Hydrological link between the Amazon River Basin and the eastern Guiana Shield on the Neotropical ichthyofauna

by

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Abstract

The seasonal inundation of the Rupununi savannas in south central Guyana allows for potential faunal exchange between the Takutu and Rupununi Rivers and ultimately between the Essequibo and Amazon Rivers. This hydrological connection unites two distinct regions in South America, the Amazon River basin to the drainages of the eastern Guiana Shield. Significant fish community differences on either side of the Rupununi portal suggest the importance of this feature on fish distributions. Therefore, in order to further investigate the influence of the Rupununi portal on fish distributions, I evaluated gene flow of five species found across the portal. This study incorporated three molecular markers: two mitochondrial genes and one nuclear gene. Population genetics of the five species varied, suggesting that the Rupununi portal is acting as a barrier to dispersal for some and a conduit for others. These patterns were based primarily on their ecology. In addition to population genetics of species across the portal, assuming a molecular clock I was able to estimate the timing of the final breakup of the proto-Berbice, thus forming the Rupununi portal. This study highlights the significance of the Rupununi portal in uniting the most species rich river in the world to a region of historical geological complexity and its role in shaping fish distributions of the Neotropical ichthyofauna.

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INTRODUCTION

Fundamental to understanding processes that govern diversification of the Neotropical ichthyofauna is the geographical context that influences speciation and how the hydrological history of river drainage patterns, in conjunction, with geological history of the area affects dispersal, divergence and extinction of lineages. The Rupununi portal, in southern Guyana, is a unique biogeographic area providing a seasonal connection between two major South American river systems. The Rupununi portal seasonally connects the Amazon River (via the Takutu-Branco-Negro Rivers) and the Essequibo River (via the Rupununi River; Figure 1.1). Both drainages have an incredibly diverse fish fauna (Reis *et al.*, 2003) and consistently are recognized as separate biogeographic provinces and areas of endemism (Géry, 1969; Weitzman and Weitzman, 1982; Lowe-McConnell, 1987; Hubert and Renno, 2006). This hydrological connection occurs from the inundation of the low-lying Rupununi savannas during the rainy season, allowing for faunal exchange between these two major drainages. Annual inundation of the Rupununi Savanna extends over 3,480km^2 with a hydroperiod of 49 days. The magnitude of the flood pulse varied over a nine-year study on floodplain inundation patterns in the Rupununi Savanna (Hamilton *et al*., 2002). Consequently, variables associated with the seasonal cycle can have a major impact on the fish communities such as availability of food resources, rate of predation, habitat partitioning, reproduction and competition.

As with many of the Guiana Shield river systems, the Rupununi portal area shares a dynamic paleogeographic history (Lundberg, 1998). The North Rupununi savanna contains a rift valley, referred to as the Takutu graben located between the Pakaraima and Kanuku mountains.

The Takutu graben is a sediment filled ENE-WSW trending rift that extends 280km long and 40km wide at the border of Brazil and Guyana, centered over the town of Lethem, Guyana (Hammond, 2005). A large endorheic lake, Lake Maracanata, filled the graben approximately 100m deep during the early Cretaceous then by the Paleogene began transitioning to a fluvial system (Crawford *et al*., 1985). This fluvial system became the main stem of the proto-Berbice, which during the majority of the Cenozoic was a large northeast flowing river that drained most of the central Guiana Shield exiting into the Atlantic somewhere between present day towns of New Amsterdam, Guyana and Nickerie, Suriname (McConnell, 1959). A series of stream capture events by the Branco shifted the drainage patterns of the upper proto-Berbice, initially the Branco captured the Cotinga and Uraricoera then subsequently capturing the Takutu and Ireng Rivers during the Pleistocene (Crawford *et al*., 1985; Gibbs & Barron, 1993). The lower proto-Berbice shifted away from present day Berbice joining the Essequibo River as evidenced by the sharp elbow curve north of the confluence with the Rupununi River.

Currently, the North Rupununi savanna occupies the former Maracanata basin, thus creating a shallow divide between the east flowing Rupununi into the Essequibo and southwest flowing Takutu into the Branco and ultimately to the Amazon River. This divide is the current location of the Rupununi portal, the physical extent to which this divide floods is referred to as Lake Amuku. Although the Rupununi portal has been suggested to have played a strong role in structuring the flora and fauna of the region (Eigenmann, 1912; Lowe-McConnell, 1975; Hoogmoed, 1979; Turner *et al.*, 2004), no study has thoroughly examined the fish communities of drainages that are linked. In order to accurately assess the role a biogeographic feature has on the local fauna, there must be solid comprehension of community structure (Ricklefs, 1987).

Studies of fish communities in the Neotropics are often hampered by taxonomic uncertainties and logistical difficulties of collecting repeated samples across multiple localities. Although important efforts were made by Planquette, Keith and Le Bail (1996, 2000) in the three-volume guide of freshwater fishes in French Guiana, few studies (Boujard, 1992; Mérigoux *et al*., 1998) have assessed fish communities in the Guiana Shield and only one study has characterized fish communities in Guyana (Lowe-McConnell, 1964). Rosemary Lowe-McConnell pioneered much of the research on the freshwater fish of the Rupununi district of Guyana. Over six years (1956-1962) she studied the fish fauna of the region, their ecology and the effects of the seasonal cycle on fish. She made several observations of fish movements at the onset of the wet and dry season; their migratory movements onto the savannas to spawn and the subsequent stranding of many fish in shallow ponds scattered throughout the savanna as the waters recede. These fish experience a physiological winter characterized by intense crowding, decreased food availability, desiccation, anoxic conditions and high rates of predation (Lowe-McConnell, 1964). Interestingly, these pond species were often observed in both Amazon and Essequibo drainages, while strictly riverine fishes were found only on the Amazon or Essequibo side. Therefore, the Rupununi portal may be a corridor of dispersal for some fish species and a barrier to dispersal for other species.

Although Lowe-McConnell's work in the Rupununi on fish communities is insightful, the number of localities and sample sizes were low. Our study has the most extensive sampling effort to date, including four expeditions in the Rupununi district with collections from the Takutu and Rupununi River drainages. The aim of this study is to provide a thorough examination of the fish community at this seasonal connection and infer the potential role the portal may have on fish distributions of this region. Extensive baseline data of distribution and

abundance of species richness is rare in Neotropical systems, particularly in the Guiana Shield. This baseline data has implications for assessment of biodiversity, diversification patterns, biogeography and conservation of the Guiana Shield ichthyofauna.

MATERIALS & METHODS

Study site and sampling

The Rupununi portal area is located between the Western and Eastern Guiana Shield regions at the border between Brazil and Guyana (Figure 1.1). Extensive sampling of similar areas in the Rupununi and Takutu River drainages was done during four expeditions to the region (2002, 2003, 2005 and 2007). Fishes were collected by a variety of methods including seine, gill net, cast net, hook and line and by hand. All collections were made during the dry season with collections made during the day and night. Localities included sites from the main river channel, tributaries, ponds, and borrow pits. Georeferencing data was recorded for each site using a handheld GPS. Individuals were sorted and identified using current taxonomic keys for the different groups and by having experts examine some taxa. Over 400 species and 55,156 specimens from over 100 localities were deposited at the Auburn University Museum (AUM) fish collection. In addition, duplicates are deposited at the University of Guyana Centre for the Study of Biodiversity, the Academy of Natural Sciences of Philadelphia (ANSP), and Southern Illinois University (SIU), but this analysis uses only AUM specimens.

Statistical evaluation

All sites were divided into their respective drainage area, ‘Rupununi’ or ‘Takutu’. Sample-based species accumulation curves were generated for both drainages using EstimateS ver. 8.2 (Colwell, 2009) including 95% confidence intervals. Both curves reached an asymptote

indicating sufficient sampling was done in order to assess fish community structure. Rarefaction was applied to the dataset in order to account for the variation in sample sizes among sites on estimates of species richness and diversity (Gotelli and Colwell, 2001). Data from each site were resampled 1,000 times without replacement to generate the rarefied estimates of species richness, diversity, and evenness (EcoSim, ver. 7.72, Gotelli and Entsminger, 2010). Species richness, Shannon diversity, and evenness were calculated for both Rupununi (n = 44) and Takutu (n = 45) sites. Differences between rarefied estimates of species richness, Shannon diversity, and evenness between fish assemblages in the Takutu and Rupununi drainages were assessed using Mann-Whitney U tests.

RESULTS

55,156 individuals of 433 species, 13 orders, and 41 families were collected during the expeditions to the Rupununi Savanna. The dominant orders in terms of species were Characiformes (44%) and Siluriformes (36%), with 192 and 155 species respectively. Perciformes ranked third with 40 species (9.3%). Gymnotiformes was represented by 24 species, while Beloniformes, Clupeiformes, Cyprinodontiformes, Osteoglossiformes, Pleuronectiformes, Rajiformes, Synbranchiformes, and Tetradontiformes were represented by less than 10 species. Of the forty-one families represented, by more than one species, the dominant families were Characidae with 93 species (21.5%), Loricariidae with 46 (10.6%) species and Cichlidae with 33 species (7.6%). See Table 1.1 for species list.

Species accumulation curves for the Rupununi and Takutu River drainages indicate that sampling for both drainages was sufficient to assess species richness and diversity (Figure 1.2) (Colwell 2009). A total of 343 species were collected from the Rupununi River with 89 species

found only in the Rupununi, a total of 344 species were collected from the Takutu River with 90 species unique to the Takutu, and 254 species were shared between the two drainages (Figure 1.3). The dominant orders in terms of species for shared species were Characiformes (48%), Siluriformes (31%) and Perciformes (9%) with 122, 79 and 25 species respectively. Species only in the Rupununi predominantly included 36 species in Characiformes (40%) and 34 species in Siluriformes (38%). Species only in the Takutu predominantly included 42 species in Siluriformes (46%) and 35 species in the Characiformes (38%).

The dominant families in species shared between the two regions were Characidae (25%), Loricariidae (10%), and Cichlidae (7.5%) with 65, 26, and 23 species respectively. Species only in the Rupununi predominantly included Characidae (15%) with 13 species, Loricariidae (12%) with 11 species, and Anostomidae (9%) with 8 species. Species only in the Takutu predominantly included Characidae (17%) with 15 species, Trichomycteridae (12%) with 11 species, Loricariidae (10%) with 9 species and Crenuchidae (8%) and Heptapteridae (8%) representing 7 species each (Table 1.2).

Several new species, cognate species (across the Rupununi portal), and endemic species were identified in this study. While there may be many more new species from these collections, listed here are Siluriformes recently identified as new species. Including, *Cetopsidium soniae* (Vari and Ferraris, 2009), *Gelanoglanis* sp. 1, *Gelanoglanis* sp. 2, *Hypostomus macushi* (Armbruster and de Souza, 2005), *Hypancistrus* sp., *Panaque* sp., *Peckoltia cavatica* (Armbruster and Werneke, 2005), *Peckoltia sabaji* (Armbruster, 2003), *Typhlobelus* sp., and *Rhinodoras armbrusteri* (Sabaj *et al*.,2008).

Cognate species are two or more geographically isolated forms that have diverged morphologically from their common ancestor. Cognate species-pairs found on either side of the

Rupununi portal (Takutu vs. Rupununi) consist of *Peckoltia cavatica* (Rupununi) and *Peckoltia braueri* (Takutu), *Auchenipterus ambyiacus* (Takutu) and *Auchenipterus demerarae* (Rupununi), *Curimata roseni* (Takutu) and *Curimata* sp. (Rupununi), *Acanthopoma* sp. (Rupununi) and *Acanthopoma sp*. 1(Takutu), *Brachioica* sp. (Rupununi) and *Brachioica* sp. 1(Takutu). It has also been suggested that *Geophagus abalios* are cognate species across the portal (H. López-Fernández, pers comm.). The following four species ranges are restricted to the Takutu-Branco drainage and considered endemic: *Peckoltia braueri*, *Hypancistrus* sp., *Typhlobelus* sp., and *Panaque* sp., while *Peckoltia cavatica* is endemic to the Rupununi River.

Rupununi River assemblages exhibited higher species richness and Shannon diversity than Takutu River assemblages, but evenness was not different between groups. A Mann-Whitney U test indicated that Species richness and Shannon diversity between the Rupununi and Takutu were significantly different (Table 1.3). Evenness measures are strongly influenced by sample size (Kvalseth, 1991), therefore the lack of differences between the groups in evenness can be attributed to use of rarefaction, which accounts for disparity in sampling sizes.

DISCUSSION

The paleogeographic history of the Rupununi portal area suggests that vicariance, isolation, and faunal exchange with secondary contact have contributed to the origins of the fauna surrounding the portal. In conjunction with paleogeological events shifting river drainages between eastern and western Guiana shields is the recent development of the Rupununi portal. The seasonal connection at the Rupununi portal brings additional complexity to understanding the processes influencing diversification, because this allows for a reconnection of portions of the ancient proto-Berbice. Therefore, two processes at work influencing fish diversity and

distribution are vicariance of the proto-Berbice break-up that was complete in the Pleistocene (or earlier) and/or recent dispersal via the Rupununi portal.

For species with ranges in the Takutu and Rupununi Rivers, a natural expectation is that this hydrological connection would allow aquatic species to freely disperse between the two drainages. Indeed, phylogeographic studies on *Potamorrhaphis*, *Cichla* and *Prochilodus* (Lovejoy and Araújo, 2000; Turner *et al*., 2004; Willis *et al*., 2007;) all support the hypothesis of dispersal through the ephemeral aquatic connection at the Rupununi portal, in addition to historical biogeographical analysis of possible dispersal routes in South American Rivers that also suggest faunistic exchange via the Rupununi portal (Hubert and Renno, 2006). In this study, we found statistically significant differences in the fish assemblages of the rivers linked by the Rupununi portal, suggesting the portal is serving as a conduit for dispersal for some species and not for other species.

Measures of community structure like species richness, diversity, and evenness can reveal important information about structure, stability and function of species assemblages. Species richnesss is the number of species in an assemblage, diversity characterizes the number of species and their relative abundance, while evenness is the variation in the abundance of individuals per species within a community. These measures provide critical baseline data in assessing biodiversity and have strong conservation implications. Assessing the differences in fish community structure in the Takutu and Rupununi Rivers enables us to infer the potential influence of the Rupununi portal on fish distributions. A Mann Whitney U test revealed significant difference in species richness and diversity between the two drainages. Below we examine differences in community structure of the two drainages to further assess why these drainages differ in diversity and richness.

There is a consistent pattern across the groupings at a higher taxonomic level, where the dominant orders are Characiformes, Siluriformes, and Perciformes (Table III). The patterns are more structured as we examine the composition at lower taxonomic levels and smaller area. The species that are found in the Takutu but not the Rupununi are mainly characids, trichomycterids and loricariids. Characids and loricariids are the dominant families in the region, so it is not surprising that the list of endemic species should include them, but the diversity of the trichomycterids deserves explanation.

Trichomycterids are commonly thought of as parasitic fishes, although they are considered to have the widest trophic adaptations among catfish families (Schaefer *et al*., 2005). Some trophic modes include hematophagy (feeding on blood) in the candirus, lepidophagy (scales), mucophagy (mucus), necrophagy (carrion) and algivory (algae). Equally remarkable is the variation of ecological habitats and elevation occupied within this family (Arratia and Menu-Marque, 1984; Fernández and Schaefer, 2003). Those found in the Takutu but not in the Rupununi are primarily psammophilic species (sand-dwelling), an adaptation that has involved complex evolutionary specializations (Zuanon *et al*., 2006). The predominant substratum of the Takutu is sand and therefore could explain the prevalence of several species. In addition, the extensive network of tributaries with sandy bottoms and shores connected to the Takutu would allow for their increased presence. Conversely, the lack of psammophilic habitat in the Rupununi portal would pose as a barrier to dispersal and thus, the decreased numbers of species found in the Rupununi. Interestingly, the hematophagic species of *Vandellia* are psammophilic, but are found on both sides of the portal, and not found in the northeastern Guiana Shield outside of the Essequibo River. The two species *Vandellia* (*V. beccari* and *V. sanguinea*) could have

ridden across the portal on a host and settled in the Rupununi as suggested by Zuanon & Sazima (2005) of dispersal in trichomycterids.

