current models of riverine population genetics. I conclude existing models of dendritic network population structure should be extended to consider out-of-network dispersal conduits.

Chapter IV further explores out-of-network fish dispersal in the Rupununi Portal of southern Guyana. The Portal has been observed to seasonally flood and allow for inter-basin fish dispersal of some species, while others remain isolated within one system or the other. I determined the genetic population structure of two fishes (*Geophagus* sp. and *Guianacara dacrya*) across the Rupununi Portal and observed inter-basin admixture that corroborates previous observations of fish dispersal. The results indicate that dispersal through ecological corridors shapes population genetic structure and generates recognizable phylogeographic patterns across corridors.

Taken together my dissertation supports the importance of out-of-network dispersal in shaping fish population structure and biogeography on several spatial and temporal scales. A comprehensive understanding of the evolutionary and ecological processes that shape the freshwater biodiversity of the Guiana Shield, and across river systems more broadly, requires further consideration of out-of-network dispersal.

I. General Introduction

Biodiversity is heterogeneously distributed across the globe, reflecting a complex array of biotic and abiotic factors that shape the biogeography and evolution of wild species (MacArthur and Wilson 1967; Simberloff 1974; Lomolino et al. 2006; Futuyma 2009). The number of species extant in a community changes over evolutionary time through processes such as diversification and immigration, which add species, or through local extinction, which decreases the number of species. The biodiversity observed in each region is reflective of those species that were able to disperse into the region, establish themselves, evolve (and sometimes diversify) *in* situ, and persist in sympatry with the local community. Similarly, the dispersal of species modifies how populations are connected within their extant ranges, with population structure existing on a spectrum from panmixia (free dispersal of individuals across their entire range), to sets of much more fragmented populations, which persist as relatively smaller, independent, relatively isolated, and potentially more vulnerable subunits.

In river systems, populations are variously connected along a network depending on the branching dendritic shape of the river system. In riverine fishes, population structure is modeled to depend on longitudinal and disproportionately downstream dispersal within the confines of the river network (Fagan 2002; Finn et al. 2011; Paz-Vinas and Blanchet 2015; Thomaz et al. 2016). However, river networks are not immutable in their architecture and drainage rearrangement is known to change the configurations of river systems over geologic time (Bishop 1995). Additionally, the hydrology of river systems changes seasonally with varied input, such as in tropical regions with large variation in precipitation inputs between the wet and dry seasons. The

changes in connectivity that come with shifting riverine hydrology allow for fish dispersal outside of a river's dendritic configuration. Out-of-network dispersal is an important mechanism connecting contemporary populations through dispersal over floodplains (de Souza et al. 2012, 2020; Stoffels et al. 2016) and in allowing range expansion into novel river systems over evolutionary time (Burridge et al. 2006; Lujan and Armbruster, 2011; Albert et al. 2018a; Waters et al. 2020). However, out-of-network dispersal of fishes remains understudied, and the degree to which inter-basin connections occur between river systems requires further characterization.

The Guiana Shield (GS) is the northern subunit of the Amazon platform in South America, characterized by a series of highland outcroppings of Paleoproterozoic rock that are separated from the southern Brazilian Shield by the Amazon graben (Gibbs and Barron 1993; Lujan and Armbruster 2011). Contemporarily, the river systems of the GS begin in the highlands as low-order riverine tributaries which are often <5 km apart, and variously drain to the mainstems of the Amazon, Orinoco, and Guianas River basins, which are hundreds to thousands of kilometers separated from one-another (Grill et al. 2019). The fishes of the GS region are incredibly biodiverse, but heterogeneously distributed across the GS, with genera either widely distributed throughout the GS, or endemic to subregions or individual river basins (Albert et al. 2011; Birindelli and Sidlauskas 2018; van der Sleen and Albert 2018). The river basins of the GS have seen substantial drainage rearrangements since the Miocene Epoch which would have drastically impacted the evolution of freshwater fishes in the region (Hoorn et al. 2010, 2022; Albert et al. 2018b). The Guiana Shield, as a region dominated by large river systems and a complex history of drainage rearrangements (Gibbs and Barron 1993), is therefore an ideal area in which to investigate how riverine connectivity shapes fish evolution and population connectivity. In this dissertation, I investigated the influences of inter-basin and out-of-network

dispersal on the biogeography and genomic population structure of riverine fishes through analysis of Guiana Shield fishes at several spatial scales.

In Chapter II, I investigated the biogeography of *Geophagus sensu stricto* across the GS Region. I analyzed a reduced-representation genomic dataset for *Geophagus* representing >20 river basins across the GS to first determine the phylogenetic relationships between extant lineages. The phylogeny in Chapter II identified several geographically widespread clades, and 31 distinct lineages. Using the phylogeny, I then used quantitative biogeographic models to reconstruct ancestral ranges for lineages of *Geophagus* to test the influence of hypothesized inter-basin corridors across the GS region through time. I identify and discuss several instances of range expansion and subsequent diversification in *Geophagus* and derive inferences into the varied influence that inter-basin conduits have had in shaping the biogeography of this widely distributed genus of South American fishes in and around the Guiana Shield.

In Chapter III, I analyzed the population structure of a Neotropical cichlid, *Krobia potaroensis*, across interdigitating river systems in the Pakaraima Mountains of western Guyana. Sister lineages of other fishes between adjacent river systems in the Pakaraimas have suggested a history of connections between the interdigitating river systems (Lehmberg et al. 2018; Lujan et al. 2019; Hayes et al. 2020). However, each river system in the Pakaraimas also harbors their own endemic species, highlighting the overall isolation and complex history of these upland systems. In analyzing the population structure of *K. potaroensis*, I detected signals of genetic admixture between the four river systems in the Pakaraimas; particularly at the most upstream sample sites. Additionally, I did not detect the general patterns of genetic diversity and differentiation that are expected based on models of population structure in dendritic networks (Paz-Vinas and Blanchet 2015; Thomaz et al. 2016). I noted that the out-of-network dispersal

that would lead to admixture between upper tributaries violates the assumptions of longitudinal within-network dispersal, and that existing models should be extended to consider alternative dispersal routes where overland conduits may be available.

Chapter IV continued the theme of investigating out-of-network fish dispersal by analyzing the population structure of two cichlids (*Geophagus sp*. and *Guianacara dacrya*) in the Rupununi Portal Region of southern Guyana. The Portal refers to the seasonal transient flooding that occurs and hydrologically connects otherwise distinct tributaries of the Branco River Basin and the Essequibo River Basin (Lowe-McConnell 1964, 1969, 1979). The differing fish communities that are present on either side of the Portal indicate that fish dispersal occurs for a subset of species (de Souza et al. 2012, 2020) and suggests that dispersal may be directionally biased. The genetic analyses in Chapter IV confirm the presence of the Portal as a contemporary dispersal route through the detection of admixture and gene flow. I further discuss the insights that genetic measures provided for the Portal in terms of the directionality of fish dispersal and the relative permeability of the inter-basin conduit when compared to the gene flow from longitudinal within-network dispersal.

This dissertation demonstrates the presence of out-of-network fish dispersal on several spatial and temporal scales. In identifying several previously unknown instances of out-of-network dispersal and demonstrating that population structure consistently deviates from the expectations of within-network models, I highlight the broad influence that out-of-network dispersal has on genetic population structure. Further, the contemporary instances of out-of-network dispersal are discussed as important analogs of the processes that shape regional biogeography over evolutionary time.

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II. Mid-Miocene Range Expansion Through Drainage Rearrangement Shapes the Species Diversity of a Neotropical Fish Genus: *Geophagus* (Subfamily: Cichlinae) in the Guiana Shield

ABSTRACT

The drainage rearrangements of river systems provide a mechanism for fishes to expand their ranges into adjacent river basins and subsequently diversify in isolation from their founding population. While drainage rearrangements have been inferred to shape the sister-species relationships observed in adjacent river systems, the cumulative effects of repeated drainage rearrangements are less understood on regional scales. The Guiana Shield is an ideal region to study the effects of drainage rearrangement on diversification processes and biogeography due to its immense river basins, extreme biodiversity, and complex history of drainage rearrangement. In this study I analyzed the genetic relationships of a widespread freshwater fish genus in the Guiana Shield, *Geophagus sensu stricto* (Family: Cichlidae), to better understand the effects of Miocene to Pleistocene drainage rearrangements in the region. I used a ddRAD approach to sequence thousands of genome-wide loci and resolved the phylogenetic relationships for clades in >20 river basins and sub-basins across the Guiana Shield. I used probabilistic biogeographic methods to model the ancestral ranges for *Geophagus* and test the likely influence of several previously hypothesized river captures. Within the Amazon and Orinoco River basins I infer a history of allopatric speciation followed by range expansion and secondary sympatry that has led to multiple co-occurring *Geophagus* within each subbasin in the region. Conversely, I identified a predominant pattern of one lineage per basin in the Guianas, which formed after the mid-

Miocene expansion of *Geophagus* from the western tributaries of the Amazon into the Guianas. I identify three corridors across the Guiana Shield that facilitated inter-basin range expansion of *Geophagus* between the Amazon, the Orinoco, and the Guianas; with two of the corridors facilitating more than one range expansion event. Further informing taxonomic studies of widespread taxa, several sister-relationships in *Geophagus* span inter-basin corridors, highlighting the complexity of the evolutionary origin of geographically widespread and taxonomically diverse fish clades in regions with long and intricate histories of drainage rearrangement. Further study into those taxa which expanded along similar corridors as *Geophagus*, or were present and unable to use those corridors, will complement our understanding of how freshwater biogeography in the Guiana Shield is shaped by hydrological connections.

INTRODUCTION

The rearrangement of river systems through topographic and erosional shifts over geologic time can have a profound effect on the diversification and biogeography of freshwater species by reconfiguring drainage networks (e.g., river capture), and allowing for range expansion and diversification (Jordan 1905; Bishop 1995; Burridge et al. 2006, 2007; Albert et al. 2018a, b). Range expansions and allopatric speciation in riverine fishes through river captures have been inferred from the observation of sister relationships between neighboring (and contemporarily isolated) systems (Jordan 1905; Nijssen 1970; Lovejoy and de Araujo 2000; Cardoso and Montoya-Burgos 2009; Lujan and Armbruster 2011; Abreu et al. 2020; Argolo et al. 2020; Waters et al. 2020; MacGuigan et al. 2022), and through species-area relationships in regions with historical large-scale river captures (Albert et al. 2018b; Val et al. 2022).

Additionally, clades of Neotropical fishes, such as cichlids (subfamily Cichlinae), are often species rich without substantial ecomorphological divergence within genera (López-Fernández et al. 2012, 2013; Arbour and López-Fernández 2014; Argolo et al. 2020;) suggesting that biogeographic processes, leading to allopatric speciation, may have a large role in the origin of Neotropical freshwater fish diversity at the species level (Albert et al. 2018b; Thomaz and Knowles 2020; Anderson et al. 2023). Despite the instrumental role that inter-basin range expansion may play in shaping freshwater biodiversity, many inter-basin corridors have only been noted for their influence on sister-clade relationships (Nijssen 1970; Lujan and Armbruster 2011; Cardoso and Montoya-Burgos 2009; Lujan et al. 2019; Waters et al. 2020), with fewer studies having evaluated the relative influence of specific hypothesized corridors on widely distributed taxa (but see Fontenelle et al. 2021; Frable et al. 2022; Cassemiro et al. 2023). Determining the relative importance of specific inter-basin corridors in shaping continental biogeography is therefore integral to understanding why certain species are widely distributed, while others remain endemic within smaller distributions.

Biogeographic patterns and species diversity are structured by species-area relationships that modify dispersal and speciation processes (increasing diversity), as well as rates of extinction (decreasing diversity; MacArthur and Wilson 1967; Schluter and Ricklefs 1993; Antonelli et al. 2018). The disproportionate biodiversity of the Neotropics, exemplified by freshwater fishes (Lundberg et al. 2000; Albert and Reis, 2011; Birindelli and Sidlauskas 2018; Albert et al. 2020) and other taxa (Lomolino et al. 2010; Antonelli and Sanmartin 2011; Brown 2014; Couvreur 2015; Pereira 2016; Rull and Carnaval 2020), has been explained under various hypotheses, including those that posit high regional rates of speciation and low rates of extinction (Jablonski et al. 2006), the accumulation of species in large areas over geologic time

(Fine and Ree 2006), or allopatric speciation due to isolation in subregions of the continent (e.g. Wallace 1889; Nores 2002). Specific to the diversity of aquatic taxa, the 'river capture hypothesis' has shown that the capture of river drainages accelerates diversification through the merging of two areas (i.e. 'geodispersal'), and that the past capture of large river drainages is a driving factor of overall riverine biodiversity (Albert et al. 2018b; Val et al. 2022). Understanding the biotic exchange facilitated by river capture may therefore be integral to understanding the disproportionate diversity of fishes in Neotropical rivers.

The link between river capture events, geodispersal, and vicariance results in the phylogenies of aquatic taxa being reflective of past drainage rearrangements. With the advent of probabilistic biogeographic modeling (Ronquist 1997; Ree and Smith 2008; Matzke et al. 2014a), it has become increasingly possible to test competing biogeographic hypotheses, distinguishing among alternative dispersal routes. Applying these probabilistic methods to clarify the complex history of drainage rearrangement (e.g., through ancestral range estimation; Matzke et al. 2014a) is giving new impetus to the study of aquatic taxa in riverine networks (Kim et al. 2020; Hughes et al. 2020; Fontenelle et al. 2021; Frable et al. 2022).

With a hydrology dominated by immense river systems, and a complex history of inter-connection and drainage rearrangement (Gibbs and Barron 1993; Latrubesse et al. 2005; Hoorn et al. 2010, 2022; Albert et al. 2018b), the South American Guiana Shield is an ideal system in which to study how inter-basin corridors affect fish biogeography. The Guiana Shield (GS) constitutes the northern part of the Amazon geologic platform (craton) in South America and is characterized by highlands of Paleoproterozoic rock, which are separated into eastern and western lobes by the Takutu graben in southern Guyana (Gibbs and Barron 1993; Lujan and Armbruster 2011). The uplands of the GS variously drain as tributaries to the Orinoco River

basin, the Amazon River basins, or to the Atlantic-versant River basins of the Guianas. The river basins of the Guiana Shield are hypothesized to have undergone successive large-scale rearrangements in river configuration, the effects of which are thought to have repeatedly influenced range expansion and diversification of freshwater fishes (Sinha 1968; Gibbs and Barron 1993; Lujan and Armbruster 2011; Lujan et al. 2018; Faustino-Fuster et al. 2021).

Several significant connections between the Orinoco, the Amazon, and the Guianas are hypothesized, with relevance to the diversification of freshwater fishes in the Miocene-Pleistocene (Lundberg et al. 1998; Hoorn et al. 2010, 2022; Albert et al. 2018). The Prone-8 model (Lujan and Armbruster 2011) conceptualizes both the contemporary and historical connections in the GS region by noting that hypothesized dispersal routes around the periphery of eastern and western lobes of the GS resemble the number eight, with corridors between (i) the Orinoco and the western Guianas (Lasso et al. 1990; Armbruster and Taphorn 2008), (ii) the Orinoco and the Rio Negro (the Casiquiare Canal corridor; Lovejoy and Araujo 2000; Willis et al. 2007; Winemiller et al. 2008), (iii) the Rio Branco and the western Guianas (the Takutu graben corridor; Lujan 2008; Fontenelle et al. 2021; Frable et al 2022), and (iv) the lower Amazon and eastern Guianas (Nijssen 1970; Cardoso and Montoya-Burgos 2009; Lemopoulos and Covain 2018). While there is evidence for each proposed corridor of the Prone-8 model, the availability and use of inter-basin corridors by widely distributed taxa is not well understood and depend on both the permeability of the corridors to fishes of differing ecologies (e.g., Winemiller et al. 2008, de Souza et al. 2012), and the geologic timing of their availability for dispersal (e.g., Lundberg et al. 1998; Lovejoy et al. 2006; Lujan and Armbruster 2011, Albert and Carvalho 2011; Albert et al. 2018). The phylogenies of widespread Guiana Shield fishes, and analyses of

their biogeographic histories, should reflect the history of relevant corridor connections between river basins.

In this chapter, I analyze the phylogenetic relationships of the genus *Geophagus sensu stricto* (Kullander 1986), a widespread Neotropical cichlid (Subfamily: Cichlinae) in the Guiana Shield Region, to reconstruct the timing and effects of drainage rearrangement on range expansions and diversification. *Geophagus* lineages are present throughout river systems in the Amazon, the Orinoco, and the Guianas (Kullander et al. 2018), and are therefore well-suited to investigating dispersal corridors between these three regions (Lujan and Armbruster 2011 and see above Prone-8 model). Taxonomic knowledge of *Geophagus* lineages remains incomplete, with 22 species currently described and many more noted as requiring formal description (Hauser and López-Fernández 2013; Lucinda et al. 2013 Chuctaya et al. 2022; Ximenes et al. 2022; and see Results/Discussion of this study). Within *Geophagus*, species lacking an infraorbital stripe are recognized to belong within the '*G. surinamensis* complex*'* (López-Fernández and Taphorn 2004), a clade within *Geophagus* which currently contains 16 of the described species (Ximenes et al. 2022; Chuctaya et al. 2022). *Geophagus* species with a complete infraorbital stripe (currently six described species) form a non-monophyletic arrangement informally referred to as the '*argyrostictus* group'.

I used a genome-wide reduced-representation genomic approach, ddRAD (Peterson et al. 2012), to sequence and analyze thousands of loci from across the genome of *Geophagus* lineages and resolve relationships at a population-level resolution. I dated the resulting phylogeny and used the biogeographic modeling software BioGeoBEARS (Matzke 2013) to estimate ancestral ranges for *Geophagus* and compare the likely influence of proposed corridors between Guiana Shield River basins on the current distribution of the genus. Understanding the influence of

proposed corridors in the Guiana Shield on a widespread fish clade will provide critical insight into the ways that drainage rearrangements have contributed to the biogeography and diversity of this region.

METHODS

Sample Collection

Field-collected samples of *Geophagus* from river basins in the Amazon, Orinoco, and Atlantic flowing rivers of the Guianas were gathered between 2008-2018 and supplemented with vouchered specimens from museum collections when possible (Fig. II-1; see table SII-1 for sampling locations and accession numbers). Sampling of *Geophagus* represents the most comprehensive geographic coverage to date from within the Guianas, with between 3 and 13 samples from every major river basin between the Mazaruni and Cuyuni River basins in western Guyana to the Maroni-Marowijne River basin on the eastern border of Suriname (n=83 within the Guianas). Sampling of rivers in French Guiana, the Orinoco and Amazon River basins is less dense, but in combination, sampling covers dozens of sub-basins representing the majority of the Guiana Shield Region (n=171).

Outgroup sampling consisted of the sister clade to *Geophagus*, including three species of *Gymnogeophagus* and one species of the *'Geophagus' steindachneri* clade (see Kullander 1986, López-Fernández et al. 2010; Ilves et al. 2018; Table SII-1). Outgroup sampling was designed to include the crown node for *Gymnogeophagus*, a genus containing the fossil

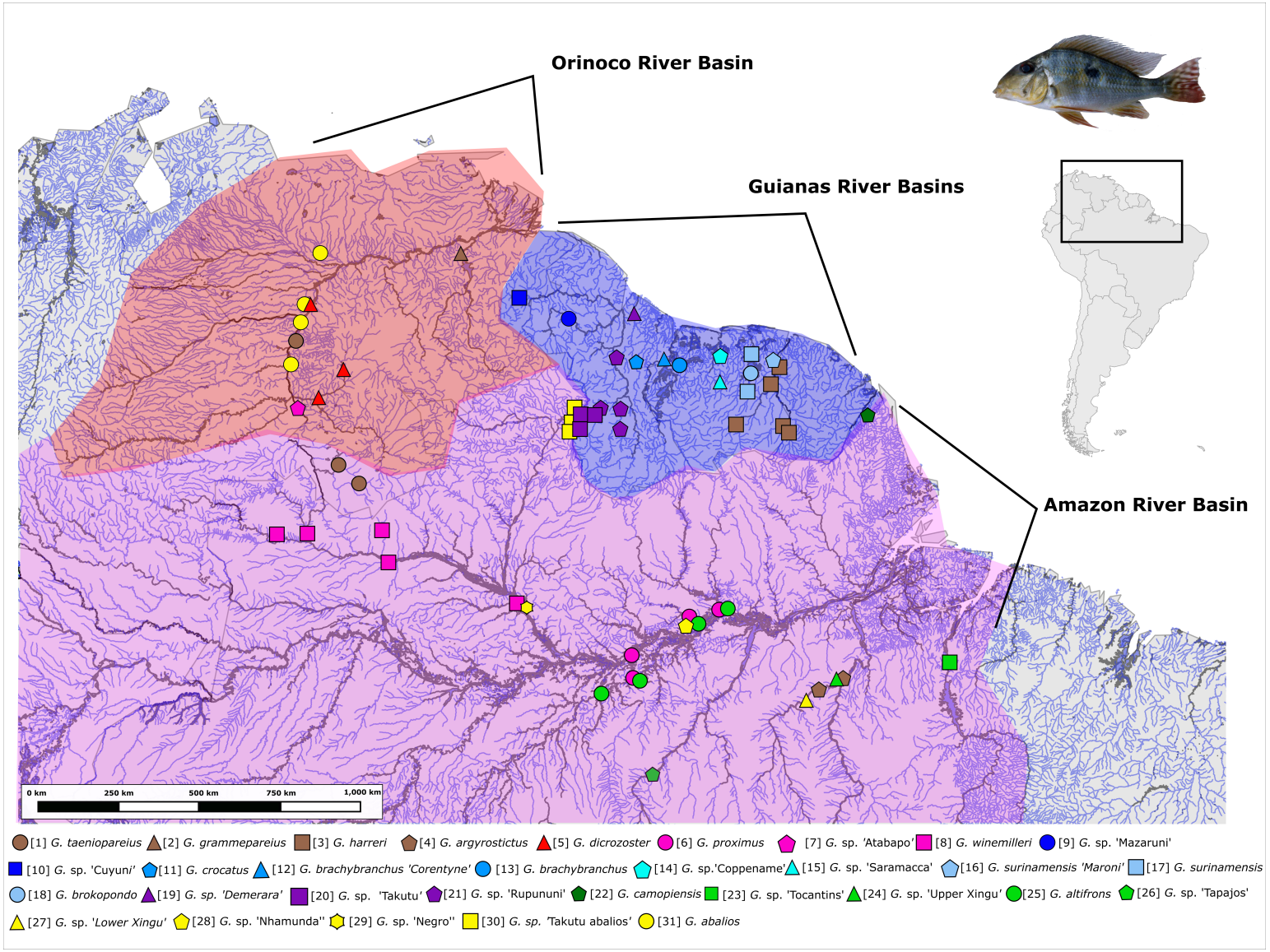


Figure II-1: Map of sampling locations for 163 *Geophagus* from the Amazon-Orinoco-Guianas region of northern South America Photo: *Geophagus dicrozoster*, H. López-Fernández.

†Gymnogeophagus eocenicus, which I also used for age calibration (see below, and Malabarba et al. 2010, 2014).

ddRAD library preparation and sequencing

Genomic DNA was extracted from all tissues using a Qiagen DNeasy Blood and Tissue Kit (Qiagen), with the addition of 35U of RNaseA (Qiagen) and quantified using a Qubit 2.0 fluorometer. Following DNA extraction and quantification, libraries were prepared for 171 *Geophagus*, six *Gymnogeophagus*, and three '*Geophagus*' *steindachneri* following a modified ddRAD protocol (Peterson et al. 2012) developed at the Marine Genomics Lab at the University of Texas, Corpus Christi, and at the Genomics Diversity Lab at the University of Michigan. Briefly, 500ng of DNA was digested for each individual for 16 hours at 37°C using 40 units each of EcoRI-HF and SphI-HF (New England BioLabs Inc). Digested DNA was cleaned using TotalPure NGS beads before ligation of barcoded adaptor sequences. Following ligation, libraries were size-selected on a PippenPrep for fragments between 375 and 525 bp in length. Illumina© adaptors were added using PCR amplification (after Peterson et al. 2012) and libraries were sequenced for paired-end 150 bp reads on either a HiSeq (Genewiz, South Plainfield, NJ) or a Novaseq Illumina sequencing platform (University of Michigan Advanced Genomics Core, Ann Arbor, MI).

ddRAD matrix assembly

RAD sequences were demultiplexed, filtered, and assembled using the *ipyrad* pipeline (version 0.9.63; Eaton and Overcast 2020). Following demultiplexing, eight *Geophagus* and one

Gymnogeophagus were removed due to low read number (<100,000 raw reads). RAD matrices were assembled using default parameters, with a clustering threshold of 0.85 and a minimum sequence depth of 6. To analyze and account for any effects of missing data (Huang and Knowles 2016,) I assembled three matrices with full sequence loci representing three different levels of missing data, with loci present in a minimum of either 41, 82, or 123 individuals.

Tree inference and time-calibration

I inferred phylogenomic trees under Maximum Likelihood (ML) and multi-species coalescent (MSC) approaches for each of the three ddRAD matrices to account for any effects of missing data. Maximum-likelihood trees were built from concatenated matrices using RAxML v8.2.12 (Stamatakis et al. 2014) under a GTR+Γ+I model, and with 1,000 bootstrap replicates to assess node support. To account for potential gene-tree to species-tree discordance (e.g., from incomplete lineage sorting (ILS), hybridization or introgression), trees were also inferred using the multispecies coalescent (MSC) model in SVDQuartets (implemented in PAUP* version 4.0a168; Swofford 2002; Chifman and Kubatko 2014) using all possible quartet combinations and assessed with 100 bootstrap replicates. Topologies derived from all three matrices were identical between all six trees (ML+MSC x 3 matrices) for all *Geophagus* clades at the river basin level (clades as defined in Fig. II-2); all topological differences observed between trees were between individuals within inferred clades (see suppl figures). Subsequent time-calibration and biogeographic analyses were then generated using the ML tree generated from the RAD loci present in at least 82 of 163 individuals (hereafter 'the ML tree').

Ultrametric time-calibrated trees were inferred using two alternative calibrations in *TREEPL*1.0 (Smith and O'Meara 2012) for the ML tree. The first time-calibrated tree

incorporated one secondary (from herein "root") calibration of 51.4 to 67.8 Ma for the crown node of the clade including *Geophagus,* '*Geophagus*' *steindachneri*, and *Gymnogeophagus*; based on the 95% Highest Posterior Density (HPD) tree for the '*Geophagus*' *brasiliensis* to '*Geophagus*' *steindachneri* + *Geophagus* clade in Matschiner et al. (2017; Suppl Fig. II-2). The second time-calibration again incorporated the root calibration from Matschiner et al. (2017) but added †*Gymnogeophagus eocenicus* (Malabarba et al. 2010) as a fossil calibration of the crown age of *Gymnogeophagus* (Malabarba et al. 2014). The age interval of the clade was set to 40.7-54.7 Ma based on the radioisotope dating of the "Faja Verde" layer of the Lumbrera formation in which †*G. eocenicus* was discovered (DeCelles et al. 2011, Malabarba et al. 2010, 2014). Ma. Inferred times of major nodes events within the focal *Geophagus* '*surinamensis*-group' were compared between the two trees, with major differences noted in the text.

Inference of biogeographic histories

I used BioGEoBEARS (Matzke et al. 2013, 2014b) to study the influence of hypothesized inter-basin corridors by inferring the most likely biogeographic histories for the *Geophagus* clade. Ancestral ranges were estimated using all six models implemented in BioGeoBEARS. BioGeoBEARS allows for model comparison using AICc among inferred ancestral distributions by contrasting three common probabilistic models of ancestral range estimation, 'DIVAlike' (after Dispersal-Vicariance Analysis; Ronquist 1997, 1998), 'DEClike' (after the Dispersal Extinction Cladogenesis model; Ree and Smith 2008), and 'BAYEAREAlike' (after Landis et al. 2013). Each of the three compared models allow for estimation of ancestral ranges along the branches of phylogenies, though with differing treatment of anagenetic (i.e., dispersal or extinction) and cladogenetic events (i.e., sympatric speciation or

vicariance). Each of the three principal models are additionally implemented with the probability of founder-event-speciation (the *j* parameter), resulting in six modeled ancestral histories that were compared by AICc. To implement BioGeoBEARS, I pruned each subtree to include one individual in each river of the 31 basin clades supported by at least 95% percent bootstrap values (all but lineage 29 were 100%, see Fig. 2). In two cases, clades with 100% bootstrap support included individuals from multiple basins: (i) *G.* 'Takutu', with individuals from the Rio Branco and the Essequibo River basins (lineage [20], Figs II-1 & II-2), and (ii) *G. abalios* lineages which occur within both the Apure and upper Orinoco rivers, defined as two areas in the analyses (sensu Dagosta and Pinna 2017; lineage [31] Figs II-1 & II-2). Ancestral range estimations were modeled for each of three, nested subtrees of *Geophagus*, which allowed us to observe the effects of different sister-taxon relationships within the genus, which sometimes have widely disjunct extant ranges, on estimates of ancestral distributions among clades. I modeled the ancestral ranges on three subtrees of the wider time-calibrated tree: (i) the entire *Geophagus* tree including the *argyrotictus* and *surinamensis*-groups (Fig. 1, lineages [1 to 31]), (ii) the *Geophagus* '*surinamensis*' group (Fig. 1, lineages [5 to 31], and (iii) only the subtree of *Geophagus* from the 'Guianas-clade' (Fig. 1, lineages [9 to 21]). Geographic ranges for extant *Geophagus* lineages were split into 16 areas delineating the relevant river basins as defined in Dagosta and Pinna (2017; See Fig. 2). The focal groups of interest were the *surinamensis*-group and the Guianas-clade, for which I had greater taxonomic and geographic sampling. When analyzing the Guianas-clade subtree (lineages [9 to 21]), which includes lineages that do not occur across the wider Guiana Shield (i.e. have ranges that exclude the Orinoco and Amazon basins), the river basins of the Guianas were subdivided into nine smaller areas (from five in the

wider analyses) representing smaller local river basins as well as the Rio Branco as a 10th area (see Figs S5 and S6).

To evaluate the influence of proposed Prone-8 corridors, ancestral ranges were estimated in BioGeoBEARS for the three subtrees described above (Fig. II-2 & Figs SII-3 to S2-6). However, I principally focus on *Geophagus* from the *surinamensis*-group (Figs 1 and 2, lineages [5 to 31]) for which I had wide geographic sampling and greater taxonomic representation, and which diversified on the relevant Miocene-Pleistocene timeline of hypothesized major drainage rearrangements. In modeling ancestral ranges, lineages were limited to occupy a maximum of two of the sixteen areas at any given time, representing the maximum range-size of extant lineages, and dispersal was only allowed between adjacent river areas. To test hypotheses of Miocene-Pleistocene dispersal within the Guiana Shield through the putative corridors of the Prone-8 hypothesis (Lujan and Armbruster 2011), as well as the transition of the Miocene Pebas megawetland to the modern configurations of the Amazon, Orinoco, and Guianas river basins (Hoorn et al. 2022), I time-stratified the analyses to allow inter-basin connections starting in the Miocene. In the analyses, prior to 11.8 Ma (Lundberg et al. 1998), the lower Amazon (Fig. 2, areas Ma, I, To, Ta, Tr, and U), the Negro-Branco (Fig. 2, areas N and B), the Guianas (Fig. 2, areas O, M, C, D, and E), and the Orinoco (Fig.2, areas A, UO, and LO) were treated as unconnected, with range expansion only allowed within each region. After 11.8 Ma, I expanded dispersal routes by modifying the BioGeoBears adjacency matrix to allow for dispersal between these areas via newly gained connectivity through the proposed Prone-8 corridors in the Guiana Shield, and by overflowing of the Purus arch, which connected western Amazonia with the lower Amazon and the Negro-Branco regions and isolated the Orinoco from the newly formed Amazon mainstem. To reflect the hypothesized inter-basin corridors I adjusted the area-adjacency matrix

to allow dispersal between (i) the lower Amazon and the eastern Guianas (areas To, Tr, I, and O; Lujan and Armbruster 2011), (ii) the lower Amazon and the Maroni (areas Tr and M; Nijssen 1970; Cardoso and Montoya-Burgos 2009; Lemopoulos and Covain 2018), (iii) the lower Amazon and the Negro-Branco (E, U, and Ma; Lundberg et al. 1998; Albert et al. 2018; Hoorn et al. 2022), (iv) the Branco and the western Guianas (B and E; Sinha 1968; Lujan and Armbruster 2011), (v) the Negro-Branco and the Orinoco (N, B, and UO, and B and LO; Lovejoy and Araujo 2000; Lujan and Armbruster 2011, Winemiller et al. 2008), and (vi) the Orinoco and Guianas (LO and E, Fig. II-2; Lovejoy and Araujo 2000; Lujan and Armbruster 2011); see Fig. II-2.

RESULTS

ddRAD demultiplexing and matrix

Individuals with <100,000 raw reads were removed as failed samples, leaving 171 samples with sequences from 163 *Geophagus*, five *Gymnogeophagus*, and three '*Geophagus*' *steindachneri* with an average of 3,009,819 (SD: 1,992,635) raw reads per individual. The concatenated matrices for loci in at least 41 individuals (6,463 loci, 1,801,886 bp), at least 82 individuals (4,393 loci, 1,268,574 bp), and at least 123 individuals (2,767 loci, 824,395 bp) had 35.6%, 23.2%, and 13.7% missing data respectively.

Tree inference and time calibration

Resulting topologies from Maximum Likelihood and MSC analyses were identical (see Fig. II-1 and Fig SII-1 and SII-2). The *Geophagus* '*surinamensis*-group' (Fig. II-2, lineages [5 to 31] and see López-Fernández and Taphorn, 2004), consisted of the Orinoco species *G. dicrozoster* (Fig.

II-1; lineage [5]) as sister species to all other *surinamensis*-group *Geophagus*. The *surinamensis*-group was, in turn, sister to a paraphyletic set of lineages that I refer to as the *Geophagus* '*argyrostictus*-group' (Fig. 2, lineages [1 to 4], and see López-Fernández and Taphorn 2004).

Within the *surinamensis*-group I recovered four clades I refer to as: Clade A: including *Geophagus proximus, G.* sp. *'*Atabapo'*,* and *G. winemilleri* (Fig. 2, lineages [6 to 8]); Clade B or Guianas Clade: all with distributions limited to basins in Guyana, Suriname and French Guiana (Figs II-1 & II-2, lineages [9 to 21]); Clade C: including *G. camopiensis* and several lineages of *G. 'altifrons' sensu lato* from within sub-basins of the eastern Amazon (Fig. II-2, lineages [22 to 26]); and Clade D, a widespread clade spanning the Orinoco, Negro and Branco river basins, as well as tributaries of the eastern Amazon (Fig. II-2, lineages [23 to 31]).

Inferred node dates were broadly similar between both time-calibrated trees (Fig. II-2, see Fig SII-3 and SII-4 for exact divergence times in both trees), with nodes slightly older in the fossil-calibrated tree. I primarily report and discuss dates for the tree that included the *†Gymnogeophagus eocenicus* crown calibration as it incorporates more information about divergence times than the single secondary calibration chronogram.

Inference of biogeographic histories

Geophagus "surinamensis" clade and Guiana Shield dispersal corridors

In the time-calibrated tree analysis, the common ancestor for clade (A+B) and (C+D) was inferred at approximately 12.7MYA, the common ancestor of Clade A and Clade B (Guianas-group) around 8.7MYA, and the common ancestor of Clade C to Clade D about 10.3MYA (Figs II-3 & SII-3). In ancestral range estimation analyses, the most likely model as determined by AICc was *BAYEAREA+j* for the analysis of the entire *Geophagus* tree (lineages [1 to 31]; Fig.

SII-3). However, the deeper nodes of this tree inferred widely disjunct range that included both Amazonian and Orinoco tributaries (Fig. S2-5); possibly due to the inclusion of the '*argyrostictus*-group' in the analyses, which has less bootstrap support in the tree. I therefore focused on the ancestral range estimation analyses of the *surinamensis*-group tree (Fig. II-2, lineages [5 to 31]), which diversified on the Miocene-timeline that corresponds to the major drainage rearrangement events in the Guiana Shield, and the formation of the contiguous Amazon.

The preferred model in the BioGeoBEARS ancestral reconstruction analysis was *DIVALike+j*, which revealed various distributional expansions in the *G. surinamensis* group between the Miocene and the Pleistocene. Before the Miocene, the ancestral node to clades ((A+B)+(C+D)) is inferred to have an ancestral range in the Rio Negro (or Rio Negro+Branco; Fig. 2); consistent with the hypothesized timing and location of Lake Pebas (Hoorn et al. 2022). After the eastward range expansion that formed clade (C+D), clade (A+B) is estimated to have continued its range around the present-day Negro and Branco Rivers before splitting into a Rio Negro + Rio Branco clade (clade A) and the Guianas-clade (clade B; Fig. 2, node 3). This range expansion is consistent with hypothesized connections between the Guianas and Branco River tributaries in the Miocene and with the hypothesized breakup sequence of the proto-Berbice paleodrainage. The proto-Berbice, a large paleo-fluvial predecessor of modern Guyana river basins, which encompassed much of the drainage area of the modern Branco, is hypothesized to have broken up through a sequence of river captures in the Pliocene to Pleistocene. (Sinha 1968; Gibbs and Barron 1993; Lujan and Armbruster 2011).

The Miocene dissolution of the Purus Arch, facilitating the connection of the Negro to lower Amazonian systems, is inferred to have allowed range expansion of *Geophagus* into the

lower Amazon and the formation of Clade (C+D); estimated as lineages with ranges in Ma and Tr (Fig. II-3, node 1). Further diversification in the lower Amazon subsequently formed clade C (areas Ma and Tr) and clade D (areas O and To). Extant *Geophagus* distributions are therefore the result of a sequence of range expansion events since the late Miocene that can be linked to major drainage rearrangements in the GS (Fig. II-2).

Finer geographic-scale analysis of Geophagus in the Guianas

To see if I could delineate range expansion processes for Clade B at a finer geographic scale than the areas delineated by Dagosta and Pinna (2017), I ran an analysis with the five areas of the Guianas split instead into nine areas corresponding to their individual river basins. However, the finer-scale analysis of Clade B (preferred model *DIVAlike+j*; Fig S2-7) failed to delineate greater detail of the post-Miocene expansion and diversification of *Geophagus* in the Guianas than the broader-scale analyses. In the finer-scale analysis the west to east pattern of range expansion, that was also observed in the broader analysis, persisted. However, the ancestral areas at each node were typically estimated as two-area ranges, and combined to infer ranges if only five areas were still delineated. Also, the finer-scale analyses typically had low confidence, with <50% modeled probability for any areas (Fig S2-7). The inability of the analyses to determine ancestral ranges at the finer geographic scale of each contemporary Guianas basin is likely due to a lack of phylogenetic signal in this part of the tree (Litsios and Salamin 2012). The pattern that emerged was very similar to the broader *surinamensis*-group analysis (with the Guianas subclade B defined within five areas), and I therefore primarily discuss the Guianas in the context of the coarser-geographic scale, that includes the entire *surinamensis*-group (Fig. II-3; clades [5 to 31]).

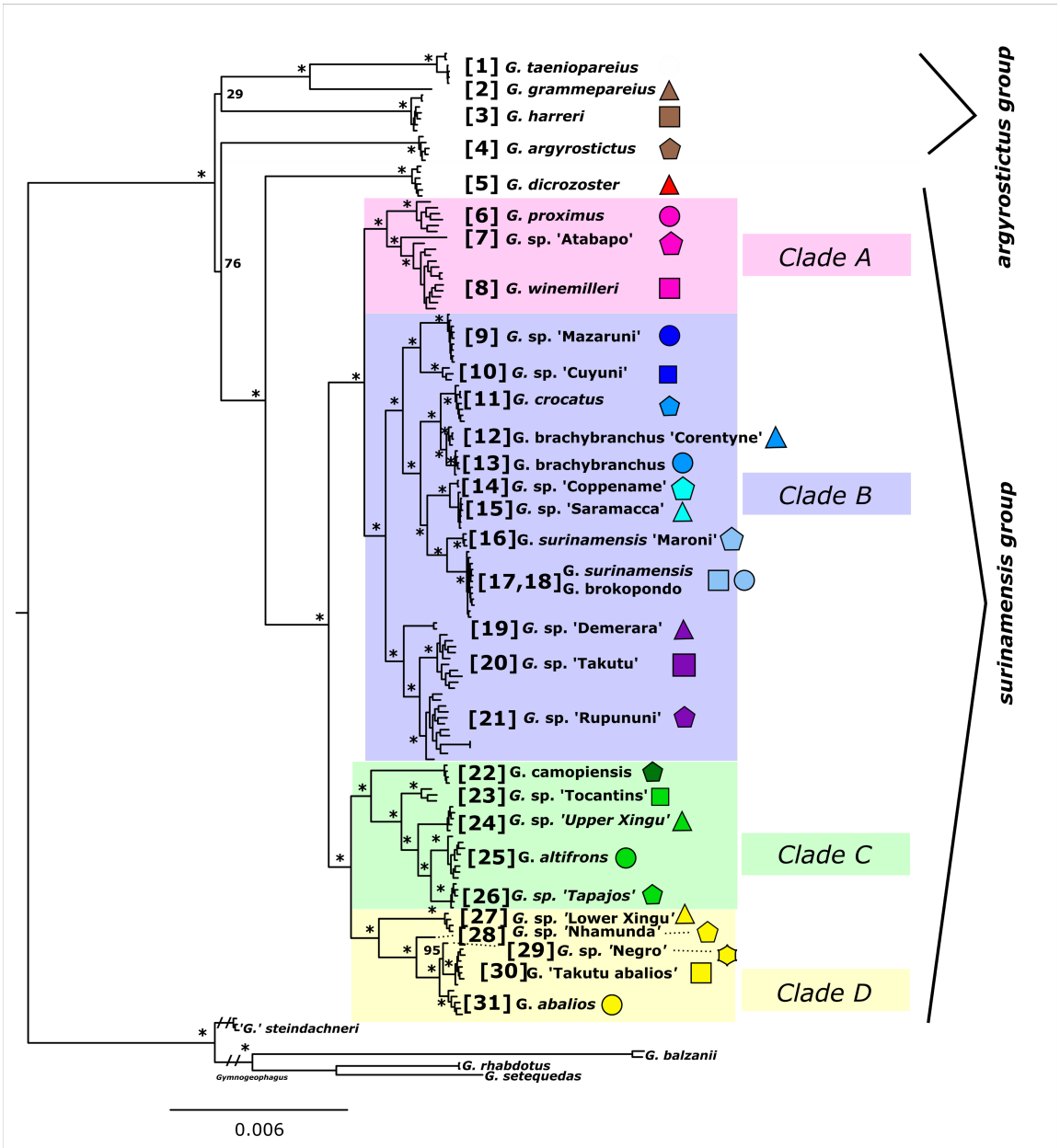


Figure II-2: Maximum-likelihood tree for 163 *Geophagus* from the Amazon-Orinoco-Guianas region of northern South America. The ML tree was generated from a concatenated matrix of 1,268,574 bp representing 4,393 loci present in at least 82 of the individuals. Clades are defined for discussion as Clade A: lineages 6 to 8; Clade B - Guianas clade: lineages 9 to 21; Clade C lineages 22 to 26; Clade D: lineages 27 to 31. All nodes with 100% bootstrap support are denoted with '*'.

Table II-1: Comparison of the six models of ancestral range estimation as implemented and compared in BioGeoBEARS for '*surinamensis group' Geophagus*, (lineages [5-31]), in the Guiana Shield Region of South America

Model	LnL	numparams	d	e	j	AICc	reference
DIVALIKE+J	-74.84	3	0.013	0.013	0.32	156.7	Matzke (2014)
DEC+J	-75.49	3	0.011	0.013	0.4	158	Matzke (2014)
BAYAREALIKE+J	-75.88	3	0.012	0.022	0.29	158.8	Matzke (2014)
DIVALIKE	-90.75	2	0.079	0.059	0	186	Ronquist (1997)
DEC	-97.27	2	0.1	0.11	0	199	Ree and Smith (2008)
BAYAREALIKE	-105.5	2	0.18	0.22	0	215.5	Landis et al. (2013)

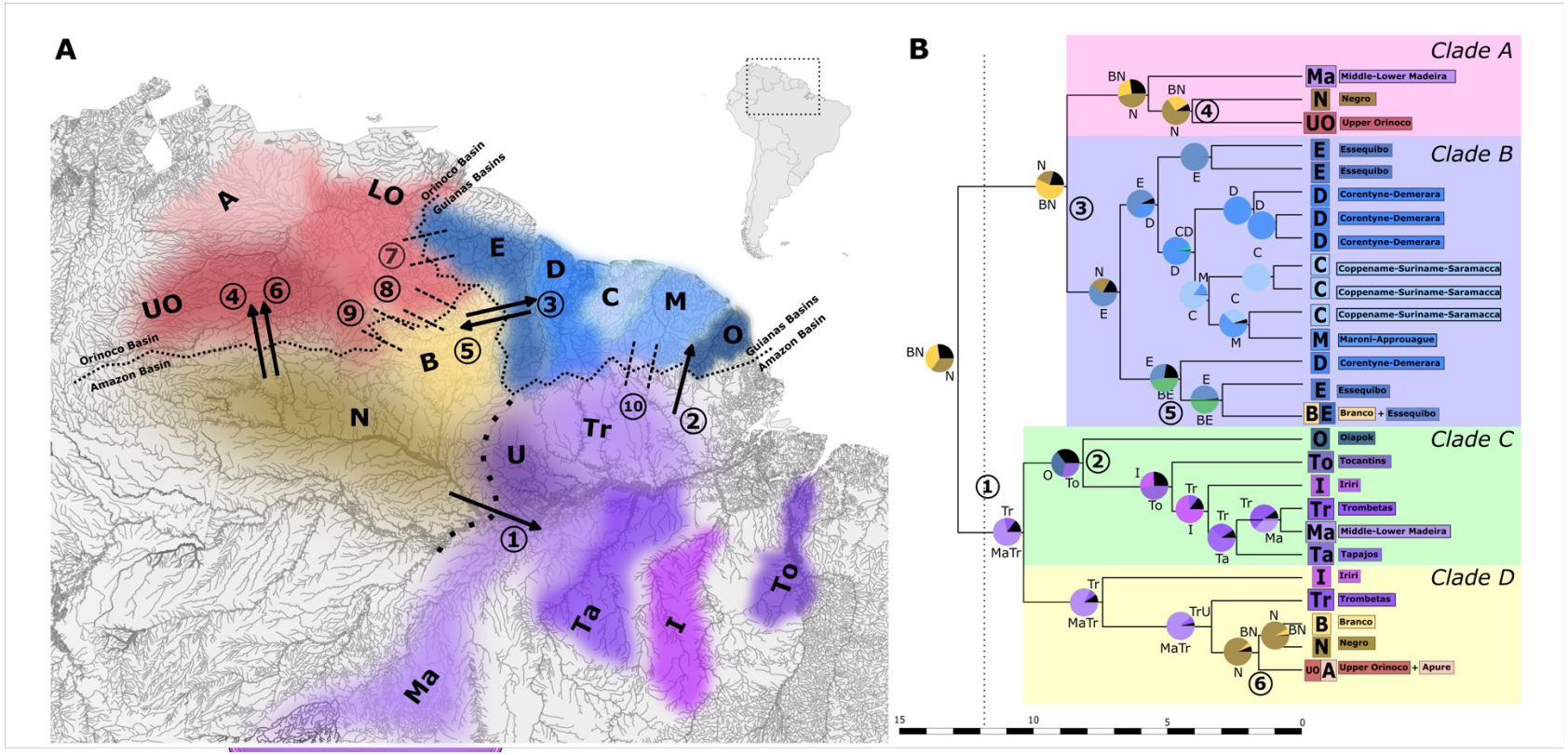


Figure II-3: Ancestral range estimations for *Geophagus sensu stricto* (*surinamensis* group) from the Guiana Shield region of northern South America. A) Map of areas defined in modeling of ancestral ranges (after Dagosta and Pinna 2017). Areas within the Orinoco River basin (red): (UO) Upper Orinoco, (A) Apure River, (LO) Lower Orinoco; Areas within the Amazon River Basin (purple): (N) Rio Negro, (B) Rio Branco, (U) Uatumá River, (Tr) Trombetas River, (Ma) Madeira River, (Ta) Tapajos River, (I) Iriri-Xingu River, (To) Tocantins River; Areas within the Guianas (blue): (E) Essequibo River basin, (D) Corentyne-Demerara, (C) Coppename-Suriname-Saramacca, (M) Maroni-Marowijne River, (O) Oyapock River. B) Ancestral range estimations from mid-Miocene for Geophagus surinamensis group [lineages 5-31] as determined under the DIVAlike+j model in BioGeoBEARS with the two most likely modeled areas at each node represented as proportions (see suppl table for exact values). *Geophagus dicrozoster* (lineage [5]) was included in the analyses but is omitted from the figure due to its split from the rest of the surinamensis group in the early Miocene (est. 20 Ma. suppl. Fig. II-1). Range expansions that are consistent with Miocene-Pleistocene hypotheses of drainage rearrangement: (1) mid-Miocene range expansion from western Amazonia (Lake Pebas) into the lower Amazon following the overflow of the Purus Arch, (2) late-Miocene range expansion between the lower Amazon and eastern Guianas, (3) late-Miocene range expansion into the Guianas from the Negro-Branco, across the Takutu graben, (4) Pliocene range expansion from the Negro to the upper Orinoco River, across the Casiquiare Canal corridor (5) Pliocene range expansion from Guianas back into the Negro-Branco, across the Takutu graben, (6) Pleistocene range expansion from the Negro to the upper Orinoco River, across the Casiquiare Canal corridor. Four further corridors were allowed in the ancestral range estimation but were not observed to have been used: A corridor between the Lower Orinoco and the Essequibo (7), a corridor between the lower Orinoco and Branco (8), a corridor between the upper Orinp and a connection between the lower Amazon and the Maroni-Marowijne (10).

DISCUSSION

The biogeographic history of Geophagus in the Guiana Shield

Geophagus in Lake Pebas and the mid-Miocene formation of the contiguous Amazon

I inferred the origin of *surinamensis*-group *Geophagus* was within western Amazonia, with substantial range expansion and diversification occurring since the mid-Miocene. Eastward range expansion and diversification from the nascent Negro-Branco into the lower Amazon is consistent with the breakdown of the Purus arch and the dissolution of Lake Pebas (Hoorn et al. 1995, 2010, 2022; Albert et al. 2018b). Range expansion and diversification of *Geophagus* then continued through the Pliocene-Pleistocene to form the extant lineages observed across the Guiana Shield (Fig. 2).

The mid-Miocene presence of *Geophagus* in western Amazonia is consistent with the timing and location of Lake Pebas. Reaching its maximum extent between 17 and 15Ma. (Hoorn et al. 2022), Lake Pebas was a large wetland system that dominated the sub-Andean area of western Amazonia from the early to mid-Miocene (Hoorn et al. 2010, 2022; Albert et al. 2018). The mid-Miocene then saw the dissolution of Lake Pebas into eastern and western fluvial precursors of the modern Amazon River; which drained most of the area now defined by the modern Amazon basin (Horton 2018; Albert et al. 2018; Hoorn et al. 2022). The western proto-Amazonian Basin drained the Putumayo, Napo, Marañón and Ucayali sedimentary basins (much of the area now defined as the western Amazon and Negro River), and drained northwest to the Atlantic Ocean through the Llanos and Apure sedimentary basins (contiguous with portions of the modern Orinoco basin; Albert et al. 2018). The eastern (and east-flowing) portions of the proto-Amazon drained the Amazonas and Marajo sedimentary basins and were separated from

the northwestern-flowing portion of the proto-Amazon by the Purus Arch (Lundberg et al. 1998; Hoorn et al. 2010, 2022; Albert et al. 2018; Fig. 2). Approximately 10 Ma. the rise of the Vaupes arch, separating the Llanos from the western Amazon, and the overflowing of the Purus arch, allowed for the connection of eastern and western proto-Amazonian subbasins, and precipitated the formation of the modern basins of the Guiana Shield Region (Lundberg et al. 1998; Albert et al. 2018; Hoorn et al. 2022). The overflowing of the Purus arch allowed *Geophagus* to expand its range from the Negro-Branco into the lower Amazon with the formation of the contiguous Amazon (estimated at 10.3 Ma. in my analyses; Clade C+D; Fig. II-3).

After the breakdown of the Purus Arch, three inter-basin corridors between the Amazon, Orinoco, and Guianas are inferred to have allowed further range expansion and diversification of *Geophagus* (Fig. II-3, (i) 2, (ii)3+5, and (iii) 4+6). First, eastern Amazonian tributaries are estimated to have been connected to the Oyapock River basin in the middle Miocene, allowing range expansion of *Geophagus* into the eastern Guianas from eastern (Fig. II-3, dispersal 2). However, this range expansion only resulted in one eastern lineage of *Geophagus* in my analyses (*G. camopiensis*; lineage [22]). A second corridor allowed two separate events of range expansion between the Branco and the Essequibo River basins into western Guyana. These connections are inferred to have occurred in the middle Miocene (establishing Clade B; Fig. II-2 and II-3 lineages [9 to 21]) and subsequently allowing Clade B to expand its range back into Branco tributaries in the Pleistocene (lineage [20]). Finally, a third corridor allowed two range expansions from the Negro River into the upper Orinoco, lineages [7] and [31] (Figs II-2 and II-3); in the Pliocene and Pleistocene respectively.

In several cases, range expansions into previously unoccupied range lead to diversification in *Geophagus.* In Clades C and D, the analyses identified 13 lineages that

emerged after range expansion into the lower Amazon from western Amazonia, including several described species (Fig. II-2 and II-3; and see appendix A). However, the ancestral range estimations did not unequivocally recover which specific subbasins were the likely ancestral ranges (i.e., the Tocantins, the Iriri-Xingu, the Madeira, or the Tapajos River basin; Fig. II-2).

I found strong evidence that the breakdown of the Purus Arch and formation of the contiguous Amazon allowed for range expansion of *Geophagus* that led to 13 extant lineages in the lower Amazon. Further, the major drainage rearrangements that started in the mid-Miocene continued to facilitate inter-basin range expansions and have led to the widespread distribution of *Geophagus* lineages*,* including a diverse clade in the Guianas (Clade B; further discussed below).

The ancestral ranges at the sub-basin level were not always discernible in my analyses. The ranges of extant *Geophagus* are themselves variable and obscured by the incomplete taxonomy of this genus. Extant *Geophagus* species have been variably described to occupy wide distributions within several basins (Bloch 1792; Kullander 1986), or more limited distributions within individual tributaries (Kullander and Nijssen 1989; Lucinda et al. 2013; Chuctaya et al. 2022). As taxonomic and biogeographic work on the genus expands, the trend has been for the recognition of larger numbers of taxa with increasingly basin-specific distributions, particularly within the '*surinamensis*-group' (Kullander et al. 1992; Hauser and López-Fernández 2013; Ximenes et al. 2022; this study). While additional taxonomic sampling of *Geophagus* from subbasins of the lower Amazon may aid in clarifying the sequence of range-expansion within this region, sister-lineages of lower-Amazonian *Geophagus* are identified in this study (Fig. 1) from as far away as the Negro River (lineage [8]), Orinoco River basin (Fig. II-2, lineages [7 & 31]) and eastern Guianas (lineage [22]), highlighting that modeling fine-scale biogeographic

histories may require denser sampling and more comprehensive regional analyses. The uncertainties I observed in the lower-Amazonian part of the tree (Clades C+D; Figs II-1 & II-2) may be due to incomplete taxonomic and geographic sampling in this part of the tree.

***Geophagus* range expansion and the origin of the Guianas Clade**

Divergence from the ancestrally distributed Lake Pebas *Geophagus* into the lower Amazon (Clade C+D) and the Rio Negro-Branco (Clade A+B), ultimately led to further diversification in the latter, starting a prolonged period of further dispersal into the Guianas. A late Miocene range expansion (est. 8.7 Ma.; Fig. 2) of *Geophagus* across the Takutu graben into the western Guianas (from the Branco to the Essequibo River basin; Fig. 2) resulted in the establishment of *Geophagus* in the Guianas (Guianas Clade, Clade B; Fig. 1 and 2). Range expansion corridors in the Takutu graben have been previously discussed in the context of a hypothesized role for the Pliocene-Pleistocene breakup of the proto-Berbice paleodrainage (Sinha 1968; Gibbs and Barron 1993; Lujan and Armbruster 2011), and for the contemporary seasonal connections between the upper-Branco and the Essequibo basins through the Rupununi Portal (Lujan and Armbruster 2011; De Souza et al. 2011, 2020).

The proto-Berbice hypothesis provides a historical mechanism for possible expansion of western Amazonian taxa into the Guianas through the Takutu graben (Sinha 1968; Gibbs and Barron 1993; Lujan 2008; Lujan and Armbruster 2011; Albert et al. 2018). The proto-Berbice was a large Atlantic-flowing fluvial system that drained much of the western Guianas region and emptied into the Atlantic near the modern-day Berbice river (Sinha 1968; Lujan and Armbruster 2011; Stokes et al. 2018; Fig. 2). The breakup of the proto-Berbice is thought to have occurred in the Pliocene-Pleistocene, through successive river captures, by the nascent eastern Amazon, of southern tributaries of the proto-Berbice near the expanding headwaters of what would become

the modern Rio Branco (Sinha 1968; McConnell 1959, 1968; Crawford 1985; Gibbs and Barron 1993; Lujan and Armbruster 2011; Stokes et al. 2018). Sister-group relationships between Negro-Branco lineages and those in southern Guyana have been highlighted as congruent with the faunal exchanges presumably facilitated by river captures associated with the breakup of the proto-Berbice (e.g. crocodilians: Bittencourt et al. 2019; Roberto et al. 2020; fishes: Lujan 2008; Fontenelle et al. 2021; Frable et al. 2022), although the timing and extent of drainage rearrangements in the region remain under ongoing study (Lujan and Armbruster 2011; Stokes et al. 2018). The inferred timing of range expansion across the Takutu graben predates currently proposed Pliocene-Pleistocene breakup of the proto-Berbice (i.e., 8.7 Ma in this study vs. 4 to 6 Ma, Fontanelle et al. 2021; or 2.3 to 3.2 Ma, Frable et al. 2022).

Beyond the time of initial entry of *Geophagus* into the Guianas, once established in the Essequibo, successive range expansions between the adjacent river basins in the Guianas appears to have started a pattern of eastward dispersal that resulted in one *surinamensis*-group *Geophagus* lineage in each of the Atlantic flowing river basins (lineages [9 to 21]; Fig. 1 and 2). Early Pliocene range expansion presumably first saw dispersal north, from the Essequibo to what are today the Mazaruni, Cuyuni and Demerara Rivers and east into the modern Berbice and into the Corentyne-Nickerie river basins (Fig. 2). Range expansion then continued east, most likely through headwater capture events around the modern Sipaliwini region of southwestern Suriname (Nijssen 1970), through the Late Pliocene and Pleistocene as *Geophagus* lineages expanded into today's Coppename, Saramacca, Suriname and Maroni-Marowijne River basins. The phylogenetic analyses highlight close associations between (i) the Takutu, Rupununi, and Essequibo Rivers (Fig. II-2, lineages [20 & 21]) (ii) the Berbice, Corentyne, and Nickerie Rivers (Fig. II-2, lineages [11, 12, & 13]), (iii) the Coppename and Saramacca Rivers (Fig. II-2,

lineages [14 & 15]), and (iv) the Suriname and Maroni-Marowijne Rivers (Fig. II-2, lineages [16, 17, & 18]), that warrant further taxonomic investigation (see Fig. 1).

The Casiquiare Canal: Geophagus range expansion between the Negro River and Orinoco River Basin

The Casiquiare Canal connection, in western Amazonia, is also apparent in my analyses, having allowed for range expansion of *Geophagus* between the Rio Negro and the Orinoco River basin (Fig. 2, nodes 4 and 6). The Casiquiare Canal is a contemporary example of ongoing river capture, with the upper Orinoco River bifurcating and diverting a portion of its flow to the Negro River. Over geologic time, the upper tributaries of the Orinoco (the Mavaca and Ocamo Rivers) are expected to be captured and become part of to the Negro River (Winemiller et al. 2008; Winemiller and Willis 2011).

Similar to the connections across the Takutu graben, *Geophagus* has dispersed across the Casiquiare corridor connection at least twice (est. 4.0 Ma. and 1.6 Ma. for lineages [7 & 8] and [29 to 31] respectively; Figs II-1 & II-2). Previous studies on the Casiquiare Canal have shown at least two range expansions in species of peacock bass cichlids (*Cichla spp.*; Willis et al. 2010), potamotrygonid stingrays (Fontenelle et al. 2021), and prochilodontid characiforms (Frable et al. 2022), consistent with what I observed in *Geophagus*. The Casiquiare Canal region therefore represents another instance where one area appears to have influenced several historical inter-basin range expansions through time, leading to historical diversification while maintaining a current connection that both informs modern processes and serves as a contemporary analog of past dispersal events.

The Guiana Shield Region as a model system for understanding riverine biogeography

That multiple inter-basin corridors are hypothesized to have connected river basins in the Guiana Shield provided a framework under which to investigate how *Geophagus* lineages came to be widely distributed and species-rich in the region (Nijssen 1970; Hoorn et al. 1995, 2010, 2022; Lovejoy and Araujo 2000; Lujan and Armbruster 2011; Albert et al. 2018; Fontenelle et al. 2022; Frable et al. 2022). My analyses demonstrate repeated instances of range expansion between upland tributaries, and corroborate several previously hypothesized corridors connecting the Amazon, the Orinoco, and the Guianas. Specifically, I determined that *Geophagus* expanded its range across connections between (i) the lower Amazon and eastern Guianas, (ii) the Rio Branco and the western Guianas, and (iii) the Rio Negro and the upper Orinoco (Fig. 2). Range expansion in the Miocene due to major drainage rearrangement, likely followed by periods of allopatry, allowed for the diversification of *Geophagus* into dozens of novel lineages (Figs II-1 & II-2). Clades of *Geophagus* that first diversified in the Miocene are modeled to have spread to much of their distributions in the Pliocene to Pleistocene, suggesting that secondary sympatry has led to observed patterns of co-distributed non-sister clades across the greater Orinoco and Amazon basins. The analyses in this study highlight several instances where the same inter-basin conduits facilitated multiple range expansions. Given the limited number of taxa examined to date and the inherent uncertainty in dating molecular phylogenies, analyses of additional taxa, as well as incorporating additional primary dating evidence (e.g., fossils when available), are needed to further clarify the timing and the number of instances in which drainage rearrangement facilitated connections between the Amazon and the GS through the Miocene-Pliocene period.

Contemporary connections between the Rupununi and Takutu Rivers (across the Rupununi Portal; de Souza 2012, 2020), and between the Negro and upper Orinoco (across the

Casiquiare Canal, Winemiller et al. 2008a; Winemiller and Willis 2011) are also apparent in my analyses (lineages [20 & 21 for the Rupununi]; and lineages [7 & 8] and lineages [29 & 30] for the Casiquiare; Figs II-1 & II-2), with extant clades sister to one another, and the additional inclusion of one Rupununi-caught *Geophagus* grouping within the Takutu-clade (Figs II-1 & II-2, lineage 20). Despite the contemporary hydrologic connections, differing fish communities are present on either side of these inter-basin conduits, with only a subset of species occurring across basins, in both systems (De Souza et al. 2012, 2020; Winemiller et al. 2008; Winemiller and Willis 2011). The Casiquiare has been noted for its connecting two systems with differing water chemistries: clearwater (the Orinoco River) and blackwater (the Negro River). Further investigation into potential abiotic and biotic filters across the Rupununi and Casiquiare (e.g., physicochemical tolerances; Willis et al. 2022) will help inform why some species are (or were) able to disperse and become widespread across inter-basin conduits, leading to some of the biogeographic patterns I unveiled in this study.

CONCLUSION

The widespread distribution of *Geophagus* and its history of range expansion between otherwise distinct river basins highlights the complexity of Guiana Shield fish biogeography. I identified 31 lineages within *Geophagus*, including many potentially requiring formal description, and demonstrated that much of this diversity arose directly following establishment of novel ranges through inter-basin corridors. In line with previous studies of GS fishes (Fontenelle et al. 2021; Frable et al. 2022), I observe drainage rearrangement as a critical process in shaping the biogeography and diversification of Guiana Shield cichlids, even when substantial

morphological differences aren't present or immediately apparent (López-Fernández et al. 2013; Arbour and López-Fernández 2014; Argolo et al. 2020).

The evolution of riverine taxa is increasingly elucidated by the lens of genomic and biogeographic analyses. The complex history of drainage rearrangements in the Guiana Shield is emblematic of how range expansions can stimulate diversification across space and time. A better understanding of the connectivity of river systems, both historically and contemporarily, is critical to the accurate characterization of freshwater fish evolution and ecology, and to the conservation and management of freshwater biodiversity (Bernos et al. 2020). This study further builds on a framework whereby the biogeographic histories of individual clades are highlighting hydrogeologic events with widespread influence on the historical biogeography and evolution of Neotropical freshwater taxa. Further investigation into which species were present, and either able or unable, to expand their ranges across inter-basin corridors will greatly improve our understanding of the biodiversity and endemism in the Neotropics.

III. Dispersal Across Headwaters Determines Fish Population Structure Between Interdigitating River Systems in the Guiana Shield Highlands

ABSTRACT

Riverine species find themselves principally confined to the dendritic networks that define river basins. Models have emerged to generalize the expectations of population structure for riverine aquatic taxa, such as freshwater fishes, but these models typically assume longitudinal within-network dispersal. However, rivers are not immutable in their configurations, and hydrologic connections change both seasonally and over geologic time. The degree to which hydrologic changes to riverine configurations allow for 'out-of-network' (i.e., overland) dispersal should be investigated, as such dispersal may affect population structure and alter the assumptions that inform current models of riverine population structure. To investigate the potential out-of-network connections between interdigitating river systems, I analyzed the genetic population structure of *Krobia potaroensis*, a cichlid fish endemic to the river systems of the Pakaraima Mountains in western Guyana, South America. I observe genetic associations between populations which do not align with contemporary river configurations, highlighting that dispersal can and has occurred between adjacent river systems. That out-of-network dispersal can occur between river systems indicates that longitudinal dispersal within-network cannot always be assumed, and that the effects that alternative dispersal routes have on population structure, genetic diversity, and genetic differentiation should be further investigated.

INTRODUCTION

Population connectivity and genetic diversity can critically influence the ecology, evolution, and persistence of species (Gilpin 1987). Genetic diversity, and how it is distributed within a heterogenous spatial environment, affect the fitness and resilience of populations and have long been incorporated into conservation planning (Gilpin and Soulé 1986; Moritz 1994; Allendorf et al. 2010; DeWoody et al. 2020). Additionally, the distribution and evolutionary trajectories of species can be highly dependent on their ability to disperse across the landscape, whether between subpopulations of the established species distribution, or to novel systems to establish a wider range (e.g., range expansion). The fragmentation of populations can also lead to disconnected and smaller subpopulations which can be more likely to differentiate in allopatry or to become locally extinct causing either extirpation or range contraction. Therefore, the connectivity of populations, both contemporarily and over geologic or evolutionary time, is critically important to the evolution and persistence of natural populations.

For riverine taxa such as fishes, river network architecture structures the connectivity of populations on contemporary timescales (Vannote et al. 1980; Meffe & Vrijenhoek, 1988; Fagan 2002; Labonne et al. 2008; Paz-Vinas et al. 2015; Thomaz et al. 2016; Blanchet et al. 2020), and shapes species distributions on macroevolutionary timescales (Gilbert 1980; Lundberg et al. 1998; Albert et al. 2011). Several models of rivers as dendritic networks have been developed to describe the varying connectivity and structuring of metapopulations from small upland tributaries down to large river mainstems (Fagan 2002; Labonne et al. 2008; Altermatt 2013; Paz-Vinas and Blanchet 2015; Thomaz et al. 2016; Tonkin et al. 2018). Breaks in river network connectivity act as barriers to dispersal within river systems or between river basins, making them a defining factor governing regional freshwater fish biogeography (Gilbert 1980). Further

extensions on the dendritic model of rivers have demonstrated that both resource and habitat availability change based on riverine architecture, and that biodiversity and species abundance are consequently modified along a river's branches and at its confluences (Bernhardt et al. 2005; Campbell Grant et al. 2007; Paz-Vinas et al. 2015; Shao et al. 2019). Increased habitat availability and habitat heterogeneity introduced in the downstream branches of river networks and at river confluences (or nodes) result in greater downstream genetic and species diversity (α-diversity, Vannote et al. 1980; Paz-Vinas et al.2015; Thomaz et al. 2016). Correspondingly, more isolated upstream systems are hypothesized to have lower genetic and α-diversity, and higher genetic differentiation than downstream reaches.

The varied structuring of fish communities and populations within river systems highlights the differing but critical influence of river network connectivity on regional biodiversity in upstream and downstream river reaches. Greater biomass and overall biodiversity are predicted and observed within the large mainstem systems of river basins (Vannote et al. 1980; Matthews 1986). However, upstream and headwater systems are also critical to regional biodiversity as they constitute most of the length of river systems (typically >70% of total river length; Leopold 1964), often contain upstream-endemic species, and thus contribute substantially to β-diversity within rivers (Finn et al. 2011).

Existing models of riverine population structure (e.g., Paz-Vinas et al. 2015; Thomaz et al. 2016) typically consider single dendritic networks, with dispersal assumed to occur only within the confines of the river embankments ('within-network' and 'intra-system'; see Box III-1). However, hydrologic connections between adjacent river systems ('inter-system') have been documented to allow some fish species to disperse between basins, connecting populations either contemporarily (e.g., Willis et al. 2010; de Souza et al. 2012, 2020; Stoffels et al. 2016) or

historically (e.g., Burridge et al. 2006; Lujan and Armbruster 2011; Thomaz and Knowles 2020; Waters et al. 2020). Expanding models of riverine population structure to appropriately consider out-of-network and inter-system dispersal requires an understanding of when and how aquatic organisms can use inter-network conduits.

Accounting for inter-network connectivity is particularly important in biogeography because river basins are not immutable in their architecture and are known to change their configurations over both ecological (e.g., transient flooding) and geological time (e.g., river capture). Such processes redefine the catchments of river basins and the configuration of upland tributaries, ultimately changing the downstream systems to which they flow (Bishop 1995). River systems may also change their flow seasonally (e.g., due to wet-season flooding/inundation) augmenting the dispersal environment within and between river systems (e.g., rapids and waterfalls being inundated or flooded forest dispersal; see Box III-1 for terms).