Anostomidae is considered to be highly diverse in the Guiana Shield (Sidlauskas and Vari, 2008), and anostomids were found to have many species present in the Rupununi but not in the Takutu. Their omnivorous diet and versatility in varying habitats could allow for their increased presence in the Rupununi drainage. Many of the anostomid species found in the Rupununi but not the Takutu are found in the Amazon basin, suggesting that the range of habitats we were able to sample in the Takutu may not be suitable for some species of anostomids to establish populations.

Several species found moving between the drainages were collected consistently at multiple sites. Many of these species, as Lowe-McConnell (1964) suggested, were found in savanna ponds as well as in the river system. Some species found in the ponds, like *Hoplias malabaricus, Pygocentrus nattereri*, *Cichla ocellaris* and *Acestrorhynchus microlepis*, have widespread distributions and are predatory fishes. This would enable them to endure conditions during the dry season in these ponds when competition for resources is high. Additionally, *Prochilodus rubrotaeniatus* is found throughout the drainages of the Guiana Shield and, not surprisingly, is among the species that occur on either side of the portal. This widespread detritivore makes long distance migrations, which would facilitate dispersal across the flooded savanna (Vari, 2004). Many of the species found between the two drainages are widely distributed, but there are also ones with smaller known ranges like *Hypostomus macushi* that were found on both sides of the portal.

Our study also suggests that the portal served as a barrier of dispersal for one-fourth of the species unique to the Rupununi and Takutu drainages. Although this may be an artifact of

collecting for some species (for example, *Roestes ogilviei* was only collected in the Rupununi, but it is known from the Amazon basin), the savanna is clearly a barrier for other species. For example, *Cichla temensis* is restricted to the Amazon side of the portal. This could be explained by habitat preference of this species, typically blackwater systems (Willis *et al*., 2007). Loricariids like *Peckoltia cavatica*, *Ancistrus leucostictus* and *Pseudancistrus megacephalus* are restricted to the Essequibo side of the portal. All three species are found in swift flow among gravel, cobble and boulders. They are often found clustered together and therefore may not disperse long distances or the inundated savannas may not provide suitable habitat for these species. The possibility of competition for niche occupancy cannot be ruled out and therefore confine them to smaller ranges.

Despite the extensive sampling in the Rupununi and Takutu over four expeditions, species accumulation curves reveal slight differences. The Takutu curve has just barely reached an asymptote, whereas the Rupununi curve has, suggesting that increased sampling in the Takutu would likely demonstrate increased species richness compared to that of the Rupununi. The Takutu and Rupununi were part of the proto-Berbice until the Amazon captured the Takutu about 2 MYA, and likely had similar faunas. The Amazon had more species than the proto-Berbice, so capture of the Takutu by the Amazon likely enriched the Takutu over the Rupununi. The Rupununi Portal has also likely enriched the fauna of the Essequibo over that of other eastern Guiana Shield rivers. The enrichment of the Takutu can especially be seen in the increased presence of trichomycterids that are found there as well as the rest of the Amazon, but not in the Rupununi.

The ecology of fishes seems to play a role in the overall pattern of distributions; however, very little is known about the specific ecologies of many of these species. There is evidence that

the ecologies of fishes affect dispersal through the Casiquiare River, which drains part of the Upper Orinoco into the Upper Negro and ultimately into the Amazon River. Examination of this connection revealed that the Casiquiare River serves as a zoogeographical filter, functioning as a corridor for some species and barrier for others (Winemiller *et al*., 2008). Distribution of fishes was based on an environmental gradient found along the Casiquiare River defined by shifts in water chemistry, habitat, and food resources. Additionally, further evidence from species specialized to rheophilic habitat, *Pseudancistrus brevispinis*, supported utilization of different mechanisms for dispersal influencing their distribution patterns in the Guiana Shield (Cardoso & Montoya-Burgos, 2009). Dispersal and diversification in *Pseudancistrus brevispinis* among adjacent basins is suggested to have occurred when marine waters receded followed by allopatric divergence during the marine incursions. Therefore, sea level fluctuations are suggested to have played a role in diversification and distribution of the Neotropical fish fauna.

Some of these factors could be driving the differences in fish assemblages across the Rupununi portal. Firstly, there are compositional differences in the drainages linked by the Rupununi portal. The Rupununi River has a larger floodplain surrounded by savanna and gallery forests, whereas the Takutu River is primarily surrounded by savanna. Steep banks are characteristic of the Takutu along many stretches of the river, which is due to tilting of basement layer of the Shield and a result of stream capture by the Branco (Gibbs and Barron, 1993). Water chemistry differences can also contribute to the differences. The Takutu River transitions between blackwater (starting at the Ireng) to whitewater as it flows into the Branco. Blackwater systems, dark in appearance due to the leaching of tannins from decaying leaves, tend to have low pH, low conductivity, and low dissolved oxygen (Val and Almeida-Val, 1995). This could pose physiological constraints on aquatic organisms. Low pH alone influences ionic balance, in

addition to other physiological processes in fishes such as osmotic balance, oxygen affinity for hemoglobin and digestion (Wilson *et al*., 1999; Matsuo and Val, 2002). Therefore, blackwater fishes are adapted to the specific environmental physicochemical characteristic of their environment (Val and Almeida-Val, 1995). The Rupununi River is a clearwater to whitewater system. There is moderate pH and dissolved oxygen concentrations in these systems, but also in whitewater portions there is sediment load when the waters levels are high, giving it a muddy appearance. In addition to the water chemistry within the drainages, waters throughout the vegetation of the inundated savanna during the rains affect differences in water chemistry (Carter, 1934; Sarmiento, 1984). Therefore, species adapted to specific characteristics of water type could find movement into another water type as a barrier to dispersal. In our study, water chemistry could explain the absence of widespread taxa that prefer whitewater (like *Leporinus friderici* and *Roestes ogliviei*) from the Takutu.

In order to survive and persist, organisms are driven to adapt, thereby developing characteristics for differing environmental conditions, acquisition of food resources and reproduction. These ongoing processes are evident in the tremendous specializations found in much of the Neotropical ichthyofauna. For instance, species that bury themselves in the sand (*Potamotrygon*, *Gymnorhamphicthys*, *Sarcoglanis* and *Vandellia*), species hidden in cavities of lateritic boulders (*Rhinodoras* and *Parotocinclus*), species associated with floating vegetation (*Apistogramma*), ones able to move over floating vegetation (*Hoplias* and *Hoplerythrinus*), and many found on submerged woody debris (many loricariids). Many of the specializations reveal the ecology of fishes have an important role in distributional patterns. Additionally, the ecology allows us to have a better understanding of the effect a biogeographic feature has on taxa (Bermingham and Martin, 1998). Closer examination of the ecologies of species at the portal

could provide more insight to specific diversifying processes driven by the Rupununi portal. Further, recent population genetics and phylogeographic studies have provided important new insights to bioeographical distributions of Neotropical fish across the Casiquiare River connection as well as the Rupununi portal (Lovejoy and Araújo, 2000; Sivasundar *et al*., 2001; Turner *et al*., 2004; Moyer *et al*., 2005; Willis *et al*., 2007) further revealing the intimate relationship of South America's geological history and evolution of the Neotropical fish fauna. Willis *et al*. (2010) points out that what is lacking in these biogeographical studies is an examination of these biogeographically important features of fishes across differing ecological requirements. Currently, we are attempting to fill this gap with a population genetics study of fishes with varying ecologies across the Rupununi portal in an attempt to determine how diverse ecologies effect gene flow. Assessing the genetics of species across the portal could reveal unsuspected diversity as found in *Pseudancistrus brevispinis*, where population genetics revealed six distinct lineages within the Guianas region (Cardoso and Montoya-Burgos, 2009).

This study has provided the most extensive assessment of the fish distributions in the Rupununi district, Guyana, while also resolving the composition of the fish fauna across the Rupununi portal. The fish community structure reveals the intrinsic role of the Rupununi portal on aquatic taxa likely influencing diversifying processes, as in cognate species. The highly structured fish fauna across the portal reveals a fauna that is constantly adapting to changes imposed by a fluctuating system of seasonal floods. The significance of the Rupununi portal on fish distributions has strong implications for conservation of this vital watershed for supporting flora and fauna.

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Table 1.1 – FISHES OF THE RUPUNUNI SAVANNA DISTRICT, GUYANA (*continued*)

TAXA	Rupununi	Takutu	Both
Beloniformes			
Belonidae			
Potamorrhaphis guianensis	-	-	✓
Pseudotylosurus microps	-	-	✓
Characiformes			
Acestrorhynchidae			
Acestrorhynchus falcirostris	-	-	✓
Acestrorhynchus microlepis	-	-	✓
Acestrorhynchus minimus	-	-	✓
Acestrorhynchus falcatus	✓	-	-
Acestrorhynchus guianensis	-	✓	-
Anostomidae			
Anostomoides laticeps	✓	-	-
Anostomus ternetzi	-	-	✓
Hypomasticus megalepis	-	✓	-
Laemolyta proxima	-	-	✓
Laemolyta taeniata	✓	-	-
Leporellus vittatus	-	-	✓
Leporinus agassizii	-	-	✓
Leporinus desmotes	-	-	✓
Leporinus fasciatus	-	-	✓
Leporinus nigrotaeniatus	-	-	✓
Leporinus alternus	✓	-	-
Leporinus friderici	✓	-	-
Leporinus maculatus	✓	-	-
Leporinus sp.	✓	-	-
Leporinus cf. *agassizii*	-	✓	-
Leporinus granti	-	✓	-
Leporinus ortomaculatus	-	✓	-
Petulanos plicatus	✓	-	-
Petulanos cf. *spiloclistron*	-	✓	-
Pseudanos irinae	✓	-	-
Schizodon cf. *vittatus*	-	-	✓
Synaptolaemus cingulatus	-	✓	-
Characidae			
Acanthocharax microlepis	✓	-	-
Acestrocephalus sardina	-	✓	-
Aphyocharacidium melandetum	-	✓	-
Aphyocharax alburnus	-	-	✓
Aphyodite grammica	-	-	✓
Charax hemigrammus	✓	-	-
Astyanax bimaculatus	-	-	✓
Astyanax fasciatus	-	-	✓
Astyanax rupununi	-	-	✓
Astyanax clavitaeniatus	-	✓	-
Brittanichthys myersi	-	-	✓
Brycon falcatus	-	-	✓
Brycon pesu	-	✓	-
Bryconamericus hyphesson	-	-	✓
Bryconamericus sp.	-	-	✓
Bryconamericus sp. "deep body"	-	-	✓
Bryconops affinis	-	-	✓
Bryconops alburnoides	-	-	✓
Bryconops caudomaculatus	-	-	✓
Catoprion mento	-	-	✓
Chalceus epakros	-	-	✓
Chalceus macrolepidotus	✓	-	-
Charax gibbosus	-	-	✓
Creagrutus maxillaris	-	-	✓
Creagrutus melanzonus	-	-	✓
Creagrutus sp.	✓	-	-
Ctenobrycon spilurus	-	-	✓
Cynopotamus essequibensis	-	-	✓
Exodon paradoxus	-	-	✓
Galeocharax guio	-	✓	-
Gephyrocharax sp.	✓	-	-
Hemigrammus analis	-	-	✓
Hemigrammus bellottii	-	-	✓
Hemigrammus cf. *schmardae*	-	-	✓
Hemigrammus cylindricus	-	-	✓
Hemigrammus iota	-	-	✓
Hemigrammus levis	-	-	✓
Hemigrammus microstomus	-	-	✓
Hemigrammus ocellifer	-	-	✓
Hemigrammus rodwayi	-	-	✓
Hemigrammus schmardae	-	-	✓
Hemigrammus stictus	-	-	✓
Hemigrammus unilineatus	-	-	✓
Hemigrammus gracilis	✓	-	-
Hemigrammus erythrozonus	-	✓	-
Hyphessobrycon bentosi	-	-	✓
Hyphessobrycon catableptus	-	-	✓
Hyphessobrycon gracilis	-	-	✓
Hyphessobrycon minor	-	-	✓
Hyphessobrycon sp.	-	-	✓
Iguanodectes spilurus	-	-	✓
Jupiaba atypindi	-	-	✓
Jupiaba pinnata	-	-	✓
Jupiaba polylepis	-	-	✓
Jupiaba scologaster	-	✓	-
Microschemobrycon callops	-	-	✓
Microschemobrycon casiquiare	-	-	✓
Microschemobrycon sp.	-	✓	-
Moenkhausia ceros	-	-	✓
Moenkhausia cf. *eigenmanni*	-	-	✓
Moenkhausia chrysargyrea	-	-	✓
Moenkhausia collettii	-	-	✓
Moenkhausia copei	-	-	✓
Moenkhausia dichroura	-	-	✓
Moenkhausia jamesi	-	-	✓
Moenkhausia lepidura "1"	-	-	✓
Moenkhausia lepidura "2"	-	-	✓
Moenkhausia oligolepis	-	-	✓
Moenkhausia sp. "ghost"	-	-	✓
Moenkhausia comma	✓	-	-
Moenkhausia sp.	✓	-	-
Moenkhausia lepidura "3"	-	✓	-
Moenkhausia megalops	-	✓	-
Moenkhausia sp. 1	-	✓	-
Odontostilbe gracilis	-	-	✓
Parapristella aubynei	-	-	✓
Phenacogaster microstictus	-	-	✓
Phenacogaster megalostictus	✓	-	-
Poptella brevispina	-	-	✓
Poptella compressa	-	-	✓
Poptella longipinnis	-	-	✓
Brachychalcinus orbicularis	-	✓	-
Pristella maxillaris	-	-	✓
Roeboides affinis	-	-	✓
Tetragonopterus argenteus	-	-	✓
Tetragonopterus chalceus	✓	-	-
Triportheus brachipomus	-	-	✓
Triportheus cf. *venezuelensis*	✓	-	-

Table 1.1 – FISHES OF THE RUPUNUNI SAVANNA DISTRICT, GUYANA (*continued*)