Analyzing instances where inter-system (and therefore out-of-network and overland) dispersal of fishes may have occurred requires sampling adjacent river systems at a regional scale, to encompass substantial proportions and the broader distribution of the focal populations. However, empirical studies of genetic population structure in rivers have principally focused on smaller geographic scales, such as subsets of river systems or individual sampling units (Shao et al. 2019); both of which would limit or preclude observation of inter-system dispersal. Further studies of species that are distributed across adjacent and interdigitating river systems are therefore critically important to inform our understanding of how overland dispersal conduits affect population distribution and structure. Differentiating the instances where out-of-network dispersal has occurred, or identifying the circumstances under which species follow models of single dendritic networks (dispersing only within-network), is critical to understand the

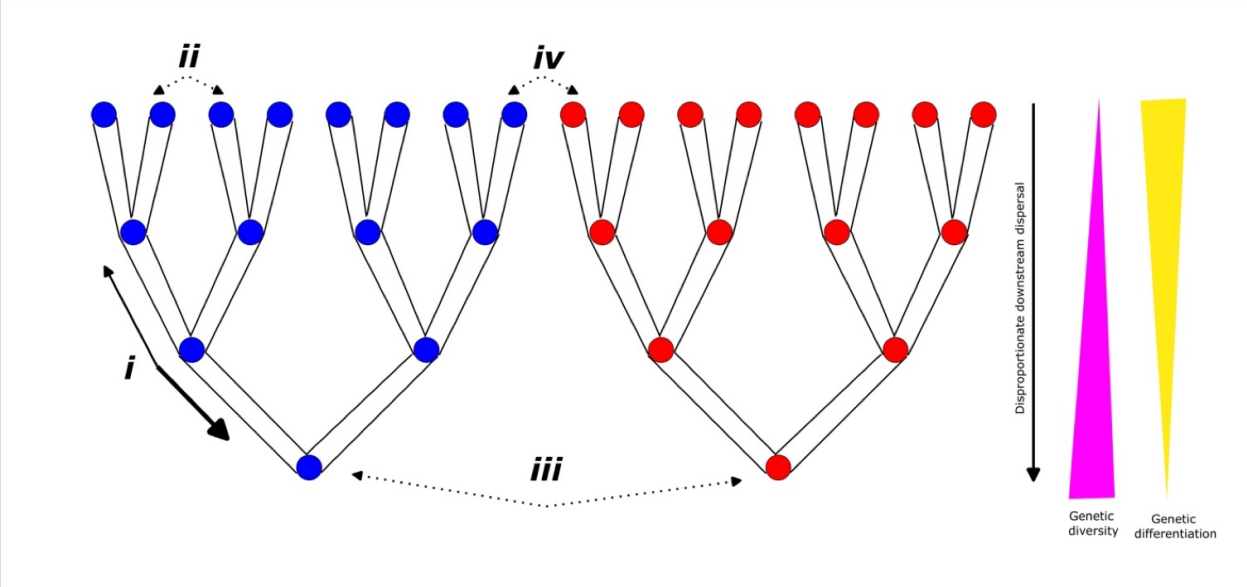
evolutionary and biogeographic history of riverine species, as well as to their conservation and management through correct delineation of population boundaries.

The Pakaraima Mountains Region in Guyana (Fig. 1) is an ideal area to study many of the aspects of riverine architecture that affect population structure. The river systems of the Pakaraimas (the upper Mazaruni, the upper Ireng, the upper Kuribrong and the upper Potaro Rivers), are all separated from lower portions of their containing river basins by large waterfalls, and consequently hold distinct uplands fish communities (Hardman et al. 2002; Alofs et al. 2014; Taphorn et al. 2017). Each of the four river systems of the Pakaraimas harbors a high proportion of endemic fishes, with species either isolated within individual river systems (e.g., 65-95% endemic fish species within the upper Mazaruni; Alofs et al. 2014), or as species that, as currently understood, are distributed throughout the upper tributaries of the Pakaraimas (e.g., *Krobia potaroensis*, Eigenmann 1912; *Gymnotus carapo*, Lehmberg et al. 2018; or *Trichomycterus cf. guianensis*, Hayes et al. 2020). Where species are not endemic to the Pakaraimas river systems, the waterfalls that separate the upper tributaries from lower reaches still represent a substantial delineating barrier from the fish communities of the lowlands (Hardman et al. 2002).

Fish dispersal, as facilitated by both intra-system and inter-system hydrologic connectivity, can be better understood by analyzing the population structure of species that exist among multiple adjacent river networks. *Krobia potaroensis* (= *'Aequidens' potaroensis*; Eigenmann 1912,) is a species of Neotropical cichlid (Subfamily: Cichlinae) putatively found throughout the river systems of the Pakaraimas. Originally described as *'Aequidens' potaroensis* (Eigenmann 1912), more recent descriptive studies have noted that *'A'. potaroensis* (along with *'A' paloemeuensis*) is intermediate in morphologic features between true *Aequidens* and the

genus *Krobia* (Kullander and Nijssen 1989). Subsequent molecular phylogenies have supported that *'A' potaroensis* belongs within a clade that includes lowland *Krobia* (and sister to other *Aequidens*; Musilová et al. 2009; López-Fernández et al. 2010; Ilves 2018), I therefore follow those authors and refer to the species as *Krobia potaroensis* throughout this paper. The presence of putative *K. potaroensis* in all four river systems of the Pakaraimas contrasts with the pattern of many species in the region which typically occur within a subset of the river systems (e.g., *Mazarunia*, López-Fernández et al. 2012; *Akawaio penak*, Maldonado-Ocampo et al. 2014; *Yaluwak primus*; Lujan et al. 2019; *Trichomycterus*, Hayes et al. 2020). *Krobia potaroensis* therefore serves as a useful model species for understanding whether (and how) the interdigitation of the Pakaraimas river systems (with tributaries <5km from one another; Grill et al. 2019) contributes to dispersal between these otherwise isolated systems.

To analyze the population structure and connectivity of *K. potaroensis* in the interdigitating river systems of the Pakarimas I used a ddRAD approach (Peterson et al. 2012) to sequence thousands of genetic loci from individuals within each of the four river systems. I used this dataset to ask: (i) Do putative *K. potaroensis* from throughout the Pakaraimas belong to a single clade? And (ii) What is the genetic population structure within and between the river systems? Answering these questions by characterizing the degree of divergence, or of ongoing gene flow, between populations of *K. potaroensis* will inform critical improvements to contemporary models of riverine genetic population, and additionally elucidate the processes that lead to multi-basin distributions of freshwater fishes on biogeographic timescales.



Box III-1: Diagrammatic model of riverine connections after Thomaz et al. (2016). Colored circles represent demes (subpopulations) within two adjacent river networks with differing intra-network and inter-network dispersal pathways: (i) 'within-network' dispersal along the defined river channel ('intra-system') with greater thickness representing an often-assumed dispersal bias downstream, (ii) 'out-of-network' dispersal between adjacent tributaries ('intra-system'; e.g. due to flooding or river capture), (iii) 'out-of-network' dispersal between adjacent mainstems of river systems ('inter-system'; e.g. across marine or estuary systems), (iv) 'out-of-network' dispersal between headwaters or tributaries of distinct river basins ('inter-system'; e.g. due to flooding or river capture). Note that differing expectations of modeled intraspecific genetic diversity and genetic differentiation at low-order streams versus mainstems would affect the genetic composition of the demes involved in out-of-network gene flow (e.g., scenarios (iii) versus (iv)).

METHODS

Sampling

A total of 79 specimens of *K. potaroensis* and selected outgroups were obtained from museum collections (Table SIII-1 and Fig. III-1). Included samples spanned as much of the upper Mazaruni, Ireng, Kuribrong, and Potaro River basins as possible with the goal of characterizing intraspecific variation within and between river systems. Outgroup sampling aimed at testing the monophyly of *K. potaroensis* along its native distribution and to explore its relationships to other described *Krobia*. Outgroup samples included four *Krobia sp.*'Middle Mazaruni' (Middle Mazaruni, Guyana), two *K. petitella* (Berbice River, Guyana; Steele and López-Fernández 2013),

two *K. guianensis* (Suriname River, Suriname; Kullander and Nijssen 1989), two *K. itanyi* (Maroni-Marowijne River; Suriname; Kullander and Nijssen 1989), two *Krobia sp*. 'Sinnamary' (Sinnamary River, French Guiana), one *K. paloemeuensis* (= *'Aequidens' paloemeuensis*, Paloemeu River, Suriname; Kullander and Nijssen 1989 and see Musilová et al. 2009), one *K. xinguensis* (Xingu River, Brazil; Kullander 2012), two *Cichlasoma bimaculatum* (Demerara River, Guyana; Linnaeus 1758), two *Aequidens tetramerus* (Rio Novo, Brazil) and two *Aequidens michaeli* (Xingu River, Brazil).

ddRAD sequencing, demultiplexing, and matrix assembly

Reduced representation libraries were sequenced for all individuals using a modified ddRAD approach (Peterson et al. 2012). DNA was first extracted using Qiagen DNeasy Blood and Tissue kits, with the addition of 35U of RNaseA (Qiagen) and quantified using a Qubit fluorometer. 500 ng of DNA was then digested for each sample using 40U of SphI-HF and 40U of EcoRI-HF (New England Biolabs). Following digestion, individual barcodes were introduced through adaptor ligation and individuals pooled into libraries of 40-48 individuals. Pooled libraries were each then size selected for fragments between 375 and 525 bp on a PippenPrep gel electrophoresis system (Sage Science). Illumina adaptors were added using PCR (protocol as in Peterson et al. 2012) and libraries were sequenced using the PE150 chemistry on a NovaSeq platform (Illumina) at the University of Michigan Advanced Genomics Core.

Following sequencing, raw sequence files were demultiplexed and matrices were assembled using *ipyrad* (version 0.9.63; Eaton and Overcast 2020). Matrices were exported for three datasets, (i) Pakaraimas, middle Mazaruni, and outgroups (n=79; hereafter the 'phylogeny-dataset'), (ii) Pakaraimas + middle-Mazaruni (as the closest outgroup to the Pakaraimas; n=63;

hereafter the '*potaroensis*-clade' dataset), and (iii) the upper Pakaraimas (n=59; hereafter the 'Pakaraimas' dataset). Matrices were exported for loci present in >50% of individuals for the phylogeny dataset (to retain more loci in outgroup samples for tree building; Wagner et al. 2014) and in >75% of individuals for the *potaroensis*-clade and Pakaraimas datasets; therefore representing loci in at least 40, 48, and 45 individuals for phylogeny dataset, the *potaroensis*-clade, and Pakaraimas-dataset respectively. Aligned sequence matrices were exported for all three datasets, while unlinked SNP matrices (one SNP per locus) were additionally exported for the *potaroensis*-clade and the Pakaraimas datasets.

Taxonomy, genetic diversity, and population structure analyses of Krobia potaroensis

Phylogeny of Krobia in the Pakaraimas

To determine whether all putative *K. potaroensis* form a clade within the Pakaraimas, I first constructed a maximum-likelihood (ML) tree for the phylogeny-dataset using RAxML (v 8.0; Stamatakis et al. 2014). The ML tree was calculated under a GTR+γ model, with 20 independent maximum-likelihood searches, and with 1000 non-parametric bootstrap replicates to assess topological robustness.

Clustering analyses and species tree analyses

To determine whether the genetic diversity of *K. potaroensis* is split into identifiable genetic subpopulations, and to relate these subpopulations to the modern river systems, I performed cluster analyses on the unlinked SNP matrices for the *potaroensis*-clade-dataset and Pakaraimas-dataset. First, I determined the most likely number of genetic clusters hierarchically using the

program *STRUCTURE* (Pritchard et al. 2000); parallelized using Strauto (v1.0; Chhatre and Emerson 2017). The number of clusters (K) was determined through comparison of 10 independent runs of K = 1-5 with 50,000 (burn-in) followed by an additional 500,000 MCMC iterations. Convergence between runs was evaluated using *Structure harvester* (Earl and VonHoldt 2012) and the most likely value of K was determined using the Evanno method, which evaluates K based on the second order rate of change of the likelihood function (Evanno et al. 2005). Independent STRUCTURE runs were combined and visualized using *CLUMPAK (*Jakobsson and Rosenberg 2007; Kopelman et al. 2015). Upon determination of the most likely K, each subpopulation was run through STRUCTURE again to determine additional substructure, until K=1 was determined most likely (Meirmans 2015).

To further examine the genetic relationships between river systems and to account for the potential incomplete-lineage sorting and gene-tree to species-tree discordance I estimated population trees for full sequences of the *potaroensis*-clade and Pakaraimas-dataset. Population trees were estimated under the multispecies coalescent model using *SVDQUARTETS* (Chifman and Kubatko 2014) as implemented in Paup (v 4.0; Swofford 2003). Trees were estimated under all possible quartets, with 1,000 bootstrap replicates to assess branch support.

Genetic diversity metrics and principal components analysis

Measures of genetic diversity were determined for the populations of *K. potaroensis* using the R packages *hierfstat* (version 0.5-11; Goudet 2005; Goudet et al. 2022) and *Adegenet* (version 2.1.10; Jombart 2008; Jombart and Ahmed 2011). I again analyzed the unlinked SNP matrices for both the *potaroensis*-clade and Pakaraimas datasets (n= 59 and 63 respectively). Measures of population subdivision (F_{ST}), observed heterozygosity (H_O), and within-population gene

diversity (H_S) were calculated for each river system overall, and by sampling-site. I further ran principal components analysis (PCA) and spatial principal components analysis (sPCA) in *Adegenet* (version 2.1.10; Jombart and Ahmed 2011) to visualize the genetic diversity within and between river systems, with missing data replaced by inferred mean values (Jombart 2008; Jombart and Ahmed 2011; Jombart and Collins 2015).

RESULTS

ddRAD sequencing, demultiplexing, and matrix-assembly

The average number of sequenced raw reads per sample was 4,593,480 bp (SD 1,649,438) for samples included in analyses (see Table SIII-2 for individual counts). The 'phylogeny dataset' had a final alignment of 2,107,645 bp representing 7,440 loci with 18.5% missing data (see Table SIII-2). The sequence-matrices used in SVDQuartets for the *potaroensis*-clade-dataset and the Pakaraimas-dataset had final alignments of 2,975,799 bp and 3,073,420 bp respectively; representing 10,366 and 10,719 loci, with 5.2 and 5.0% missing data respectively. The unlinked SNP matrices exported for the *potaroensis*-clade-dataset and the Pakaraimas-dataset were represented by 7,938 and 5,100 SNPs respectively; and with 5.9% and 6.4% missing genotypes respectively (Table SIII-2).

Taxonomy, genetic diversity, and population structure analyses of Krobia potaroensis

Phylogeny of Krobia in the Pakaraimas

I found strong bootstrap support (>95%) in the RAxML tree for three broader clades in the analyzed taxa (Fig. III-2): (i) the chosen outgroup taxa (*Aequidens* and *Cichlasoma*), (ii) the

'lowland *Krobia*' group (*K. xinguensis*, *K. paloemeuensis*, K. sp. 'Sinnamary', *K. itanyi, K. petitella,* and *K. guianensis*), and (iii) the '*potaroensis*-clade', formed by all individuals of *K. sp*. 'Middle Mazaruni' and *K. potaroensis* (Ireng+Kuribrong+Upper-Mazaruni+Upper-Potaro; Figs III-1 and III-2). The short branch lengths between *K. sp*. 'Middle Mazaruni' and *K. potaroensis* in the Pakaraimas river systems provide evidence for a closer relationship between these clades than previously known, strongly suggesting that lowland *Krobia* from the middle Mazaruni river of Guyana do not share a common evolutionary history with the rest of the lowland *Krobia* species (*K. guiananensis*; Regan 1905) found throughout lowland Guyana and Suriname (Taphorn et al. 2022). Among samples within the Pakaraimas river systems, only the Ireng River and upper Mazaruni River samples grouped into highly supported clades (100 bootstrap support), both nested within a poorly supported wider clade of upper Potaro and Kuribrong samples (Fig. III-3), indicating a recent evolutionary relationship between these clades. Population genetic analyses were separately performed on (i) the *potaroensis*-clade dataset: to include measures of population genetic analyses of the Pakaraimas rivers relative to their sister clade in the Middle Mazaruni River, (n=63; the '*potaroensis*-clade' datatset), and (ii) the dataset that included only samples from within the Pakaraimas (n=59; the 'Pakaraimas' dataset).

Species tree and clustering analyses

Species tree analyses by SVDQuartets (Fig. III-3A-i and Fig. S3-1) further supported the Middle Mazaruni *Krobia (K.* "Middle Mazaruni" heretofore*)* as a separate clade sister to a monophyletic grouping of all *K. potaroensis* samples from the rivers of the Pakaraimas highlands. Within *K. potaroensis*, both the *potaroensis*-clade and Pakaraimas datasets grouped the upper Mazaruni, the upper Ireng, and the upper Potaro rivers as monophyletic with respect to each other and

forming a moderately supported (>70% bootstrap support) clade sister to a non-monophyletic arrangement from the Kuribrong river (Fig. III-3A-i). Further subdivision within river systems was not supported by the SVDQuartets tree.

Cluster analyses in STRUCTURE recovered each analyzed river system as a distinct population (Fig. III-3). First, the middle Mazaruni river system was determined as distinct relative to the upland Pakaraimas drainages (Fig. III-3A; 3B-i, K=2) with samples in the Pakaraimas sorting entirely with the first cluster (100% cluster 1) and the middle Mazaruni with the second cluster (100% cluster 2). The second STRUCTURE run (the Pakaraimas dataset, excluding middle Mazaruni samples; Fig. III-3B-ii) separated the Kuribrong relative to the other three Pakaraima river systems (K=2). In this second run (n=59) the Mazaruni, Ireng, and Potaro Rivers clustered almost entirely in group 1 (>95% cluster 1) while the Kuribrong River individuals were shared among both cluster 1 and cluster 2 (or historical populations); with individuals falling 16-78% with cluster 1, and 22-84% with cluster 2. In this second analysis the Potaro River mostly grouped with the Mazaruni and Ireng (>92% cluster 1), but with a 5-8% membership in cluster 2 (Fig. III-3B-ii). The third STRUCTURE run excluded Kuribrong samples (n=54) and again found K=2 to be the most likely number of ancestral populations (Fig. III-3B-iii). This third STRUCTURE run mostly separated the Mazaruni river (87-100% cluster 1) from the Ireng and Potaro Rivers (50-100% cluster 2). Additionally, the cluster analyses revealed that the two most upstream Mazaruni sites partially grouped with the Potaro and Ireng (11 and 12% cluster 2; Fig. III-3B-iii). The final STRUCTURE runs then showed an association between the upper Potaro and upper Ireng Rivers (Fig. III-3B-iv&v, n=16 n=9 respectively; (K=2), with the main genetic

break being at a waterfall that separates the most downstream Ireng samples (n=3) from Ireng and Potaro samples further upstream.

Genetic diversity metrics and Principal components analysis

Measures of genetic diversity and genetic distance in the Pakaraimas-dataset (n=59) were consistent with the population structure observed in the STRUCTURE analyses (Tables III-1 and S3-2). Between river systems F_{ST} values ranged from 0.03 (between the upper Ireng and upper Potaro, and between the Upper Mazaruni and upper Potaro) to 0.12 (between the Kuribrong and the upper Mazaruni, and between the Kuribrong and Upper Ireng; Table SIII-2). Measures of observed heterozygosity (H_O) and mean gene diversity (H_S) were moderate for the upper Mazaruni (H_O=0.049; H_S=0.051), the upper Ireng (H_O=0.045; H_S=0.059), slightly higher in the upper Potaro (H_O=0.087; H_S=0.084), and highest within the Kuribrong (H_O=0.17; H_S=0.226); suggesting more genetically diverse (and a potentially larger and older population) within the Kuribrong relative to the other rivers of the Pakaraimas. Genetic diversity ranged by sampling site (Table I) but did not appear to follow the expected pattern of the downstream-increase-of genetic diversity model (DIGD; Paz-Vinas et al. 2015; Thomaz et al. 2016).

The principal components analysis (PCA) further corroborated the findings of the other analyses in general patterns of intra-system and inter-system genetic diversity and population structure (Fig. III-4). Greater genetic variation between river systems than within river systems is apparent in the case of all river systems except the Kuribrong (Fig. III-4); despite similar geographic distances between samples within and between river systems (Fig. 1).

The spatial principal components analysis (sPCA, Fig. III-5) allowed for the analyses of principal components when controlling for the distance between samples (calculated via Moran's

I). In the sPCA analysis, two groups were apparent in the analyzing the first two PCs: one group corresponding to the upper Mazaruni, and the other group corresponding to the Kuribrong, the Potaro and the Ireng together (Fig. III-5). Additionally, though the sPCA analysis does not model the distance along the river, and instead models a network of linear distances that cross land, the observed relationships between populations were still suggestive of the topography of the region and riverine interdigitation in shaping fish dispersal routes. Interestingly, the two samples in the upper Mazaruni that showed association with the upper Potaro in the STRUCTURE analyses again showed association with the other river systems with similar scores to the other (non-Mazaruni) genetic group along both PC1 and PC2 (Fig. III-3 and III-5).

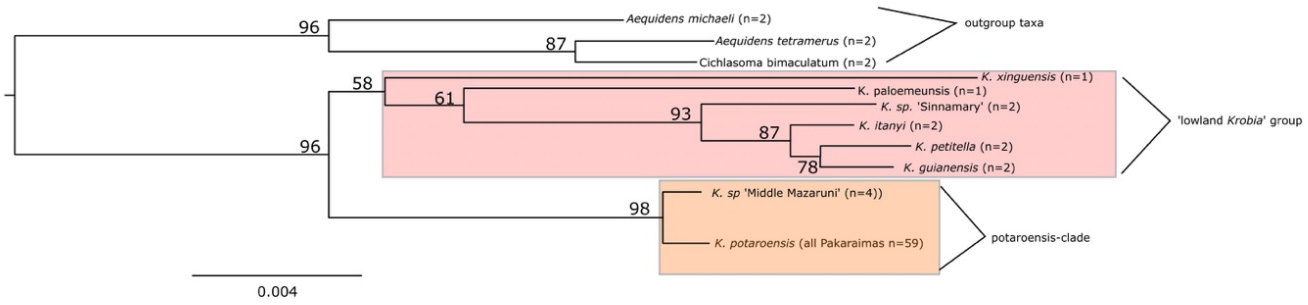


Figure III-1: Phylogeny of *Krobia* and selected outgroups. Tree generated from RAxML analysis of 2,107,645 bp representing 7,440 loci.

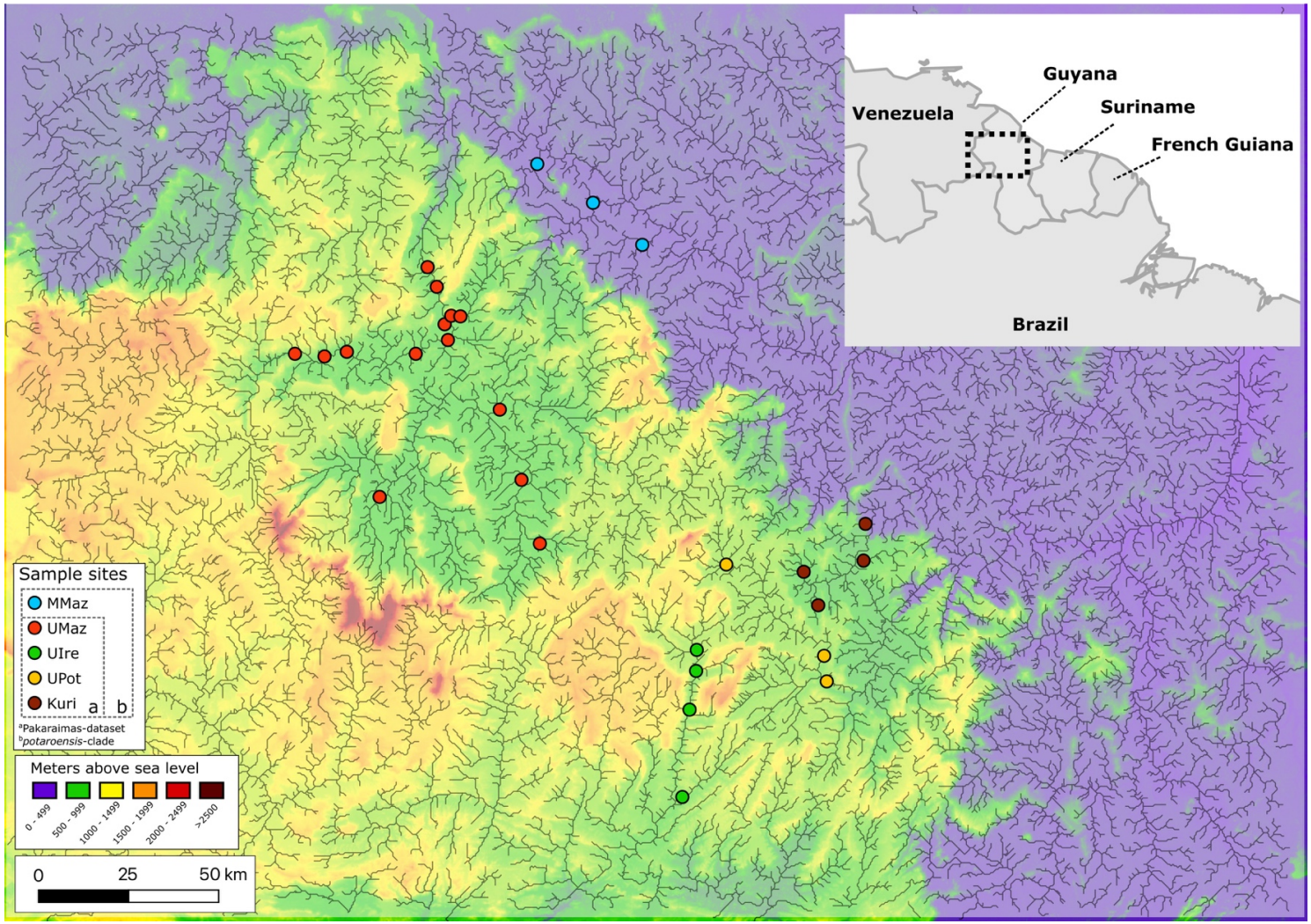


Figure III-2: Sampling sites for *Krobia potaroensis* (n=59) from the river systems of the Pakaraima Mountains and sister *Krobia* sp. 'Middle-Mazaruni' (n=4). Map generated in QGIS v3 with topography layers derived from NASA Shuttle Radar Topography Mission Global 1 arc second (2013), and river layers from the free-flowing rivers dataset of Grill et al. (2019). MMaz=middle Mazaruni, UMaz= upper Mazaruni, UIre= upper Ireng, UPot = upper Potaro, and Kuri= Kuribrong.

Table III-1: Observed heterozygosity at each site in the river systems of the Pakaraimas from upstream (low order tributaries) to more downstream sites. See Table SIII-1 for GPS sites of each site. Highest and lowest values within each river system are underlined.

Kuribrong upstream	**Potaro upstream**	**Ireng upstream**	**Mazaruni upstream**
0.0817 (Kuri-04)	0.078 (Upot-03)	0.0516 (UIre-04)	0.0407 (UMaz-14)
0.1706 (Kuri-03)	0.0877 (Upot-02)	0.0432(UIre-03)	0.0548 (UMaz-13)
0.2445 (Kuri-02)	0.0856 (Upot-01)	0.0642 (UIre-02)	0.053 (UMaz-12)
0.2661 (Kuri-01)		0.0285 (UIre-01)	0.056 (UMaz-11)
			0.0456 (UMaz-10)
			0.0459 (UMaz-09)
			0.0511 (UMaz-08)
			0.0509 (UMaz-07)
			0.0454 (UMaz-06)
			0.0533 (UMaz-05)
			0.0498 (UMaz-04)
			0.0476 (UMaz-03)
			0.0421 (UMaz-02)
			0.0407 (UMaz-01)
Kuribrong downstream	**Potaro downstream**	**Ireng downstream**	**Mazaruni downstream**

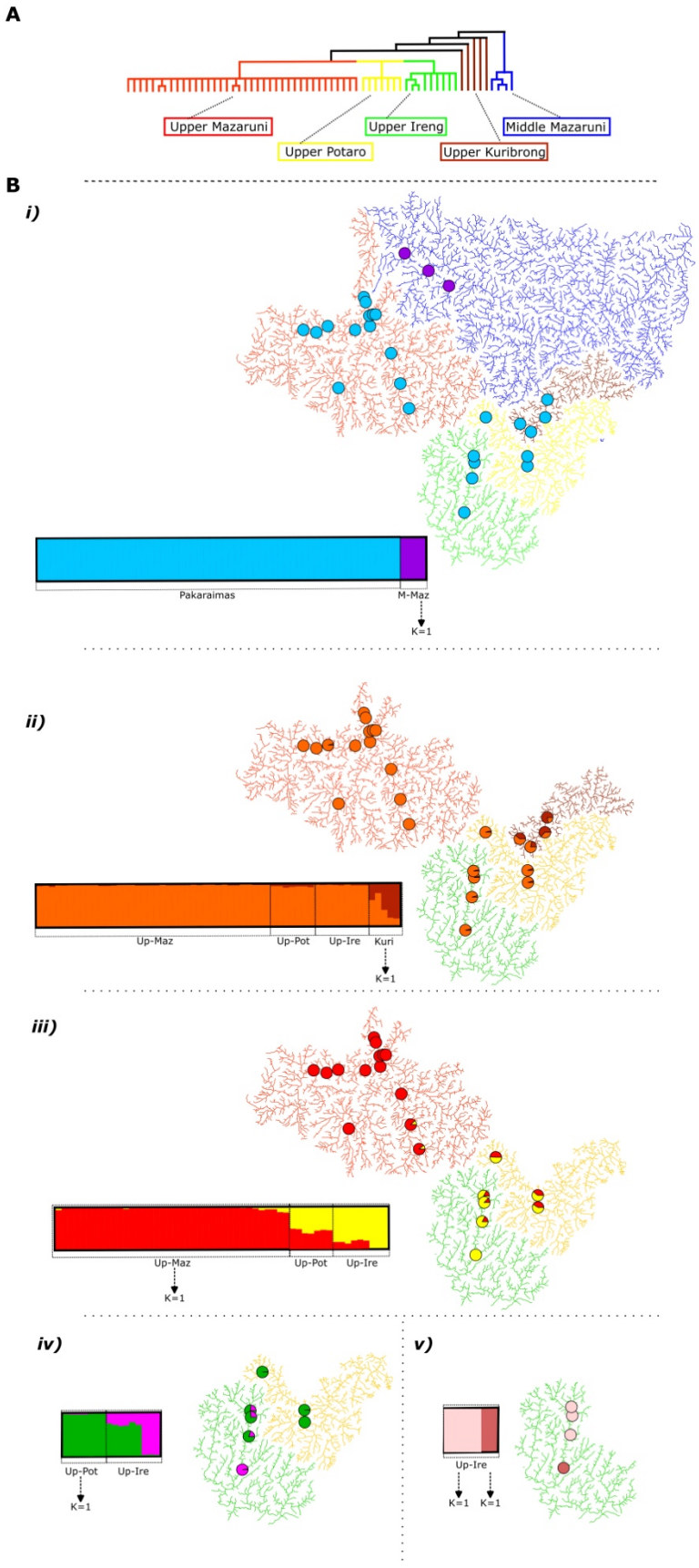

A
Upper Mazaruni
Upper Potaro
Upper Ireng
Upper Kuribrong
Middle Mazaruni
B
i)
Pakaraimas
M-Maz
K=1
ii)
Up-Maz
Up-Pot
Up-Ire
Kuri
K=1
iii)
Up-Maz
Up-Pot
Up-Ire
K=1
iv)
Up-Pot
Up-Ire
K=1
v)
Up-Ire
K=1
K=1

Figure III-3: Species-tree and cluster-analysis for *Krobia potaroensis* in the Pakaraimas region and middle-Mazaruni. A) Simplified SVDQuartets tree showing branches with strong bootstrap support (>70%), tree colors: red = upper-Mazaruni, yellow = upper-Potaro, green = upper-Ireng, brown = Kuribrong, and blue = middle-Mazaruni; B) [i] to [v] results of hierarchical STRUCTURE analyses and corresponding map of sample distributions with: [i] *K. potaroensis* from the rivers of the Pakaraimas (upper-Mazaruni, upper-Ireng, upper-Potaro, and Kuribrong) and samples from the middle-Mazaruni (K=2; n=63), [ii] samples from river systems of the Pakaraimas (K=2; n=59), [iii] K. potaroensis from the upper-Mazaruni, upper-Potaro, and upper-Ireng (K=2; n= 54), [iv] K. potaroensis from the upper-Potaro and upper-Ireng (K=2; n=16), and [v] K. potaroensis from the upper-Ireng (K=2; n=9).). BMaps corresponding to analyses in panel A showing K. potaroensis sample locations within the river systems for analyses, coloring of river systems: red=upper-Mazaruni, brown=Kuribrong, yellow=upper-Potaro, and green=upper-Ireng. Pie-charts show proportional cluster-membership for representative samples from each sampling site.

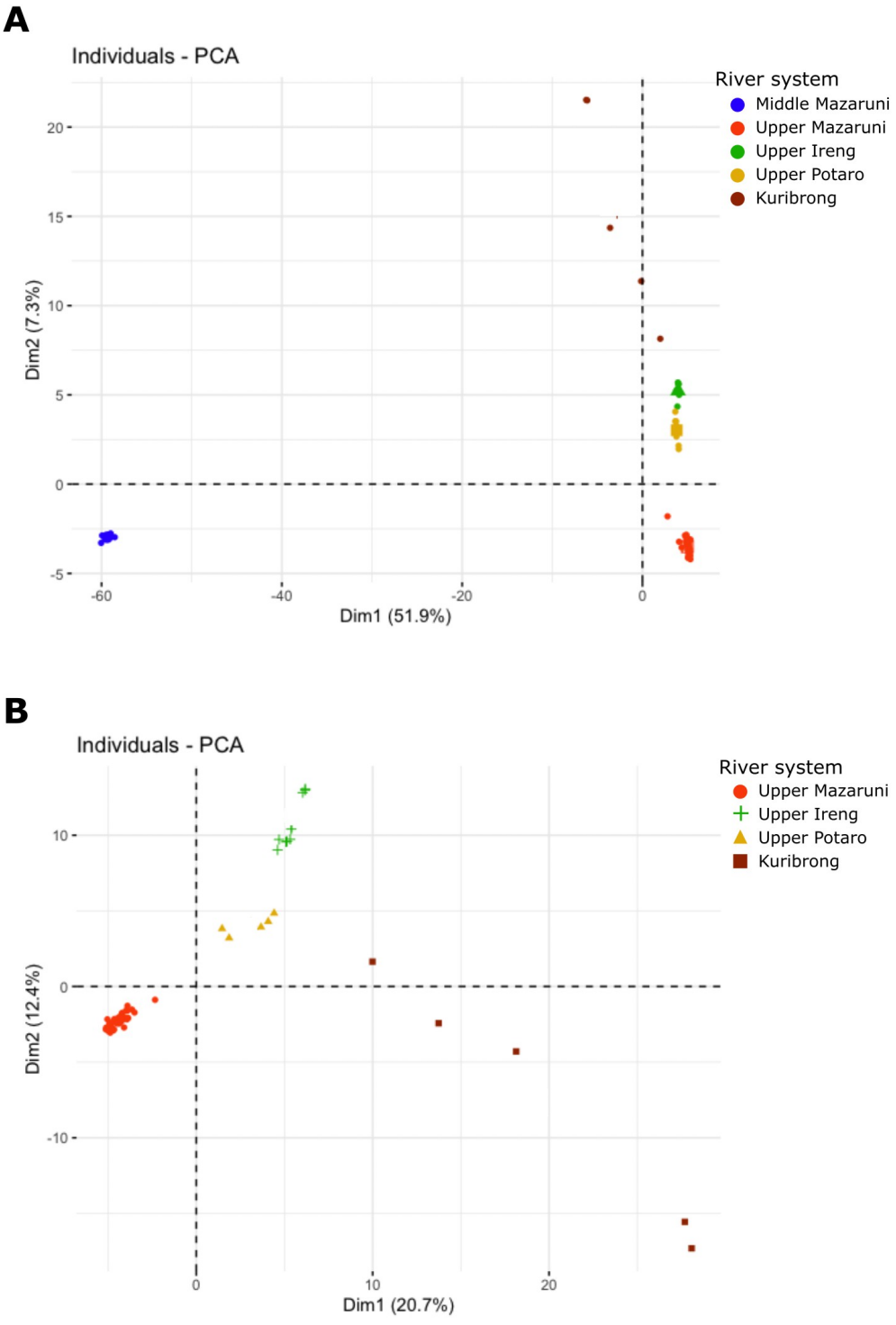


Figure III-4: Genetic principal components analysis for A) the *'potaroensis*-clade' (n=63) and B) the Pakaraimas-dataset of Krobia potaroensis (n=59), representing 7,938 and 5,100 SNPs respectively.