TAXA	Rupununi	Takutu	Both
Triportheus albus	-	✓	-
Triportheus rotundatus	-	✓	-
Chilodontidae			
Caenotropus labyrinthicus	-	-	✓
Chilodus punctatus	-	-	✓
Crenuchidae			✓
Characidium hasemani	-	-	✓
Characidium pteroides	-	-	✓
Characidium steindachneri	-	-	✓
Characidium zebra	-	-	✓
Characidium sp.	-	✓	-
Crenuchidae sp.	-	✓	-
Elachocharax junki	-	-	✓
Elachocharax geryi	-	✓	-
Melanocharacidium dispilomma	-	-	✓
Melanocharacidium nigrum	-	-	✓
Melanocharacidium depressum	-	✓	-
Melanocharacidium pectorale	-	✓	-
Melanocharacidium sp.	-	✓	-
Microcharacidium eleotrioides	-	✓	-
Ctenoluciidae			
Boulengerella cuvieri	-	-	✓
Boulengerella lucius	-	-	✓
Curimatidae			
Curimata cyprinoides	-	-	✓
Curimata roseni	-	-	✓
Curimata sp.	✓	-	-
Curimatopsis crypticus	-	-	✓
Cyphocharax festivus	-	-	✓
Cyphocharax leucostictus	-	-	✓
Cyphocharax microcephalus	-	-	✓
Cyphocharax spilurus	-	-	✓
Cyphocharax sp.	✓	-	-
Psectrogaster ciliata	✓	-	-
Psectrogaster essequibensis	✓	-	-
Steindachnerina planiventris	-	-	✓
Cynodontidae			
Cynodon gibbus	✓	-	-
Hydrolycus armatus	-	-	✓
Roestes ogilviei	✓	-	-
Erythrinidae			
Erythrinus erythrinus	-	-	✓
Hoplerythrinus unitaeniatus	-	-	✓
Hoplias malabaricus	-	-	✓
Hoplias sp.	-	-	✓
Hoplias aimara	✓	-	-
Gasteropelecidae			
Carnegiella strigata	-	-	✓
Hemiodontidae			
Bivibranchia fowleri	-	✓	-
Hemiodus quadrimaculatus	✓	-	-
Hemiodus argenteus	-	-	✓
Hemiodus semitaeniatus	-	-	✓
Hemiodus sp.	-	-	✓
Hemiodus unimaculatus	-	-	✓
Hemiodus thayeria	✓	-	-
Lebiasinidae			
Copella nattereri	-	-	✓
Nannostomus eques	-	-	✓
Nannostomus harrisoni	-	-	✓
Nannostomus marginatus	-	-	✓
Nannostomus trifasciatus	-	-	✓
Nannostomus unifasciatus	-	-	✓
Nannostomus beckfordi	✓	-	-
Nannostomus minimus	✓	-	-
Pyrrhulina filamentosa	-	-	✓
Parodontidae			
Parodon bifasciatus	-	-	✓
Parodon nasus	-	✓	-
Prochilodontidae			
Prochilodus rubrotaeniatus	-	-	✓
Semaprochilodus insignis	-	✓	-
Serrasalmidae			
Metynnis argenteus	-	-	✓
Metynnis luna	-	-	✓
Metynnis sp.	✓	-	-
Metynnis hypsauchen	-	✓	-
Metynnis lippincottianus	-	✓	-
Myleus pacu	-	-	✓
Myleus sp.	-	-	✓
Myleus setiger	✓	-	-
Myloplus rubripinnis	-	-	✓
Mylossoma aureum	-	✓	-
Prosomyleus rhomboidalis	✓	-	-
Pygocentrus nattereri	-	-	✓
Pygopristis denticulatus	-	-	✓
Pristobrycon striolatus	-	-	✓
Serrasalmus eigenmanni	-	-	✓
Serrasalmus rhombeus	✓	-	-
Clupeiformes			
Engraulidae			
Amazonsprattus scintilla	-	✓	-
Anchovia surinamensis	✓	-	-
Anchoviella guianensis	-	-	✓
Anchoviella sp.	-	-	✓
Anchoviella sp. 1	✓	-	-
Anchoviella sp. 2	✓	-	-
Anchoviella sp. 3	✓	-	-
Jurengraulis juruensis	-	-	✓
Cyprinodontiformes			
Rivulidae			
Rivulus sp.	-	-	✓
Rivulus stagnatus	-	✓	-
Gymnotiformes			
Apteronotidae			
Apteronotus albifrons	-	-	✓
Apteronotus sp.	-	-	✓
Platyurosternarchus macrostomus	-	-	✓
Sternarchorhynchus oxyrhynchus	-	✓	-
Gymnotidae			

Table 1.1 – FISHES OF THE RUPUNUNI SAVANNA DISTRICT, GUYANA (*continued*)

TAXA	Rupununi	Takutu	Both
Electrophorus electricus	-	✓	-
Gymnotus sp.	-	-	✓
Hypopomidae			
Brachyhypopomus brevirostris	-	-	✓
Brachyhypopomus sp.	-	-	✓
Brachyhypopomus pinnicaudatus	✓	-	-
Brachyhypopomus bullockii	✓	-	-
Brachyhypopomus sp. 2	✓	-	-
Hypopygus lepturus	-	-	✓
Microsternarchus sp.	✓	-	-
Steatogenys elegans	-	-	✓
Rhamphichthyidae			
Gymnorhamphichthys hypostomus	-	-	✓
Gymnorhamphichthys rondoni	-	-	✓
Gymnorhamphichthys rosamariae	-	-	✓
Gymnorhamphichthys sp. "fused saddles"	-	✓	-
Gymnorhamphichthys sp. "small saddle"	✓	-	-
Rhamphichthys marmoratus	-	-	✓
Rhamphichthys rostratus	✓	-	-
Sternopygidae			
Eigenmannia microstoma	-	-	✓
Eigenmannia virescens	-	-	✓
Rhabdolichops electrogrammus	-	✓	-
Osteoglossiformes			
Osteoglossidae			
Osteoglossum bicirrhosum	-	-	✓
Perciformes			
Cichlidae			
Acarichthys heckelii	-	-	✓
Acaronia nassa	-	-	✓
Acaronia sp.	-	✓	-
Aequidens tetramerus	-	-	✓
Aequidens potaroensis	-	✓	-
Apistogramma ortmanni	-	-	✓
Apistogramma rupununi	-	-	✓
Apistogramma steindachneri	-	-	✓
Apistogramma sp.	-	✓	-
Biotodoma cupido	-	-	✓
Chaetobranchopsis orbicularis	-	✓	-
Chaetobranchus flavescens	-	-	✓
Cichla ocellaris	-	-	✓
Cichlasoma bimaculatum	-	-	✓
Crenicichla alta	-	-	✓
Crenicichla lugubris	-	-	✓
Crenicichla reticulata	-	-	✓
Crenicichla saxatilis	-	-	✓
Crenicichla strigata	-	-	✓
Crenicichla wallacii	-	-	✓
Crenicichla acutirostris	✓	-	-
Geophagus sp.	-	-	✓
Geophagus sp. "takutu 2"	-	-	✓
Geophagus surinamensis	-	-	✓
Geophagus sp. "essequibo"	✓	-	-
Geophagus sp. "yupukari"	✓	-	-
Geophagus sp. "takutu"	-	✓	-
Guianacara sphenozona	-	-	✓
Guianacara dacrya	-	-	✓
Heros severus	✓	-	-
Mesonauta guyanae	-	-	✓
Satanoperca jurupari	-	-	✓
Satanoperca leucosticta	-	-	✓
Eleotridae			
Microphilypnus amazonicus	-	-	✓
Sciaenidae			
Pachypops fourcroi	-	-	✓
Pachypops sp.	✓	-	-
Pachypops trifilis	✓	-	-
Petilipinnis gurnniens	-	-	✓
Plagioscion squamosissimus	✓	-	-
Plagiscion surinamensis	✓	-	-
Pleuronectiformes			
Achiridae			
Achirus achirus	-	✓	-
Hypoclinemus mentalis	-	-	✓
Soleonasus finis	-	-	✓
Rajiformes			
Potamotrygonidae			
Paratrygon aeriba	✓	-	-
Potamotrygon orbignyi	-	-	✓
Potamotrygon hystrix	-	✓	-
Siluriformes			
Aspredinidae			
Amaralia hypsiura	-	-	✓
Bunocephalus amaurus	-	-	✓
Bunocephalus sp.	-	-	✓
Bunocephalus verrucosus	✓	-	-
Hoplomyzon atrizona	-	✓	-
Xyliphius sp.	-	✓	-
Auchenipteridae			
Ageneiosus piperatus	✓	-	-
Auchenipterichthys thoracatus	✓	-	-
Auchenipterus demerarae	✓	-	-
Auchenipterus ambylacas	-	✓	-
Centromochlus reticulatus	-	-	✓
Gelanoglanis sp.	-	✓	-
Glanidium leopardum	-	-	✓
Tatia intermeida	-	-	✓
Trachelyopterus galeatus	-	-	✓
Trachelyopterus sp.	-	✓	-
Trachycorystes obscurus	✓	-	-
Callichthyidae			
Corydoras bondi	-	-	✓
Corydoras melanistius	-	-	✓
Corydoras sipaliwini	-	-	✓
Corydoras blochi	-	✓	-
Corydoras sp. "plain"	-	✓	-
Megalechis personata	-	-	✓
Megalechis picta	✓	-	-

Table 1.1 – FISHES OF THE RUPUNUNI SAVANNA DISTRICT, GUYANA (*continued*)

TAXA	Rupununi	Takutu	Both
Megalechis thoracata	-	✓	-
Cetopsidae			
Cetopsidae sp. 1	-	-	✓
Cetopsidium soniae	-	✓	-
Cetopsidium roae	-	-	✓
Cetopsis cf. *montana/axelrodi*	-	✓	-
Denticetopsis macilenta	-	-	✓
Denticetopsis iwokrama	-	✓	-
Doradidae			
Acanthodoras cataphractus	✓	-	-
Acanthodoras spinosissimus	✓	-	-
Amblydoras affinis	-	-	✓
Doras carinatus	✓	-	-
Doras micropoeus	✓	-	-
Hassar sp.	-	-	✓
Hassar sp. 1	-	-	✓
Leptodoras hasemani	-	-	✓
Leptodoras linnelli	-	-	✓
Leptodoras praelongus	-	✓	-
Nemadoras leporhinus	-	-	✓
Nemadoras trimaculatus	-	-	✓
Nemadoras sp.	-	✓	-
Opsodoras sp.	✓	-	-
Opsodoras ternetzi	-	✓	-
Oxydoras niger	-	-	✓
Platydoras hancockii	-	-	✓
Rhinodoras armbrusteri	-	-	✓
Scorpiodoras affinis	-	-	✓
Trachydoras cf. *steindachneri*	-	-	✓
Trachydoras brevis	✓	-	-
Trachydoras pseudomicrostomus	-	✓	-
Heptapteridae			
Brachyrhamdia heteropleura	-	-	✓
Cetopsorhamdia insidiosa	-	✓	-
Chasmocranus cf. *tapanahoniensis*	✓	-	-
Chasmocranus longior	✓	-	-
Chasmocranus sp.	✓	-	-
Goeldiella eques	✓	-	-
Imparfinis hasemani	-	-	✓
Mastiglanis sp. "no spot"	-	-	✓
Mastiglanis sp. "spot"	-	-	✓
Phenacorhamdia sp. "slim"	✓	-	-
Phenacorhamdia sp. "short anal fin"	-	✓	-
Pimelodella cristata	-	-	✓
Pimelodella megalops	-	-	✓
Pimelodella sp. 2	✓	-	-
Pimelodella sp. "dark"	-	✓	-
Pimelodella sp. "ireng"	-	✓	-
Pimelodella sp.	-	✓	-
Rhamdella leptosoma	-	✓	-
Rhamdia quelen	-	-	✓
Rhamdia sp.	-	✓	-
Loricariidae			
Ancistrus circle E	-	-	✓
Ancistrus leucostictus	✓	-	-
Ancistrus sp.	✓	-	-
Ancistrus sp. 2	-	✓	-
Ancistrus nudiceps	-	✓	-
Farlowella acus	-	-	✓
Farlowella nattereri	-	-	✓
Farlowella reticulata	-	-	✓
Farlowella rugosa	-	-	✓
Harttia platystoma	✓	-	-
Hemiodontichthys acipenserinus	✓	-	-
Hypancistrus sp.	-	✓	-
Hypoptopoma guianense	-	-	✓
Hypoptopoma thoracatum	✓	-	-
Hypoptopoma sp.	-	✓	-
Hypostomus hemiurus	-	-	✓
Hypostomus macushi	-	-	✓
Hypostomus squalinus	-	-	✓
Hypostomus taphorni	-	-	✓
Lasiancistrus schomburgkii	-	-	✓
Limatulichthys griseus	-	-	✓
Lithoxus lithoides	-	-	✓
Loricaria cataphracta	-	-	✓
Loricaria sp.	-	-	✓
Loricaria sp. 1	-	✓	-
Loricariichthys brunneus	✓	-	-
Loricariichthys microdon	✓	-	-
Loricariichthys sp.	✓	-	-
Panaque sp.	-	✓	-
Parotocinclus britskii	-	-	✓
Peckoltia sabaji	-	-	✓
Peckoltia cavatica	✓	-	-
Peckoltia braueri	-	✓	-
Pseudacanthicus leopardus	-	-	✓
Pseudancistrus nigrescens	-	-	✓
Pseudancistrus megacephalus	✓	-	-
Pseudoloricaria laeviuscula	-	✓	-
Rhadinoloricaria macromystax	-	✓	-
Rineloricaria fallax	-	-	✓
Rineloricaria lanceolata	-	-	✓
Rineloricaria sp.	-	-	✓
Rineloricaria sp. 1	-	-	✓
Rineloricaria sp. 2	-	-	✓
Rineloricaria stewarti	✓	-	-
Spatuloricaria sp.	-	-	✓
Sturisoma monopelte	-	-	✓
Pimelodidae			
Hemisorubim platyrhynchos	-	-	✓
Hypophthalmus edentatus	✓	-	-
Megalonema platycephalum	-	-	✓
Pimelodus albofasciatus	-	-	✓
Pimelodus blochii	-	-	✓
Pimelodus ornatus	-	-	✓
Pimelodus sp.	-	-	✓
Pseudoplatystoma fasciatum	-	-	✓
Sorubim elongatus	-	-	✓
Sorubim lima	✓	-	-
Pseudopimelodidae			
Batrochoglanis villosus	-	-	✓
Microglanis poecilus	-	-	✓
Microglanis secundus	-	-	✓
Pseudopimelodus bufonius	-	-	✓
Pseudopimelodus sp.	✓	-	-

Table 1.1 – FISHES OF THE RUPUNUNI SAVANNA DISTRICT, GUYANA (*continued*)

TAXA	Rupununi	Takutu	Both
Trichomycteridae			
Acanthopoma sp. 2	✓	-	-
Acanthopoma sp.	-	✓	-
Branchioica sp. 1	✓	-	-
Branchioica sp.	-	✓	-
Haemomaster sp.	-	✓	-
Haemomaster venezeualae	-	✓	-
Henonemus punctatus	-	-	✓
Homodiatus sp.	-	-	✓
Ituglanis sp.	-	✓	-
Ochmacanthus flabelliferus	-	-	✓
Ochmacanthus sp. 2	-	-	✓
Ochmacanthus sp.	-	✓	-
Paracanthopoma sp.	-	-	✓
Paracanthopoma sp. 1	-	✓	-
Pygidianops eigennmanni	-	✓	-
Sarcoglanis simplex	-	✓	-
Stegophilus sp.	-	✓	-
Trichomycterus sp.	-	-	✓
Typhlobelus sp.	-	✓	-
Vandellia beccarii	-	-	✓
Vandellia sanguinea	-	-	✓
<u>Synbranchiformes</u>			
Synbranchidae			
Synbranchus marmoratus	-	-	✓
<u>Tetraodontiformes</u>			
Tetraodontidae			
Colomesus psittacus	-	-	✓

TABLE 1.2. Dominant Orders and Families found in Rupununi, Takutu, Shared and Overall.

Taxonomic Level	Rupununi	Takutu	Shared	Overall
Dominant Orders	Characiformes 40% (36)	Siluriformes 46% (42)	Characiformes 48% (122)	Characiformes 44% (192)
	Siluriformes 38% (34)	Characiformes 38% (35)	Siluriformes 31% (79)	Siluriformes 36% (155)
	Perciformes 8% (8)	Perciformes 6% (6)	Perciformes 10% (26)	Perciformes 9.3% (40)
Dominant Families	Characidae 15% (13)	Characidae 17% (15)	Characidae 25% (65)	Characidae 22% (93)
	Loricariidae 12% (11)	Trichomycteridae 12% (11)	Loricariidae 10% (26)	Loricariidae 11% (46)
	Anostomidae 9% (8)	Loricariidae 10% (9)	Cichlidae 9% (13)	Cichlidae 7.6% (33)

TABLE 1.3. Results from Mann-Whitney U tests comparing species diversity measures between the Rupununi and Takutu drainages.

DIVERSITY MEASURES	RUPUNUNI (N=45)	TAKUTU (N=44)	Ustat	P-value
Mean Richness	37.72	29.16	1283	0.015*
Mean Shannon's diversity	2.59	2.38	1244	0.035*
Mean Evenness	0.73	0.72	1095	0.388

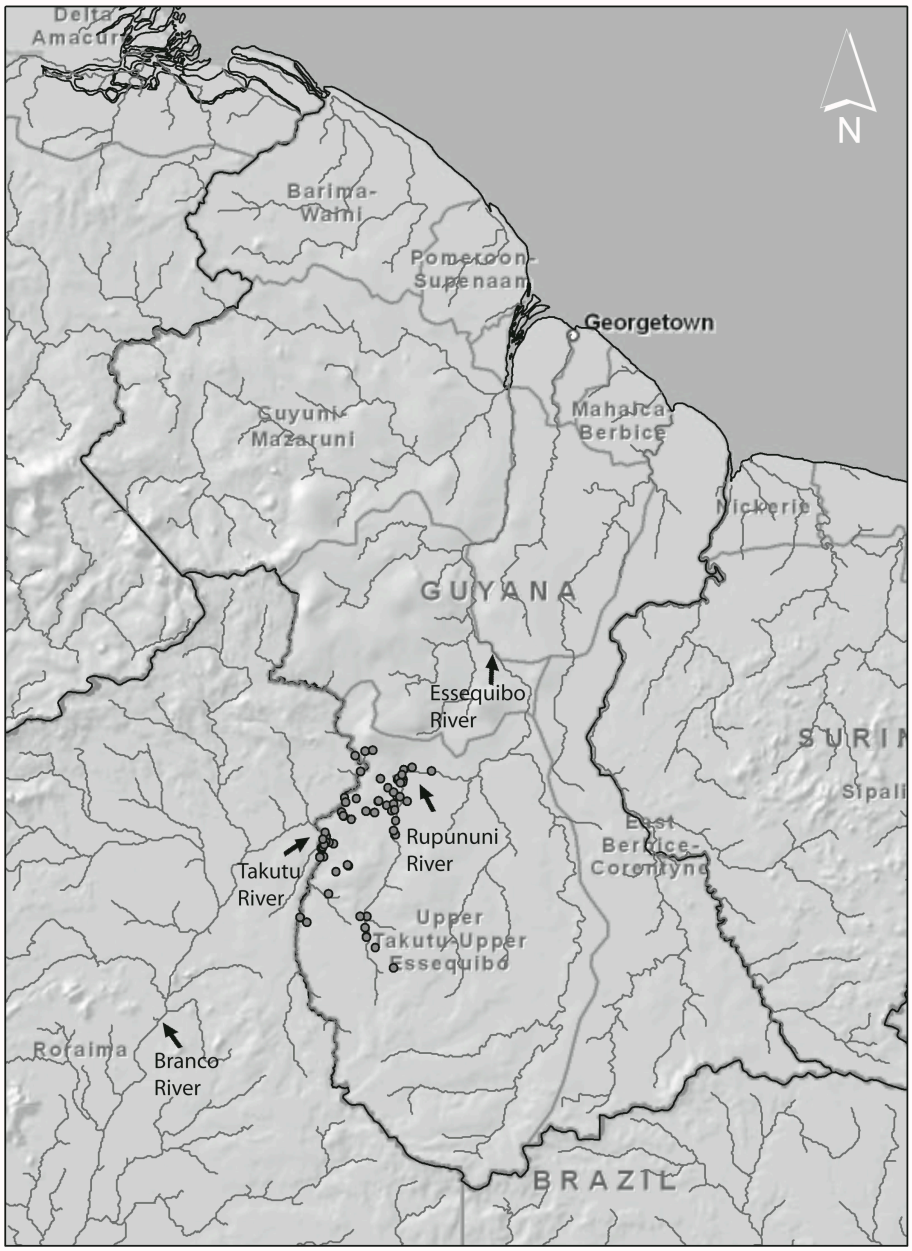

Figure 1.1. Collection sites in the Rupununi and Takutu River drainages.

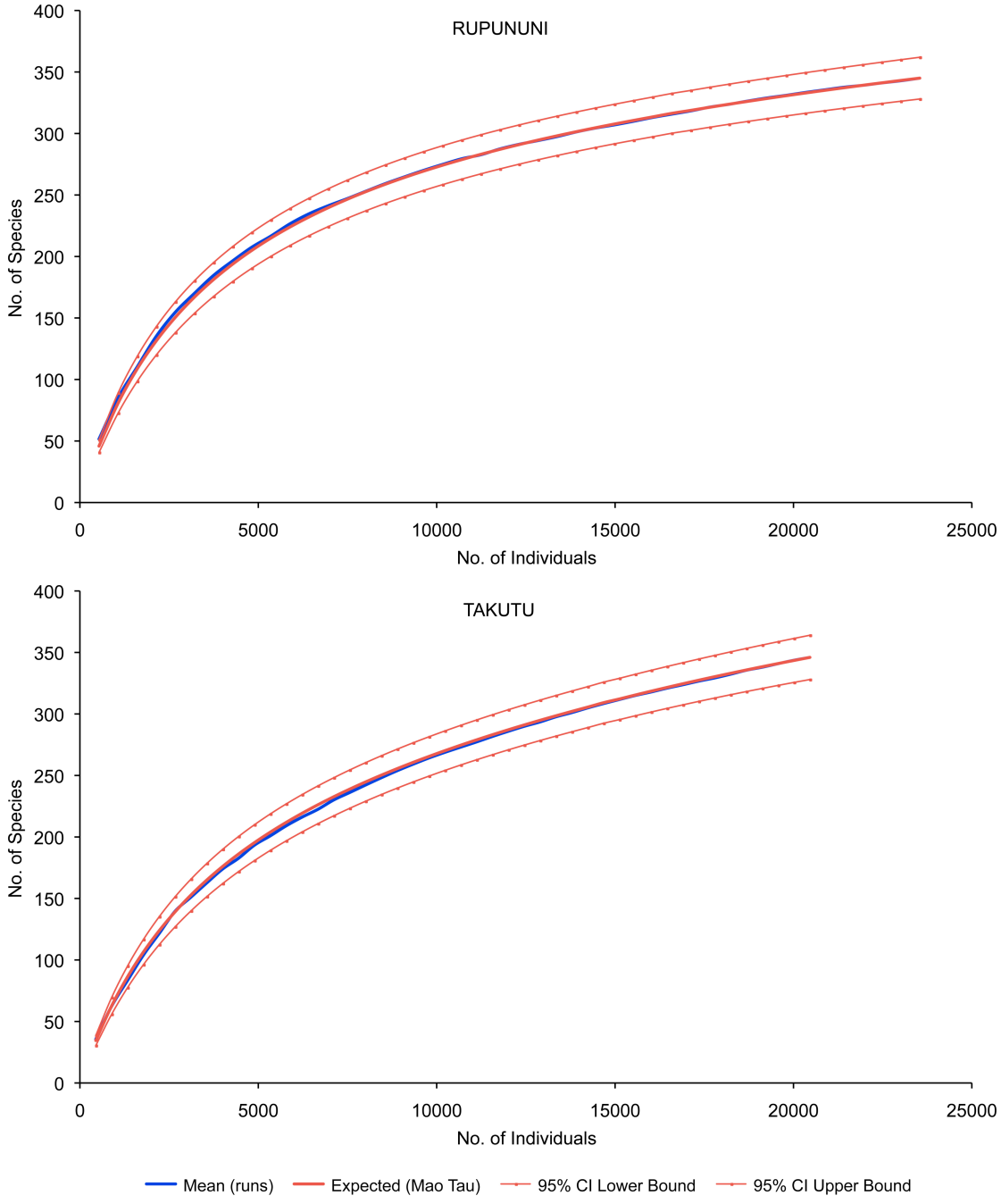

Figure 1.2. Species accumulation curves for Rupununi and Takutu River drainages. Mao Tau is expected estimates at infinite number of randomizations. Dotted lines denote the upper and lower bound of 95% confidence intervals.

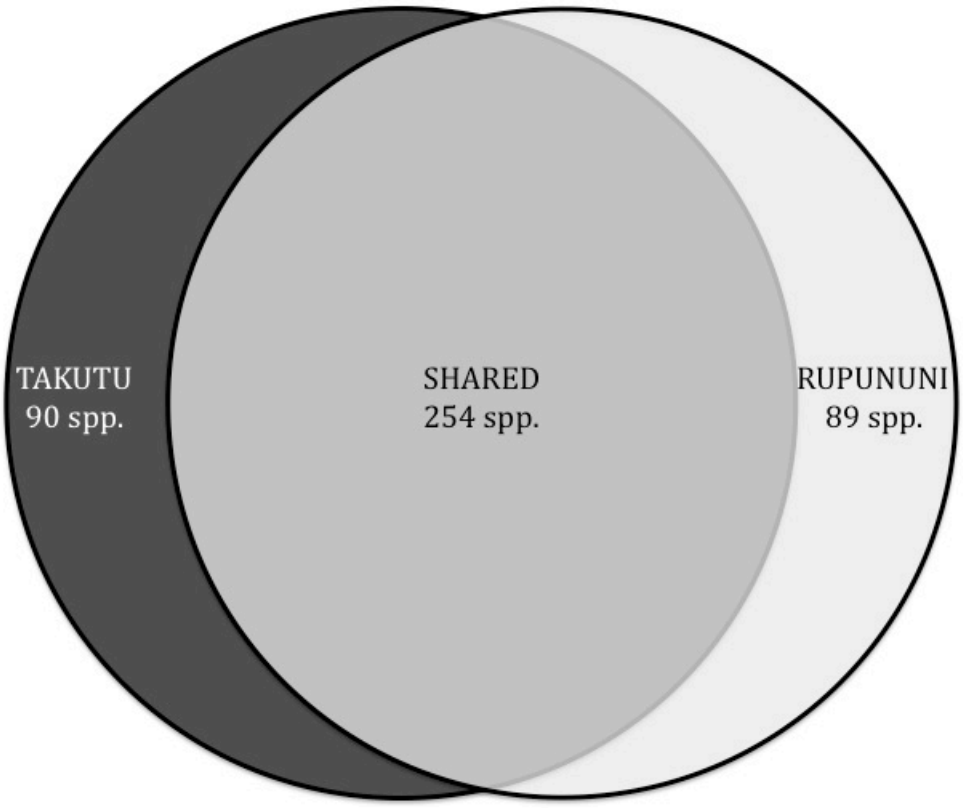

Figure 1.3. Venn diagram of fish distributions in Rupununi and Takutu Rivers.

CHAPTER 2 – The role of the North Rupununi wetlands on gene flow and dispersal in fish populations

INTRODUCTION

The North Rupununi wetlands are an expansive network of ephemeral ponds, oxbow lakes, depression lakes, and creeks across the vast savannas of south-central Guyana. Bordered by two main river drainages, the Takutu and Rupununi Rivers, the wetlands are intimately associated with the nearby forests, savannas, and indigenous people. These wetlands are an important site for migration and spawning of many aquatic taxa, especially during the wet season when the annual rains allows for a connection between the drainages of Amazonas and the Guiana Shield. Globally, this is the only known seasonal connection uniting two extremely diverse species faunas (Reis et. al 2003, Albert et. al 2011).

The unique biogeographic corridor of the Rupununi wetlands for aquatic taxa appears to offer opportunities for gene flow and dispersal between fish populations of the Amazon River basin and the Essequibo River drainage of the Guiana Shield. The significance of uniting a historically ancient drainage system to a younger Amazon drainage has implications for understanding the evolutionary processes governing the diversification of the Neotropical ichthyofauna. In addition to this present hydrological connection, there is the paleogeographic history of the region's river drainage patterns that are unlike the modern day configurations. The Rupununi wetlands are located in a rift valley that was once filled by an endorheic lake, Lake Maracanata, during the Cretaceous. In the early Paleogene, Lake Maracanata began transitioning into a fluvial system known as the proto-Berbice, which was the main drainage system through this rift valley during much of the Cenozoic. A series of stream capture events during the

Pliocene (Chapter 3) fragmented the proto-Berbice altering the configuration of drainages and creating a shallow divide between east flowing Rupununi into the Essequibo and southwest flowing Takutu into the Branco and ultimately to the Amazon River. Currently, the North Rupununi savannas and its associated wetlands occupy this shallow divide.

The Rupununi region has likely played a role in both the vicariance and dispersal of fishes between the Amazon and the Essequibo. The fragmentation of the proto-Berbice likely isolated previously widespread taxa, resulting in allopatric sister lineages. More recently, the origination of the Rupununi wetland connection may have subsequently allowed dispersal (range expansion) or gene flow between the Amazon and Essequibo. Current fish distributions reflect both of these possibilities, for instance, sister species *Peckoltia braueri* and *P. cavatica* have allopatric distributions in, respectively, the Amazon and Essequibo; these distributions could be explained by the proto-Berbice vicariant event. Other species such as *Hypostomus macushi*, *Arapaima gigas*, *Osteoglossum bicirrhosum*, *Peckoltia sabaji* have distributions in the Amazon and Essequibo and these broad distributions could be due to dispersal through the Rupununi wetlands.

For species ranges that currently encompass the Amazon and Essequibo, a natural expectation is that the flooded Rupununi wetlands could allow gene flow between these two basins. No phylogeographic study has assessed the role of this hydrological connection on gene flow, although studies have speculated the Rupununi wetlands as a corridor for dispersal (Lovejoy & de Araújo 2000; Willis et. al 2007). Therefore, here I investigate the role of the Rupununi wetlands on gene flow for five taxa with ranges in the Amazon and Essequibo basins using three molecular markers (two mitochondrial and one nuclear). Taxa included in this study were *Hypostomus squalinus*, *Ancistrus* sp. 'white spot', *Geophagus surinamensis*, *Hoplias*

malabaricus, and *Potamorrhaphis guianensis*, which were selected to incorporate taxa with varying ecologies. The sampling scheme was focused in the Takutu and Rupununi River drainages bordering the Rupununi wetlands, in addition to sampling in the wetlands. The goal of this study is to determine the Rupununi wetlands' role in gene flow and genetic differentiation for populations of these five taxa. In addition to distinguishing between historical and contemporary gene flow.

A sense of urgency surrounds research in the Rupununi savannas due to impending threats by development, oil drilling, logging, gold/diamond mining and agriculture. Amerindian communities are intimately connected to the Rupununi wetlands, forests, savannas, and associated wildlife for resources of food, products to construct homes, canoes, crafts and the medicinal properties. The health of this ecosystem is vital for the persistence of biological and cultural communities. Fish provide Amerindians with over 60% of their protein (Watkins et. al 2010), and the Rupununi wetlands are vital in the spawning for many fish species. Understanding the role these wetlands play in the genetic diversity and distribution of fishes could prove crucial for preservation of a fauna and culture so intimately intertwined.

MATERIALS & METHODS

Study site and sampling

The Rupununi portal area is located between the Western and Eastern Guiana Shield regions at the border between Brazil and Guyana (Figure 1). Extensive sampling in the Rupununi and Takutu River drainages was done during four expeditions to the region (2002, 2003, 2005 and 2007). Samples from expeditions to the Orinoco drainage in Venezuela were also included in this study. Fishes were collected by a variety of methods including seine, gill net, cast net, hook and line and by hand. All collections were made during the dry season with

collections made during the day and night. Localities included sites from the main river channel, tributaries, ponds, and borrow pits. Georeferencing data was recorded for each site using a handheld GPS. Muscle tissue was removed and preserved in 95% ethanol in the field. These tissue samples were stored in a -80°C freezer prior to DNA analysis. Voucher specimens were individually tagged and preserved in 10% buffered formalin then transferred to 70% ethanol. Specimens were deposited in museum collections at Auburn University Museum, and duplicates have been deposited in the museum at the University of Guyana Centre for the Study of Biodiversity.