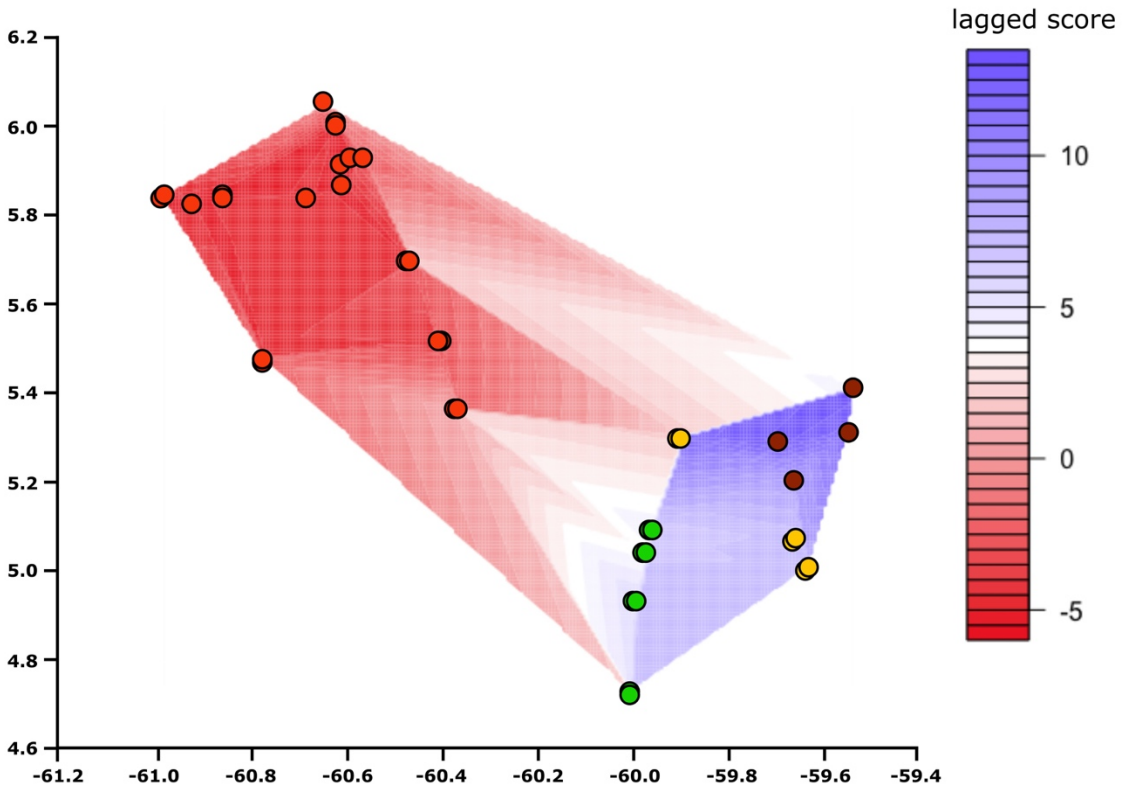


Figure III-5: Spatial principal components analysis for the Pakaraimas-dataset K. potaroensis with longitude and latitude represented on the x and y axes respectively. Lagged values for principal components are represented as genetic clines along vectors connecting samples to one another with similar colors representing local genetic structure and an apparent western (Red, Mazaruni) genetic group and eastern (Blue, Ireng, Potaro, Kuribrong) group. Network of connections determined via calculation of Delauney connection network.

DISCUSSION

Krobia in the uplands and lowlands of the Guiana Shield

Putative *K. potaroensis* throughout the river systems of the Pakaraimas were confirmed by the phylogenetic analyses to belong to one closely related clade, confirming there is only one species of *Krobia* in the Pakaraimas uplands (Fig. III-1). As part of the wider '*potaroensis*-clade', the ML-tree then places *K. potaroensis* within an evolutionary context of upland and lowland *Krobia* that requires further attention.

While the representation of *Krobia* in my analyses (Fig. III-1) is incomplete across the Guianas, I highlight the need for additional descriptive analyses of *Krobia* from the middle Mazaruni (Guyana) and the Sinnamary rivers (French Guiana). Additionally, supporting the previous findings of Musilová et al. (2008; 2009), *K. potaroensis* and *K. paloemeuensis* were both confirmed to belong within *Krobia*, rather than their original tentative assignments to '*Aequidens'*. However, *K. potaroensis* and *K. paloemeuensis* were not found to be sister-taxa, and the shared intermediate morphologic characteristics that led to their original taxonomic classification should perhaps be revisited (Kullander and Nijssen 1989).

The fishes of the Pakaraimas can be generally characterized as highly endemic, with many species and genera found only within their river systems (Hardman et al. 2002; López-Fernández et al. 2012; Alofs et al. 2014; Maldonado-Ocampo et al. 2014; Taphorn et al. 2017; Hayes et al. 2020). That *K. potaroensis* (along with middle Mazaruni *Krobia*) form a sister clade to all other analyzed *Krobia*, and with deep branch lengths (Fig. III-1), is suggestive of a long period of isolation in the uplands of the Pakaraimas and a previously undocumented biogeographic relationship between cichlids from the upland and lowland regions of the western Guiana Shield. This upland '*potaroensis*-clade' of *Krobia* is sister to more eastern 'lowland' species in the ML-tree, suggesting historical separation between upland and lowland clades. A similar topology was observed in the phylogeny of the knifefish *Gymnotus carapo* which has an upland Pakaraimas clade (along with the upper Berbice River) that was sister to a clade of lineages found throughout the more eastern lowlands of the Guiana Shield (Lehmberg et al. 2018). However, in contrast to the long branch lengths I observe between upland and lowland *Krobia*, Lehmberg et al. (2018) highlighted the relatively shallow divergence between upland and lowland *G. carapo* that may be linked to its long-distance dispersal and ability to surpass

elevational river barriers. Contrasting the potentially recent isolation of upland *G. carapo*, the isolation of several endemic catfishes (superfamily: Loricariodea; Rafinesque 1815) above the waterfalls of the Pakaraimas have been hypothesized to predate the uplift of planation surfaces in the Oligocene (Lundberg et al. 2007; Taphorn et al. 2010; Armbruster and Taphorn 2011; Lujan and Armbruster 2011). Taken together, the biogeography of the modern Pakaraimas appears to have been shaped by range expansions of fishes into its river systems at drastically differing geological periods. Further, the presence of each of these clades in multiple river systems highlights that the connections between river systems across the Pakaraimas are either reoccurring or persistent, otherwise they would have not led to the observed multi-river distributions of species that arrived in the region at markedly differing times.

Beyond its utility in further understanding the biogeographical history of the Pakaraimas, my analyses support *Krobia*'s likely value in wider regional biogeographic analyses. The 'lowland clade' of *Krobia* (Fig. III-1) highlights associations between the eastern Amazon (*K. xinguensis*), the uplands of southern Suriname (*K. paloemeuensis*), and the coastal rivers of the Guianas (*K. guianensis*, *K. petitella*, *K. itanyi* and *K.* sp. 'Sinnamary') that warrant further investigation of the evolutionary history and dispersal pathways across the Guiana Shield. *Krobia* may be well suited to further investigating hypothesized historical connections between the lower Amazon and southern Suriname that have led to sister clade relationships among fishes across the region (Nijssen 1970; Cardoso and Montoya-Burgos 2009).

Genetic population structure of Krobia and modeling of riverine connectivity

The genetic population structure I observe in *K. potaroensis* demonstrates likely dispersal and gene flow between otherwise distinct river systems. Across analyses, I see genetic clines that

connect upland interdigitating tributaries (Figs III-3 to III-5). Analyses inferred connections between all four river systems, with the upper Potaro detected as an intermediary river system. This intermediate position of the upper Potaro, which interdigitates with the three other upland river systems, was detected in the analyses that do not incorporate spatial information (STRUCTURE and PCA; Figs III-3 and III-4 respectively) as well as in the spatially explicit sPCA analysis (Fig. III-5). The mechanisms that lead to overland dispersal, and result in genetic populations that extend beyond the bounds of individual river networks, should be further characterized, and incorporated into models of riverine population structure.

The existing models of riverine population structure provide a set of expectations with which to compare my empirical results. I identify several instances in which *K. potaroensis* exhibits dispersal and gene flow along routes which were not previously considered in simulation studies of riverine population structure, but which may nonetheless have substantial impact on the population structure of riverine fishes. In Box III-1, I highlighted the expectations of a generalized model of riverine populations with greater genetic diversity downstream and greater genetic differentiation upstream (after the gene flow models of Paz-Vinas et al. 2015 and Thomaz et al. 2016). Meta-analyses of riverine populations support a general pattern of DIGD, with the degree of population isolation, genetic diversity, and genetic differentiation modified by riverine branching and configuration, the dispersal ability of differing fish species, and the degree of asymmetry to dispersal (Paz-Vinas et al. 2015; Comte and Olden 2018). However, I did not find strong evidence of DIGD for *K. potaroensis* in the Pakaraimas, other than potentially in the Kuribrong River (for which I had only five samples, Table I). That genetic diversity of *K. potaroensis* is relatively uniform within each river system may be due to several factors I observed in the wider set of Pakaraimas rivers. Out-of-network dispersal between the

interdigitating river systems of the Pakaraimas (Box III-1-iv) challenges the expectation that most upstream riverine tributaries are the least connected subpopulations within a river. Even if instances of gene flow between interdigitating river systems are rare, that it occurs at all has implications for the long-term persistence of upland populations (e.g., through genetic rescue; Whiteley et al. 2015).

Seasonal flooding provides a mechanism by which populations in otherwise distinct river systems could disperse out-of-network. Seasonal flooding has been observed to allow dispersal of fishes in several systems in the Guiana Shield, such as the Rupununi Portal in southern Guyana (de Souza et al. 2012; 2020). The success of species in dispersing across flooded systems has been linked to their life history strategies (Stoffels et al. 2016), and inter-system flooding has led to the dispersal of some species and their distributions spanning multiple river systems, while other species remain endemic to one river system or the other (de Souza et al. 2012). Past meta-analyses of genetic population structure in river systems have highlighted the likely importance of morphological and life-history traits in facilitating dispersal and gene flow (Comte and Olden 2018). However, the biotic and abiotic determinants of out-of-network dispersal and its effects on genetic population structure are still incompletely understood.

The signals of out-of-network dispersal are most obvious when they provide evidence for the connection of otherwise distinct river systems (Box III-1-iv), and the admixture of distinct ancestral populations (clusters) can be detected. I observed these signatures in *K. potaroensis*, indicating gene flow and admixture between the upper tributaries of each of the four Pakaraimas river systems (Figs III-3 to III-5). Interestingly, the ability to disperse between river systems likely involves the same hydrologic mechanisms (i.e., transient flooding) that would allow out-of-network dispersal and gene flow within a river system (Box III-1-ii). The possibility of

alternative dispersal routes, beyond the classic longitudinal within-system dispersal, presents a mechanism by which otherwise isolated upland river systems (e.g., by rapids or waterfalls) can be reached by more-downstream populations or by those in adjacent river systems. The analyses in this study lend credence to the notion that additional consideration should be paid to connections between upland tributaries both within a river system and between distinct river systems (Box III-1-ii and iv), and that more complex models that incorporate these dispersal routes should provide more realistic and useful representations of riverine population structure. Not least, beyond illuminating biogeographic and evolutionary mechanisms generating and maintaining riverine biodiversity, such models would introduce powerful new information and nuance to the toolbox of aquatic conservation and management.

CONCLUSION

The hydrological connections that structure the populations *of K. potaroensis* explain its distribution in each of the river systems of the Pakaraimas and have implications to its conservation along with that of the wider and increasingly threatened fish communities of the region (e.g., Lujan et al. 2013; Alofs et al. 2014; Taphorn et al. 2022). The multi-river distribution of *K. potaroensis* is consistent with recent studies of *Trichomycterus* catfishes and the knifefish *G. carapo* which were found to each have genetic lineages that occur within multiple river basins in the Pakaraimas (Lehmberg et al. 2018; Hayes et al. 2020). Further understanding the abiotic and biotic factors that have led to these species becoming widespread in the region, while a large proportion of other fishes remain endemic to a subset of the rivers (e.g., 67-95% endemic species in the upper Mazaruni; Alofs et al. 2014), is critical to understanding the evolution of species, their persistence, and consequently to their conservation.

Consistent with previous studies, the upper Mazaruni River was highlighted in my analyses as potentially the most isolated river system relative the others (Fig. III-5). That the upper Mazaruni may be less connected to the other river systems may explain the extreme patterns of endemism that characterizes its fish fauna; and casts a sobering light on the urgency of enacting conservation measures to protect one of the most distinct riverine systems of the Neotropics as it is broadly and swiftly transformed by expanding mining pressures (Alofs et al. 2014; Taphorn et al. 2022).

The inter-network connections I characterized in the Pakaraimas have both regional conservation value as well as broader value to models of riverine populations. The conservation of endemic species in the Pakaraimas rivers and elsewhere, depend on accurate characterization of their populations, the resilience of populations facing anthropogenic disturbance, and available habitat. More broadly, the synthesis of models of contemporary riverine population structure with the models of historical biogeography and hydrogeology are critical in understanding the interplay between contemporary population structure, evolutionary processes, and historical biogeography.

IV. Genetic Population Structure of Two Fish Species in a Transiently Flooding River System: Dispersal Out-of-Network Connects Distinct Rivers in the Rupununi Portal of Guyana

ABSTRACT

River network architecture structures the populations of riverine species such as fishes. Models used to study the dendritic network of rivers typically assume a downstream increase in genetic diversity based on aquatic dispersal longitudinally within a river. However, in floodplain systems the assumption of longitudinal within-network dispersal is often violated. Seasonal flooding in one such system, the Rupununi Portal of southern Guyana, has been observed to allow fish dispersal between the tributaries of two otherwise distinct river basins. The Rupununi Portal is therefore an ideal system in which to study how the dispersal of fishes across flooding systems affects the genetic structure of populations. I analyzed the genetic population structure of two species of Neotropical cichlid fishes found across the Rupununi Portal, *Geophagus sp.* and *Guianacara dacrya*, to determine any effects of out-of-network dispersal on genetic population structure. The analyses of genetic population structure confirmed dispersal of both species across the Portal, corroborating the active role of one previously known dispersal conduit. I also identified a second, southern conduit for inter-network dispersal by *Guianacara dacrya*. I note that a set of waterfalls on the Rewa River represented a much greater barrier to dispersal and gene flow than being present in different river basins across the flood-prone Portal connection. This study clarifies the permeability of flooded systems to fish dispersal, and how the movement

of fishes between river systems on ecological timescales cascades to affect their population structure and biogeography over multiple generations.

INTRODUCTION

The distributions of species are shaped, among other things, by dispersal, which affects their population structure, ability to access novel range and habitat, and consequently their evolutionary trajectories and resilience to extinction (MacArthur and Wilson 1967; Simberloff 1974; Lomolino et al. 2006; Kool et al. 2013). The distribution of aquatic species along the length of a river network depends on their longitudinal dispersal capacity, and is modulated by physical barriers to dispersal, (e.g., rapids, waterfalls), habitat availability, and nutrient input variation along the river system, among others (Pringle 2000, 2003; Ramirez et al. 2008, Winemiller et al. 2008a). Earlier models of riverine communities focused on biodiversity patterns apparent in upstream versus downstream systems as linked to nutrient input and its downstream flow and processing (Vannote et al. 1980), before discussion of riverine biodiversity and community dynamics shifted to the importance of rivers as dendritic networks (Meffe and Vrijenhoek, 1988; Campbell Grant et al. 2007; Paz-Vinas et al. 2015; Fagan 2002; Paz-Vinas and Blanchet 2015; Thomaz et al. 2016; Blanchet et al. 2020). Several studies have highlighted that species, including fishes, are variably present within riverine networks based on contemporary ecological factors such as habitat availability, seasonality, interspecific competition, and life history strategies (Vannote et al. 1980; Winemiller and Rose 1992, Arrington et al. 2005, Arrington and Winemiller 2006; Finn et al. 2011).

Similar to its structuring of biodiversity at the community level, the architecture and ecology of a river system also affect fishes at the population level. The genetic structure of

aquatic populations varies along a river system depending on how connected subpopulations are to one another (Finn et al. 2007; Paz-Vinas and Blanchet 2015; Thomaz et al. 2016). The effect of varying habitat availability and the hydrology of dendritic architecture has been conceptually generalized as shaping a pattern of 'downstream increase in genetic diversity' (DIGD) in riverine populations (Paz-Vinas et al. 2015; Thomaz et al. 2016). The implications of having genetically less diverse and more differentiated populations upstream have in turn shaped discussions about the contrasts between smaller and larger river systems, and the differing approaches that should be taken in conserving upstream versus downstream fish communities (Fagan 2002; Finn et al. 2011; Paz-Vinas et al. 2015; Thomaz et al. 2016; Blanchet et al. 2020). Models such as DIGD, and its corresponding pattern of greater differentiation upstream have served as a useful set of expectations and because they fit the observed patterns of diversity within many river systems and for many species. However, the model's assumptions — mainly that riverine configurations are fixed, and that dispersal occurs only longitudinally within rivers— do not reflect all cases of either riverine or fish population dynamics.

Erosional processes, for example, are known to change riverine configurations and can, through river capture, change the catchments of upstream tributaries and the mainstems to which they flow (Bishop 1995). Additionally, seasonal changes to river connectivity (e.g., through flooding) can alter the connections within otherwise dendritic systems or connect distinct adjacent systems (i.e., inter-basin connections). Changing configurations of river systems, over both geologic time (e.g., through erosion) and contemporarily (e.g., due to seasonal flooding), facilitate fish dispersal and range expansion (Albert et al.2018; Thomaz and Knowles 2020; Waters et al. 2020; MacGuigan et al. 2022). Dispersal between adjacent river systems is a well-studied mechanism of fish range expansion through which species come to inhabit multiple river

basins (Lovejoy and De Araújo 2000; Winemiller et al. 2008a, Willis et al. 2010, 2012; Albert et al. 2018; Waters et al. 2020). While inter-basin dispersal is predominantly discussed on macroevolutionary timescales, there are several examples of more recent inter-basin fish dispersal that serve as contemporary examples for how out-of-network movement seeds range expansion and diversification (e.g., Echelle 2008; Turner et al. 2004; Willis et al. 2010, 2015). While DIGD has proven useful as a model for many species, especially when out-of-network dispersal can be reasonably discounted, it may better serve as a null expectation for riverine species, to be rejected when species disperse overland (e.g., during flooding) or when changes to riverine architecture shuffle the expected connections of upstream tributaries to higher-order stems downstream. Further characterizing the circumstances under which out-of-network gene flow occurs is critical to informing more complex and realistic models of riverine population structure. Accurate understanding of population structuring along and across river basins should help clarify the processes that shape the diversification and biogeography of species over macroevolutionary time and is critical to inform the conservation of contemporary populations.

The Rupununi Portal (hereafter 'the Portal') in southern Guyana, South America (Fig. IV-1) is an ideal system in which to study the potential influence of inter-basin dispersal on population structure. The Portal lies along the low-lying Takutu graben (McConnell 1968; Sinha 1968; Gibbs and Barron 1993) within the Rupununi savannahs region of southern Guyana, which defines an area of seasonal flooding and connection between tributaries of the Rio Branco (Amazon River Basin) and the Rupununi and Rewa Rivers (Essequibo River Basin) (Hamilton et al. 2002; Junk et al. 2011)). Long proposed as a corridor between the Amazon basin and the Atlantic flowing Essequibo (e.g., Lowe-McConnell 1964, 1969, 1979), recent analyses of fishes found across the Portal support the idea that seasonal flooding creates a conduit for fish dispersal

between the adjacent river systems (de Souza et al. 2012). Fish dispersal across the Portal has been specifically observed to occur two sites: 'Lake Amuku' which refers to a seasonally-flooding area within the northern part of the Rupununi savannahs, and the 'Sand Creek Portal' which occurs further south (de Souza et al. 2020, see Fig. IV-1 and IV-2). However, while 254 species of fishes were shared between both systems, a further 90 and 89 species were found to only exist within the Takutu (Rio Branco basin) and Rupununi, respectively (de Souza et al. 2012). That the Portal serves as a conduit for some species but as a barrier for others is consistent with past studies of interspecific dispersal across floodplains and the potential effects of ecological factors as filters for dispersal (Winemiller et al. 2008; Willis and Winemiller 2011; Stoffels et al. 2016). To date, however, no analyses have addressed how dispersal across the Rupununi portal might shape the structure of fish populations on either side of the divide.

In this chapter I investigated the genetic population structure of two fish species in order to further understand the dispersal and gene flow patterns of fishes across the river systems of the Rupununi Portal. I sequenced thousands of loci for two cichlid species (subfamily: Cichlinae) found within both the Takutu and Rupununi systems; *Geophagus sp.* and *Guianacara dacrya* (hereafter *Geophagus* and *Guianacara* respectively; Arbour and López-Fernández 2011; Taphorn et al. 2022). I analyzed the genetic population structure to determine: (i) is there a signature of genetic admixture and gene flow between the adjacent Takutu and Rupununi river systems, consistent with the Portal connection? (ii) what can be determined about the permeability of the Portal to fish dispersal in comparing measures of gene flow within-network to measures of gene flow between river networks? And (iii) how does the genetic population structure of fishes in the Portal region fit or deviate from expectations of riverine population structure?

METHODS

Sample acquisition

Samples of *Geophagus* and *Guianacara* (n= 39 and 35 respectively*)* from the Rupununi Portal Region were field- collected in 2018 following methods approved by the Institutional Animal Care and Use Committee of the University of Michigan (PRO00010134) and supplemented with vouchered tissue samples from museum collections (see Table SIV-1). In several cases, sampling sites were identical for both represented species, particularly along the Rewa River (a tributary of the Rupununi River; Fig. IV-1). The sample set represents several upstream tributaries of the Takutu, Rupununi, Kuyuwini, and Essequibo River systems at locations where these river systems interdigitate with one-another, allowing for investigation of the finer-scale relationships of dispersal and gene flow in this region known for out-of-network fish dispersal (de Souza et al. 2012, 2020).

Library preparation and matrix assembly

DNA was extracted for each tissue using a Qiagen DNeasy blood and tissue kit, with the addition of 5U of RNaseA, and quantified on a Qubit 3 fluorometer. Following extraction genomic libraries were prepped using a modified ddRAD protocol (Peterson et al. 2012). Briefly, 500ng of DNA was digested for each individual for 16 hours at 37°C using 40 units each of EcoRI-HF and SphI-HF (New England BioLabs Inc). Following digestion, the DNA was cleaned using TotalPure NGS beads and barcoded adaptor sequences were ligated to each sample. Following ligation, libraries were size-selected on a PippenPrep for fragments between 375 and 525 bp in

length and Illumina© adaptors were added using PCR amplification (after Peterson et al. 2012). Samples were then pooled, and libraries were sequenced for paired-end 150 bp reads on a Novaseq Illumina sequencing platform (University of Michigan Advanced Genomics Core, Ann Arbor, MI).

Illumina reads were demultiplexed using the ipyrad pipeline (version 0.9.63; Eaton and Overcast 2020). Upon demultiplexing, all samples with >100,000 raw reads were included, and matrices were aligned for loci present in at least 75% of samples; present in a minimum of 29 and 27 of the *Geophagus* and *Guianacara* samples respectively. Loci were aligned in *ipyrad* using standard parameters (0.85 clustering threshold) and a single SNP was exported for each locus.

Bayesian clustering

Genetically distinct populations were first characterized for all samples using the Bayesian clustering program STRUCTURE (v 2.3.4; Pritchard et al. 2000; Hubisz et al. 2009) to infer the most likely number of ancestral populations (or clusters, K). Ten independent runs of 50,000 (burn-in) + 500,000 iterations were estimated in *STRUCTURE* for each value of K (from one to six) with runs parallelized using *Strauto* (v1.0; Chhatre and Emerson 2017). Independent *STRUCTURE* runs were compared using the Evanno method (Evanno et al. 2005) as determined in *Structure harvester* (Earl and vonHoldt 2012), and runs were combined using *CLUMPAK* (Jakobsson and Rosenberg 2007; Kopelman et al. 2015).

Genetic diversity and differentiation

I characterized the genetic diversity and genetic distance of both *Geophagus* and *Guianacara* across the Rupununi Portal using the R packages *hierfstat* (version 0.5-11; Goudet 2005; Goudet et al. 2022) and *Adegenet* (version 2.1.10; Jombart 2008a; Jombart and Ahmed 2011). Observed heterozygosity (H_O), mean gene diversity (H_S), and pairwise-FST (Weir and Cockerham 1984; Takezaki and Nei 1996) were determined for samples at two levels (i) by river basin (either Takutu-Branco or Rupununi-Essequibo) or (ii) by individual sampling site (site locations in Figs IV-1&IV-2 and Table SIV-1).

Multivariate analyses

To further quantify and represent the genetic diversity and structure of the analyzed populations in the Rupununi Portal Region I analyzed *Geophagus* and *Guianacara* using genetic principal components analysis (PCA) and spatial principal components analysis (sPCA) as implemented in *Adegenet* (version 2.1.10; Jombart 2008a; Jombart and Ahmed 2011). Principal components analysis allows for a reduction of genetic variability in large datasets to a few multivariate dimensions (principal components) that best explain the main genetic patterns of variation and the analysis is commonly applied to reveal ancestral relationships that can be related to geographic relationships (Reich et al. 2008). Spatial principal components analysis then incorporates the GPS locations for each sample, and accounts for the effects of spatial autocorrelation between samples, through a spatial weighting matrix. Spatial autocorrelation, the similarity of samples due solely to their being near one another, can then be accounted for (Jombart et al. 2008b). The sPCA analyses therefore reveal local and regional patterns of spatial genetic variation that can be used to infer the genetic variability that is due to factors other than baseline isolation-by-distance (IBD; Wright 1943). If dispersal and gene flow are easier along

within-river connections versus between river basins, this should be further apparent in the sPCA genetic clines between samples.

RESULTS

Library preparation and matrix assembly

After sequencing, one *Geophagus* sample was removed due to low sequence reads (<100,000), leaving a total of 38 *Geophagus* and 35 *Guianacara* samples with an average of 3,470,499 (SD = 1,866,294) and 4,953,193 (SD = 1,734,866) raw reads per individual respectively (see Table SIV-2). Upon aligning and filtering loci, the exported matrices for *Geophagus* and *Guianacara* contained 7396 and 8986 unlinked SNPs respectively with 7.8% and 6.3% missing data, respectively (Table SIV-2).

Bayesian clustering

The most likely number of genetic clusters (i.e., ancestral populations) in the Rupununi Portal differed between *Geophagus* and *Guianacara*. However, I observed complementary patterns in their ancestral populations across the portions of river where their sampling overlapped. Three genetic clusters were inferred for *Geophagus* (K=3) with one each for the Takutu River, most of the Rupununi and Rewa Rivers, and for three samples that occurred furthest upstream in the Rewa River, above a set of waterfalls (site 15; Fig. IV-2), respectively. Interestingly, one downstream Rupununi River individual (at site 8; Fig. IV-2) clustered entirely with the Takutu River samples, indicating a possible conduit for dispersal across the Portal. Site 8 is within the Rupununi River basin, but at a site directly adjacent to Lake Amuku. Lake Amuku refers to an

area of seasonal flooding across the northern Takutu and Rupununi Rivers and has been identified as a conduit for fish dispersal; the first confirmed 'portal' site (De Souza et al. 2012, 2020).

In *Guianacara* four clusters were inferred (K=4; Fig. IV-1 & Fig. IV-2B), albeit highly congruent with the geographic relationships observed in *Geophagus*. Cluster 1 mostly corresponded to the Takutu River; cluster 2 comprised most of the Rupununi+Rewa Rivers.; cluster three corresponded with a set of upstream waterfalls in the Rewa River (site 15), but these samples clustered with sites in the Kuyuwini River, south and outside of the Rewa basin (sites 16 and 17; Fig. IV-2B), for which I had no *Geophagus* samples. The two most upstream (southernmost) Takutu samples also showed partial assignment to the Kuyuwini cluster (sites 2 and 4, Fig. IV-2). This widespread third cluster in *Guianacara* may indicate gene flow between the Kuyuwini and the upper Rewa, the Kuyuwini and the southern Takutu, and more generally identify a widely distributed genetic cluster that appears absent from the middle and lower Rewa River. The fourth cluster in *Guianacara* included two samples from the Konawaruk River, a tributary of the middle Essequibo just south of the Pakaraima Mountains (site 19). Interestingly, the one *Guianacara* sample from the Ireng River (a northern tributary of Branco, which meets the Takutu, Fig. IV-2B: site 6) showed some assignment to northern cluster 4 (membership coefficient = 0.27). The Ireng *Guianacara* sample is the geographically closest sample to the Konawaruk samples (Fig. IV-2B: site 19) sample, but in river systems separated by the Pakaraima Mountains.

Genetic diversity and differentiation

Geophagus genetic diversity, as measured by observed heterozygosity, was similar between the Takutu and Rupununi River systems (H_O: 0.14 and 0.15 respectively). When delineated by individual sampling site (Fig. IV-1B&IV-2; and Table SIV-3), measures of genetic diversity for *Geophagus* were mostly uniform throughout the Portal (H_O from 0.14 to 0.16; see Table SIV-3). Exceptions to this pattern were the furthest upstream (southern) site in the Takutu (site 1; H_O=0.096) and the furthest upstream site in the Rewa (above the waterfalls; site 15, H_O=0.013), which showed markedly lower genetic diversity than was detected at other sites. For *Geophagus*, measures of genetic distance (F_{ST}) ranged between sampling sites from 0.09 and 0.14 in the Takutu and typically between 0.05 and 0.10 in the Rupununi-Rewa indicating a similar level of genetic distance along the course of each river basin (Table SIV-4).

Meanwhile, patterns of genetic distance for *Geophagus* across the Portal corroborate the results of STRUCTURE analyses. First, *Geophagus* individuals sampled adjacent to Lake Amuku in both the Takutu and Rupununi, had among the lowest measures of genetic distance (F_{ST}=0.08 between sites 5 and 8; Figs IV-1B&IV-2); suggestive of the Rupununi Portal conduit (de Souza et al. 2012, 2020). The single *Geophagus* individual sampled from the farthest north Rupununi side (site 8, Fig. IV-1B&IV-2) also showed greater genetic distance to nearby Rupununi sites (F_{ST} of 0.14 and 0.15) relative to the closest upstream and downstream sites respectively (Fig. IV-1B&IV-2, sites 7 and 9), suggesting that this individual may be a recent Takutu migrant. Overall, the genetic distance between Takutu River sampling sites and most Rupununi River sampling sites ranged from 0.08 to 0.18, indicating a similar level of genetic distance between the systems as was observed within each system (Table SIV-4). Measures of genetic distance for *Geophagus* also corroborated the pattern observed for cluster 3 at the upper Rewa River sites (above the set of waterfalls, site 15, Fig. IV-1B&IV-2). The upper Rewa

showed a genetic distance (F_{ST}) of 0.15 to 0.18 relative to lower and middle Rewa sampling sites, representing 50-80% higher genetic distance than is observed throughout the entire length of the lower to middle Rewa (>60 km of sampled river) despite <10km separating sites below and above the waterfalls.

In *Guianacara* I saw corroborating patterns of genetic diversity (Table SIV-3) and genetic distance (Table SIV-5) to those observed in *Geophagus*, and again in line with the STRUCTURE results. Observed heterozygosity (H_O) by sampling site ranged from 0.07 to 0.12 in the Takutu, from 0.08 to 0.15 in the Rupununi-Rewa, 0.10 at both sites in Kuyuwini, and 0.10 in the Konawaruk. Once again, the above-waterfall site in the Rewa (site 15) showed the lowest genetic diversity of Rewa samples (H_O=0.08 relative to 0.14 to 0.15 at other Rupununi-Rewa sites). Genetic distance in the Kuyuwini and upper Rewa were low (F_{ST} of 0.07 and 0.08; see Table SIV-5) relative to F_{ST} between the upper Rewa (site 15), and lower Rewa sites (between 0.11 and 0.12 for site 15 relative to sites 9, 11, 12, and 14). That the genetic distances for both *Geophagus* and *Guianacara* were on a similar scale between river systems as they were within each river system indicates that dispersal across the Portal due to flooding facilitates comparable gene flow as is observed from longitudinal dispersal within each river system.

Multivariate analyses

The genetic clusters identified in the STRUCTURE analyses were also apparent in the PCA analyses (Fig. IV-3). The first two PC axes explain 16.3% and 10.2% of the observed variation for the *Geophagus* analysis, respectively (Fig. IV-3A). The first two PC axes explained 20.5% and 13.2% of the variation for the *Guianacara* analysis (Fig. IV-3B). Three groups separate along the first two PCs in the PCA of *Geophagus*: (i) one that corresponds entirely to Takutu

samples, (ii) one that corresponds to the Rupununi and the lower- to middle-Rewa River below the waterfalls, and (iii) the upper Rewa (Fig. IV-1&IV-2, site 15). Again, one Rupununi sample (site 8, Fig. IV-1&IV-2) grouped with Takutu samples. The genetic variation along PC1 seems to be largely explained by the distinctness of the Takutu and Rupununi River populations, while PC2 separates populations above and below the Rewa waterfalls and highlights their role as a barrier to dispersal and gene flow.

The genetic groupings in the PCA of *Guianacara* are consistent with those observed in *Geophagus*, but once again broadening the regional representation of population structure due to the wider geographic sampling. The same genetic groups of (i) Takutu River, (ii) Rupununi and lower- to middle-Rewa, and (iii) upper-Rewa (Fig.IV-1&IV-2, site 15; above waterfall) that were apparent in *Geophagus* are identifiable in *Guianacara*. Additionally, the southern samples from the Kuyuwini grouped with upper-Rewa samples (Fig. IV-3B), suggesting an additional genetic population in the southern part of the region. The genetic variation along PC1 therefore seems to be largely explained by the break in gene flow due to the waterfalls in the Rewa River, with very little genetic distance observed between the Kuyuwini and upper-Rewa. The second PC axis separates different river systems with obvious gaps between samples from the Takutu, the Rupununi-Rewa, and the Konawaruk. Conspicuously, the Ireng sample (a Rio Branco tributary like the Takutu, site 6, Fig. IV-1&IV-2) is separated from Takutu samples on both PCs (Fig. IV-3B). The identification of a fourth cluster including the Konawaruk samples (site 19, Fig. IV-1&IV-2) and partially the sole Ireng sample, is suggestive of further connections between the northern Rupununi and the Essequibo mainstem through low elevation drainages draining or surrounding the Pakaraima mountains.