Taxa

Taxa examined for this study consisted of five species with widespread distributions found in abundance across the Rupununi portal of varying life history and ecology. These taxa include: Loricariidae - *Hypostomus squalinus, Ancistrus* 'white spot' sp.; Cichlidae - *Geophagus surinamensis*; Erythrinidae – *Hoplias malabaricus*; Belonidae – *Potamorrhaphis guianensis*.

Loricariidae

Hypostomus squalinus (lentic) is found in the Orinoco, Essequibo, and Negro drainages, and is typically a lowland species found in fairly swift sandy streams. *Ancistrus* 'white spot' sp. (lotic) is found in clear water streams in fast flowing waters, where it is closely associated with rocks and driftwood in streams, and it actually represents a complex of species.

Cichlidae

Geophagus surinamensis (lotic) are distributed in both the Essequibo and Negro drainages in main river channels.

Erythrinidae

Hoplias malabaricus (lentic) has a large distribution and is found throughout all the rivers of South America in varying habitats (Oyakawa, 1990), although ongoing research indicates that it may consist of multiple undescribed species. *Hoplias malabaricus* are predatory fish and suggested in playing an instrumental role in the dynamics of the complex food web of tropical rivers.

Belonidae

Potamorrhaphis guianensis (lentic) are insectivorous and piscivorous needlefishes with a widespread distribution (Orinoco, Essequibo, and Amazon) and are more often found in backwater lakes and streams than in major rivers.

DNA isolation, PCR and sequencing

DNA extractions were made from ethanol preserved tissues using proteinase K digestion followed by protein precipitation. Nucleic acids were precipitated using 95% ethanol at -20° C. Pellets were washed in 70% ethanol, dried and resuspended in 20μl of ddH_20. DNA sequences of two mitochondrial genes, cytochrome b gene and CO1 gene and one nuclear gene, first intron of the S7 ribosomal protein were generated by PCR amplification using primers specific to each taxa (Table 2.1). PCRs for each taxa were conducted in a PTC-100^{TM} thermocycler (MJ Research) under the following conditions: initial denaturing step of 94° C for 3 min, 34 cycles of 94° C for 30s, 45° C for 30s, 72° C for 45s and a final extension of 72° C for 5 min. Amplifications were visualized via electrophoresing 3μL of PCR product in a 1% agarose gel. Amplified products were sequenced by High-Throughput Genomics Unit (HTGU) genomics facility at the University of Washington. All sequences were aligned using SEQUENCHER 4.1.4 (Gene Codes Corporation, Ann Arbor, MI).

Genetic variation, population structure and migration

Genetic diversity measures nucleotide (π) and haplotype (*h*) diversity estimates for all taxa were calculated following methods of Nei (1987) in the DNASP 5 (Librado & Rozas, 2009). Genetic differentiation between populations were assessed using pairwise ϕ_{ST} statistics (based on haplotype frequency and molecular divergence) and exact tests (based on haplotype frequency distributions only) in the program ARLEQUIN 3.0 (Excoffier et. al, 2005). An analysis of molecular variance (AMOVA; Excoffier et. al 1992) was conducted in ARLEQUIN 3.0 (Excoffier et. al, 2005) to assess the spatial distribution of genetic variation in all taxa. For the AMOVA, ϕ-statistics provided an estimate of the distribution of molecular variance at three levels: (i) among the drainages (ϕ_{CT}); (ii) among populations within drainages (ϕ_{SC}), and; (iii) within populations (ϕ_{ST}). Tamura and Nei model of DNA sequence evolution with among-site variation (TN+G; Gamma = 0.59), as selected by the Akaike information criterion (AIC) in JModelTest version 0.1.1 (Posada 2008) was used for pairwise ϕ_{ST} statistics and AMOVAs with 10000 permutations to determine significance. Mantel tests for each species were conducted in program R version 2.13.1 (2008) using the vegan package (Oksanen et. al 2007) to test for correlations between geographic distance and genetic distance. Partial mantel tests were also conducted using a binary matrix to test correlations between genetic distance and the Rupununi portal. Significance of mantel and partial mantel tests were assessed using 10000 permutations.

Haplotype relationships and demographic analyses

The relationships among haplotypes of all taxa were assessed using maximum likelihood in MEGA5 (Tamura et. al 2011). For ML analyses the best-fit models of sequence evolution was estimated using the Akaike information criterion (AIC) in JModelTest version 0.1.1 (Posada 2008) (Table 2.2). Support for nodes were evaluated by calculating bootstrap values using 1000 pseudoreplicates. I expect that reciprocally monophyletic groups of haplotypes in the Takutu

and Rupununi Rivers would indicate long-term isolation between populations, while para- or polyphyletic haplotype lineages could suggest recent gene flow, or recent dispersal of a species from one basin or the other. Additionally, the relationships of haplotypes were visualized using a haplotype network in the program TCS version 1.21 (Clement et al. 2000). The analysis was conducted under default settings, which provides the 95% parsimoniously plausible connections between haplotypes.

Tajima's *D* (Tajima 1989) tests were conducted to determine whether patterns of each taxa sequence variation (for CO1, Cyt b, and S7) were consistent with the predictions of a neutral model. Significance of the neutrality tests for all taxa was assessed by 10,000 permutations using ARLEQUIN 3.0 (Excoffier et. al, 2005). To test whether the sequence data from each population deviated from what is expected under a sudden expansion model, Harpending's raggedness index (*Hri*, Harpending, 1994) was calculated. *Hri* is based on mismatch distributions (the frequency distributions of pairwise differences among all haplotypes in a sample) and significance was assessed by 10,000 permutations in ARLEQUIN 3.0. A significant *Hri* value ($P < 0.05$) is taken as evidence for rejecting the sudden expansion model (Schneider & Excoffier, 1999).

RESULTS

Genetic diversity

Mitochondrial genes Cytochrome Oxidase I (CO1), Cytochrome b (Cyt b) and a nuclear marker, first intron of the S7 ribosomal protein were sequenced in this study for *Ancistrus* 'white spot' sp., *Hypostomus squalinus*, *Geophagus surinamensis*, *Hoplias malabaricus*, and *Potamorrhaphis guianensis*. Number of individuals, length of gene fragment and other attributes of the data for all taxa are listed in (See Appendix 1, 2 and 3). Nucleotide (π) diversity and

Haplotype (*h*) diversity values for all species were consistent for all populations across the three genes, with the exception of *H. squalinus*, which had exceptionally low values compared to other species (Table 2.3). *Hypostomus squalinus* also showed considerably lower number of haplotypes as compared to other species.

Haplotype networks and trees per species

Ancistrus 'white spot' sp.

The final alignment for protein coding cytochrome oxidase I gene for *Ancistrus* 'white spot' sp. included 669 bp from 42 individuals (18=Rupununi, 17=Takutu, 7=Venezuela). Cytochrome b gene alignment resulted in 1107 bp from 45 individuals (21=Rupununi, 18=Takutu, 6=Venezuela). There were no ambiguous chromatogram readings, frameshifts or stop codons observed in the data, suggesting these sequences are not likely pseudogenes. The CO1 sequences collapsed to 16 haplotypes with no haplotypes shared between the Rupununi and Takutu drainages. Eight haplotypes were only in the Rupununi, four only in the Takutu and four only in Venezuela drainages. Cytochrome b sequences collapsed into 19 haplotypes. One haplotype was shared between Rupununi and Takutu, ten haplotypes only in the Rupununi, four only in the Takutu, and four only in Venezuela. The final alignment of the first intron of the S7 ribosomal protein gene resulted in 640bp for 39 individuals (14=Rupununi, 19=Takutu, 6=Venezuela). S7 sequences collapsed into 19 haplotypes, no haplotypes were shared between Rupununi and Takutu, nine haplotypes were only in the Rupununi, six haplotypes only in the Takutu and four haplotypes only in Venezuela (Figure 2.1a-c).

The Maximum likelihood (ML) analysis of haplotype relationships from three independent ML analyses for CO1, Cyt b and S7 resulted in a single tree with similar optimal scores (best lnL -1708.09, lnL -4090.27, lnL -1375.90), bootstraps were terminated at 1000 runs

(Figure 2.1a-c). ML analyses were run under TrN for CO1 and GTR for Cyt b and S7, as selected by AIC. The phylogeny of haplotypes recovered a highly structured topology (CO1, Cyt b, &S7) indicating that *Ancistrus* 'white spot' sp. is likely not all one species. Therefore further population analyses were not conducted for this species since it appears we are dealing with multiple species, and will be referred to hereafter as *Ancistrus* 'white spot' spp.

Hypostomus squalinus

The final alignment of protein coding COI gene for *H. squalinus* included 673 bp from 67 individuals (39=Rupununi, 25=Takutu, 3=Venezuela) and 1152 bp from 68 individuals (37=Rupnuni, 26=Takutu, 5=Venezuela) for the cytochrome *b* gene. There were no ambiguous chromatogram readings, frameshifts or stop codons observed in the data, suggesting these sequences are not likely pseudogenes. The CO1 sequences collapsed to six haplotypes in the Rupununi and Takutu as visualized in the haplotype network (Figure 2.3a-c). Two haplotypes were shared between the two drainages, while four haplotypes were only found in the Takutu. Cytochrome b sequences collapsed into six haplotypes, one haplotype was shared between Rupnuni and Takutu, one haplotype only in the Rupununi, four haplotypes in the Takutu and two haplotypes in Venezuela drainages. The final alignment of the first intron of the S7 ribosomal protein gene resulted in 623bp for 38 individuals (27=Rupununi, 11=Takutu). S7 sequences collapsed into nine haplotypes, one haplotype was shared between Rupununi and Takutu, five haplotypes were only in the Rupununi, and three haplotypes only in the Takutu.

Maximum likelihood (ML) analysis of haplotype relationships from three independent ML analyses resulted in a single tree with similar optimal scores (best lnL -1241.13, lnL -1954.44, and lnL -833.35) for CO1, Cyt b and S7 respectively (Figure 2.4a-c). Bootstraps were terminated at 1000 runs. ML analyses were run under HKY model for all three genes. In this

phylogeny, haplotypes in the Rupununi and Takutu did not form reciprocally monophyletic groups, instead the phylogeny suggests that *Hypostmus squalinus* is a panmictic population across the Rupununi portal. This topology was recovered with the three molecular markers.

Geophagus surinamensis

The final alignment for protein coding gene CO1 and Cyt b for *Geophagus surinamensis* resulted in 678bp for 49 individuals (25=Rupununi, 22=Takutu, 2=Venezuela) and 1047bp for 51 individuals (25=Rupununi, 26=Takutu), respectively. There were no ambiguous chromatogram readings, frameshifts or stop codons observed in the data, suggesting these sequences are not likely pseudogenes. The CO1 sequences collapsed to 14 haplotypes. One haplotype shared between Rupununi and Takutu drainages, eight haplotypes only in the Rupununi, three haplotypes only in the Takutu, and two haplotypes in Venezuela. Cytochrome b sequences collapsed to 11 haplotypes in the Rupununi and Takutu. One haplotype is shared between Rupununi and Takutu, six haplotypes are found only in Takutu and three haplotypes are found only in the Rupununi. The final alignment of the first intron of the S7 ribosomal protein gene resulted in 604bp for 59 individuals (28=Rupununi, 30=Takutu, 1=Venezuela). S7 sequences collapsed into 10 haplotypes, three haplotypes were shared between Rupununi and Takutu, four haplotypes were only in the Rupununi, two haplotypes only in the Takutu and one haplotype in Venezuela. The haplotype network for CO1 and S7 suggested three independent lineages, while Cyt b suggested two lineages (Figure 2.5a-c). The two lineages in the Cyt b haplotype network and tree could be due to the fact that the individuals in the third lineages were not amplified for Cyt b.

Maximum likelihood (ML) analysis of haplotype relationships from three independent ML analyses resulted in a single tree with similar optimal scores (best lnL -1930.92, lnL -

2217.64 and lnL -1423.83) for CO1, Cyt b and S7 respectively (Figure 2.6a-c). Bootstraps were terminated at 1000 runs. ML analyses were run under HKY model of sequence evolution for CO1, Cyt b and S7 as selected by AIC. The phylogeny of haplotypes for CO1 and S7 contained three separate clades suggesting three separate lineages. One clade was panmictic with shared Rupununi and Takutu haplotypes, a second clade with only Rupnuni haplotypes and the third clade with only Takutu haplotypes. The phylogeny of haplotypes formed two major clades, one with shared haplotypes between Rupununi and Takutu and the second clade with only haplotypes found in the Takutu. The haplotype relationships of *Geophagus surinamensis* based on the phylogeny and network for CO1, Cyt b, and S7 supported similar results.

Hoplias malabaricus

The final alignment for protein coding gene CO1 and Cyt b for *Hoplias malabaricus* resulted in 672bp for 56 individuals (27=Rupununi, 27=Takutu, 2=Venezuela) and 996bp for 51 individuals (25=Rupununi, 24=Takutu, 2=Venezuela), respectively. There were no ambiguous chromatogram readings, frameshifts or stop codons observed in the data, suggesting these sequences are not likely pseudogenes. The CO1 sequences collapsed to 12 haplotypes, two haplotypes were shared between the Rupununi and Takutu, four haplotypes only in the Rupununi, five only the Takutu and one haplotype among the two Venezuela individuals. Cytochrome b sequences collapsed to 13 haplotypes, two haplotypes were shared between the Rupununi and Takutu, eight haplotypes only in the Takutu and 3 haplotypes only in the Rupununi. The final alignment of the first intron of the S7 ribosomal protein gene resulted in 704bp for 19 individuals (11=Rupununi, 8=Takutu). One haplotype is shared between Rupununi and Takutu, seven haplotypes only in the Rupununi and three only in the Takutu (Figure 2.7a-c).

Maximum likelihood (ML) analysis of haplotype relationships from three independent ML analyses resulted in a single tree with similar optimal scores (best lnL -1880.40, lnL -2419.62, and lnL -2143.67) for CO1, Cyt b and S7 respectively (Figure 2.8a-c). Bootstraps were terminated at 1000 runs. ML analyses were run under HKY model for CO1 and GTR model for Cyt b and S7 as selected by AIC. The phylogeny of haplotypes formed one large panmictic clade with Rupnuni and Takutu haplotypes and two separate clades with only Takutu haplotypes. Haplotype networks also supports these three lineages. Interestingly, within the panmicitc clade in the S7 phylogeny is a clade with strong support of all Takutu haplotypes and one Rupununi, suggesting a possible recent dispersal back into the Takutu.

Potamorrhaphis guianensis

The final alignment for protein coding gene CO1 and Cyt b for *Potamorrhaphis guianensis* resulted in 660bp for 76 individuals (39=Rupununi, 34=Takutu, 3=Venezuela) and 777bp for 63 individuals (28=Rupununi, 33=Takutu, 2=Venezuela), respectively. There were no ambiguous chromatogram readings, frameshifts or stop codons observed in the data, suggesting these sequences are not likely pseudogenes. The CO1 sequences collapsed to eight haplotypes, two haplotypes were shared between Rupununi and Takutu, three haplotypes only in the Rupununi, two haplotypes only in the Takutu and two haplotypes in Venezuela. Cytochrome b sequences collapsed into 12 haplotypes, one haplotype was shared between Rupununi and Takutu, five haplotypes were only in the Rupununi, five haplotypes only in the Takutu and one haplotype in Venezuela. The final alignment of the first intron of the S7 ribosomal protein gene resulted in 746bp for 58 individuals (27=Rupununi, 29=Takutu, 2=Venezuela). Nine haplotypes were recovered with two shared between the Rupununi and Takutu, seven only in the Rupununi and two in Venezuela (Figure 2.9a-c).

Maximum likelihood (ML) analysis of haplotype relationships from three independent ML analyses resulted in a single tree with similar optimal scores (best lnL -2024.35, lnL -2557.61 and lnL -1035.36) for CO1, Cyt b and S7 respectively (Figure 2.10a-c). Bootstraps were terminated at 1000 runs. ML analyses were run under GTR model of sequence evolution for CO1 and Cyt b and TrN for S7 as selected by AIC. The phylogeny of haplotypes recovered one large panmictic clade with haplotypes shared between Rupununi and Takutu.