The sPCA analyses highlighted the associations between the identified clusters once spatial autocorrelation had been factored out (Fig. IV-4). In the *Geophagus* sPCA there was a grouping of Takutu samples (in blue) and a grouping of Rupununi samples (in red, Fig. IV-4A). However, the connection across the Rupununi Portal is apparent in Portal-adjacent Rupununi sites associating with Takutu samples (specifically at site 7, see Fig. IV-4A).

In *Guianacara*, the genetic clines most obviously separate two genetic groups, one that includes the Takutu, Rupununi, and lower Rewa, and one that includes the upper Rewa and the Kuyuwini (Fig. IV-4B). The lower Takutu and lower-Rupununi samples may therefore have a good amount of the observed genetic distance explained by spatial autocorrelation and my sampling, and otherwise constitute an inter-basin Portal population (i.e., differentiation by 'isolation-by-distance', IBD; Wright 1943).

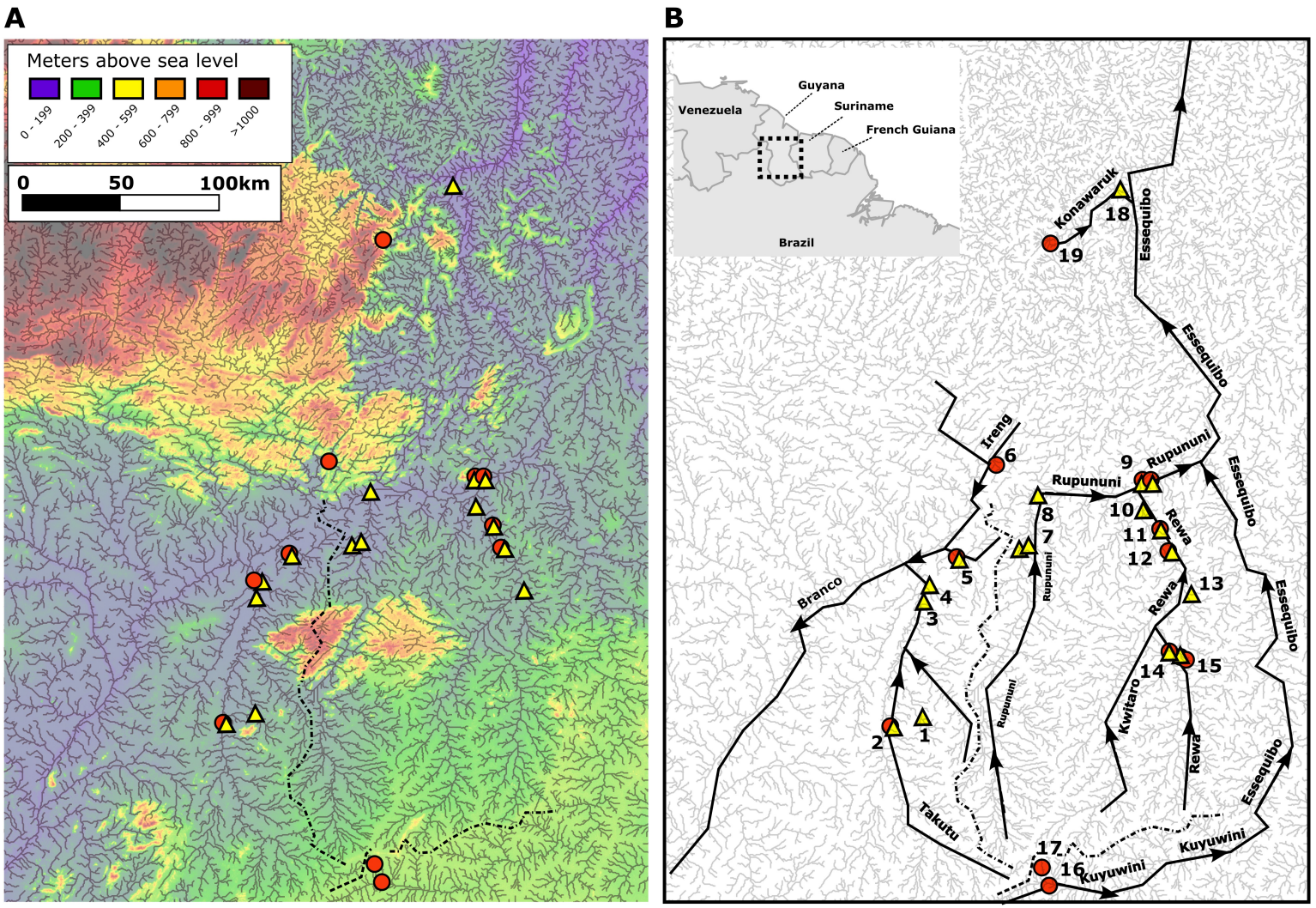


Figure IV-1: Sampling distribution for *Geophagus* (n=38, yellow triangles) and *Guianacara* (n=35, red circles) in the Rupununi Portal Region of southern Guyana. A) sampling sites in relation to the topography of the Region. B) Simplified representation of the major river systems sampled in my analyses, and relative direction of water-flow indicated with arrows along the river systems between sampling sites; 1 to 19 indicate sample sites. Dashed lines delineate the Branco River Basin (Takutu and Ireng Rivers, west of the line) from the Essequibo River Basin (Rupununi and Rewa Rivers, east of the line) and delineate the Kuyuwini River from tributaries of the Takutu and Rupununi.

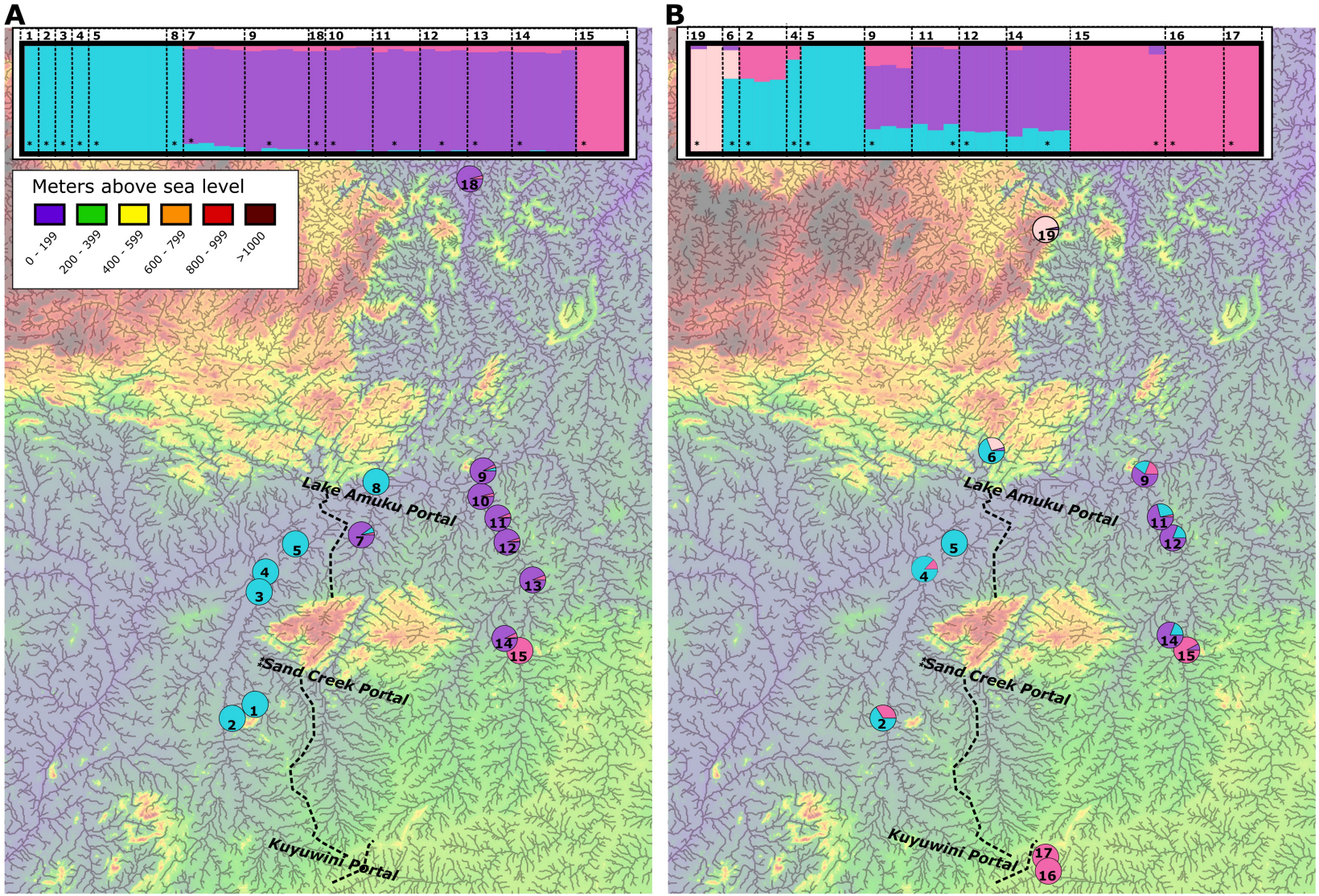


Figure IV-2: Cluster assignment from STRUCTURE analysis of A) *Geophagus* (n=38) and B) *Guianacara* (n=35) within the Rupununi Portal Region of southern Guyana. Clusters assignments of all individuals are shown at the top, with numbers corresponding to sampling sites as shown Figure 1. Pie charts show cluster assignment of one sample from each site, the individual representing that site is shown in the above panel, indicated with *. Sand Creek Portal (de Souza et al. 2020) = ⁑, which was not detected in my analyses, possibly due to the low sampling adjacent to that site.

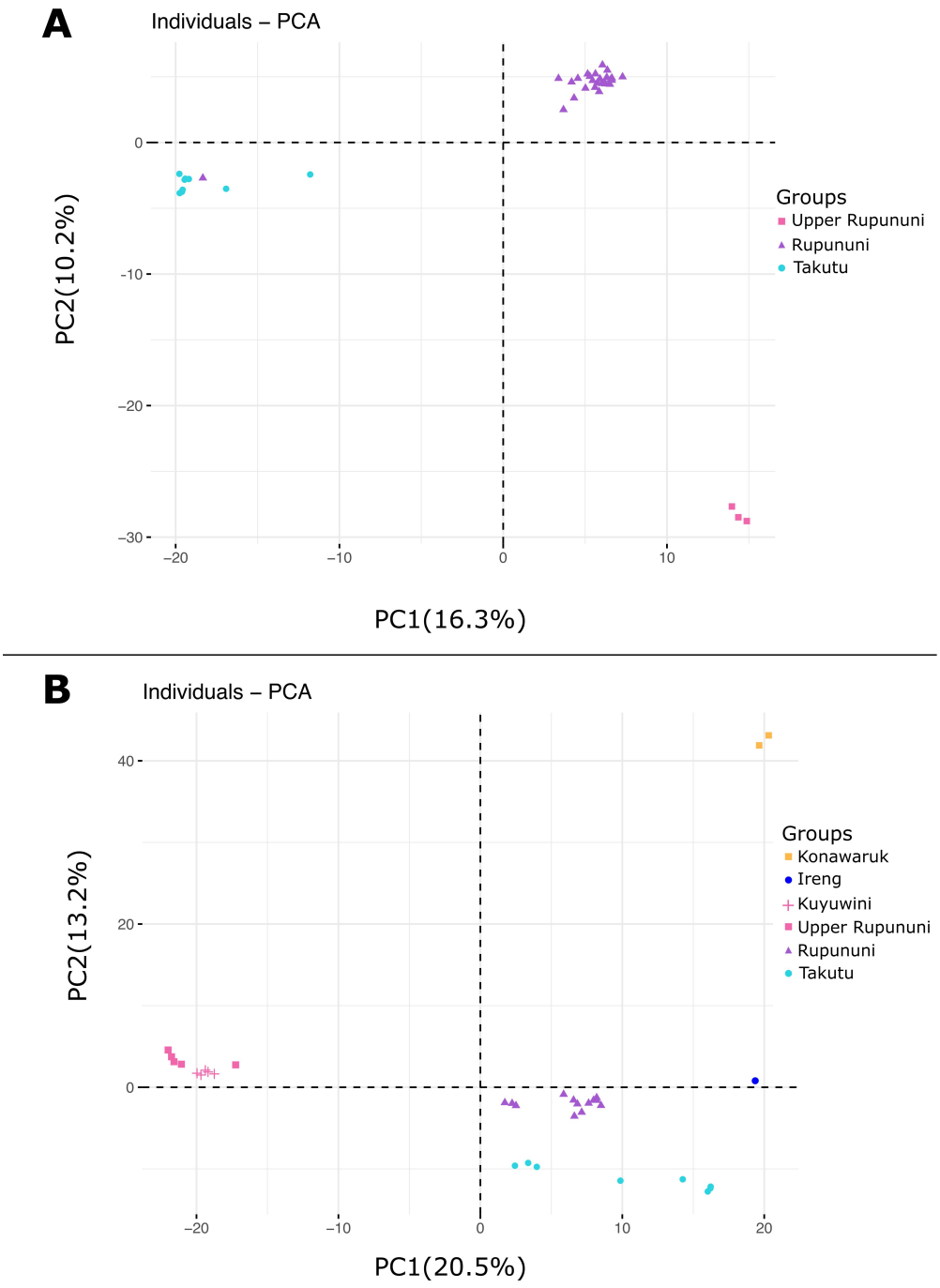


Figure IV-3: Genetic principal components analysis for A) *Geophagus sp.* and B) *Guianacara dacrya* individuals from the Rupunini Portal Region of southern Guyana; based on 7,396 and 8,986 unlinked SNPs respectively.

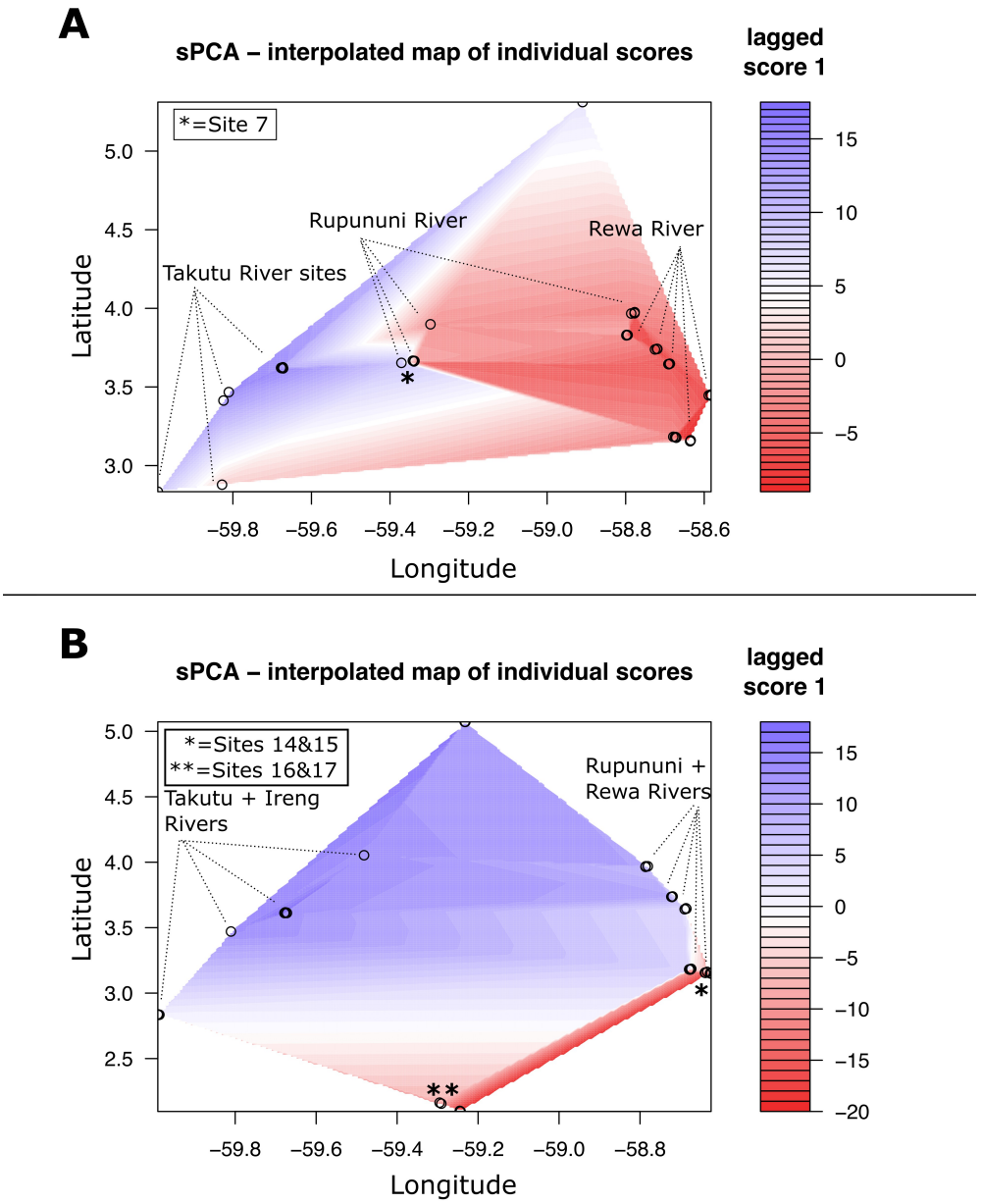


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Figure IV-4: Genetic spatial principal components analysis for A) *Geophagus sp.* and B) *Guianacara dacrya* individuals from the Rupunini Portal Region of southern Guyana; based on 7,396 and 8,986 unlinked SNPs respectively. Genetic clines are indicated by similar coloration indicating more genetically similar samples. Lagged scores as representation of principal components with statistical noise caused by spatial autocorrelation removed. * and ** highlight sites that are discussed for their out-of-network associations indicating dispersal and gene flow.

DISCUSSION

I analyzed the population structure of two genera of South American fishes in the Guiana Shield region to understand the effects of inter-basin connectivity at various time scales on the distribution of diversity in and across river networks. I found clear signals of spatial and temporal effects of inter-network connection and isolation across an ecologically dynamic region that serves as a permeable barrier between two large South American river basins.

Genetic admixture across the Rupununi Portal: multiple conduits lead to bidirectional inter-basin fish dispersal.

I identify signatures of inter-basin dispersal across the Portal in two areas, a more northern site, connecting populations of both *Geophagus* and *Guianacara*, and a more southern site, connecting *Guianacara* populations between the Kuyuwini and southern Takutu. First, the signatures of a northern conduit are congruent with earlier evidence of Lake Amuku as a contemporary conduit for fish dispersal (de Souza et al. 2012; 2020). I found evidence for dispersal of both *Geophagus* and *Guianacara* in the form of gene flow and admixture between the Takutu River and the Rupununi River systems, and the closest genetic associations (in all analyses) indicate that this occurs near sites 5, 7, and 8, which are adjacent to Lake Amuku (Figs IV-1 and IV-2). The signatures of admixture at the more northern conduit also indicated a west-to-east direction of dispersal for both *Geophagus* and *Guianacara* with a proportion of the

Takutu ancestral population found within Rupununi samples of both species, but no signature of the Rupununi ancestral population detected within Takutu individuals (Fig. IV-2).

Second, the analyses of *Guianacara* revealed a possible inter-basin conduit further south in the Portal Region, between southern tributaries of the Takutu and the Kuyuwini River (Figs IV-1 and IV-2). Signatures of admixture as well as the sPCA analyses group the upper Rewa, the Kuyuwini, and southern Takutu samples. However, the genetic distance between the southern Takutu samples and the Kuyuwini (F_{ST} = 0.12 to both Kuyuwini sites; Table SIV-4) were slightly higher than the genetic distance between the southern Takutu and Rupununi-Rewa sites (F_{ST} = 0.10; Table SIV-4). The partial assignment of southern Takutu samples to the southern Kuyuwini+upper-Rewa group is indicative of east-to-west dispersal at this more southern Portal.

The two conduits I identified in this study add to previous work that identified multiple conduits across the Portal (de Souza et al. 2012, 2020). However, while I noted that the results support the presence of the northern corridor, which is consistent with Lake Amuku as a conduit, I did not have samples adjacent to the southern 'Sand Creek Portal', and its influence was therefore not apparent in my analyses. The potential southern conduit between the Kuyuwini and southern Takutu which I identified may be related to the Sand Creek Portal or may represent a novel 'Kuyuwini' corridor. There may therefore be several flooded points in the Portal region that serve as inter-basin conduits. Systematic geographic and taxonomic sampling is required to further inform which flooded regions allow fish dispersal, and to what degree. If confirmed, the presence of several conduits would serve as an invaluable set of model systems in which to

analyze the both the biotic and abiotic determinants of dispersal and gene flow between adjacent river systems.

The support I present for the directionality of dispersal of fishes across the Portal represents novel information about these regional inter-basin conduits. The biotic and abiotic factors that allow some species to disperse across the Portal, with established populations in both river basins, are not immediately clear and require further characterization. However, the biotic factors that allow some species to disperse between river basins while other species are unable is most useful if it can be placed under a generalized framework that can be applied to other systems globally.

Inter-basin and intra-basin gene flow across the Portal Region: the permeability of the Portal to fish dispersal.

The sampling of both *Geophagus* and *Guianacara* from across the river systems of the Rupununi Portal allowed for the comparison of genetic diversity and genetic distance as they vary within- and between each river system and to relate this variation to known hydrologic features of the Portal. The genetic population structures observed in *Geophagus* and *Guianacara* are consistent with many patterns in past studies of Neotropical fishes, such as the importance of waterfalls as barriers (e.g., Tatarenkov et al. 2013; Prado et al. 2018; Apolinário-Silva et al. 2021).

That waterfalls represent a substantial barrier to fish dispersal is well established (Ricklefs and Schluter 1993; Kruse et al. 1997; Dias et al. 2013). Waterfalls often represent

points with high species turnover and marking significant genetic distance between their upstream and downstream habitats (e.g., Hardman 2002; Dias et al. 2013; da Silva et al. 2019; Lujan et al. 2020; Ebner et al. 2021). Additionally, when waterfalls isolate smaller, low-order systems, the long-term persistence of species may be threatened by limited habitat-availability and isolation.

In cases where waterfalls isolate smaller low-order river systems, as I observed in the upper Rewa River, further understanding the connectivity between interdigitating river systems is necessary in properly defining geographic populations. While the upper Rewa populations of both *Geophagus* and *Guianacara* appear by some initial metrics to be isolated, the wider sampling of *Guianacara* allowed us to identify a genetic population that spans the Kuyuwini River and upper Rewa. The connection between the Kuyuwini and upper Rewa has not previously been characterized and highlights the ways that drainage rearrangement and inter-basin gene flow can result in population structure that deviates from model expectations. The extent to which this connection facilitated dispersal of other species should be investigated with further genetic characterization of species present in both systems.

In analyzing the genetic population structure of *Geophagus* and *Guianacara*, I demonstrated that upstream fish populations, such as those in the upper Rewa, may not always be entirely isolated. While waterfalls represent substantial barriers to longitudinal fish dispersal, out-of-network dispersal between upper tributaries presents a set of alternative routes by which populations could be connected. When riverine insects (with aerial life stages) were observed to disperse overland, patterns of DIGD were absent (Blanchet et al. 2020). Further informing

models for fish dispersal out-of-network could therefore be informed by overland dispersals of semi-aquatic species, such as salamanders (Grover and Wilbur 2002; Rissler et al. 2004), freshwater shrimp (Hurwood and Hughes 2001), and aquatic insects (Petersen et al. 2004; Macneale et al. 2005; Blanchet et al. 2020). My findings that upper tributaries may be more connected to one another than previously appreciated fit into a wider set of literature that asks how low-order stream systems may act as refuges for species persistence (May et al. 2017; Moy et al. 2018; Allan et al. 2021).

The biogeography of fishes in the Rupununi Portal

The genetic population structures of *Geophagus* and *Guianacara* across the Portal highlight how fish dispersal between distinct river basins can lead, for some species, to the establishment of novel range. Globally, low-order tributaries of distinct river basins often come into close proximity with one another within upland regions (Grill et al. 2019). However, numerous questions remain on how, and for which species, hydrologic conduits facilitate out-of-network dispersal. I presented evidence of gene flow between adjacent populations, for a system that has previously had its inter-basin dispersals characterized using ecological methods (de Souza et al. 2012, 2020). The genetic characterization of the Rupununi Portal as a contemporary case of inter-basin range expansion serves as a useful analog for range expansion across inter-basin conduits on macroevolutionary timescales.

The Rupununi Portal is a model system in which to further our understanding of fish dispersals between river basins as they shape to the biogeography of riverine fishes on multiple temporal scales. Past studies have highlighted the importance of life history strategy, habitat requirements, physicochemical tolerances, and inherent dispersal ability as possible determinants of success for fishes establishing novel range (Winemiller et al. 2008a,b; Stoffels et al. 2016; Comte and Olden 2018; Willis et al. 2022). However, the fish communities of the Portal demonstrate the complexity of the biological determinants of dispersal, with several congeners displaying contrasting patterns of distribution. Within cichlids there are several instances where species with seemingly comparable life history strategies and ecologies are differently present across the Portal. Two species of *Cichla* are present on each side of the Portal, *C. temensis* and *C. ocellaris* on the Takutu side of the Portal and *C. cataractae* and *C. ocellaris* on the Rupununi side (Willis et al. 2007; Sabaj et al. 2020). Meanwhile *Geophagus* has one known species in both river systems (*Geophagus* sp., this study), and a second species that occurs only on the Takutu side of the Portal. The further study of species-pairs across the Portal presents an intriguing set of systems in which to understand the determinants of inter-basin dispersal.

Inter-basin dispersal in the Rupununi Portal Region has had a recurring role in shaping the biogeography of the Guiana Shield (Lujan and Armbruster 2011; Fontenelle et al. 2021; Frable et al. 2022). The Rupununi Portal Region exists within the Takutu graben, a low relief between uplands of the Pakaraima and Kanuku, Kamoa, and Acarai Mountains through which large paleodrainages are thought to have connected precursors of contemporary river basins (Sinha 1968; Gibbs and Barron 1993; Lujan and Armbruster 2011). The Takutu graben harbored

tributaries of the proto-Berbice throughout the Miocene, and its breakup through river capture is the hypothesized mechanism for range expansion of numerous fishes between the Amazon, Orinoco, and Guianas river basins (Sinha 1968; Lundberg et al. 1998; Lujan and Armbruster 2011). The range expansion of *Geophagus* through the Takutu graben was shown in Chapter II to lead to the diversification of several lineages within the Guianas, and its widespread distribution across the entire region. Those species that were unable to expand their ranges would therefore have been denied that opportunity to diversity allopatrically in adjacent basins. Contemporary patterns of dispersal across the Rupununi are therefore an intriguing analog to the processes that shaped the biogeography of the Guiana Shield, and with the Rupununi itself central in both cases.

CONCLUSION

I identify several instances of out-of-network dispersal that have occurred in the Rupununi Portal Region of southern Guyana. I discussed the detection of out-of-network (or 'overland') dispersal as a deviation from typical longitudinal fish dispersal within riverine dendritic networks. That out-of-network dispersal routes facilitate gene flow therefore necessitates reevaluation of the null expectations of population structure in dendritic networks (e.g. DIGD).

The Portal connections in the Rupununi will continue to serve as a critical model system for understanding inter-basin corridors. However, studying additional systems is also critical to understanding riverine population structure. The Casiquiare Canal connection of western

Amazonia, the Atabapo River connection to the Negro, and the Madeira to Paraguay River connection of eastern Bolivia are all riverine systems in which further investigation could elucidate when riverine population structure should consider out-of-network as a dimension (Hamilton et al. 2002). Beyond the likely biological underpinnings of the fishes that are dispersing, studying multiple systems will allow further investigation into the abiotic factors that influence riverine population structure. Water chemistry, water flow characteristics, and water body type have all been variously investigated for their influences on fish dispersal (Winemiller et al. 2008a; Araújo et al. 2017; Chea et al. 2020; Willis et al. 2022). The Rupununi Portal Region and the Casiquiare Canal system represent two systems where major shifts in water chemistry and habitat availability have been determined important environmental filters to fish dispersal highlighting once again their critical utility to future studies (Willis et al. 2007, 2022; Winemiller et al. 2008a; Winemiller and Willis 2011; Araújo et al. 2017; de Souza et al. 2020).

V. General Conclusions

SUMMARY

In this dissertation, I analyzed the genetic relationships of riverine fishes and highlighted the impacts of inter-basin and out-of-network dispersal on several spatial and temporal scales. First, **Chapter II** highlighted how drainage rearrangements between the river basins of the Guiana Shield facilitated range expansions for a *Geophagus* and shaped its biodiversity across the region. The analyses of *Geophagus* lineages support the existence of several previously hypothesized inter-basin corridors in the Guiana Shield, and further demonstrates the disproportionate influence that a few of the corridors had in shaping the biogeography of this genus. Inter-basin range expansions followed by isolation and diversification are observed to have occurred repeatedly throughout the Miocene to Pleistocene, demonstrating that dispersal between river basins is a repeating mechanism shaping diversification and biogeography.

I then analyzed the population structure of fish species in two different regions to clarify how gene flow between adjacent upland tributaries on contemporary timescales structures populations. In **Chapter III**, analyses of genetic population structure for *Krobia potaroensis* detected admixture and gene flow between the interdigitating river systems of the Pakaraima Mountains systems. Additionally, the populations of *K. potaroensis* were not observed to be structured as is expected by models of dendritic river systems. **Chapter IV** explored the theme of out-of-network dispersal through analyses of two cichlids in the transiently flooding Rupununi Portal. I demonstrated genetic admixture between *Geophagus sp.* and *Guianacara dacrya*

populations across the Portal connection, confirming previous ecological studies of this region. Further, the analyses of genetic diversity and genetic distance for both species along a substantial length of each river systems allowed for the contextualization of inter-basin genetic measures relative to intra-basin gene flow. Altogether, the chapters of this dissertation demonstrate that the phylogenetic relationships and the patterns of genetic diversity for riverine fishes are continuously shaped by both the intra-basin and inter-basin architecture of river systems.

KEY IMPLICATIONS AND TAKEWAYS

This dissertation highlights the ways that inter-basin hydrologic connections facilitate fish dispersal on both evolutionary and contemporary timescales. I highlight several inter-basin dispersals that are detectable at the genetic population level and in the phylogenies of Guiana Shield cichlids. I demonstrate that multiple inter-basin conduits were available over geologic time, but that certain conduits disproportionately shape the diversity of GS cichlids, relative to other conduits.

On the contemporary scale, individual river systems are considered the geographic units for riverine fishes and are modeled as distinct. I demonstrated that inter-basin dispersal is shaping the genetic population structure of GS fishes in two regions, with gene flow apparent between distinct and interdigitating rivers. In analyzing admixture and gene flow both out-of-network and within-network I note that the permeability and directionality varies between inter-basin conduits and between species. I further highlighted the ways that out-of-network dispersal violates the assumptions of genetic population models for riverine systems, and therefore the

need to extend these models when out-of-network dispersal has shaped contemporary population structure.

The spread of genetic diversity across geographic space shapes the evolutionary trajectories of wild species. This dissertation elucidates one of the mechanisms that shapes the biogeography and extreme biodiversity of Guiana Shield fishes. The macroevolutionary influences of drainage rearrangement and range expansion on the biogeography of the Guiana Shield (**Chapter II**) are better understood through the lens of contemporary connections (**Chapters III and IV**) and vice versa.

FUTURE DIRECTIONS

In this dissertation, I established that cichlids from several genera disperse between adjacent river systems when hydrologic connections are available. However, that the species analyzed in this dissertation were able to disperse between river basins does not resolve why some species are able to disperse while other sympatric species are not. I proposed that future studies of inter-basin dispersal employ a framework such as the periodic table of niches (Winemiller et al. 2015) to categorize fishes in a generalizable way when analyzing both the species that can disperse between networks and the species that cannot.

The Guiana Shield Region has several inter-basin conduits that vary in their permeabilities to dispersal (Lowe-McConnell 1964, 1979; Gibbs and Barron 1993; Hamilton et al. 2002; Winemiller et al. 2008a; Araújo et al. 2017; de Souza et al 2012, 2020). The differing genetic structuring of fishes along and between river systems shapes the success of species in

becoming widespread and diverse over evolutionary time, and this genetic structure is affected by out-of-network dispersal.

The rivers of the Guiana Shield harbor a disproportionate amount of freshwater fish diversity globally and are therefore a critical set of systems in which to further understand how riverine connection drives diversification and how it isolates endemic lineages (Birindelli and Sidlauskas 2018). The biogeography of Guiana Shield fishes is such that species are variously distributed (Albert et al. 2011) and differentially vulnerable to extirpation. Analogous to how genetic diversity and population structure shapes evolutionary trajectories, it also bolsters resilience to anthropogenic disturbances. The results of this dissertation provide crucial information in the general delineation of genetic populations in rivers, which is critical to the development of conservation and management strategies for riverine fishes.

Appendices

Appendix A: Taxonomic Insights into *Geophagus sensu stricto*

A.1.0. Biogeography and phylogenetics of described species of *Geophagus*

In the context of cryptic morphologic diversity, phylogenomic analyses have allowed for unprecedented resolution of intrageneric relationships (e.g., Willis et al. 2014; Willis 2017; Argolo et al. 2020). Consistent with other analyses of Neotropical cichlids, I observe cladogenesis in *Geophagus sensu stricto* without substantial ecomorphological diversification (López-Fernández et al 2013; Arbour and López-Fernández 2014); highlighting the importance of range expansion and isolation to regional biodiversity.

A pattern of multiple co-occurring non-sister clades of *Geophagus* was identified for lineages of Geophagus sampled from sub-basins of the Amazon and Orinoco, and a contrasting pattern of (typically) one lineage per-basin was observed within the river basins of the Guianas; the two exceptions in the Guianas are non-sister lineages *G. harreri* and *G. surinamensis* 'Maroni' (lineages [3 and 16] respectively) both occurring in the Maroni-Marowijne River Basin, and non-sister lineages *G.* 'Takutu sp. 1' and *G.* 'Takutu sp. 2' (lineages [20 and 30] respectively) both occurring in the Takutu River (Fig. III-1).

In several instances clades in my analyses are noted to correspond to identified species. However, there are several instances where novel lineages are identified as sister to described

species. Further descriptive studies incorporating the genomic data I present, along with morphological analyses, should resolve whether widespread clades represent multiple species or substantial isolation-by-distance (Wright 1943).

For described species of *Geophagus* in my analyses, there is strong support in the phylogenomic trees for clades representing (i) *G. taeniopareius* (lineage [1]; Orinoco River; Kullander and Royero 1992), (ii) *G. grammepareius* (lineage [2]; Orinoco River; Kullander and Taphorn 1992), (iii) *G. harreri* (lineage [3]; Maroni River; Gosse 1976), (iv) *G. argyrostictus* (lineage [4]; Xingu River; Kullander 1991), (v) *G. dicrozoster* (lineage [5]; Orinoco River; López-Fernández and Taphorn 2004), (vi) *G. proximus* (lineage [6]; Madeira, Nhamunda, and Trombetas Rivers; Castelnau 1855), (vii) *G. winemilleri* (lineage [8]; Negro and Casiquiare Rivers; López-Fernández and Taphorn 2004), (viii) *G. crocatus* (lineage [11]; Berbice River; Hauser and López-Fernández 2013), (ix) *G. brachybranchus* (lineage [13]; Nickerie River; Kullander and Njissen 1989), (x) *G. surinamensis* (lineage [17]; Suriname River; Bloch 1794), (xi) *G. camopiensis* (lineage [22]; Oyapock River; Pellegrin 1903), (xii) *G. altifrons* (lineage [25]; Madeira and Nhamunda Rivers; Heckel 1840), and (xiii) *G. abalios* (lineage [31]; Orinoco, River; López-Fernández and Taphorn 2004). My analyses therefore support the identities of at least 13 of the described species of *Geophagus*; five within the Guianas including *G. harreri* and *G. camopiensis*, and an additional eight in the wider GS Region (not including *G. brokopondo*; discussed below). Additionally, I note that there are several instances where species descriptions of *Geophagus* within the Amazon Basin occurred after the collection of specimens included in this study. I note below those instances where lineages in the analyses correspond geographically with newly described species.

The deepest nodes of the ML tree represent groups for which I have fewer representative samples (i.e. 'the *argyrostictus* group'; Fig. II-2, lineages [1-4]). However, my analyses support many of the previously delineated relationships within *Geophagus* and clarify others. The designation of *Geophagus* into either those in the '*argyrostictus* group' or the '*surinamensis* group' (López-Fernández and Taphorn 2004) is supported with the represented '*argyrostictus'* lineages (lineages [1-4]) falling as a sister arrangement (though not monophyletic) to a '*surinamensis* group' clade (lineages [5-31]).

Within the '*surinamensis* group' *G. dicrozoster (*lineage [5]) is sister to all other sampled *Geophagus* (Clades A-D). Clade A consists of *G. proximus*, *G.* 'Atabapo', and *G. winemilleri* (Fig. II-2, lineages [6-8]). I note that *G.* 'Atabapo' as a sister-lineage to *G. winemilleri* in the Atabapo river system could represent either a range extension of *G. winemilleri* (and therefore widespread intraspecific variation) or a novel species requiring taxonomic description.

Within the Guianas, Clade B (Fig. II-2, lineages [9-21) was identified to contain multiple undescribed putative species of *Geophagus*. There are currently four described species of '*surinamensis* group' *Geophagus* in the Guianas (Fig. II-2): *G. brachybranchus* (Kullander and Njissen 1989; lineage [13]), *G. brokopondo* (Kullander and Njissen 1989; lineage [18]), *G. crocatus* (Hauser and López-Fernández 2013; lineage [11]), and *G. surinamensis* (Bloch 1794; Kullander and Njissen 1989; lineage [17]). Two other species of *Geophagus* are also found within more eastern river systems of the Guianas: *G. harreri* (lineage [3]; *argyrostictus*-group) and *G. camopiensis* (lineage [22]; Clade C) though they are not a part of Clade B and are inferred to have expanded to their current ranges through separate biogeographic events (Fig. II-2).

A.1.2. Synonymizing *Geophagus surinamensis* and *G. brokopondo*

Our phylogenomic analyses of the two described *Geophagus* species in the Suriname River (*G. surinamensis* [lineage 17] and *G. brokopondo* [lineage 18]) supports synonymizing these species. Representatives of *G. brokopondo* are entirely paraphyletic with *G. surinamensis* in the Suriname River. Samples of *G. surinamensis* from the Suriname River, both upstream and downstream of the Brokopondo reservoir were included in the analyses; along with several *G. brokopondo* samples (Fig. II-1 and II-2). The geographic bound of *G. surinamensis* may still require further investigation, with populations of *G. surinamensis* from the Maroni River, the adjacent river basin to the east of the Suriname River, identified as a separate clade from those in the Suriname River (lineages [16-18]).

A.1.3. Indications of novel *Geophagus* species requiring formal description

Several additional instances of potentially undescribed diversity within the Guianas-clade (Clade B) *Geophagus* are apparent in my analyses (Figs II-1 and II-2). Clades identified in the analyses for (i) the Mazaruni River (lineage [9]), (ii) the Cuyuni River (lineage [10]), (iii) the Corentyne River (lineage [12]), (iv) the Coppename River (lineage [14], (v) the Saramacca River (lineage [15]), (vi) the Maroni River (lineage [16]), (vii) the Demerara River (lineage [19]), (viii) the Essequibo and Rupununi Rivers (lineage [20]), and (ix) the Takutu River (lineage [21]) all require further study and potential description as putatively distinct species. Further morphological investigations are necessary to clarify where unidentified lineages correspond to a morphologically distinguishable species (of the nine identified undescribed clades within the Guianas), or whether a more conservative number of species should be described, with several species spanning more than one river basin.

Outside of the Guianas further undescribed diversity in *Geophagus* can be seen in Clades C and D, where phylogenomic relationships reaffirm several described species, as well as again highlighting instances where formal descriptions are warranted (Figs II-1 and II-2). Undescribed clades were identified for Orinoco and Amazon *Geophagus* sequenced from (i) the Tocantins River (lineage [23]), (ii) the Xingu River (lineages [24 & 27]), (iii) the Tapajos River (lineage [26]), (iv) the Nhamunda River (lineage [28]), (v) the Negro River (lineage [29]), and (vi) the Branco River (lineage [30]). Based on their sister-relationships with described species, some of these clades may represent intraspecific variation of widespread species such as *G. altifrons* (lineages [24 to 27], and G. abalios (lineages [28 to 31]). However, widespread geographic sampling and morphological analyses may be necessary to determine whether there are distinguishable boundaries between putative species, or whether there is a continuum of intraspecific genetic variation due to isolation-by-distance.

Recent studies on the diversity of *Geophagus*, including novel species descriptions, are aiding in the characterization of this widespread and diverse genus. The description of two *Geophagus* species from the Tocantins River (*G. neambi* and *G. sveni*; Lucinda et al. 2013) and one from the Tapajos River (*G. pyrocephalus*; Chuctaya et al. 2022) is geographically consistent with samples I analyzed from these river systems (Figs II-1 and II-2; lineages [23] and [26] for the Tocantins and Trombetas respectively). However, with multiple co-occurring species of *Geophagus* within each river system, and defining characteristics often only present in adult individuals, it can be difficult to definitively determine the species of *Geophagus* using morphological characters alone. I note that the sister-relationship in my analyses of *Geophagus* 'Tocantins' to *G. altifrons* lineages is consistent with analyses from Ximenes et al. (2022), corresponding to their '*G. altifrons* Araguaia-Tocantins', and distinct from *G. sveni* which is also

in their study. However, *G. neambi* is not definitively identified in either Ximenes et al. or this study, so its relationship to the Tocantins lineage and to other *Geophagus* remains unclear.

The recent description of *G. pyrocephalus* from the Tapajos River (Chuctaya et al. 2022) corresponds to relationships observed in my trees. The analysis of cytB relationships by Chuctaya et al. places *G. pyrocephalus* as sister to *G. neambi* (a Tocantins lineage), and that these two species fall within a clade that is sister to *G. abalios.* The C and D clades correspond to the Chuctaya et al. tree topology with my *G.* 'Tocantins' clade (lineage [23]) and *G.* 'Tapajos' clade (lineage [26]) falling within clade C, and sister to Clade D which includes *G. abalios* and *G.* 'Takutu sp. 2'. Other lineages within my analyses were not included in Chuctaya et al. (2022), and further morphological analyses would be illustrative. However, the phylogenetic analyses in this study are consistent with the possibility that *G*. 'Tocantins' and *G*. 'Tapajos' (lineages [23 & 26] respectively) may be *G. neambi* and *G. pyrocephalus* respectively.

Ximenes et al. (2022) represents a well-sampled study of Amazonian *Geophagus* that corroborates many of the relationships I report here. In line with the mtDNA analyses of Ximenes et al. (2022), the RADseq analyses support: that (i) *G. dicrozoster* is sister to all other analyzed lineages of *Geophagus* within the 'surinamensis group' (Figs II-1 and II-2; lineage [5]), (ii) *G. proximus*, and *G. winemilleri* are sister lineages (Figs II-1 and II-2; lineages [6 & 8]), (iii) *G. altifrons* Araguia-Tocantins (G 'Tocantins'; Figs II-1 and II-2; lineage [23]) is sister to all other lineages of *G. altifrons* (lineages [24 to 26]).

The omission of certain Amazonian samples from my analyses, and the omission of Orinoco and Guianas samples from Ximenes et al. (2022) obscures definitive species identification in several instances. For example, the inclusion of samples from the eastern Guianas and Orinoco in my analyses (*G. camopiensis*, *G. abalios*, and *G*. 'Atabapo' lineages [22,

31, & 7] respectively) highlights a close relationship between Amazonian *Geophagus* and lineages in the wider Guiana Shield that is not observed in Ximenes et al. (2022). The wider *G. abalios* group in this study (lineages [27 to 31]) corresponds to the '*Geophagus* sp. 1' of Ximenes et al. (2022). However, their study did not include Orinoco localities from which *G. abalios* is described (López-Fernández and Taphorn 2004). Due to the history of widespread range expansion and diversification of *Geophagus* in the GS region, inclusion of samples from throughout the entire region is necessary to associate Amazonian lineages with species described from the Orinoco. Similarly, and illustrative of where gaps in the sampling of this study obscures species identification, Ximenes et al.'s '*Geophagus* sp. 3', '*Geophagus* sp 4', '*Geophagus* sp 5', '*Geophagus.* sp. 6' are not easily distinguished in my analyses. '*Geophagus* sp. 3' may correspond to the lineage I identify as *G*. sp. 'Lower Xingu aff. altifrons' (lineage [27]), though the absence of *G. mirabilis* and *G. megasema* from my analyses limits the comparisons to the tree of Ximenes et al (2022). Similarly, '*Geophagus* sp 4', '*Geophagus* sp 5', '*Geophagus*. sp. 6', are part of the '*argyrostictus* group' for which I have less dense sampling and are consequently absent from my analyses. With sister-relationships spanning hundreds of kilometers of river (e.g., Clade D, lineages [27 to 31]; and *G.* 'Atabapo' + *G. winemilleri*, lineages [7 & 8]), caution is necessary in delineating novel species that may otherwise be representative of widespread isolation-by-distance (Willis 2017).

The need for further descriptive studies of Neotropical fishes is widely acknowledged, with several thousand species estimated to still require formal description (Birindelli and Sidlauskas 2018). I identify within *Geophagus* difficulties that are emblematic of what can arise from incomplete taxonomic and geographic sampling. A complete taxonomy of Neotropical freshwater fishes is integral to understanding their evolutionary history and conserving their

biodiversity and will be ameliorated with continuing descriptive studies that incorporate both genetic and morphological information.

Appendix B: Supplementary Tables and Figures

Table SII-1: Museum collection information for Geophagus sensu stricto and selected outgroups used in phylogenetic and biogeographic analyses. Museum abbreviations: ROM=Royal Ontario Museum, UMMZ = University of Michigan Museum of Zoology, ANSP = Academy of Natural Sciences of Philadelphia at Drexel University, HV=Henrique Varella, MHNG=Natural History Museum of Geneva

MUSEUM COLLECTION	TISSUE SAMPLE NUMBER	GENUS	SPECIES	RIVER SYSTEM	LATITUDE	LONGITUDE	SAMPLE NAME IN ANALYSES
ROM	T08072	Geophagus	crocatus	Berbice River	4.91	-58.24	Cro-ber-1
ROM	T08073	Geophagus	crocatus	Berbice River	4.91	-58.24	Cro-ber-2
ROM	T08074	Geophagus	crocatus	Berbice River	4.91	-58.24	Cro-ber-3
ROM	T08164	Geophagus	crocatus	Berbice River	5.15	-58.20	Cro-ber-4
ROM	T08165	Geophagus	crocatus	Berbice River	5.15	-58.20	Cro-ber-5
ROM	T08166	Geophagus	crocatus	Berbice River	5.15	-58.20	Cro-ber-6
ROM	T08167	Geophagus	crocatus	Berbice River	5.15	-58.20	Cro-ber-7
ROM	T19360	Geophagus	brokopondo	Brokopondo Reservoir	4.92	-55.13	Bok-bok-1
ROM	T19361	Geophagus	brokopondo	Brokopondo Reservoir	4.92	-55.13	Bok-bok-2
ROM	T19362	Geophagus	brokopondo	Brokopondo Reservoir	4.92	-55.13	Bok-bok-3
ROM	T19363	Geophagus	brokopondo	Brokopondo Reservoir	4.92	-55.13	Bok-bok-4
ROM	T17404	Geophagus	winemilleri	Cano Negro	6.12	-67.50	G-sp-ori-1
ROM	T14664	Geophagus	altifrons	Canumã, lower Madeira	-4.01	-59.10	Alt-can-1
ROM	T14665	Geophagus	altifrons	Canumã, lower Madeira	-4.01	-59.10	Alt-can-2
ROM	T12325	Geophagus	abalios	Cinaruco River	8.16	-67.00	G-sp-cin-1
ROM	T18882	Geophagus	brachybranchus	Corantijn River	5.06	-57.32	Brc-cor-1
ROM	T18893	Geophagus	brachybranchus	Corantijn River	5.06	-57.32	Brc-cor-2
ROM	T18894	Geophagus	brachybranchus	Corantijn River	5.06	-57.32	Brc-cor-3
ROM	T18895	Geophagus	brachybranchus	Corantijn River	5.06	-57.32	Brc-cor-4
ROM	T20485	Geophagus	mazaruni	Cuyuni-Mazaruni	6.22	-60.15	Maz-olc-1
ROM	T20486	Geophagus	mazaruni	Cuyuni-Mazaruni	6.22	-60.15	Maz-olc-2
ROM	T20487	Geophagus	mazaruni	Cuyuni-Mazaruni	6.22	-60.15	Maz-olc-3
ROM	T20488	Geophagus	mazaruni	Cuyuni-Mazaruni	6.22	-60.15	Maz-olc-4
ROM	T20814	Geophagus	sp	Demerara River	6.50	-58.21	Dem-dem-1
ROM	T20815	Geophagus	sp	Demerara River	6.50	-58.21	Dem-dem-2

ROM	T08695	Geophagus	sp	Takutu River	3.62	-59.67	G-sp-tak-tak-1
ROM	T08699	Geophagus	sp	Takutu River	3.62	-59.67	G-sp-tak-tak-2
ROM	T08701	Geophagus	sp	Takutu River	3.62	-59.67	G-sp-tak-tak-3
ROM	T08703	Geophagus	sp	Takutu River	3.62	-59.67	G-sp-tak-tak-4
ROM	T18126	Geophagus	sp	Takutu River	5.31	-58.91	G-sp-ese-1
ROM	T15835	Geophagus	sp	Katuwao River	2.87	-59.83	G-sp-tak-kat-1
ROM	T23184	Geophagus	sp	Kwein Kwein Creek	4.45	-55.76	Sar-kkc-1
ROM	T23185	Geophagus	sp	Kwein Kwein Creek	4.45	-55.76	Sar-kkc-2
ROM	T14666	Geophagus	proximus	Lake Canacari - Urubu River	-3.07	-58.35	Pro-can-1
ROM	T14675	Geophagus	altifrons	Lake Grande	-3.71	-58.25	Alt-lgr-1
ROM	T14677	Geophagus	altifrons	Lake Grande	-3.71	-58.25	Alt-lgr-2
ROM	T14680	Geophagus	proximus	Lake Grande	-3.71	-58.25	Pro-lgr-1
ROM	T14681	Geophagus	proximus	Lake Grande	-3.71	-58.25	Pro-lgr-2
ROM	T11820	Geophagus	harreri	Maroni River	3.18	-54.18	Har-mar-1
ROM	T11824	Geophagus	harreri	Maroni River	3.18	-54.18	Har-mar-2
ROM	T18763	Geophagus	harreri	Marowijne River	4.65	-54.43	Har-mar-3
ROM	T19840	Geophagus	harreri	Marowijne River	4.91	-54.44	Har-mar-4
ROM	T20035	Geophagus	surinamensis	Marowijne River	4.90	-54.48	Sur-mar-1
ROM	T20036	Geophagus	surinamensis	Marowijne River	4.90	-54.48	Sur-mar-2
ROM	T20037	Geophagus	surinamensis	Marowijne River	4.90	-54.48	Sur-mar-3
ROM	T20340	Geophagus	mazaruni	Mazaruni River	6.22	-60.15	Maz-maz-1
ROM	T20341	Geophagus	mazaruni	Mazaruni River	6.22	-60.15	Maz-maz-2
ROM	T21413	Geophagus	mazaruni	Mazaruni River	6.29	-60.23	Maz-maz-3
ROM	T14702	Geophagus	dicrozoster	Negro River	-0.42	-65.02	G-sp-neg-3
ROM	T14703	Geophagus	sp	Negro River	-2.62	-60.95	G-sp-neg-4
ROM	T14704	Geophagus	sp	Negro River	-2.62	-60.95	G-sp-neg-5
ROM	T14695	Geophagus	sp	Negro River	0.11	-67.33	G-sp-neg-1
ROM	T14696	Geophagus	sp	Negro River	0.11	-67.33	G-sp-neg-2
ROM	T14709	Geophagus	altifrons	Nhamunda River	-2.19	-56.71	Alt-nha-1
ROM	T14710	Geophagus	altifrons	Nhamunda River	-2.19	-56.71	Alt-nha-2
ROM	T14705	Geophagus	proximus	Nhamunda River	-2.19	-56.71	Pro-nha-1
ROM	T14706	Geophagus	proximus	Nhamunda River	-2.19	-56.71	Pro-nha-2
ROM	T14708	Geophagus	proximus	Nhamunda River	-2.19	-56.71	Pro-nha-3
ROM	T19228	Geophagus	brachybranchus	Nickerie River	4.90	-56.96	Brc-nik-1
ROM	T19256	Geophagus	brachybranchus	Nickerie River	4.90	-56.96	Brc-nik-2

ROM	T19257	Geophagus	brachybranchus	Nickerie River	4.90	-56.96	Brc-nik-3
ROM	T19258	Geophagus	brachybranchus	Nickerie River	4.90	-56.96	Brc-nik-4
ROM	T19259	Geophagus	brachybranchus	Nickerie River	4.90	-56.96	Brc-nik-5
ROM	T08644	Geophagus	sp	Pirara River	3.62	-59.67	G-sp-tak-pir-1
ROM	T08649	Geophagus	sp	Pirara River	3.62	-59.67	G-sp-tak-pir-2
ROM	T08653	Geophagus	sp	Pirara River	3.62	-59.67	G-sp-tak-pir-3
ROM	T08667	Geophagus	sp	Pirara River	3.62	-59.67	G-sp-tak-pir-4
ROM	T08668	Geophagus	sp	Pirara River	3.62	-59.67	G-sp-tak-pir-5
ROM	T13930	Geophagus	winemilleri	Rio Atabapo	3.78	-67.63	Win-ata-1
ROM	T09078	Geophagus	taeniopareius	Río Cataniapo	5.60	-67.61	Tae-ori-1
ROM	T11626	Geophagus	dicrozoster	Rio Cinaruco	6.56	-67.42	Dic-cin-1
ROM	T11627	Geophagus	dicrozoster	Rio Cinaruco	6.56	-67.42	Dic-cin-2
ROM	T11666	Geophagus	grammepareius	Rio Claro	7.92	-63.09	Gra-car-1
ROM	T15011	Geophagus	sp	Rio Iriri	-3.81	-52.62	Xin-iri-2
ROM	T15012	Geophagus	sp	Rio Iriri	-3.81	-52.62	Xin-iri-3
ROM	T09730	Geophagus	abalios	Río Marieta	5.02	-66.36	G-sp-ori-3
ROM	T14734	Geophagus	sp	Rio Negro	0.42	-65.02	G-sp-neg-6
ROM	T14735	Geophagus	sp	Rio Negro	0.42	-65.02	G-sp-neg-7
ROM	T14729	Geophagus	winemilleri	Rio Negro	0.42	-65.02	G-sp-neg-8
ROM	T14730	Geophagus	winemilleri	Rio Negro	0.42	-65.02	G-sp-neg-9
ROM	T14731	Geophagus	winemilleri	Rio Negro	0.42	-65.02	Win-neg-3
ROM	T14732	Geophagus	winemilleri	Rio Negro	0.42	-65.02	Win-neg-4
ROM	T14733	Geophagus	winemilleri	Rio Negro	0.42	-65.02	Win-neg-5
ROM	T15324	Geophagus	sp	Rio Novo	-4.47	-53.67	Xin-nov-1
ROM	T15325	Geophagus	sp	Rio Novo	-4.47	-53.67	Xin-nov-2
ROM	T15326	Geophagus	sp	Rio Novo	-4.47	-53.67	Xin-nov-3
ROM	T15327	Geophagus	sp	Rio Novo	-4.47	-53.67	Xin-nov-4
ROM	T09896	Geophagus	abalios	Río Orinoco	4.92	-67.83	G-sp-ori-2
ROM	T11894	Geophagus	taeniopareius	Rio Siapa	2.12	-66.47	Tae-sia-1
ROM	T09321	Geophagus	dicrozoster	Río Ventuari	3.98	-67.06	Dic-ven-1
ROM	T09574	Geophagus	dicrozoster	Río Ventuari	4.75	-66.37	Dic-ven-2
ROM	T09576	Geophagus	dicrozoster	Río Ventuari	4.75	-66.37	Dic-ven-3
ROM	T06833	Geophagus	sp	Rupununi River	3.65	-59.37	G-sp-rup-rup-2
ROM	T06962	Geophagus	sp	Rupununi River	3.66	-59.34	G-sp-rup-rup-3
ROM	T06963	Geophagus	sp	Rupununi River	3.66	-59.34	G-sp-rup-rup-4

ROM	T06964	Geophagus	sp	Rupununi River	3.66	-59.34	G-sp-rup-rup-5
ROM	T11935	Geophagus	sp	Rupununi River	3.89	-59.29	G-sp-rup-rup-1
ROM	T22693	Geophagus	sp	Saramacca River	4.06	-55.90	Sar-sar-1
ROM	T22767	Geophagus	sp	Saramacca River	4.19	-55.89	Sar-sar-2
ROM	T22768	Geophagus	sp	Saramacca River	4.19	-55.89	Sar-sar-3
ROM	T22769	Geophagus	sp	Saramacca River	4.19	-55.89	Sar-sar-4
ROM	T22770	Geophagus	sp	Saramacca River	4.19	-55.89	Sar-sar-5
ROM	T23402	Geophagus	surinamensis	Suriname River	5.53	-55.05	Sur-sur-1
ROM	T23403	Geophagus	surinamensis	Suriname River	5.53	-55.05	Sur-sur-2
ROM	T23404	Geophagus	surinamensis	Suriname River	5.53	-55.05	Sur-sur-3
ROM	T23405	Geophagus	surinamensis	Suriname River	5.53	-55.05	Sur-sur-4
ROM	T11939	Geophagus	sp	Takutu River	3.41	-59.82	G-sp-tak-tak-7
ROM	T15921	Geophagus	sp	Takutu River	2.84	-59.99	G-sp-tak-tak-5
ROM	T12048	Geophagus	sp	Takutu River	3.36	-59.83	G-sp-tak-tak-8
ROM	T11938	Geophagus	sp	TakutuRiver	3.47	-59.81	G-sp-tak-tak-6
ROM	T21381	Geophagus	mazaruni	Tamakay Creek	6.34	-60.29	Maz-tam-1
ROM	T21403	Geophagus	mazaruni	Tamakay Creek	6.34	-60.29	Maz-tam-2
ROM	T14719	Geophagus	sp	Tapajos River	-6.23	-57.74	G-sp-tap-1
ROM	T14720	Geophagus	sp	Tapajos River	-6.23	-57.74	G-sp-tap-2
ROM	T14721	Geophagus	sp	Tapajos River	-6.23	-57.74	G-sp-tap-3
ROM	T14722	Geophagus	sp	Tapajos River	-6.23	-57.74	G-sp-tap-4
ROM	T14723	Geophagus	sp	Tapajos River	-6.23	-57.74	G-sp-tap-5
ROM	T14670	Geophagus	altifrons	Tocantins at Baião	-3.13	-49.68	Alt-toc-1
ROM	T14671	Geophagus	altifrons	Tocantins at Baião	-3.13	-49.68	Alt-toc-2
ROM	T14672	Geophagus	altifrons	Tocantins at Baião	-3.13	-49.68	Alt-toc-3
ROM	T14712	Geophagus	proximus	Trombetas River	-1.77	-55.87	Pro-tro-1
ROM	T14713	Geophagus	proximus	Trombetas River	-1.77	-55.87	Pro-tro-2
ROM	T14715	Geophagus	proximus	Trombetas River	-1.77	-55.87	Pro-tro-3
ROM	T14693	Geophagus	dicrozoster	Uaupes River	0.09	-68.18	G-sp-uau-1
ROM	T10762	Geophagus	argyrostictus	Xingu River	-1.53	-52.24	Arg-xin-1
ROM	T15005	Geophagus	sp	Xingu River	-3.81	-52.62	Xin-xin-1
ROM	T15006	Geophagus	sp	Xingu River	-3.81	-52.62	Xin-xin-2
ROM	T15007	Geophagus	sp	Xingu River	-3.81	-52.62	Xin-xin-3
ROM	T13104	Geophagus	steindachneri				G-stein-1
ROM	T13106	Geophagus	steindachneri				G-stein-2
ROM	T13108	Geophagus	steindachneri				G-stein-3

ROM	T11753	Gymnogeophagus	balzanii				Gym-bal-1
ROM	T13212	Gymnogeophagus	balzanii				Gym-bal-2
ROM	T11751	Gymnogeophagus	rhabdotus				Gym-rha-1
ROM	T11752	Gymnogeophagus	rhabdotus				Gym-rha-2
ROM	T11847	Gymnogeophagus	setequedas				Gym-set-2
ROM	T11630	Geophagus	abalios	Cinaruco River	6.53	-67.42	Aba-lag-1
ROM	T11631	Geophagus	abalios	Cinaruco River	6.53	-67.42	Aba-lag-2
MHNG	192.270	Geophagus	harreri	Maroni River	2.8585833	-53.98	Har-mar-5
UMMZ	UMMZ-ICH 00637	Geophagus	sp	Rewa River	3.64	-58.69	Rew-rew-1
UMMZ	UMMZ-ICH 00638	Geophagus	sp	Rewa River	3.64	-58.69	Rew-rew-2
UMMZ	UMMZ-ICH 01084	Geophagus	sp.	Rewa River	3.16	-58.64	Rew-rew-3
UMMZ	UMMZ-ICH 01085	Geophagus	sp.	Rewa River	3.16	-58.64	Rew-rew-4
UMMZ	UMMZ-ICH 00343	Geophagus	surinamensis	Suriname River	4.50	-55.33	Sur-sur-5
UMMZ	UMMZ-ICH 00344	Geophagus	surinamensis	Suriname River	4.50	-55.33	Sur-sur-6
UMMZ	UMMZ-ICH 00345	Geophagus	surinamensis	Suriname River	4.50	-55.33	Sur-sur-7
UMMZ	UMMZ-ICH 00346	Geophagus	surinamensis	Suriname River	4.50	-55.33	Sur-sur-8
ROM	T11672	Geophagus	sp. Cuyuni	Rio Cuyuni	6.72	-61.61	G-sp-cuy-1
ROM	T11673	Geophagus	sp. Cuyuni	Rio Cuyuni	6.72	-61.61	G-sp-cuy-2
ROM	T11674	Geophagus	sp. Cuyuni	Rio Cuyuni	6.72	-61.61	G-sp-cuy-3
UMMZ	UMMZ-ICH 01183	Geophagus	sp.	Rupununi River	3.97	-58.79	G-sp-rup-rup-7
ANSP	185178	Geophagus	sp.	Siapa River	1.60	-65.72	G-sp-sia-1
ANSP	43557	Geophagus	taeniopareius	Siapa River	1.45	-65.65	Tae-sia-2
MHNG	172.110	Geophagus	harreri	Maroni River	3.37	-55.43	Har-mar-6
MHNG	172.120	Geophagus	harreri	Maroni River	3.37	-55.43	Har-mar-7
UMMZ	UMMZ-ICH 00262	Geophagus	surinamensis	Coppename River	5.12	-55.84	Sur-cop-1
UMMZ	UMMZ-ICH 00263	Geophagus	surinamensis	Coppename River	5.12	-55.84	Sur-cop-2
UMMZ	UMMZ-ICH 00836	Geophagus	sp.	Rewa River	3.45	-58.59	Rew-rew-5
UMMZ	UMMZ-ICH 00837	Geophagus	sp.	Rewa River	3.45	-58.59	Rew-rew-6
HV	61525	Geophagus	cf. altifrons	Amazon	3.31	-59.92	G-sp-tak-bra-1
HV	61526	Geophagus	cf. altifrons	Amazon	3.31	-59.92	G-sp-tak-bra-2
HV	82694	Geophagus	surinamensis	Oiapoque	3.81	-51.81	G-sp-oya-1
HV	82695	Geophagus	surinamensis	Oiapoque	3.81	-51.81	G-sp-oya-2
HV	82696	Geophagus	surinamensis	Oiapoque	3.81	-51.81	G-sp-oya-3
HV	82697	Geophagus	surinamensis	Oiapoque	3.81	-51.81	G-sp-oya-4

ROM	T15181	Geophagus	argyrostictus	Rio Iriri	-4.00	-53.24	Arg-xin-2
ROM	T15183	Geophagus	argyrostictus	Rio Iriri	-4.00	-53.24	Arg-xin-3
ROM	T15518	Geophagus	argyrostictus	Rio Xingu	-3.65	-52.38	Arg-xin-4
ROM	T15519	Geophagus	argyrostictus	Rio Xingu	-3.65	-52.38	Arg-xin-5
ROM	T09079	Geophagus	taeniopareius	Río Cataniapo	5.60	-67.61	Tae-cat-1
ROM	T11896	Geophagus	taeniopareius	Rio Siapa	2.12	-66.47	Tae-sia-3

Table SII-2: Sequence and exported-matrix information for ddRAD library of Geophagus sensu stricto and selected outgroups used in phylogenetic and biogeographic analyses.

Sample name	Raw reads	Loci in 41 of 163 matrix (of 6,463)	Loci in 82 of 163 matrix (of 4,393)	Loci in 123 of 163 matrix (of 2,767)
Aba-lag-1	3,866,483	3,972	3,317	2,493
Aba-lag-2	1,319,281	2,751	2,421	2,077
Alt-can-1	2,594,964	5,315	3,943	2,634
Alt-can-2	1,424,785	5,093	3,851	2,627
Alt-lgr-1	1,167,509	4,236	3,443	2,551
Alt-lgr-2	999,434	3,960	3,319	2,499
Alt-nha-1	3,494,854	4,843	3,806	2,635
Alt-nha-2	3,796,143	5,174	3,929	2,628
Alt-tap-5	1,698,723	4,633	3,684	2,596
Alt-toc-1	1,124,318	3,613	3,106	2,441
Alt-toc-2	800,027	3,355	2,921	2,388
Alt-toc-3	861,939	3,622	3,062	2,416
Arg-xin-1	1,063,176	3,541	2,852	2,114
Arg-xin-2	5,203,041	4,121	3,175	2,203
Arg-xin-3	4,060,861	4,170	3,175	2,171
Arg-xin-4	6,557,200	3,895	3,220	2,236
Arg-xin-5	6,531,169	4,015	3,200	2,208
Bok-bok-1	3,623,134	4,682	3,852	2,674
Bok-bok-2	3,592,009	4,422	3,740	2,666
Bok-bok-3	4,114,137	4,864	3,933	2,678
Bok-bok-4	3,220,137	4,471	3,730	2,662
Brc-cor-1	3,496,073	4,416	3,617	2,588
Brc-cor-2	6,129,726	5,472	4,127	2,684
Brc-cor-3	1,426,654	3,802	3,236	2,501
Brc-cor-4	1,916,618	4,245	3,577	2,614
Brc-nik-1	2,767,489	4,488	3,701	2,640
Brc-nik-2	2,932,209	4,680	3,788	2,652
Brc-nik-3	3,278,656	4,615	3,775	2,663
Brc-nik-4	3,492,539	4,840	4,003	2,692

Brc-nik-5	3,217,633	4,638	3,908	2,694
Cro-ber-1	2,879,880	4,579	3,877	2,699
Cro-ber-2	2,053,934	4,273	3,698	2,669
Cro-ber-3	2,703,839	5,052	4,064	2,700
Cro-ber-4	2,824,833	4,346	3,763	2,686
Cro-ber-5	3,081,763	4,598	3,893	2,701
Cro-ber-6	2,435,363	4,384	3,785	2,689
Cro-ber-7	2,525,661	4,458	3,809	2,689
Dem-dem-1	3,538,474	4,773	3,904	2,670
Dem-dem-2	3,124,561	4,610	3,863	2,669
Dic-cin-1	4,216,910	4,258	3,484	2,409
Dic-cin-2	5,493,720	4,448	3,515	2,396
Dic-ven-1	1,106,287	2,700	2,399	1,991
Dic-ven-2	876,188	2,508	2,215	1,851
Dic-ven-3	4,946,234	4,298	3,481	2,409
G-sp-cin-1	3,821,009	4,463	3,701	2,638
G-sp-cuy-1	8,910,024	5,077	3,940	2,579
G-sp-cuy-2	6,906,029	5,052	3,915	2,569
G-sp-cuy-3	8,917,401	4,949	3,932	2,577
G-sp-ese-1	2,415,299	5,054	4,026	2,694
G-sp-neg-1	1,957,606	4,115	3,519	2,580
G-sp-neg-2	477,167	2,525	2,276	1,859
G-sp-neg-3	371,460	2,040	1,820	1,513
G-sp-neg-4	1,150,200	2,466	2,115	1,794
G-sp-neg-5	2,921,757	5,489	4,130	2,672
G-sp-neg-6	3,597,880	4,777	3,797	2,635
G-sp-neg-7	2,225,914	4,296	3,453	2,503
G-sp-neg-8	776,485	2,666	2,283	1,826
G-sp-neg-9	281,803	1,872	1,616	1,346
G-sp-ori-1	1,669,634	3,922	3,319	2,559
G-sp-ori-2	4,897,490	4,594	3,729	2,624
G-sp-ori-3	3,298,025	3,887	3,297	2,382
G-sp-oya-1	4,891,627	4,526	3,618	2,508

G-sp-oya-2	4,149,066	4,545	3,610	2,502
G-sp-oya-3	7,456,717	4,888	3,690	2,490
G-sp-oya-4	5,432,175	4,391	3,580	2,509
G-sp-rup-rup-1	1,728,787	4,026	3,504	2,604
G-sp-rup-rup-2	2,139,819	4,690	3,988	2,705
G-sp-rup-rup-3	2,528,152	4,505	3,852	2,687
G-sp-rup-rup-4	2,671,689	4,770	3,978	2,690
G-sp-rup-rup-5	1,084,906	3,364	3,076	2,468
G-sp-rup-rup-7	1,717,477	3,991	3,424	2,484
G-sp-sia-1	8,692,396	3,626	3,067	2,127
G-sp-tak-bra-1	4,658,500	4,853	3,670	2,538
G-sp-tak-bra-2	5,122,912	4,942	3,737	2,539
G-sp-tak-kat-1	3,352,988	4,884	3,965	2,654
G-sp-tak-pir-1	2,658,694	4,325	3,730	2,648
G-sp-tak-pir-2	3,322,043	4,923	3,845	2,634
G-sp-tak-pir-3	2,336,750	4,863	4,050	2,692
G-sp-tak-pir-4	3,695,259	4,829	4,066	2,690
G-sp-tak-pir-5	2,297,189	4,356	3,797	2,681
G-sp-tak-tak-1	3,533,046	4,383	3,711	2,640
G-sp-tak-tak-2	4,243,014	5,001	4,008	2,678
G-sp-tak-tak-3	3,396,926	4,704	3,837	2,647
G-sp-tak-tak-4	3,511,668	5,026	4,083	2,679
G-sp-tak-tak-5	3,834,968	5,481	4,127	2,644
G-sp-tak-tak-6	390,081	2,425	2,235	1,857
G-sp-tak-tak-7	958,803	3,448	3,071	2,414
G-sp-tak-tak-8	673,087	2,713	2,431	2,095
G-sp-tap-1	1,290,294	4,007	3,316	2,513
G-sp-tap-2	1,899,477	4,780	3,719	2,604
G-sp-tap-3	2,348,840	4,638	3,687	2,613
G-sp-tap-4	4,862,976	5,321	3,958	2,631
G-sp-uau-1	1,972,944	3,900	3,435	2,596
G-stein-1	1,458,940	1,307	1,186	1,002
G-stein-2	1,259,010	1,396	1,234	1,007