Population structure per species

Ancistrus spp.

The 42, 45, and 39 individuals of *Ancistrus* 'white spot' sp. sequenced for CO1, Cyt b, and S7, respectively, resulted in substantial genetic variation resulting in five independent lineages. Therefore, population level analysis was not performed on these species, as there were not enough individuals of each lineage in the analysis.

Hypostomus squalinus

Significant genetic differentiation was observed between populations across the Rupuununi portal with mitochondrial marker CO1 (Table 2.4a-l). A Mantel test to determine if there is a correlation between genetic distance and geographic distance detected no significant correlation. Whereas, a partial Mantel resulted in a strong significant correlation (r=0.899, P=0.0004) between genetic distance and the Rupununi portal (Table 2.5). The analysis of molecular variance (AMOVA) for *H. squalinus* populations found a significant proportion of the observed genetic variation occurring among Takutu and Rupununi populations (~85%; ϕ_{CT} = 0.847; P = 0.0001), and a lesser extent within populations (~17%; ϕ_{ST} = 0.834; P = 0.000) (Table 2.6a-l). No significant genetic differences were found with Cyt b with ϕ_{ST} statistics, exact tests or AMOVA. No significant genetic differentiation with ϕ_{ST} statistics or exact tests was detected

using nuclear marker S7 for *H. squalinus*. The AMOVA for *H. squalinus* populations found as significant proportion of the variation within populations (for S7: ~68; ϕ_{ST} = 0.317; P = 0.022), and to a lesser extent among populations within group (~35%; ϕ_{FC} = 0.336; P = 0.030).

Geophagus surinamensis

Significant genetic differentiation was observed in populations of *G. surinamensis* in CO1, Cyt b and S7. The three markers resulted in similar results and therefore are discussed below together (values from analyses for each marker reported in Table 2.4a-l). Populations with significant genetic differentiation were between population 0710 (Takutu River) and populations 0703 and 0738 (Rupununi River); between population 0731 and populations 0703, 0701, 0738, 0504, 0711, 0727, 0710, 0722. Additionally, significant genetic differentiation was found between population 0504 and population 0738.

The AMOVA for populations of *G. surinamensis* found a significant proportion of the variation for CO1, Cyt b, and S7 to be within groups (for CO1: ~70%; ϕ_{ST} = 0.300; P = 0.002), and to a lesser extent among populations within groups (for CO1: ~29%; ϕ_{SC} = 0.289; P = 0.002). The proportion of variation as revealed by Cyt b was relatively evenly distribution across the three levels of population structure, but also indicating significance between Takutu and Rupununi populations (Table 2.6a-l). A Mantel and partial Mantel test to assess the correlation of genetic distance and geographic distance or genetic distance and the Rupununi portal indicated no significant correlations.

Hoplias malabaricus

Significant genetic differentiation was observed for several populations of *Hoplias malabaricus* in the Rupununi with both mitochondrial markers CO1 and Cyt b. Populations with significant genetic differentiation were 0508 & 0739, 0740; 0740 & 0726, the localities of

populations that varied were on either side of the Rupununi portal. Interestingly, one pair of populations (0508 & 0730) both on the Takutu side of the Rupununi portal emerged as significantly different with ϕ_{ST} statistics and exact tests. The AMOVA found a significant proportion of the variation of *H. malabaricus* to be within populations in CO1 (~70%; ϕ_{ST} = 0.299; P = 0.003), and to a lesser extent by among populations within groups (~26%; ϕ_{SC} = 0.273; P = 0.005), no significant differences were found among populations in the Takutu versus the Rupununi (~4%; ϕ_{CT} = 0.035; P = 0.061). These results were also supported with Cyt b and S7 (values reported in Table 2.6a-l). A Mantel test and partial Mantel test found no correlations between genetic distance and geographic distance or genetic distance and the Rupununi portal. No significant genetic differentiation was detected by ϕ statistics or exact tests with nuclear marker S7, which could be due to the low numbers of samples for this marker.

Potamorrhaphis guianensis

Significant genetic differentiation was observed in populations of *P. guianensis*. Specifically, population 0731 found in a creek between Lake Amuku and Pirara Creek that drains into the Takutu during the floods (local Amerindians, pers.comm) had the most significant differences among all three molecular markers to populations on the Rupununi side of the portal (0521, 0503, 0517, 0701, 0738, 0516) (Table 2.4a-l). There was significant genetic differentiation between population 0731 and population 0510 (main-stem Takutu River near Lethem). Two populations on the Rupununi side (0516 & 0521) showed significant genetic differentiation in CO1 and Cyt b.

The AMOVA for *P. guianensis* for CO1, Cyt b and S7 found a significant proportion of the variation within populations (for CO1: ~80; ϕ_{ST} = 0.200; P = 0.006), and to a lesser extent within populations among groups (for CO1: ~21%; ϕ_{SC} = 0.209; P = 0.029) (Table XX, values

for CO1, Cyt b and S7). A Mantel and partial Mantel test found no correlation between genetic distance and geographic distance or genetic distance and the Rupununi portal (Table 2.5)

Demography of fish populations in the Rupununi

Neutrality tests using Tajima's *D* were negative in the majority of the populations across all taxa and significant in three populations (*G. surinamensis* Takutu, Cytb; *P. guianensis* Takutu, Cyt b; *H. squalinus* Takutu, Cyt b) (Table 2.3). Significant negative values in neutrality tests are an indication of rare polymorphisms in populations, which can suggest positive selection or a population that has experienced a recent population expansion (Tajima 1989; Fu 1997). The Harpending's raggedness index (*Hri*) of mismatch distributions for all taxa was not significant suggesting that a sudden population expansion model cannot be rejected for the populations in this study.

DISCUSSION

The Rupununi portal is an important biogeographic feature that allows for a hydrological connection between the world's most species rich fauna and the Essequibo River drainage of the ancient Guiana Shield. This unique feature is the only known seasonal connection between drainages comprising such great biodiversity globally. The combination of historical vicariant events, isolation, and recent dispersal with secondary contact has contributed to the origins of these faunas. Critical conservation concerns that surround the Rupununi wetlands with the impending threats of development, oil drilling, gold/diamond mining and logging have generated a sense of urgency in understanding the role these wetlands play in maintaining and generating the biodiversity present. Assessing the genetic diversity of fish species inhabiting the expanse of the Rupununi portal connection provides a powerful tool in evaluating the role of the Rupununi portal in gene flow. This study of five species using three molecular markers (two mtDNA and

one nuclear) provides evidence for the Rupununi portal acting both as a corridor and a barrier for gene flow between the Amazon River basin and the Essequibo River, thus critical in structuring the aquatic taxa of the region.

For the five taxa I examined, I discovered that all taxa, with the exception of *Ancistrus* sp. 'white spot', shared haplotypes across the Rupununi portal. Including paraphyletic and polyphyletic distributions in mitochondrial markers, suggesting a relatively recent co-ancestry. For instance, *Hypostomus squalinus* phylogeny of haplotypes for Cyt b resulted in one single haplotype that was shared with all individuals in the Takutu River and Rupununi River. While CO1 and S7 also resulted in one large panmictic clade with multiple haplotypes shared between drainages. Significant genetic differentiation in the populations of *H. squalinus* with CO1 suggests that despite these shared haplotypes, there is strong evidence for the Rupununi portal's influence on the genetic structure for this species. Population 0710 & 0713 located in the main channel of the Takutu River showed significant genetic differentiation from eight populations in the Rupununi (all located at different sites in the main channel of the Rupununi). Additionally, population 0701 (Rupununi River population) showed significant genetic differentiation from three populations in the Takutu, population 0719 (Takutu River population) from three populations in the Takutu, population 0707 (Rupununi River population) from three populations in the Takutu and population 0717 (Rupununi River, Dadanawa) from population 0719 (Takutu River, N of Sand Creek). The common thread among these populations differences are they are all found in localities in the main channel of either the Takutu or Rupununi River, therefore suggesting that the conditions of the Rupununi portal may not be suitable for this species and acts a barrier to dispersal between these drainages. Further supporting the Rupununi portal's role in the genetic differentiation of *H. squalinus* is a Mantel test that did not find a significant

correlation of isolation by distance, but a highly significant correlation of genetic distance and the Rupununi portal as shown in a partial Mantel.

Lovejoy & Araújo (2000) suggested that the freshwater needlefish *Potamorrhaphis guianensis* dispersed via the Rupununi portal, although no samples from the Rupununi were available for their study. Mitochondrial markers CO1 and Cyt b in this study corroborated their hypothesis with haplotypes being shared between the two drainages and neutrality tests suggesting recent population expansion. Interestingly, nuclear marker S7 resulted in a reciprocally monophyletic group of haplotypes in the Takutu and Rupununi suggesting long-term isolation or mitochondrial introgression obscuring resolution. The split between these two groups could have resulted from the fragmentation of the proto-Berbice, causing populations to become isolated until the recent development of the Rupununi portal that allowed for dispersal. Significant genetic differentiation was observed in populations of *P. guianensis* with mitochondrial markers CO1 and Cyt b, population 0731 (Creek between Lake Amuku and Pirara creek) differed from six populations located in the main channel of the Rupununi and Takutu Rivers. The variation found between these populations could be indicative of adaptation to different habitats. Population 0731 is a population found in the middle of the Rupununi portal, where the water systems of the savanna are closely associated with vegetation. This close association with vegetation has been shown to alter water chemistry (Carter, 1934; Sarmiento, 1984). The difference in water chemistry in the savanna and nearby main river channels could pose as a barrier to dispersal for some species, hence driving divergence between populations. Differentiation in populations of *P. guianensis* with nuclear marker S7 indicated a split between population 0731 and six populations in the main channel Rupununi River. The split between

these populations could also support the hypothesis that these populations were isolated upon the fragmentation of the proto-Berbice.

Hoplias malabaricus has a widespread distribution in all of the Neotropics and is found in a variety of habitats. Therefore, finding haplotypes shared between the Rupununi and Takutu was not surprising. Interestingly, both mitochondrial and nuclear markers resulted in a clade of haplotypes only found in the Takutu drainage. These Takutu haplotypes shared a relationship with individuals from the Orinoco drainage in Venezuela and were basal in all analyses, which supports an ancient split during the fragmentation of the proto-Berbice and recent dispersal via the Rupununi portal. The Orinoco and Amazon are connected by the Río Casiquiare, which drains much of the upper Orinoco into the Negro. This connection may have allowed Orinoco haplotypes into the Amazon basin, but they have not yet crossed into the Rupununi. Significant genetic differentiation was found in *H. malabaricus* population 0508 with two populations in the Rupununi (0739 &0740) and a population (0730) located in a borrow pit near the Takutu River. Population 0508 contains the haplotypes in the clade unique to the Takutu and is located in the Pirara head, which is the exact site of the Takutu connection to the Rupununi portal. Pirara head is considered to have unique habitat characteristics as compared to the main-stem Takutu River or even Pirara creek (Don Stewart, pers. comm.; pers. obs). These unique haplotypes in the Takutu may not have moved into the Rupununi yet or further sampling of this unique haplotype in addition to morphological examination may result in determining its taxonomic status.

Unlikely to disperse across the expansive Rupununi savannas, due to their affinity to main river channels and swift current, is *Geophagus surinamensis*. Despite their affinity to these habitat conditions, shared haplotypes were found in Rupununi and Takutu populations with all three markers. In addition to a clade with shared haplotypes, was a Rupununi clade and Takutu

clade with unique haplotypes, again supported by all three markers. The analyses of haplotype relationships suggest three independent lineages. Moreover, examination of the population structure of *G. surinamensis* found significant genetic differentiation of population 0731 located in a creek between Lake Amuku and Pirara head to eight populations in the main channel of the Takutu and Rupununi drainages. The variation could be due to marked differences in habitat characteristics between the main channel and the creek located within the savanna, which is deeply associated with vegetation of the savannas influencing habitat characteristics (Carter, 1934; Sarmiento, 1984). Another striking difference, supported by all three markers, is the genetic variation between *G. surinamensis* population 0504 and 0738. These two populations are both located in the main channel of the Rupununi near Massara and are only 50 meters apart. It is unlikely these populations have not come into contact and the substantial amount of support for the genetic variation between these two populations suggests these are likely two sympatric species.

Maximum likelihood and parsimony analyses for mitochondrial and nuclear markers in *Ancistrus* sp. 'white spot', suggests multiple independent lineages originally considered to be one lineage. All haplotypes were unique to the Rupununi or the Takutu drainages. These lineages are being described along with morphological examination as *Ancistrus nudiceps*, *A. leucostictus*, *A. lithurgicus*, *Ancistrus* sp. 'circle A' and *Ancistrus* sp. 'circle E' (Taphorn et. al, in prep). Mitochondrial markers CO1 and Cyt b recovered a clade with paraphyletic *A. nudiceps* and *A. leucostictus*, and a clade with monophyletic *A. lithurgicus*, *A.* sp. 'circle A' and *A.* sp. 'circle E'. All five putative species were each recovered as monophyletic in the S7 topology. The difference between the results for the mitochondrial and nuclear markers suggests that there

may have been mitochondrial introgression between *A. nudiceps* + *A. leucostictus,* and that the mitochondrial markers are insufficient at evaluating the relationships between the species.

Shared haplotypes across the Rupununi portal in Takutu and Rupununi River drainages is indicative of gene flow between fish populations in this study, *Ancistrus* excepted. There are several factors that can be influencing whether populations are experiencing persistent versus intermittent gene flow. Fluctuations in the flood pulse of the annual rains is likely the strongest influence on movement of aquatic taxa across the portal. Annual inundation of the Rupununi Savanna extends over 3,480km^2 with a hydroperiod of 49 days, and a nine-year study found significant variation in the magnitude of the floods (Hamilton *et al*., 2002). Perhaps water systems on the periphery of the flooded savannas are only connected during high flood events, like those experienced in 2011, allowing enough flow for fish movement. Consequently, variables associated with the seasonal cycle can have a major impact on the fish communities such as availability of food resources, rate of predation, habitat partitioning, reproduction and competition.

The loricariid, *Hypostomus squalinus*, is most often found in the main river channel of the Takutu and Rupununi Rivers. Gene flow was evident in all three markers for *H. squalinus* with haplotypes found across the portal, although CO1 showed significant population structure across the Rupununi portal. Difficult to demonstrate and hotly debated in evolutionary biology is divergence in the presence of gene flow, despite recent convincing examples (Niemiller et al 2008; Rundle & Nosil 2005; Nosil 2008). A small amount of gene flow is required to keep two populations from diverging (Llopart et al 2005), although divergence can happen at some genes, even if there is gene exchange for other genes (Hey 2006). Therefore, divergence in the face of gene flow implies natural selection is playing a role in the divergence process (Endler 1977;

Coyne & Orr 2004), which could explain the difference we see in the results for *H. squalinus* with different molecular markers.

Ecological divergence is likely to be a key factor in facilitating speciation with gene flow. Due to the high degree of ecological niches available in the extent of the Rupununi portal, the ecology of aquatic taxa offers a strong explanation for the structuring of fish populations. For example, *Ancistrus* 'white spot' spp. are difficult to distinguish morphologically, but have garnered enough genetic differentiation to be considered distinct species. The divergence of these morphologically similar species is likely due to their affinity to rheophilic habitats and close association to rocky substrates. Following either the Evolutionary or Biological species concept, the Amazon and Essequibo populations would be separate species. A recent study found six independent lineages across the drainages of Suriname and French Guiana in populations of *Pseudancistrus brevispinis* specialized to rheophilic habitats (Cardoso & Montoya-Burgos, 2009). There may be many other examples of cryptic diversity to be found in South American Rivers associated with rheophilic habitat worthy to be explored. Molecular studies in South America are in there infancy, and studies such as these can inform taxonomic questions. This study suggests that rheophilic species across the Rupununi portal should be examined for cryptic diversity, with the expectation that their ecological requirements would drive divergence between populations despite the potential for contemporary or historical gene flow.