G-stein-3	1,345,811	1,728	1,410	1,041
Gra-car-1	4,305,010	3,542	3,070	2,239
Gym-bal-1	1,737,725	970	899	776
Gym-bal-2	523,969	827	731	627
Gym-rha-1	1,690,265	1,137	1,021	882
Gym-rha-2	1,450,457	1,024	949	845
Gym-set-2	1,041,597	1,145	986	815
Har-mar-1	6,127,490	4,150	3,309	2,278
Har-mar-2	1,698,531	2,847	2,417	1,945
Har-mar-3	6,011,512	4,141	3,332	2,310
Har-mar-4	1,111,194	3,276	2,762	2,104
Har-mar-5	6,236,502	3,948	3,172	2,196
Har-mar-6	2,390,201	3,529	2,992	2,191
Har-mar-7	9,712,459	3,933	3,203	2,193
Maz-maz-1	2,498,827	4,710	3,963	2,683
Maz-maz-2	2,111,063	4,471	3,848	2,682
Maz-maz-3	4,041,123	4,967	3,967	2,649
Maz-olc-1	2,389,640	4,225	3,689	2,665
Maz-olc-2	1,888,511	4,446	3,767	2,663
Maz-olc-3b	3,169,243	4,220	3,573	2,626
Maz-olc-4	1,879,076	4,239	3,630	2,647
Maz-tam-1	3,054,338	4,321	3,671	2,646
Maz-tam-2	989,358	3,779	3,304	2,515
Pro-can-1	767,695	3,442	2,956	2,272
Pro-lgr-1	995,993	4,033	3,418	2,553
Pro-lgr-2	605,412	3,865	3,289	2,455
Pro-nha-1	965,460	4,069	3,208	2,434
Pro-nha-2	1,059,671	4,198	3,494	2,555
Pro-nha-3	1,180,736	4,168	3,493	2,590
Pro-tro-1	697,833	3,558	3,077	2,403
Pro-tro-2	1,392,050	3,949	3,388	2,544
Pro-tro-3	1,303,670	4,773	3,661	2,588
Rew-rew-1	576,388	2,508	2,231	1,858

Rew-rew-2	3,564,687	4,530	3,766	2,561
Rew-rew-3	7,048,834	4,933	3,869	2,529
Rew-rew-4	2,343,560	4,239	3,524	2,485
Rew-rew-5	2,822,030	4,378	3,661	2,543
Rew-rew-6	2,406,227	3,946	3,445	2,516
Sar-kkc-1	6,044,370	5,524	4,115	2,662
Sar-kkc-2	2,514,050	4,457	3,747	2,673
Sar-sar-1	2,208,694	5,366	4,070	2,661
Sar-sar-2	3,076,439	4,395	3,726	2,668
Sar-sar-3	2,628,136	4,721	3,843	2,671
Sar-sar-4	1,965,286	4,100	3,479	2,620
Sar-sar-5	373,809	2,286	2,038	1,751
Sur-cop-1	5,742,204	4,976	3,883	2,573
Sur-cop-2	6,139,358	4,578	3,810	2,568
Sur-mar-1	839,932	3,736	3,219	2,457
Sur-mar-2	1,287,925	3,976	3,358	2,494
Sur-mar-3	3,097,183	4,600	3,861	2,662
Sur-sur-1	4,698,303	4,968	4,002	2,660
Sur-sur-2	4,273,611	4,867	4,000	2,686
Sur-sur-3	3,559,169	4,936	3,988	2,671
Sur-sur-4	2,465,912	4,603	3,809	2,680
Sur-sur-5	6,766,477	4,910	3,890	2,589
Sur-sur-6	4,204,770	4,705	3,792	2,558
Sur-sur-7	5,130,536	5,021	3,876	2,567
Sur-sur-8	6,096,442	4,937	3,869	2,561
Tae-cat-1	6,213,154	3,850	3,109	2,134
Tae-ori-1	4,738,903	4,025	3,254	2,250
Tae-sia-1	2,585,156	3,290	2,895	2,216
Tae-sia-2b	7,206,826	3,806	3,156	2,171
Tae-sia-3	9,107,794	3,771	3,108	2,138
Win-ata-1	2,141,954	4,673	3,799	2,625
Win-neg-3	2,737,221	4,822	3,837	2,638
Win-neg-4	3,256,049	4,988	3,936	2,671

Win-neg-5	2,074,439	4,685	3,757	2,623
Xin-iri-2	1,058,174	3,374	3,019	2,494
Xin-iri-3	1,226,551	3,664	3,245	2,556
Xin-nov-1	1,548,861	3,507	3,157	2,537
Xin-nov-2	2,536,674	4,109	3,533	2,602
Xin-nov-3	1,610,954	4,175	3,496	2,584
Xin-nov-4	2,378,318	5,070	3,851	2,616
Xin-xin-1	1,142,375	3,822	3,302	2,560
Xin-xin-2	1,552,582	4,123	3,547	2,604
Xin-xin-3	1,571,285	4,746	3,775	2,633

Figure SII-1: Maximum likelihood tree generated from a concatenated matrix of 1,801,886 bp representing 6,463 loci present in at least 41 of the individuals. Clades are defined as in Figures II-1 and II-2 of Chapter 2

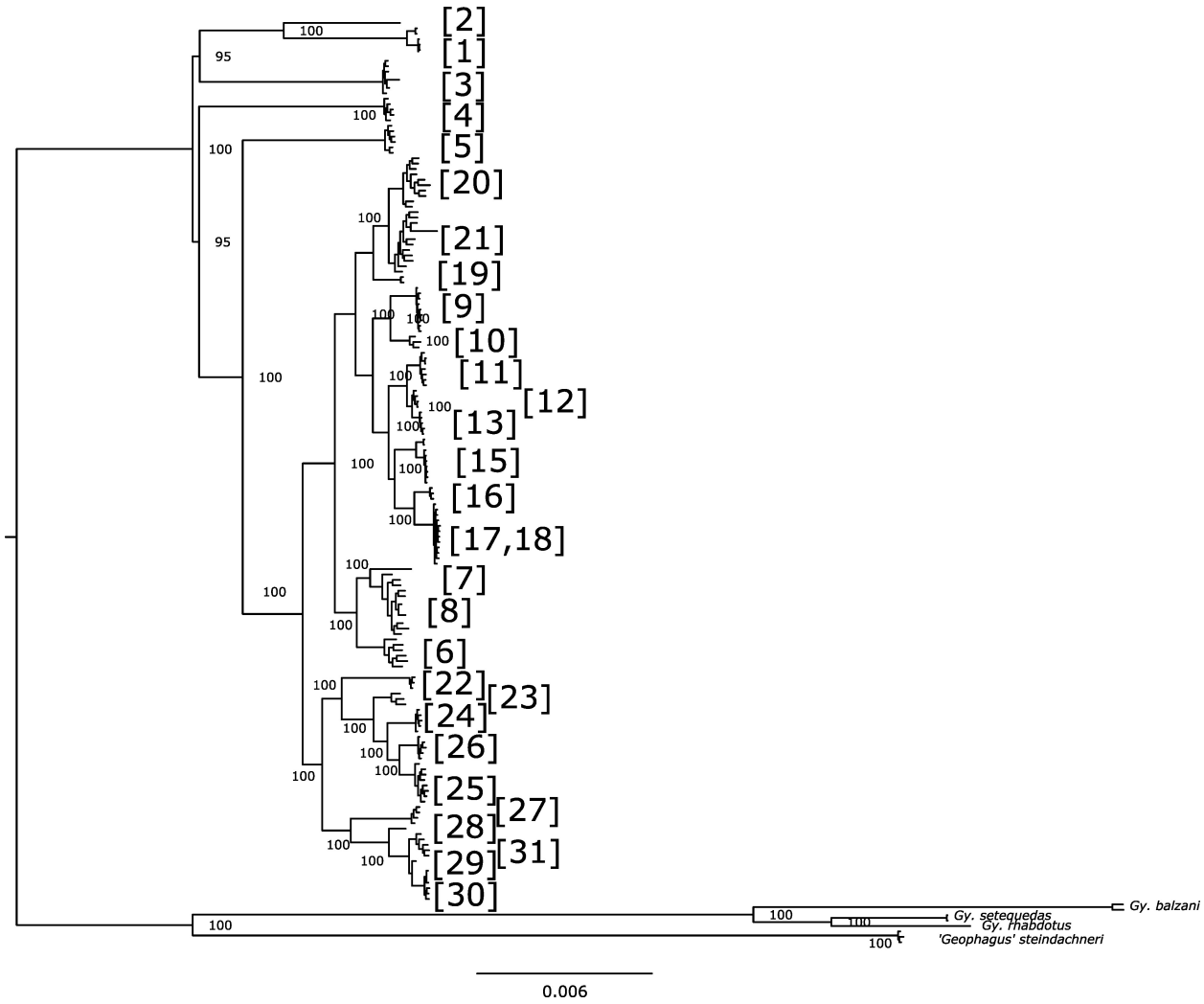

Figure SII-2: Maximum likelihood tree generated from a concatenated matrix of 824,395 bp representing 2,767 loci present in at least 123 of the individuals. Clades are defined as in Figures II-1 and II-2 of Chapter 2

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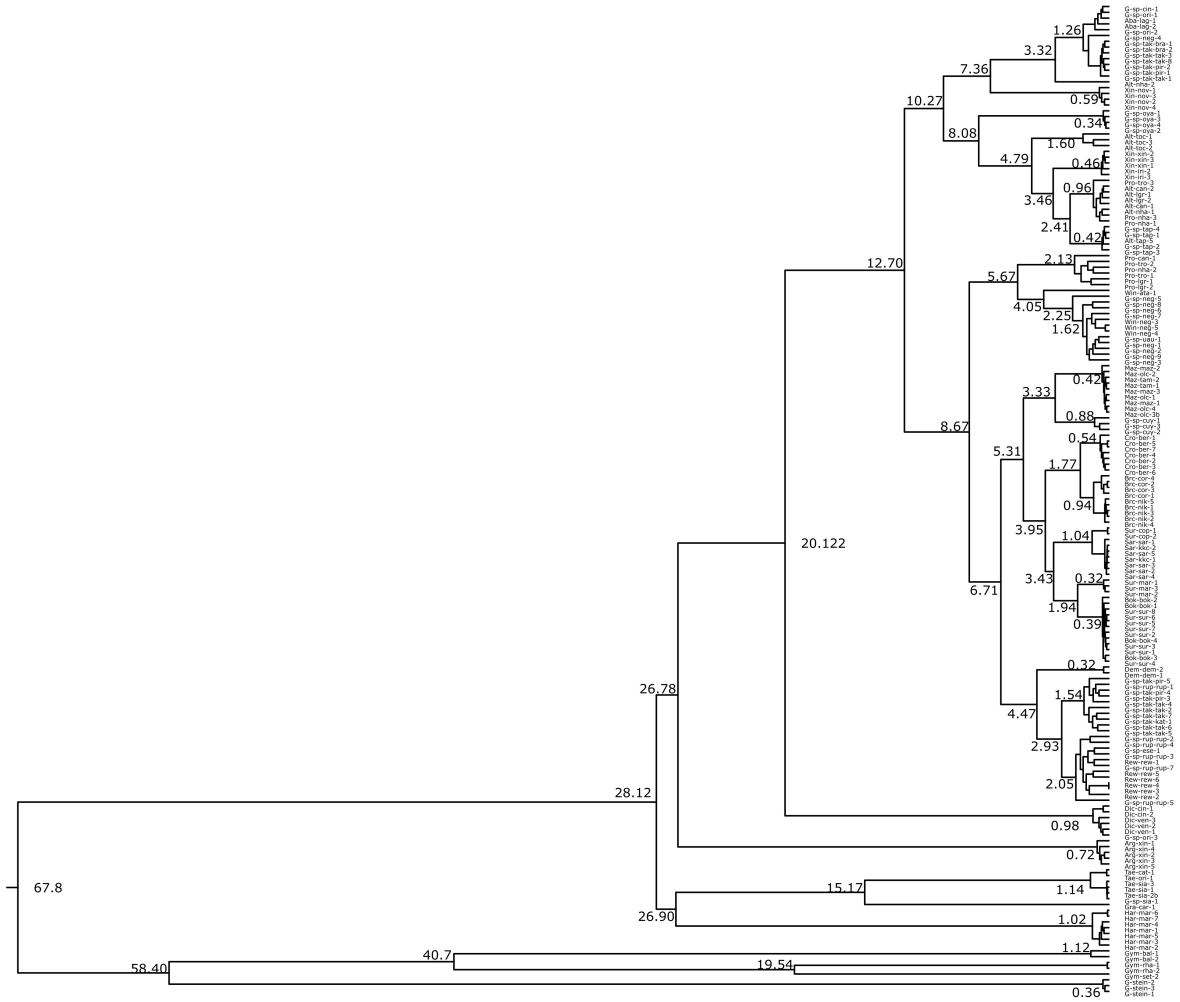

Figure SII-3: Time-calibrated phylogeny of *Geophagus sensu stricto*. Time calibrations for TreePL calibration that included both a root secondary calibration of between 51.4 and 67.8 Ma and a calibration for the crown of *Gymnogeophagus* of between 40.7 and 54.7 Ma. Times are indicated at nodes in MY.

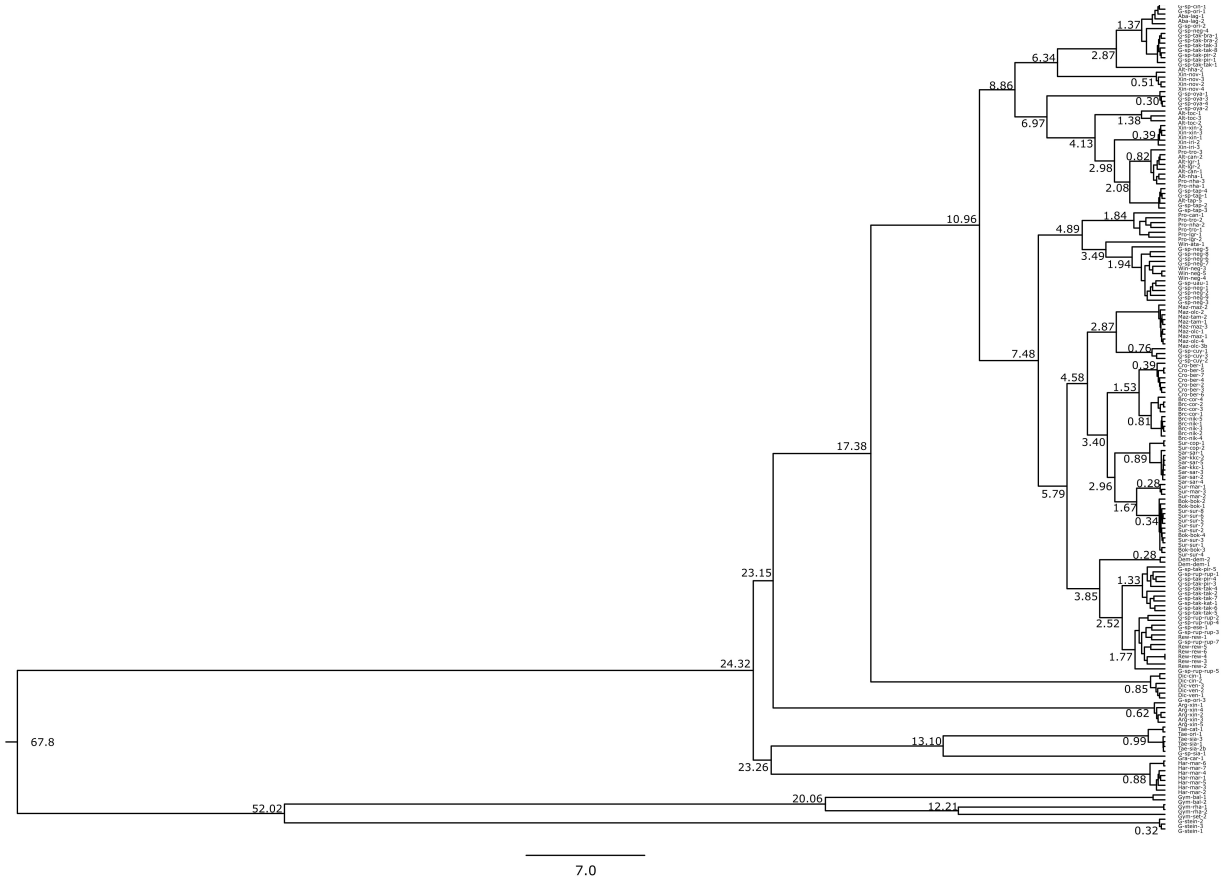

Figure SII-4: Time-calibrated phylogeny of Geophagus sensu stricto. Time calibration for TreePL calibration using only the secondary root calibration of 51.4 to 67.8 Ma. Times are indicated at nodes in MY

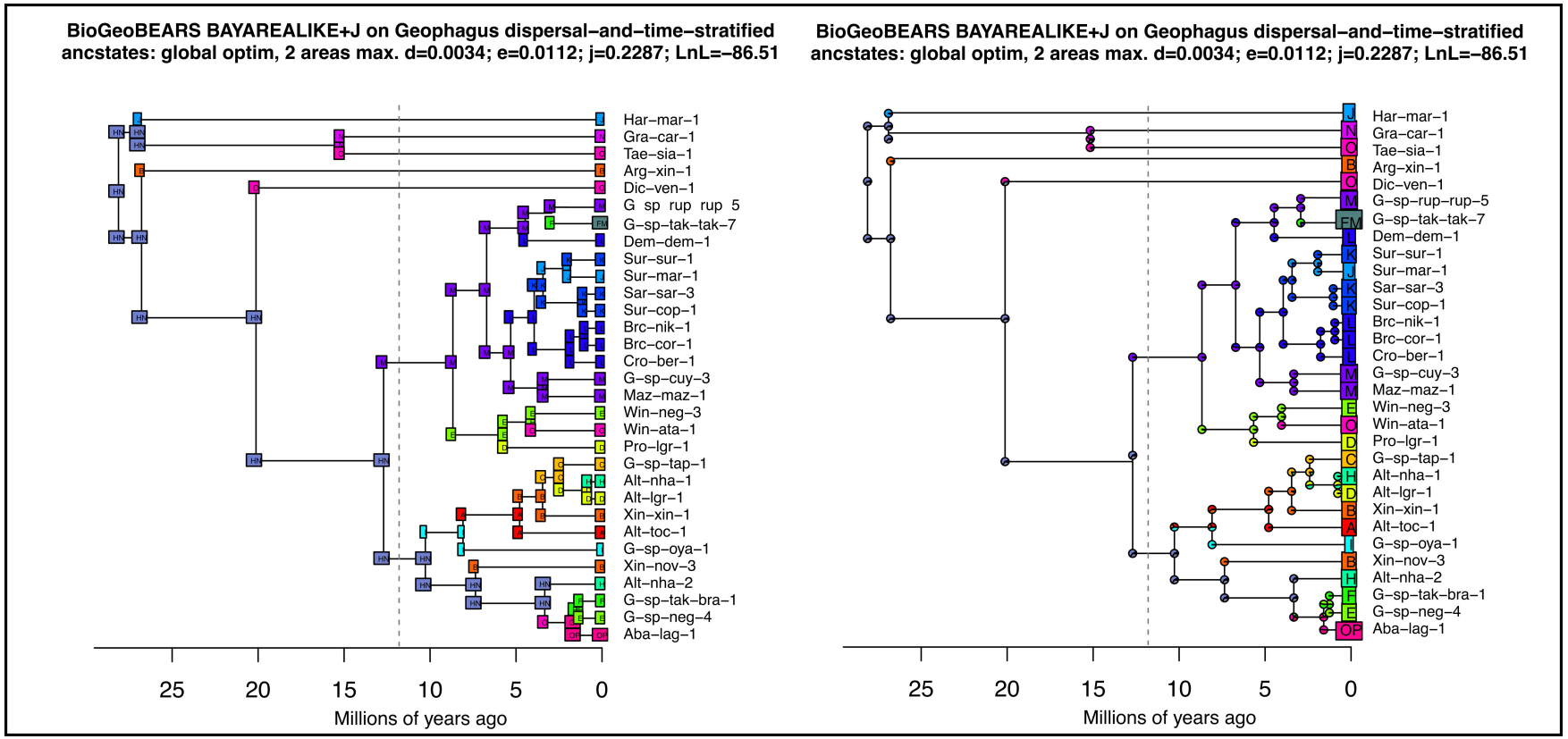

Figure SII-5: BioGeoBEARS output for preferred model by AICc BAYEAREA+j. Ancestral range estimation was performed for the entire Geophagus sensu stricto tree with outgroup Gymnogeophagus and 'Geophagus' steindachneri excluded. Max areas was set to 2 and dispersal probability was equal, but only allowed between adjacent river basins; prior to 11.8Ma lower Amazon and Negro River basin systems were not considered connected (dotted line), and the corridors between the Amazon, Orinoco, and Guianas were not considered open. The details of model comparison by AICc as well as the probability of each ancestral range are included in the corresponding supplementary excel file. Letters shown correspond to areas A = Tocantins River; B = Iriri-Xingu Rivers; C = Tapajos River; D = Middle-Lower Madeira River; E = Negro River; F = Branco River; G = Urubu-Uatuma River; H = Trombetas River; I = Oiapok River; J = Maroni-Approuague Rivers; K = Coppename-Suriname-Saramacca Rivers; L = Corentyne-Demerara Rivers; M = Essequibo River; N = Lower Orinoco River; O = Upper Orinoco River; P = Apure River.

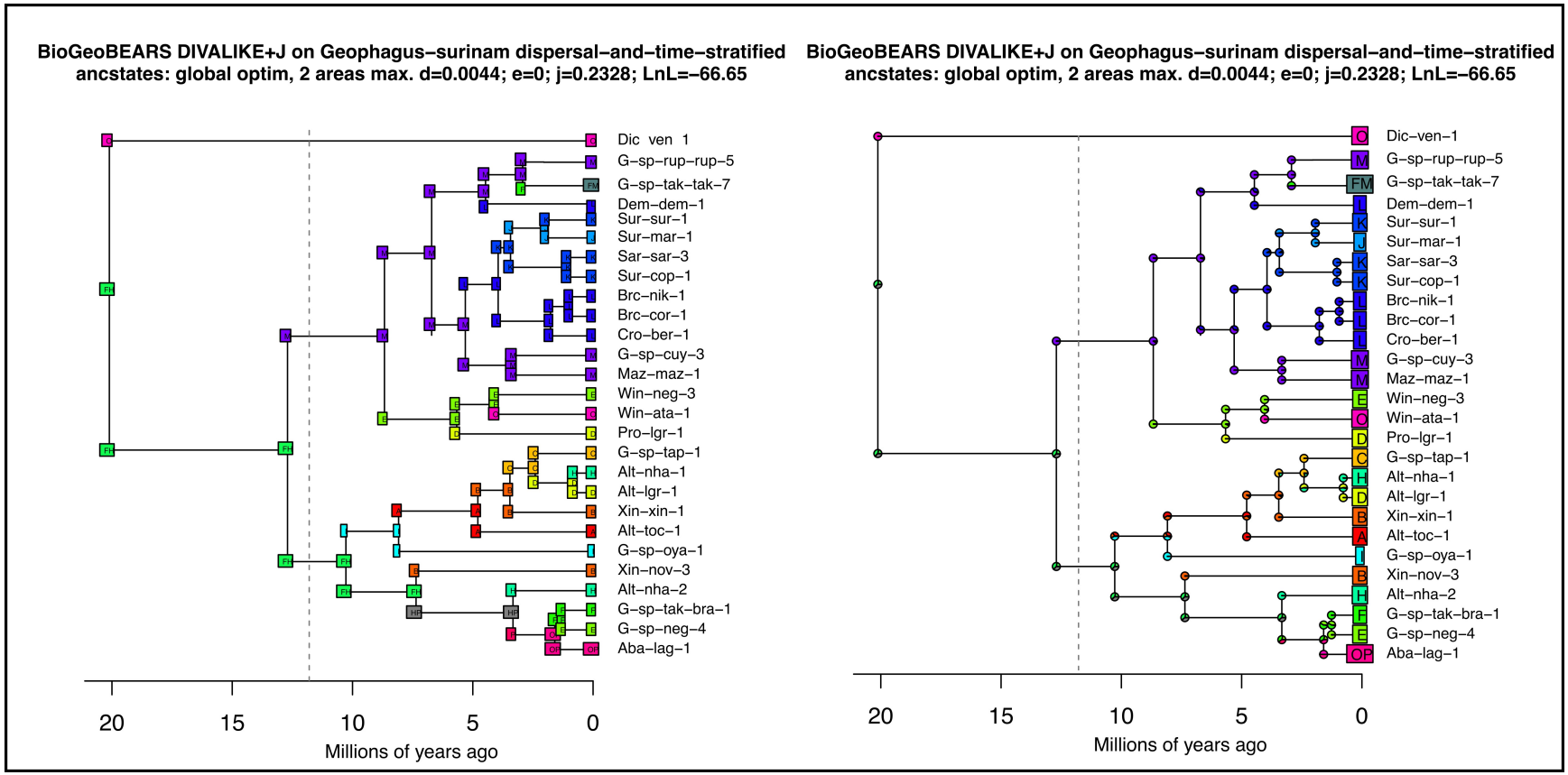

Figure SII-6: BioGeoBEARS output for preferred model by AICc DIVAlike+j. Ancestral range estimation was performed for the Geophagus 'surinamensis-group' subtree with outgroup Gymnogeophagus, 'Geophagus' steindachneri, and 'argyrostictus-group' Geophagus excluded. Max areas was set to 2 and dispersal probability was equal, but only allowed between adjacent river basins; prior to 11.8Ma lower Amazon and Negro River basin systems were not considered connected (dotted line). The details of model comparison by AICc as well as the probability of each ancestral range are included in the corresponding supplementary excel file. Letters shown correspond to areas A = Tocantins River; B = Iriri-Xingu Rivers; C = Tapajos River; D = Middle-Lower Madeira River; E = Negro River; F = Branco River; G = Urubu-Uatuma River; H = Trombetas River; I = Oiapok River; J = Maroni-Approuague Rivers; K = Coppename-Suriname-Saramacca Rivers; L = Corentyne-Demerara Rivers; M = Essequibo River; N = Lower Orinoco River; O = Upper Orinoco River; P = Apure River.

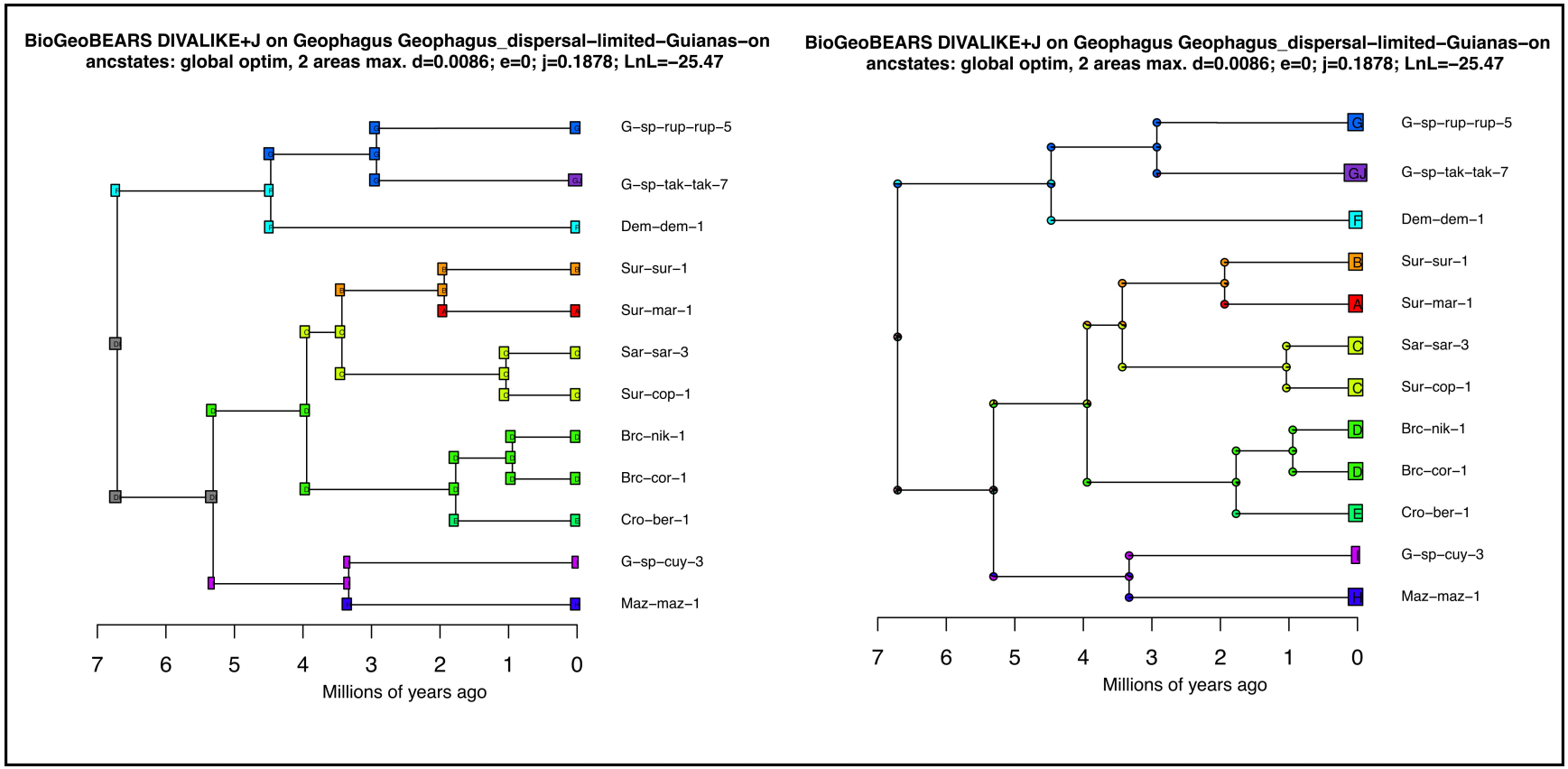

Figure SII-7:.BioGeoBEARS output for preferred model by AICc DIVAlike+j. Ancestral range estimation was performed for the Geophagus 'Clade B: Guianas-group' subtree with outgroup Gymnogeophagus and 'Geophagus' steindachneri, 'argyrostictus-group' Geophagus, and Clades A, C, and D excluded. Max areas was set to 2 and dispersal was equal, but only allowed between adjacent river basins as shown in Figure 3A. The details of model comparison by AICc as well as the probability of each ancestral range are included in the corresponding supplementary excel file. Letters shown correspond to areas A = Maroni River; B = Suriname River; C = Coppename-Saramacca Rivers; D = Corentyne-Nickerie Rivers; E = Berbice River; F = Demerara River; G = Essequibo River; H = Mazaruni River; I = Cuyuni River; J = Takutu River.

Table SIII-1: Museum collection information for *Krobia potaroensis* and selected outgroups used in phylogenetic and population genetic analyses. ***Kr-pot-maz-1 sample had an uncertain GPS coordinates within the upper Mazaruni, and was therefore excluded from site-specific analyses (sPCA, genetic distance and genetic diversity measures). Museum abbreviations: AUM=Auburn University Museum, ROM=Royal Ontario Museum, UMMZ = University of Michigan Museum of Zoology.

MUSEUM COLLECTION	TISSUE SAMPLE NUMBER	SPECIES	RIVER SYSTEM - Site ID	LATITUDE	LONGITUDE	SAMPLE NAME IN ANALYSES
AUM	6533	Krobia potaroensis	Upper Potaro River - UPot-03	5.30	-59.90	Kr-pot-kuri-1
AUM	6534	Krobia potaroensis	Upper Potaro River - UPot-03	5.30	-59.90	Kr-pot-kuri-2
AUM	10108	Krobia potaroensis	Upper Ireng River - UIre-01	4.73	-60.01	Kr-pot-ire-1
AUM	10109	Krobia potaroensis	Upper Ireng River - UIre-01	4.73	-60.01	Kr-pot-ire-2
AUM	10110	Krobia potaroensis	Upper Ireng River - UIre-01	4.73	-60.01	Kr-pot-ire-3
ROM	T21920	Krobia sp. 'Middle Mazaruni'	Middle Mazaruni	6.30	-60.37	Kr-gui-maz-1
ROM	T21858	Krobia sp. 'Middle Mazaruni'	Middle Mazaruni	6.11	-60.11	Kr-gui-maz-2
ROM	T21865	Krobia sp. 'Middle Mazaruni'	Middle Mazaruni	6.11	-60.11	Kr-gui-maz-3
ROM	T06031	Krobia potaroensis	Upper Mazaruni (main channel) - uncertain GPS***			Kr-pot-maz-1
ROM	T06132	Krobia potaroensis	Upper Mazaruni (main channel) - UMaz-11	5.69	-60.47	Kr-pot-maz-2
ROM	T06133	Krobia potaroensis	Upper Mazaruni (main channel) - UMaz-11	5.69	-60.47	Kr-pot-maz-3
ROM	T06017	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-12	5.51	-60.41	Kr-pot-maz-4
ROM	T06018	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-12	5.51	-60.41	Kr-pot-maz-5
ROM	T06055	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-13	5.36	-60.37	Kr-pot-maz-6
ROM	T14541	Krobia potaroensis	Kuribrong River - Kuri-02	5.31	-59.55	Kr-pot-kuri-3
ROM	T17202	Krobia potaroensis	Upper Potaro River - UPot-02	5.07	-59.65	Kr-pot-pot-1
ROM	T17203	Krobia potaroensis	Upper Potaro River - UPot-02	5.07	-59.65	Kr-pot-pot-2
ROM	T17204	Krobia potaroensis	Upper Potaro River - UPot-02	5.07	-59.65	Kr-pot-pot-3
AUM	10283	Krobia potaroensis	Upper Ireng River - UIre-04	5.09	-59.97	Kr-pot-ire-4
ROM	T17134	Krobia potaroensis	Upper Potaro River - UPot-01	5.01	-59.64	Kr-pot-pot-4

ROM	T17135	Krobia potaroensis	Upper Potaro River - UPot-01	5.01	-59.64	Kr-pot-pot-5
AUM	10330	Krobia potaroensis	Upper Ireng River - Ulre-02	4.93	-60.00	Kr-pot-ire-5
AUM	10132	Krobia potaroensis	Upper Ireng River - Ulre-03	5.04	-59.98	Kr-pot-ire-6
AUM	10286	Krobia potaroensis	Upper Ireng River - Ulre-04	5.09	-59.97	Kr-pot-ire-7
AUM	10324	Krobia potaroensis	Upper Ireng River - Ulre-02	4.93	-60.00	Kr-pot-ire-8
ROM	T06221	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-7
ROM	T06222	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-8
ROM	T14570	Krobia potaroensis	Kuribrong River (Grass Falls Creek Potaro) - Kuri-01	5.41	-59.54	Kr-pot-kuri-5
ROM	T17233	Krobia potaroensis	Kuribrong River - Kuri-03	5.28	-59.70	Kr-pot-kuri-6
ROM	T17295	Krobia potaroensis	Kuribrong River - Kuri-04	5.21	-59.67	Kr-pot-kuri-7
UMMZ	T01366	Krobia potaroensis	Upper Mazaruni - UMaz-06	5.87	-60.61	Kr-pot-maz-10
UMMZ	T01394	Krobia potaroensis	Upper Mazaruni - UMaz-08	5.84	-60.87	Kr-pot-maz-9
UMMZ	T01393	Krobia potaroensis	Upper Mazaruni - UMaz-08	5.84	-60.87	Kr-pot-maz-11
UMMZ	T01638	Krobia potaroensis	Upper Mazaruni - UMaz-02	6.00	-60.63	Kr-pot-maz-12
UMMZ	T01365	Krobia potaroensis	Upper Mazaruni - UMaz-06	5.87	-60.61	Kr-pot-maz-13
ROM	T06160	Krobia potaroensis	Upper Mazaruni (Waruma Creek) - UMaz-14	5.48	-60.78	Kr-pot-maz-18
ROM	T06161	Krobia potaroensis	Upper Mazaruni (Waruma Creek) - UMaz-14	5.48	-60.78	Kr-pot-maz-16
ROM	T06162	Krobia potaroensis	Upper Mazaruni (Waruma Creek) - UMaz-14	5.48	-60.78	Kr-pot-maz-14
ROM	T06163	Krobia potaroensis	Upper Mazaruni (Waruma Creek) - UMaz-14	5.48	-60.78	Kr-pot-maz-17
ROM	T06164	Krobia potaroensis	Upper Mazaruni (Waruma Creek) - UMaz-14	5.48	-60.78	Kr-pot-maz-15
ROM	T06216	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-21
ROM	T06217	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-19
ROM	T06218	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-20

ROM	T06219	Krobia potaroensis	Upper Mazaruni (Membaru Creek) - UMaz-03	5.93	-60.59	Kr-pot-maz-22
ROM	T06027	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-12	5.51	-60.41	Kr-pot-maz-23
ROM	T06056	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-13	5.36	-60.37	Kr-pot-maz-24
ROM	T06057	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-13	5.36	-60.37	Kr-pot-maz-25
ROM	T06029	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-12	5.51	-60.41	Kr-pot-maz-26
ROM	T14569	Krobia potaroensis	Kuribrong River (Grass Falls Creek Potaro) - Kuri-01	5.41	-59.54	Kr-pot-kuri-8
AUM	10133	Krobia potaroensis	Upper Ireng River - Ulre-03	5.04	-59.98	Kr-pot-ire-9
UMMZ	T01546	Krobia potaroensis	Upper Mazaruni - UMaz-04	5.93	-60.57	Kr-pot-maz-28
UMMZ	T01547	Krobia potaroensis	Upper Mazaruni - UMaz-04	5.93	-60.57	Kr-pot-maz-32
UMMZ	T01653	Krobia potaroensis	Upper Mazaruni - UMaz-01	6.05	-60.65	Kr-pot-maz-33
UMMZ	T01507	Krobia potaroensis	Upper Mazaruni - UMaz-09	5.83	-60.93	Kr-pot-maz-36
UMMZ	T01508	Krobia potaroensis	Upper Mazaruni - UMaz-09	5.83	-60.93	Kr-pot-maz-34
ROM	T06028	Krobia potaroensis	Upper Mazaruni (Kukui River) - UMaz-12	5.51	-60.41	Kr-pot-maz-37
UMMZ	T01685	Krobia potaroensis	Upper Mazaruni - UMaz-05	5.92	-60.61	Kr-pot-maz-38
UMMZ	T01384	Krobia potaroensis	Upper Mazaruni - UMaz-07	5.83	-60.69	Kr-pot-maz-30
ROM	T21452	Krobia sp. 'Middle Mazaruni'	Middle Mazaruni	6.21	-60.23	Kr-gui-maz-4
UMMZ	T01492	Krobia potaroensis	Upper Mazaruni - UMaz-10	5.84	-60.99	Kr-pot-maz-31
UMMZ	T01493	Krobia potaroensis	Upper Mazaruni - UMaz-10	5.84	-60.99	Kr-pot-maz-29
UMMZ	T01494	Krobia potaroensis	Upper Mazaruni - UMaz-10	5.84	-60.99	Kr-pot-maz-35
UMMZ	T01637	Krobia potaroensis	Upper Mazaruni - UMaz-02	6.00	-60.63	Kr-pot-maz-27

Table SIII-2: Sequence and exported-matrix information for ddRAD library of *Krobia potaroensis* in the Pakaraima Mountains region of western Guyana.

Pakaraimas *Krobia* raw reads	RAW sequences	loci in 40of79 matrix, of 7440 loci	Loci in Potaroensis-clade-matrix, (of 10,366 loci)	Loci in Pakaraimas-matrix, (of 10,719 loci)
Kr-pot-ire-1	6,378,120	7,056	9,990	10,328
Kr-pot-ire-2	3,645,888	6,883	9,857	10,180
Kr-pot-ire-3	5,124,906	6,815	9,931	10,267
Kr-pot-ire-4	5,922,926	7,195	10,116	10,454
Kr-pot-ire-5	5,216,391	7,111	10,045	10,378
Kr-pot-ire-6	6,268,663	7,182	10,144	10,477
Kr-pot-ire-7	6,594,801	7,202	10,114	10,453
Kr-pot-ire-8	4,878,220	7,072	10,057	10,388
Kr-pot-ire-9	3,940,074	7,224	10,207	10,544
Kr-pot-kuri-1	6,609,263	6,838	9,825	10,161
Kr-pot-kuri-2	5,734,180	6,961	9,929	10,251
Kr-pot-kuri-3	5,130,148	6,540	9,702	10,009
Kr-pot-kuri-5	4,082,697	6,976	9,894	10,205
Kr-pot-kuri-6	6,447,444	7,113	10,021	10,354
Kr-pot-kuri-7	4,526,840	7,091	9,962	10,291
Kr-pot-kuri-8	5,436,436	6,699	9,882	10,194
Kr-pot-maz-1	4,391,940	6,460	9,553	9,876
Kr-pot-maz-10	2,734,302	6,625	9,752	10,078
Kr-pot-maz-11	296,963	3,301	5,001	5,136
Kr-pot-maz-12	4,942,429	7,050	10,151	10,492
Kr-pot-maz-13	4,000,596	6,830	9,993	10,332
Kr-pot-maz-14	9,112,588	7,211	10,142	10,478
Kr-pot-maz-15	8,607,287	7,216	10,108	10,445
Kr-pot-maz-16	5,937,153	7,118	9,974	10,312
Kr-pot-maz-17	4,313,542	7,013	9,988	10,320
Kr-pot-maz-18	7,333,518	7,106	9,994	10,325
Kr-pot-maz-19	1,264,601	5,558	8,511	8,793
Kr-pot-maz-2	4,045,943	6,588	9,687	10,006
Kr-pot-maz-20	2,282,707	6,512	9,641	9,956

Kr-pot-maz-21	4,074,678	7,049	10,150	10,495
Kr-pot-maz-22	3,679,880	6,874	9,972	10,315
Kr-pot-maz-23	4,778,490	7,012	10,140	10,494
Kr-pot-maz-24	3,113,271	6,567	9,786	10,114
Kr-pot-maz-25	4,435,093	6,920	10,087	10,420
Kr-pot-maz-26	1,952,138	6,490	9,658	9,969
Kr-pot-maz-27	2,481,378	6,822	10,020	10,358
Kr-pot-maz-28	3,646,134	7,163	10,227	10,570
Kr-pot-maz-29	3,951,534	7,206	10,235	10,584
Kr-pot-maz-3	4,422,300	6,448	9,656	9,969
Kr-pot-maz-30	3,755,238	7,172	10,236	10,583
Kr-pot-maz-31	2,333,245	6,850	10,040	10,371
Kr-pot-maz-32	2,953,041	6,925	10,111	10,451
Kr-pot-maz-33	3,044,090	7,009	10,171	10,516
Kr-pot-maz-34	3,636,834	7,077	10,212	10,552
Kr-pot-maz-35	3,075,871	6,934	10,123	10,456
Kr-pot-maz-36	2,044,799	6,555	9,758	10,077
Kr-pot-maz-37	2,746,900	7,008	10,142	10,481
Kr-pot-maz-38	843,605	4,975	7,690	7,922
Kr-pot-maz-4	3,708,270	6,422	9,555	9,869
Kr-pot-maz-5	6,138,830	7,029	10,052	10,384
Kr-pot-maz-6	6,086,339	6,976	9,961	10,302
Kr-pot-maz-7	5,006,611	7,175	10,119	10,461
Kr-pot-maz-8	7,003,216	7,154	10,146	10,487
Kr-pot-maz-9	4,723,318	6,967	10,113	10,447
Kr-pot-pot-1	4,894,502	6,984	9,991	10,327
Kr-pot-pot-2	4,928,987	7,177	10,095	10,432
Kr-pot-pot-3	5,915,726	7,200	10,140	10,478
Kr-pot-pot-4	5,509,254	7,093	9,987	10,322
Kr-pot-pot-5	5,538,129	7,165	10,059	10,395
Kr-sp-maz-1	6,784,803	6,323	9,433	
Kr-sp-maz-2	6,056,200	6,681	9,565	
Kr-sp-maz-3	5,396,006	6,090	9,216	

Kr-sp-maz-4	7,198,546	6,709	9,596	
Kr-pett-berb-1	4,781,057	3,355		
Kr-pett-berb-2	3,850,776	3,342		
Kr-xin-1	4,908,717	3,609		**40of79 matrix**
Ae-sp-xin-1	5,833,697	2,479		snps matrix size: (79, 99922), 15.23% missing sites.
Ae-sp-xin-2	5,831,358	2,690		sequence matrix size: (79, 2107645), 18.51% missing sites.
Ae-tet-nov-1	2,860,914	2,228		
Ae-tet-nov-2	4,445,167	2,536		
Cich-bim-1	3,095,322	2,512		**48of63 matrix**
Cich-bim-2	4,272,750	2,627		snps matrix size: (63, 22665), 6.95% missing sites.
Kr-gui-sur-1	4,926,591	3,778		sequence matrix size: (63, 2975799), 5.21% missing sites.
Kr-gui-sur-2	4,333,213	3,787		unlinked snps (63, 7398), 5.90% missing sites
Kr-gui1-sinn-1	4,011,375	3,326		
Kr-gui1-sinn-2	5,642,545	3,389		**45of59 matrix**
Kr-ita-mar-1	6,054,820	3,532		snps matrix size: (59, 11296), 8.00% missing sites.
Kr-ita-mar-4	2,033,215	3,127		sequence matrix size: (59, 3073420), 4.95% missing sites.
Kr-paloe-1	4,945,624	3,669		unlinked snps (59, 5100), 6.37% missing sites

Table SIII-3: Genetic distance (F_{ST}) for *Krobia potaroensis.* between sampling sites in the Pakaraima Mountains of western Guyana (sites numbered as in Table SIII-1). brown = sites within the Kuribrong River, green= sites within the upper Ireng River, red = sites in the upper Mazaruni River, and yellow=sites in the upper Potaro River. Within-river genetic distances are bordered by a single line, while between-river genetic distances are bordered with a double line.

	Kuri-01	Kuri-02	Kuri-03	Kuri-04	Ulre-01	Ulre-02	Ulre-03	Ulre-04	UMaz-01	UMaz-02	UMaz-03	UMaz-04	UMaz-05	UMaz-06	UMaz-07	UMaz-08	UMaz-09	UMaz-10	UMaz-11	UMaz-12	UMaz-13	UMaz-14	UPot-01	UPot-02
Kuri-01	x																							
Kuri-02	0.24	x																						
Kuri-03	0.19	0.18	x																					
Kuri-04	0.21	0.19	0.13	x																				
Ulre-01	0.23	0.21	0.15	0.12	x																			
Ulre-02	0.21	0.19	0.12	0.10	0.05	x																		
Ulre-03	0.21	0.19	0.13	0.10	0.05	0.03	x																	
Ulre-04	0.21	0.19	0.12	0.09	0.05	0.02	0.03	x																
UMaz-01	0.23	0.21	0.14	0.12	0.09	0.07	0.07	0.07	x															
UMaz-02	0.22	0.20	0.13	0.11	0.08	0.06	0.06	0.06	0.03	x														
UMaz-03	0.21	0.19	0.13	0.10	0.08	0.05	0.06	0.05	0.02	0.02	x													
UMaz-04	0.22	0.20	0.13	0.10	0.08	0.05	0.06	0.06	0.03	0.02	0.02	x												
UMaz-05	0.25	0.22	0.15	0.13	0.09	0.07	0.08	0.08	0.04	0.03	0.03	0.03	x											
UMaz-06	0.22	0.20	0.13	0.11	0.08	0.06	0.06	0.06	0.03	0.02	0.02	0.02	0.03	x										
UMaz-07	0.23	0.20	0.13	0.11	0.09	0.06	0.07	0.07	0.03	0.03	0.02	0.02	0.03	0.03	x									
UMaz-08	0.23	0.21	0.14	0.11	0.08	0.06	0.07	0.06	0.04	0.03	0.02	0.03	0.04	0.03	0.03	x								
UMaz-09	0.22	0.20	0.13	0.11	0.08	0.06	0.06	0.06	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.03	x							
UMaz-10	0.22	0.19	0.13	0.10	0.08	0.05	0.06	0.05	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	x						
UMaz-11	0.22	0.20	0.13	0.11	0.08	0.06	0.06	0.06	0.03	0.03	0.02	0.02	0.04	0.03	0.03	0.03	0.03	0.02	x					
UMaz-12	0.21	0.19	0.13	0.10	0.07	0.05	0.06	0.05	0.03	0.02	0.01	0.02	0.03	0.02	0.03	0.03	0.02	0.02	0.02	x				
UMaz-13	0.21	0.19	0.13	0.10	0.07	0.05	0.06	0.05	0.03	0.02	0.02	0.02	0.04	0.02	0.03	0.03	0.02	0.02	0.02	0.02	x			
UMaz-14	0.21	0.19	0.12	0.10	0.08	0.05	0.06	0.05	0.03	0.02	0.01	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	x		
UPot-01	0.20	0.18	0.11	0.09	0.07	0.04	0.05	0.04	0.06	0.05	0.04	0.05	0.07	0.05	0.06	0.06	0.05	0.05	0.05	0.04	0.04	0.04	x	
UPot-02	0.11	0.17	0.11	0.09	0.07	0.04	0.05	0.04	0.06	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.03	x
UPot-03	0.21	0.19	0.12	0.10	0.07	0.05	0.05	0.05	0.06	0.05	0.04	0.05	0.06	0.05	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.03

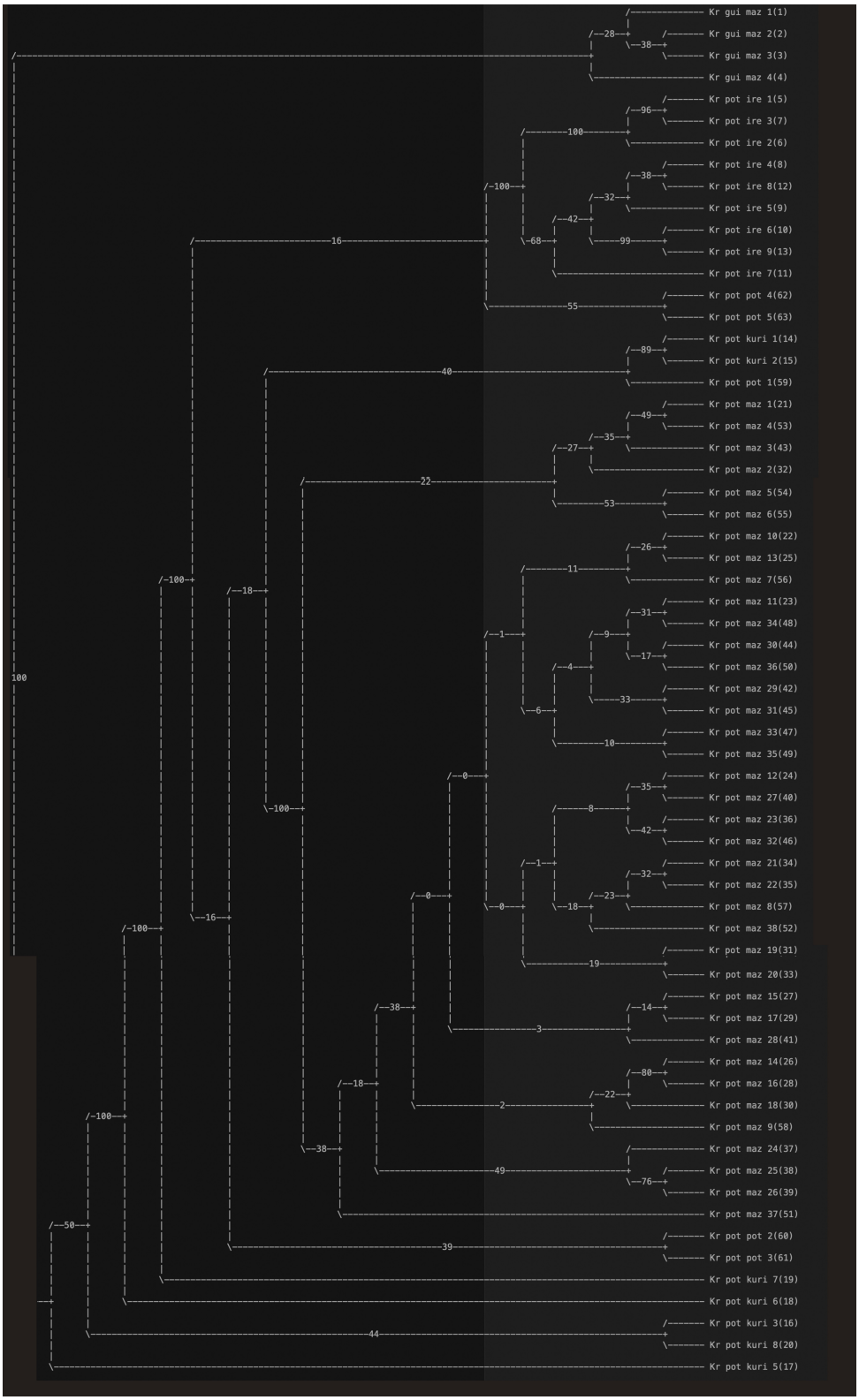

Figure SIII-1: SVDQuartets tree for *Krobia potaroensis* individuals in the Pakaraima Mountains of western Guyana. Sample names are as in Table SIII-1. Tree was generated using a matrix of 2,975,799 bp from 10,366 loci. Robustness of relationships at each node was assessed through 1000 bootstrap replicates.

Table SIV-1: Museum collection information for *Geophagus sp.* and *Guianacara dacrya* analyzed across the Rupununi Portal of southern Guyana. Museum abbreviations:, ROM=Royal Ontario Museum, UMMZ = University of Michigan Museum of Zoology, ANSP = Academy of Natural Sciences of Philadelphia at Drexel University

Museum Collection	Tissue ID	Species	River	Latitude	Longitude	RAD sequence - sample name
UMMZ	T00497	Geophagus sp.	Rewa River	3.832	-58.796	Rew-rew-10
UMMZ	T00498	Geophagus sp.	Rewa River	3.832	-58.796	Rew-rew-15
UMMZ	T00499	Geophagus sp.	Rewa River	3.832	-58.796	Rew-rew-17
UMMZ	T00557	Geophagus sp.	Rewa River	3.738	-58.721	Rew-rew-8
UMMZ	T00558	Geophagus sp.	Rewa River	3.738	-58.721	Rew-rew-12
UMMZ	T00559	Geophagus sp.	Rewa River	3.738	-58.721	Rew-rew-13
UMMZ	T00637	Geophagus sp.	Rewa River	3.644	-58.688	Rew-rew-1
UMMZ	T00638	Geophagus sp.	Rewa River	3.644	-58.688	Rew-rew-2
UMMZ	T00639	Geophagus sp.	Rewa River	3.644	-58.688	Rew-rew-14
UMMZ	T00836	Geophagus sp.	Rewa River	3.449	-58.586	Rew-rew-5
UMMZ	T00837	Geophagus sp.	Rewa River	3.449	-58.586	Rew-rew-6
UMMZ	T00838	Geophagus sp.	Rewa River	3.449	-58.586	Rew-rew-9
UMMZ	T00933	Geophagus sp.	Rewa River	3.182	-58.676	Rew-rew-7
UMMZ	T00934	Geophagus sp.	Rewa River	3.182	-58.676	Rew-rew-18
UMMZ	T00978	Geophagus sp.	Rewa River	3.179	-58.675	Rew-rew-19
UMMZ	T00979	Geophagus sp.	Rewa River	3.179	-58.675	Rew-rew-16
UMMZ	T01084	Geophagus sp.	Rewa River	3.158	-58.636	Rew-rew-3
UMMZ	T01085	Geophagus sp.	Rewa River	3.158	-58.636	Rew-rew-4
UMMZ	T01086	Geophagus sp.	Rewa River	3.158	-58.636	Rew-rew-11
UMMZ	T01183	Geophagus sp.	Rupununi River	3.966	-58.786	G-sp-rup-rup-7
UMMZ	T01196	Geophagus sp.	Rupununi River	3.972	-58.779	G-sp-rup-rup-8
UMMZ	T01197	Geophagus sp.	Rupununi River	3.972	-58.779	G-sp-rup-rup-9
UMMZ	T01198	Geophagus sp.	Rupununi River	3.972	-58.779	G-sp-ruprup-10
ROM	T06833	Geophagus sp.	Rupununi River	3.654	-59.368	G-sp-rup-rup-2
ROM	T06962	Geophagus sp.	Rupununi River	3.663	-59.341	G-sp-rup-rup-3
ROM	T06963	Geophagus sp.	Rupununi River	3.663	-59.341	G-sp-rup-rup-4
ROM	T06964	Geophagus sp.	Rupununi River	3.663	-59.341	G-sp-rup-rup-5
ROM	T08653	Geophagus sp.	Pirara River	3.621	-59.675	G-sp-tak-pir-3
ROM	T08667	Geophagus sp.	Pirara River	3.621	-59.675	G-sp-tak-pir-4

ROM	T08668	Geophagus sp.	Pirara River	3.621	-59.675	G-sp-tak-pir-5
ROM	T08699	Geophagus sp.	Takutu River	3.621	-59.675	G-sp-tak-tak-2
ROM	T08703	Geophagus sp.	Takutu River	3.621	-59.675	G-sp-tak-tak-4
ROM	T11935	Geophagus sp.	Rupununi River	3.895	-59.293	G-sp-rup-rup-1
ROM	T11938	Geophagus sp.	TakutuRiver	3.470	-59.810	G-sp-tak-tak-6
ROM	T11939	Geophagus sp.	Takutu River	3.414	-59.821	G-sp-tak-tak-7
ROM	T15835	Geophagus sp.	Katuwao River	2.875	-59.831	G-sp-tak-kat-1
ROM	T15921	Geophagus sp.	Takutu River	2.836	-59.991	G-sp-tak-tak-5
ROM	T18126	Geophagus sp.	Takutu River	5.315	-58.906	G-sp-ese-1
UMMZ	T00567	Guianacara dacrya	Rewa River	3.738	-58.721	Gu-dac-rew-14
UMMZ	T00568	Guianacara dacrya	Rewa River	3.738	-58.721	Gu-dac-rew-5
UMMZ	T00569	Guianacara dacrya	Rewa River	3.738	-58.721	Gu-dac-rew-8
UMMZ	T00646	Guianacara dacrya	Rewa River	3.644	-58.688	Gu-dac-rew-3
UMMZ	T00647	Guianacara dacrya	Rewa River	3.644	-58.688	Gu-dac-rew-4
UMMZ	T00648	Guianacara dacrya	Rewa River	3.644	-58.688	Gu-dac-rew-16
UMMZ	T00941	Guianacara dacrya	Rewa River	3.182	-58.676	Gu-dac-rew-11
UMMZ	T00942	Guianacara dacrya	Rewa River	3.182	-58.676	Gu-dac-rew-9
UMMZ	T00980	Guianacara dacrya	Rewa River	3.179	-58.675	Gu-dac-rew-13
UMMZ	T00981	Guianacara dacrya	Rewa River	3.179	-58.675	Gu-dac-rew-7
UMMZ	T01013	Guianacara dacrya	Rewa River	3.151	-58.625	Gu-dac-rew-15
UMMZ	T01014	Guianacara dacrya	Rewa River	3.151	-58.625	Gu-dac-rew-6
UMMZ	T01015	Guianacara dacrya	Rewa River	3.151	-58.625	Gu-dac-rew-12
UMMZ	T01091	Guianacara dacrya	Rewa River	3.158	-58.636	Gu-dac-rew-1
UMMZ	T01092	Guianacara dacrya	Rewa River	3.158	-58.636	Gu-dac-rew-2
UMMZ	T01093	Guianacara dacrya	Rewa River	3.158	-58.636	Gu-dac-rew-10
UMMZ	T01180	Guianacara dacrya	Rupununi River	3.966	-58.786	Gu-dac-rup-1
UMMZ	T01181	Guianacara dacrya	Rupununi River	3.966	-58.786	Gu-dac-rup-2
UMMZ	T01205	Guianacara dacrya	Rupununi River	3.972	-58.779	Gu-dac-rup-3
ROM	T06605	Guianacara dacrya	Takutu River	3.613	-59.676	Gu-dac-tak-1
ROM	T06606	Guianacara dacrya	Takutu River	3.613	-59.676	Gu-dac-tak-2
ROM	T06608	Guianacara dacrya	Takutu River	3.613	-59.676	Gu-dac-tak-6
ROM	T06609	Guianacara dacrya	Takutu River	3.613	-59.676	Gu-dac-tak-7
ROM	T11943	Guianacara dacrya	Takutu River	3.470	-59.810	Gu-dac-ess-1

ROM	T12052	Guianacara dacrya	Ireng River	4.054	-59.485	Gu-sp-ire-1
ROM	T14935	Guianacara dacrya	Takutu River	2.836	-59.990	Gu-dac-tak-4
ROM	T14962	Guianacara dacrya	Takutu River	2.836	-59.990	Gu-dac-tak-5
ROM	T14966	Guianacara dacrya	Takutu River	2.836	-59.990	Gu-dac-tak-3
ANSP	T16142	Guianacara dacrya	Moshiwuau Creek	2.159	-59.293	Gu-dac-mosh-1
ANSP	T16143	Guianacara dacrya	Moshiwuau Creek	2.159	-59.293	Gu-dac-mosh-2
ANSP	T16599	Guianacara dacrya	Kuyuwini River	2.097	-59.243	Gu-dac-kuy-1
ANSP	T16633	Guianacara dacrya	Kuyuwini River	2.097	-59.243	Gu-dac-kuy-2
ROM	T17633	Guianacara dacrya	Konawaruk-Essequibo	5.069	-59.231	Gu-dac-kon-1
ROM	T17634	Guianacara dacrya	Konawaruk-Essequibo	5.069	-59.231	Gu-dac-kon-2
ANSP	T185177	Guianacara dacrya	Kuyuwini River	2.097	-59.243	Gu-dac-kuy-3

Table SIV-2: Sequence and exported matrix information for *Geophagus sp.* and *Guianacara dacrya* samples from the Rupununi Portal Region of southern Guyana. Light blue = sites within the Takutu River system, purple= sites within the Rupununi River, pink = sites in the upper Rewa River above Bamboo Falls and sites in the Kuyuwini River, orange= sites in the Konawaruk River.

RAD sequence - sample name	Raw reads	SNPs in Geophagus dataset (of 7,396)	SNPs in Guianacara dataset (of 8,986)
G-sp-tak-kat-1	3,352,988	7,016	-
G-sp-tak-pir-3	2,336,750	7,147	-
G-sp-tak-pir-4	3,695,259	7,165	-
G-sp-tak-pir-5	2,297,189	7,035	-
G-sp-tak-tak-2	4,243,014	7,076	-
G-sp-tak-tak-4	3,511,668	7,150	-
G-sp-tak-tak-5	3,834,968	7,050	-
G-sp-tak-tak-6	390,081	4,483	-
G-sp-tak-tak-7	958,803	5,959	-
G-sp-ese-1	2,415,299	7,137	-
G-sp-rup-rup-1	1,728,787	6,583	-
G-sp-rup-rup-2	2,139,819	7,123	-
G-sp-rup-rup-3	2,528,152	7,027	-

G-sp-rup-rup-4	2,671,689	7,139	-
G-sp-rup-rup-5	1,084,906	6,104	-
G-sp-rup-rup-7	1,717,477	6,495	-
G-sp-rup-rup-8	6,549,856	7,196	-
G-sp-rup-rup-9	2,689,455	7,067	-
G-sp-ruprup-10	2,865,410	7,125	-
Rew-rew-1	576,388	4,301	
Rew-rew-2	3,564,687	6,756	-
Rew-rew-5	2,822,030	6,725	-
Rew-rew-6	2,406,227	6,548	-
Rew-rew-7	1,880,879	6,937	-
Rew-rew-8	6,043,821	7,138	-
Rew-rew-9	1,612,602	6,843	-
Rew-rew-10	5,727,189	7,166	-
Rew-rew-12	6,023,956	7,142	-
Rew-rew-13	6,800,666	7,146	-
Rew-rew-14	5,170,269	7,179	-
Rew-rew-15	5,553,327	7,161	-
Rew-rew-16	3,365,013	7,213	-
Rew-rew-17	4,568,718	7,155	-
Rew-rew-18	6,610,447	7,173	-
Rew-rew-19	5,733,284	7,203	-
Rew-rew-3	7,048,834	6,790	-
Rew-rew-4	2,343,560	6,578	-
Rew-rew-11	3,015,489	7,019	-
Gu-dac-tak-1	3,506,262	-	8,573

Gu-dac-tak-2	5,078,663	-	8,673
Gu-dac-tak-3	5,025,377	-	8,623
Gu-dac-tak-4	4,475,091	-	8,584
Gu-dac-tak-5	6,330,731	-	8,744
Gu-dac-tak-6	1,593,731	-	7,665
Gu-dac-tak-7	4,249,330	-	8,618
Gu-sp-ire-1	6,262,405	-	8,529
Gu-dac-ess-1	4,371,751	-	8,492
Gu-dac-rup-1	3,012,904	-	8,088
Gu-dac-rup-2	2,090,409	-	7,612
Gu-dac-rup-3	5,371,302	-	8,629
Gu-dac-rew-3	6,270,677	-	8,608
Gu-dac-rew-4	6,552,527	-	8,634
Gu-dac-rew-5	6,258,208	-	8,586
Gu-dac-rew-7	1,968,102	-	7,934
Gu-dac-rew-8	6,170,698	-	8,673
Gu-dac-rew-9	6,261,250	-	8,645
Gu-dac-rew-10	1,132,116	-	8,544
Gu-dac-rew-11	5,305,893	-	8,554
Gu-dac-rew-13	1,184,542	-	8,646
Gu-dac-rew-14	6,081,059	-	8,707
Gu-dac-rew-16	6,121,490	-	8,738
Gu-dac-rew-1	5,990,034	-	8,428
Gu-dac-rew-2	6,094,325	-	8,740
Gu-dac-rew-6	7,030,408	-	8,548
Gu-dac-rew-12	6,211,653	-	6,785

Gu-dac-rew-15	6,207,197	-	7,880
Gu-dac-kuy-1	6,022,206	-	8,557
Gu-dac-kuy-2	2,804,988	-	8,266
Gu-dac-kuy-3	5,466,750	-	8,428
Gu-dac-mosh-1	6,626,294	-	8,594
Gu-dac-mosh-2	5,952,180	-	8,266
Gu-dac-kon-1	6,306,765	-	8,400
Gu-dac-kon-2	3,974,450	-	8,170

Table SIV-3: Measures of observed heterozygosity (H_O) for *Geophagus sp.* and *Guianacara dacrya* samples from the Rupununi Portal Region. Sample sites numbered as in Figure 1. Light blue = sites within the Takutu River system, purple= sites within the Rupununi River, pink = sites in the upper Rewa River above Bamboo Falls and sites in the Kuyuwini River, orange= sites in the Konawaruk River.

Sample site	Geophagus H_o	Guianacara H_O
Site01	0.10	N/A
Site02	0.14	0.09
Site03	0.15	N/A
Site04	0.15	0.08
Site05	0.15	0.07
Site06	N/A	0.12
Site07	0.16	N/A
Site08	0.15	N/A
Site09	0.17	0.14
Site10	0.16	N/A
Site11	0.17	0.15
Site12	0.17	0.16
Site13	0.16	N/A
Site14	0.16	0.14
Site15	0.01	0.08
Site16	N/A	0.10
Site17	N/A	0.10
Site18	0.15	N/A
Site19	N/A	0.10

Table SIV-4: Genetic distance (F_{ST}) for *Geophagus sp.* between sampling sites in the Rupununi Portal Region (numbered as in Fig. IV-1). Light blue = sites within the Takutu River system, purple= sites within the Rupununi River, pink = site in the upper Rewa River above Bamboo Falls. Within-river genetic distances are bordered by a single line, while between-river genetic distances are bordered with a double line. *Site 18 while a site within the same Rupununi-Essequibo basin is geographically outside the Rupununi Portal Region.

	Site01	Site02	Site04	Site03	Site05	Site07	Site08	Site09	Site18*	Site10	Site11	Site12	Site13	Site14
Site01	x													
Site02	0.13	x												
Site04	0.14	0.12	x											
Site03	0.14	0.12	0.12	x										
Site05	0.11	0.09	0.09	0.09	x									
Site07	0.16	0.15	0.15	0.15	0.10	x								
Site08	0.14	0.13	0.13	0.13	0.08	0.14	x							
Site09	0.17	0.15	0.15	0.15	0.10	0.05	0.15	x						
Site18*	0.21	0.19	0.19	0.20	0.15	0.10	0.19	0.09	x					
Site10	0.18	0.16	0.16	0.16	0.11	0.06	0.16	0.06	0.10	x				
Site11	0.17	0.15	0.16	0.16	0.11	0.06	0.15	0.05	0.10	0.06	x			
Site12	0.18	0.16	0.16	0.17	0.12	0.07	0.16	0.06	0.11	0.07	0.07	x		
Site13	0.18	0.16	0.16	0.16	0.11	0.06	0.16	0.06	0.10	0.07	0.06	0.07	x	
Site14	0.17	0.15	0.15	0.16	0.10	0.05	0.15	0.05	0.10	0.06	0.05	0.06	0.05	x
Site15	0.25	0.24	0.23	0.24	0.20	0.15	0.24	0.15	0.18	0.16	0.15	0.16	0.15	0.14

Table SIV-5 : Genetic distance (F_{ST}) for *Guianacara dacrya* between sampling sites in the Rupununi Portal Region (numbered as in Fig. IV-1). Light blue = sites within the Takutu River system, purple= sites within the Rupununi River, pink = sites in the upper Rewa River above Bamboo Falls and sites in the Kuyuwini River, orange= sites in the Konawaruk River. Within-river genetic distances are bordered by a single line, while between-river genetic distances are bordered with a double line.

	Site02	Site05	Site06	Site04	Site09	Site11	Site12	Site14	Site15	Site16	Site17
Site02	x										
Site05	0.09	x									
Site06	0.17	0.11	x								
Site04	0.08	0.08	0.17	x							
Site09	0.10	0.11	0.17	0.13	x						
Site11	0.10	0.10	0.16	0.12	0.06	x					
Site12	0.10	0.11	0.17	0.13	0.07	0.05	x				
Site14	0.10	0.11	0.17	0.12	0.07	0.05	0.05	x			
Site15	0.12	0.16	0.23	0.17	0.11	0.12	0.12	0.12	x		
Site16	0.12	0.17	0.23	0.17	0.11	0.13	0.14	0.14	0.07	x	
Site17	0.12	0.17	0.24	0.17	0.12	0.13	0.14	0.14	0.08	0.04	x
Site19	0.27	0.26	0.25	0.29	0.24	0.23	0.23	0.23	0.28	0.29	0.30

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