The variability in the genetic structure observed for the five taxa in this study emphasize the complexity of the North Rupununi wetlands in shaping the biodiversity of the region. Paleogeographic events that shifted river drainages through the North Rupununi wetlands during the fragmentation of the proto-Berbice have left their imprint in the DNA of these species. Whereas, current processes at work that appear to be shaping genetic differentiation of the five

taxa studied in the Rupnuni wetlands seem to be associated with their ecology. Therefore, the seasonal connection in the Rupununi appears to function as a corridor for those aquatic species that can sustain the variability in habitat conditions throughout its expanse. These species may not move through the portal each season (as potentially evidenced in *Hypostomus squalinus*), but conditions may be adequate for them during some years. Additionally, the complex history of the Amazon basin elsewhere may be shaping patterns of genetic diversity. The Casiquiare River in Venezuela has allowed faunal exchange between the Orinoco and Amazon, which has in turn increased the potential diversity of the Takutu. This relatively recent Amazon-Orinoco exchange may not yet have enhanced the genetic diversity of the Essequibo.

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Table 2.1. Primers used for PCR and sequencing in this study.

Gene fragment	Primer name	Primer sequence	Source
CO1	LCO1490	5‘-GGT CAA CAA ATC ATA AAG ATA TTG G-3‘	Hebert et al. 2003
	HCO2198	5‘-TAA ACT TCA GGG TGA CCA AAA AAT CA-3‘	Hebert et al. 2003
	FISHF2	5’ –TCGACTAATCATAAAGATATCGGCAC- 3’	Ward et al. 2005
	FISHR2	5’ –ACTTCAGGGTGACCGAAGAATCAGAA-3’	Ward et al. 2005
CYTB	GLU-2	5’-AACCACCGTTGTTATTCAACTA-3’	Hardman 2005
	PRO-R1	5’-TAGTTTAGTTTAGAATTCTGGCTTTGG-3’	Hardman 2005
	GLUDG	5’ –CGAAGCTTGACTTGAARAACCAYCGTTG-3’	Lovejoy & Araújo 2000
	CB3	5’ –GGCAAATAGGAARTATCATTC- 3’	Lovejoy & Araújo 2000
	L14841	5’ –AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3’	López-Fernández et. al 2005
	H15915	5’-AACTGCCAGTCATCTCCGGTTTACAAGAC-3’	López-Fernández et. al 2005
S7-1st intron	S7RPEX1F	5’ –TGGCCTCTTCCTTGGCCGTC- 3’	Chow & Hazama 1998
	S7RPEX2R	5’ –AACTCGTCTGGCTTTTCGCC- 3’	Chow & Hazama 1998
	Lori_S7_R	5’ –GCTGCTGWACATGGCCTATCGG- 3’	This study
	Lori_S7_F	5’ –CGTCGTTGACTGAGGTGGGTTAG- 3’	This study

Table 2.2. Sequence evolution model as selected by AIC in Jmodeltest.

JMODELTEST	Model	Likelihood Scores (-lnL)	AIC
CO1			
Ancistrus sp. 'white spot'	TrN+I+G	1725.89	3656.24
Potamorrhaphis guianensis	GTR+G	1939.97	4205.95
Geophagus surinamensis	HKY+I+G	1972.57	4161.14
Hoplias malabaricus	HKY+G	1878.32	3981.36
Hypostomus squalinus	T92	1159.96	2586.71
CYTB			
Ancistrus sp. 'white spot'	GTR+I+G	4067.23	8330.47
Potamorrhaphis guianensis	GTR+G	2406.74	5079.47
Geophagus surinamensis	HKY+G	2192.69	4593.6
Hoplias malabaricus	GTR+G	2402.22	5020.44
Hypostomus squalinus	HKY	1955.09	4184.67
S7			
Ancistrus sp. 'white spot'	GTR+G	1542.24	3258.48
Potamorrhaphis guianensis	TrN+I+G	1271.35	2804.7
Geophagus surinamensis	HKY	1478.03	3196.04
Hoplias malabaricus	GTR+G	2173.77	4453.53
Hypostomus squalinus	F81+I	881.85	1919.55

Table 2.3. Diversity measures and results of neutrality tests for species listed below in the Rupununi Savanna.

		Diversity measures (CO1, Cytb, S7)				Neutrality tests (CO1, Cytb, S7)	
Species	**Population**	***n***	***nh***	**p**	***h***	**Tajima's D**	***Hri***
Geophagus surinamensis	Takutu	22, 26, 30	4, 4, 5	0.08020, 0.0943, 0.04438	0.54113, 0.81538, 0.74253	2.00128, 2.59162**, 1.57098	0.3598, 0.0602, 0.0980
	Rupununi	25, 25, 28	9, 8, 7	0.04121, 0.0019, 0.0320	0.72000, 0.35667, 0.79894	-0.62059, -0.20781, -0.47664	0.0631, 0.3876, 0.0960
Potamorrhaphis guianensis	Takutu	34, 32, 30	3,6,2	0.03475, 0.03730, 0.0008	0.21925, 0.29234, 0.51494	-1.22838, -1.99705*, 1.62104	0.5816, 0.3377, 0.2661
	Rupununi	41, 28, 27	5,6,7	0.01253, 0.01962, 0.0035	0.43537, 0.69312, 0,76068	-2.85601***, -2.77663***, 1.27457	0.1390, 0.1709, 0.1391
Hoplias malabaricus	Takutu	26, 25, 9	6, 10, 4	0.08621, 0.05358, 0.12883	0.73846, 0.85000, 0.58333	0.28223, 0.25842, -1.51449	0.2503, 0.0602, 0.2801
	Rupununi	27, 25, 12	6, 5, 8	0.00309, 0.00413, 0.01615	0.54986, 0.36333, 0.89394	-1.62321, -1.32006, -0.56516	0.0838, 0.3975, 0.0567
Hypostomus squalinus	Takutu	25, 26, 11	6, 5, 3	0.00091, 0.00035, 0.00259	0.42667, 0.30000, 0.49091	-1.53784, -1.99937*, -0.4852	0.1589, 0.0552, 0.6940
	Rupununi	39, 37, 26	1, 2, 6	0 , 0.00005, 0.00220	0 , 0.05405, 0.42769	0, -1.13092, 0.49156	n/a, 0.7984, 0.6462

n, number of sampled individuals; nh, number of recovered haplotypes; p, nucleotide diversity; h, haplotype diversity; Hri, Harpending's raggedness index.
*P < 0.05; **P < 0.01; *** P < 0.001

Table 2.4a. Pairwise differentiation tests for populations of *Potamorrhaphis guianensis* with CO1. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0731	GUY0722	GUY0728	GUY0510	GUY0517	GUY0521	GUY0503	GUY0516	GUY0522	GUY0701	GUY0738
GUY0731	-	0.07164±0.0025	1.00000±0.0000	0.01108±0.0009*	0.06111±0.0021	0.00816±0.0009*	0.02613±0.0013*	0.33075±0.0055	0.07034±0.0023	1.00000±0.0000	0.23672±0.0045
GUY0722	0.92593	-	0.33028±0.0019	1.00000±0.0000	0.09699±0.0021	0.11238±0.0034	0.18763±0.0051	0.27956±0.0027	1.00000±0.0000	0.24907±0.0025	0.39256±0.0051
GUY0728	-0.33207	1.000	-	0.46717±0.0017	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.33596±0.0022	-1.00000±1.0000	1.00000±0.0000
GUY0510	0.61188*	-0.33333	0.11111	-	0.15190±0.0030	0.10967±0.0037	0.16911±0.0041	0.13270±0.0034	0.19856±0.0021	0.42817±0.0018	0.42976±0.0027
GUY0517	0.17557	0.61111	-0.19431	0.18343	-	0.62186±0.0012	1.00000±0.0000	0.30125±0.0025	0.10136±0.0015	1.00000±0.0000	1.00000±0.0000
GUY0521	0.39897*	0.46429	-0.01266	0.14219	-0.072	-	0.78603±0.0026	0.20915±0.0027	0.11148±0.0024	0.48949±0.0011	1.00000±0.0000
GUY0503	0.20298*	0.48889	-0.17347	0.11824	-0.096	-0.073	-	0.43689±0.0033	0.17687±0.0038	1.00000±0.0000	1.00000±0.0000
GUY0516	0.08793	0.66667	-0.30435	0.17757	-0.031	0.082	-0.027	-	0.27902±0.0040	1.00000±0.0000	0.67354±0.0028
GUY0522	0.92593	1.000	1.000	0.33333	0.611	0.464	0.489	0.667	-	0.25230±0.0036	0.39669±0.0033
GUY0701	-0.19795	1.000	0.000	0.250	-0.068	0.098	-0.052	-0.154	1.000	-	1.00000±0.0000
GUY0738	0.24172	0.500	-0.26316	0.06667	-0.218	-0.187	-0.191	-0.094	0.500	-0.091	-

* Significant P-values (<0.05).

Table 2.4b. Pairwise differentiation tests for populations of *Potamorrhaphis guianensis* with Cyt b. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0731	GUY0510	GUY0728	GUY0722	GUY0503	GUY0517	GUY0738	GUY0516	GUY0521
GUY0731	-	0.03662±0.0026*	1.00000±0.0000	0.10671±0.0057	0.00391±0.0007*	0.00864±0.0016*	0.27296±0.0070	0.40896±0.0087	0.00023±0.0001*
GUY0510	0.559*	-	1.00000±0.0000	1.00000±0.0000	0.20227±0.0058	0.69589±0.0042	1.00000±0.0000	0.27725±0.0035	0.42671±0.0031
GUY0728	-0.963	-0.500	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0722	0.860	0.000	1.000	-	0.10971±0.0026	0.61686±0.0059	0.50616±0.0022	0.32650±0.0032	0.33294±0.0050
GUY0503	0.343*	0.012	-0.429	0.286	-	1.00000±0.0000	1.00000±0.0000	0.35214±0.0035	0.62912±0.0008
GUY0517	0.346*	-0.067	-0.500	0.143	-0.099	-	1.00000±0.0000	1.00000±0.0000	0.72793±0.0038
GUY0738	0.249	-0.071	-1.000	0.333	-0.191	-0.167	-	0.65048±0.0025	0.68210±0.0027
GUY0516	0.050	0.118	-1.000	0.600	0.048	0.021	-0.104	-	0.04506±0.0010*
GUY0521	0.640*	0.053	0.000	0.200	-0.078	-0.038	-0.014	0.286	-

* Significant P-values (<0.05).

Table 2.4c. Pairwise differentiation tests for populations of *Potamorrhaphis guianensis* with S7 (1st intron). Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0731	GUY0728	GUY0521	GUY0503	GUY0516	GUY0517	GUY0701	GUY0738
GUY0731	-	1.00000±0.0000	0.00091±0.0002*	0.00113±0.0003*	0.00493±0.0003*	0.00045±0.0002*	0.04596±0.0005*	0.00282±0.0002*
GUY0728	-0.361	-	0.50169±0.0040	0.40217±0.0035	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0521	0.223*	-0.091	-	0.79079±0.0020	0.81199±0.0029	0.30000±0.0047	0.76432±0.0031	0.78710±0.0051
GUY0503	0.222*	-0.053	-0.106	-	0.79264±0.0041	0.12543±0.0027	0.53794±0.0064	0.28261±0.0033
GUY0516	0.190	-0.191	-0.100	-0.134	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0517	0.241	-0.143	0.024	0.085	-0.128	-	1.00000±0.0000	1.00000±0.0000
GUY0701	0.189	-0.200	-0.059	-0.081	-0.136	-0.130	-	1.00000±0.0000
GUY0738	0.228	-0.200	-0.172	-0.012	-0.091	-0.091	-0.071	-

* Significant P-values (<0.05).

Table 2.4d. Pairwise differentiation tests for populations of *Geophagus surinamensis* with CO1. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0504	GUY0703	GUY0738	GUY0701	GUY0517	GUY0709	GUY0732	GUY0711	GUY0727	GUY0722	GUY0710	GUY0508	GUY0510	GUY0712	GUY0723	GUY0728	GUY0731
GUY0504	-	0.16209±0.0040	0.02402±0.0011*	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.39984±0.0043	0.02820±0.0014*	0.39730±0.0037	0.39683±0.0042	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.01377±0.0011*
GUY0703	0.181	-	0.73066±0.0036	0.73238±0.0062	0.49932±0.0067	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.83465±0.0054	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.50251±0.0066	1.00000±0.0000	0.00248±0.0005*
GUY0738	0.240	-0.064	-	0.39931±0.0063	0.19885±0.0035	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.64568±0.0037	1.00000±0.0000	0.55568±0.0042	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.20219±0.0041	1.00000±0.0000	0.00043±0.0002*
GUY0701	0.000	-0.126	-0.029	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.33387±0.0023	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.03294±0.0011*
GUY0517	0.000	0.286	0.361	0.000	-	1.00000±0.0000	1.00000±0.0000	0.33000±0.0018	0.20332±0.0015	0.33386±0.0022	0.49978±0.0036	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.14460±0.0010
GUY0709	0.000	-0.667	-0.438	-1.000	1.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	0.14399±0.0017
GUY0732	0.000	-0.667	-0.438	-1.000	1.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	0.14255±0.0018
GUY0711	0.368	-0.138	-0.023	0.000	1.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33360±0.0026	-1.00000±1.0000	0.03655±0.0009*
GUY0727	0.579*	0.057	0.142	0.385	1.000	0.000	0.000	0.000	-	-1.00000±1.0000	0.43020±0.0024	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.19967±0.0015	-1.00000±1.0000	0.00475±0.0005*
GUY0722	0.368	-0.138	-0.023	0.000	1.000	0.000	0.000	0.000	0.000	-	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33606±0.0021	-1.00000±1.0000	0.03517±0.0012*
GUY0710	0.167	-0.122	-0.032	-0.200	0.333	-1.000	-1.000	-0.200	0.111	-0.200	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.49581±0.0026	1.00000±0.0000	0.01071±0.0006*
GUY0508	0.000	-0.667	-0.438	-1.000	1.000	0.000	0.000	0.000	0.000	0.000	-1.000	-	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	0.14285±0.0012
GUY0510	0.000	-0.667	-0.438	-1.000	1.000	0.000	0.000	0.000	0.000	0.000	-1.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	0.14816±0.0017
GUY0712	0.000	-0.667	-0.438	-1.000	1.000	0.000	0.000	0.000	0.000	0.000	-1.000	0.000	0.000	-	1.00000±0.0000	-1.00000±1.0000	0.14145±0.0011
GUY0723	0.000	0.286	0.361	0.000	1.000	1.000	1.000	1.000	1.000	1.000	0.333	1.000	1.000	1.000	-	1.00000±0.0000	0.14233±0.0017
GUY0728	0.000	-0.667	-0.438	-1.000	1.000	0.000	0.000	0.000	0.000	0.000	-1.000	0.000	0.000	0.000	1.000	-	0.14400±0.0015
GUY0731	0.676*	0.619*	0.627*	0.806*	1.000	1.000	1.000	1.000*	1.000*	1.000*	0.793*	1.000	1.000	1.000	1.000	1.000	-

* Significant P-values (<0.05).

Table 2.4e. Pairwise differentiation tests for populations of *Geophagus surinamensis* with Cyt b. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0731	GUY0726	GUY0727	GUY0711	GUY0710	GUY0508	GUY0712	GUY0722	GUY0723	GUY0728	GUY0510	GUY0738	GUY0703	GUY0701	GUY0705	GUY0709	GUY0732	GUY0504
GUY0731	-	0.12589±0.0025	0.00045±0.0001*	0.02734±0.0010*	0.00822±0.0006*	0.12686±0.0021	0.12417±0.0019	0.02852±0.0011*	0.12696±0.0026	0.12261±0.0015	0.12682±0.0019	0.00008±0.0001*	0.00093±0.0002*	0.02688±0.0009*	0.02747±0.0012*	0.12293±0.0019	0.12735±0.0019	0.12518±0.0011
GUY0726	1.000	-	0.14222±0.0014	1.00000±0.0000	0.50262±0.0046	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.25063±0.0042	0.25422±0.0028	1.00000±0.0000	0.33253±0.0022	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0727	0.753*	0.467	-	1.00000±0.0000	0.23620±0.0036	0.42681±0.0020	0.43023±0.0014	0.21273±0.0020	0.14261±0.0038	0.14416±0.0027	0.14092±0.0024	0.20831±0.0049	0.31697±0.0038	0.28320±0.0041	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.14257±0.0023
GUY0711	0.831*	0.000	-0.404	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.42992±0.0067	0.42075±0.0026	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0710	0.816*	0.333	0.250	-0.200	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.49458±0.0024	0.49594±0.0044	0.50693±0.0047	0.01286±0.0012*	0.01861±0.0017*	0.39752±0.0020	0.20260±0.0022	0.49633±0.0023	0.50384±0.0024	0.49889±0.0022
GUY0508	1.000	1.000	0.200	-1.000	-1.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.26800±0.0062	0.25333±0.0045	1.00000±0.0000	0.33320±0.0022	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0712	1.000	1.000	0.200	-1.000	-1.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24738±0.0081	0.24967±0.0040	1.00000±0.0000	0.33341±0.0027	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0722	0.831*	0.000	0.205	-0.333	-0.615	-1.000	-1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.07680±0.0041	0.08892±0.0030	1.00000±0.0000	0.33725±0.0030	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0723	1.000	1.000	0.467	0.000	0.333	1.000	1.000	0.000	-	1.00000±0.0000	1.00000±0.0000	0.24587±0.0083	0.24800±0.0031	1.00000±0.0000	0.33297±0.0016	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0728	1.000	1.000	0.467	0.000	0.333	1.000	1.000	0.000	1.000	-	1.00000±0.0000	0.25296±0.0029	0.24209±0.0046	1.00000±0.0000	0.33325±0.0017	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0510	1.000	1.000	0.467	0.000	0.333	1.000	1.000	0.000	1.000	1.000	-	0.25536±0.0038	0.25085±0.0030	1.00000±0.0000	0.33537±0.0013	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0738	0.791*	0.655	0.053	0.137	0.573*	0.655	0.655	0.539	0.655	0.655	0.655	-	1.00000±0.0000	0.43659±0.0068	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.25587±0.0020
GUY0703	0.857*	0.714	0.053	0.161	0.597*	0.714	0.714	0.565	0.714	0.714	0.714	-0.108	-	0.42124±0.0061	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24989±0.0018
GUY0701	0.831*	0.000	-0.015	-0.333	0.208	0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.161	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0705	1.000*	1.000	-0.091	0.000	0.571	1.000	1.000	0.500	1.000	1.000	1.000	-0.276	-0.313	0.000	-	-1.00000±1.0000	-1.00000±1.0000	0.33443±0.0015
GUY0709	1.000	1.000	-0.600	-1.000	0.333	1.000	1.000	0.000	1.000	1.000	1.000	-0.900	-1.000	-1.000	0.000	-	-1.00000±1.0000	1.00000±0.0000
GUY0732	1.000	1.000	-0.600	-1.000	0.333	1.000	1.000	0.000	1.000	1.000	1.000	-0.900	-1.000	-1.000	0.000	0.000	-	1.00000±0.0000
GUY0504	1.000	1.000	0.467	0.000	0.333	1.000	1.000	0.000	1.000	1.000	1.000	0.620	0.667	0.000	1.000	1.000	1.000	-

* Significant P-values (<0.05).

Table 2.4f. Pairwise differentiation tests for populations of *Geophagus surinamensis* with S7 (1st inron). Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0703	GUY0738	GUY0504	GUY0701	GUY0705	GUY0709	GUY0517	GUY0727	GUY0710	GUY0723	GUY0711	GUY0722	GUY0508	GUY0712	GUY0726	GUY0728	GUY0731
GUY0703	-	0.30825±0.0034	0.76335±0.0025	0.18846±0.0056	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.37501±0.0052	0.65133±0.0026	1.00000±0.0000	0.25916±0.0043	0.75257±0.0041	1.00000±0.0000	0.62566±0.0055	0.37867±0.0066	0.62780±0.0038	0.00009±0.0001*
GUY0738	-0.027	-	0.07702±0.0030	0.04449±0.0019*	1.00000±0.0000	0.14431±0.0016	0.13998±0.0024	0.14498±0.0017	0.16525±0.0033	0.14303±0.0025	0.21056±0.0026	0.28551±0.0046	0.14358±0.0017	1.00000±0.0000	0.14182±0.0023	1.00000±0.0000	0.00062±0.0002*
GUY0504	0.069	0.194*	-	0.01681±0.0013*	0.34416±0.0049	1.00000±0.0000	1.00000±0.0000	0.17514±0.0040	1.00000±0.0000	1.00000±0.0000	0.38546±0.0036	1.00000±0.0000	1.00000±0.0000	0.49844±0.0052	0.16046±0.0034	0.50617±0.0037	0.00000±0.0000*
GUY0701	-0.221	0.055	-0.036	-	0.81013±0.0024	0.66801±0.0042	0.66393±0.0050	1.00000±0.0000	0.21123±0.0045	0.67103±0.0054	0.04375±0.0013*	0.53285±0.0076	0.66348±0.0057	0.67299±0.0055	0.66718±0.0053	0.66724±0.0076	0.00160±0.0004*
GUY0705	-0.133	-0.520	0.100	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.59713±0.0036	1.00000±0.0000	0.49922±0.0020	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.02787±0.0008*
GUY0709	-0.133	-0.520	0.100	0.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.12278±0.0018
GUY0517	0.190	0.309	-0.500	0.000	1.000	1.000	-	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.12295±0.0009
GUY0727	0.113	0.238*	0.243	-0.404	0.467	0.467	0.467	-	0.50200±0.0038	1.00000±0.0000	0.27758±0.0049	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.12503±0.0022
GUY0710	0.011	-0.183	0.194	0.045	-1.000	-1.000	0.333	0.250	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.49709±0.0035	1.00000±0.0000	0.00841±0.0010*
GUY0723	-0.133	-0.520	0.100	0.000	0.000	0.000	1.000	0.467	-1.000	-	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.12422±0.0023
GUY0711	0.073	0.023	0.188*	-0.091	-0.100	-0.100	0.267	0.050	-0.154	-0.100	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.27637±0.0018	1.00000±0.0000	0.00067±0.0002*
GUY0722	-0.106	-0.242	0.069	-0.333	-1.000	-1.000	0.000	-0.015	-0.615	-1.000	-0.424	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.02852±0.0016*
GUY0508	-0.133	-0.520	0.100	0.000	0.000	0.000	1.000	0.467	-1.000	0.000	-0.100	-1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.12351±0.0015
GUY0712	0.056	0.156	0.100	-1.000	1.000	1.000	1.000	-0.600	0.000	1.000	-0.467	-1.000	1.000	-	1.00000±0.0000	-1.00000±1.0000	0.12586±0.0017
GUY0726	0.190	0.309	0.100	0.000	1.000	1.000	1.000	0.467	0.333	1.000	0.267	0.000	1.000	1.000	-	1.00000±0.0000	-1.00000±1.0000
GUY0728	0.056	0.156	0.100	-1.000	1.000	1.000	1.000	-0.600	0.000	1.000	-0.467	-1.000	1.000	0.000	1.000	-	0.12568±0.0018
GUY0731	0.595*	0.595*	0.616*	0.831*	1.000	1.000	1.000	0.753*	0.816*	1.000	0.659*	0.831*	1.000	1.000	0.000	1.000	-

* Significant P-values (<0.05).

Table 2.4g. Pairwise differentiation tests for populations of *Hypostomus squalinus* with CO1. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0701	GUY0702	GUY0703	GUY0705	GUY0707	GUY0709	GUY0732	GUY0718	GUY0717	GUY0734	GUY0509	GUY0710	GUY0713	GUY0719	GUY0722	GUY0723
GUY0701	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.03628±0.0018*	0.00037±0.0001*	0.00209±0.0003*	0.01165±0.0008*	0.03494±0.0021*	0.08583±0.0021
GUY0702	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.09786±0.0012	0.00692±0.0009*	0.01290±0.0014*	0.09760±0.0013	0.10061±0.0026	0.40122±0.0018
GUY0703	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.06534±0.0021	0.00189±0.0003*	0.00437±0.0006*	0.02918±0.0003*	0.06565±0.0019	0.14058±0.0015
GUY0705	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.06849±0.0016	0.00242±0.0005*	0.00532±0.0007*	0.02977±0.0011*	0.06946±0.0015	0.14096±0.0020
GUY0707	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.00949±0.0006*	0.00000±0.0000*	0.00001±0.0000*	0.00174±0.0002*	0.00989±0.0007*	0.02538±0.0007*
GUY0709	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33472±0.0014	0.04399±0.0012*	0.03458±0.0009*	0.09870±0.0010	0.33457±0.0026	0.40101±0.0024
GUY0732	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33822±0.0018	0.04776±0.0020*	0.03618±0.0015*	0.09918±0.0012	0.32995±0.0031	0.39751±0.0015
GUY0718	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	0.33187±0.0018	0.34143±0.0081	0.28525±0.0060	0.25252±0.0025	1.00000±0.0000	1.00000±0.0000
GUY0717	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	0.06889±0.0010	0.00197±0.0003*	0.00510±0.0003*	0.02953±0.0006*	0.06727±0.0022	0.14293±0.0014
GUY0734	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.32936±0.0022	0.32731±0.0046	0.28795±0.0038	0.25403±0.0018	1.00000±0.0000	1.00000±0.0000
GUY0509	1.000*	1.000	1.000	1.000	1.000*	1.000	1.000	1.000	1.000	1.000	-	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0710	0.737*	0.664*	0.694*	0.694*	0.820*	0.623*	0.623*	0.536	0.694*	0.536	-0.246	-	1.00000±0.0000	1.00000±0.0000	0.53081±0.0037	0.66222±0.0041
GUY0713	0.833*	0.771*	0.797*	0.797*	0.897*	0.735*	0.735*	0.667	0.797*	0.667	-0.304	-0.070	-	1.00000±0.0000	0.46832±0.0044	0.58057±0.0027
GUY0719	1.000*	1.000	1.000*	1.000*	1.000*	1.000	1.000	1.000	1.000*	1.000	0.000	-0.110	-0.154	-	0.40025±0.0025	1.00000±0.0000
GUY0722	0.806*	0.647	0.724	0.724	0.904*	0.500	0.500	0.000	0.724	0.000	0.000	0.011	0.094	0.250	-	1.00000±0.0000
GUY0723	0.676	0.500	0.579	0.579	0.817*	0.368	0.368	0.000	0.579	0.000	-0.200	-0.090	-0.059	0.000	-0.200	-

* Significant P-values (<0.05).

Table 2.4h. Pairwise differentiation tests for populations of *Hypostomus squalinus* with Cyt b. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0701	GUY0702	GUY0703	GUY0705	GUY0707	GUY0709	GUY0717	GUY0732	GUY0718	GUY0734	GUY0521	GUY0522	GUY0504	GUY0517	GUY0503	GUY0509	GUY0710	GUY0713	GUY0719	GUY0722	GUY0723	GUY0502
GUY0701	-	-1.00000±1.0000	0.45645±0.0007	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	0.45668±0.0028	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0702	0.000	-	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0703	0.040	-0.290	-	1.00000±0.0000	0.41548±0.0017	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.80380±0.0042	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0705	0.000	0.000	-0.290	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0707	0.000	0.000	0.073	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	0.19120±0.0023	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0709	0.000	0.000	-1.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0717	0.000	0.000	-0.053	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0732	0.000	0.000	-0.290	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0718	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0734	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0521	0.000	0.000	-0.290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0522	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	0.66267±0.0041	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0504	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0517	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0503	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0509	0.000	0.000	-0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0710	0.027	-0.246	-0.086	-0.246	0.051	-0.857	-0.045	-0.246	-0.857	-0.857	-0.246	-0.857	-0.857	-0.857	-0.857	-0.110	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0713	0.100	-0.200	-0.078	-0.200	0.131	-0.800	0.014	-0.200	-0.800	-0.800	-0.200	-0.800	-0.800	-0.800	-0.800	-0.059	-0.059	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0719	0.000	0.000	-0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.110	-0.059	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0722	0.000	0.000	-0.290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.246	-0.200	0.000	-	-1.00000±1.0000	-1.00000±1.0000
GUY0723	0.000	0.000	-0.132	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.110	-0.059	0.000	0.000	-	-1.00000±1.0000
GUY0502	0.000	0.000	-1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.857	-0.800	0.000	0.000	0.000	-

* Significant P-values (<0.05).

Table 2.4i. Pairwise differentiation tests for populations of *Hypostomus squalinus* with S7 (1st intron). Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0703	GUY0717	GUY0702	GUY0707	GUY0701	GUY0705	GUY0732	GUY0718	GUY0734	GUY0709	GUY0509	GUY0713	GUY0710	GUY0723	GUY0719
GUY0703	-	0.39855±0.0017	0.39911±0.0025	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.24752±0.0012	0.25142±0.0019	0.39818±0.0026	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.25114±0.0024	0.25015±0.0011
GUY0717	0.250	-	1.00000±0.0000	0.39845±0.0043	0.10679±0.0018	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.14588±0.0023	1.00000±0.0000	1.00000±0.0000
GUY0702	0.250	-0.500	-	1.00000±0.0000	0.28609±0.0016	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.33592±0.0017	1.00000±0.0000	1.00000±0.0000
GUY0707	-0.043	0.032	-0.231	-	0.49203±0.0015	1.00000±0.0000	1.00000±0.0000	0.33348±0.0011	0.33258±0.0018	0.22713±0.0034	1.00000±0.0000	1.00000±0.0000	0.51390±0.0020	0.11040±0.0012	0.11122±0.0021
GUY0701	0.000	0.423	0.474	0.063	-	-1.00000±1.0000	-1.00000±1.0000	0.16601±0.0018	0.16373±0.0016	0.28690±0.0018	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.16541±0.0024	0.16478±0.0013
GUY0705	0.000	0.077	0.000	-0.171	0.000	-	-1.00000±1.0000	0.33230±0.0029	0.33423±0.0027	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33472±0.0017	0.33176±0.0021
GUY0732	0.000	0.077	0.000	-0.171	0.000	0.000	-	0.33006±0.0014	0.33285±0.0020	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	0.33264±0.0022	0.33161±0.0015
GUY0718	1.000	-0.500	-1.000	0.429	1.000	1.000	1.000	-	-1.00000±1.0000	1.00000±0.0000	0.33246±0.0022	1.00000±0.0000	0.19980±0.0023	1.00000±0.0000	1.00000±0.0000
GUY0734	1.000	-0.500	-1.000	0.429	1.000	1.000	1.000	0.000	-	1.00000±0.0000	0.33395±0.0022	1.00000±0.0000	0.20313±0.0016	1.00000±0.0000	1.00000±0.0000
GUY0709	0.000	-0.500	-1.000	-0.714	0.000	0.000	0.000	1.000	1.000	-	1.00000±0.0000	1.00000±0.0000	0.33231±0.0014	1.00000±0.0000	1.00000±0.0000
GUY0509	0.250	-0.200	-0.333	0.059	0.474	0.000	0.000	0.000	0.000	-1.000	-	-1.00000±1.0000	-1.00000±1.0000	0.33614±0.0024	0.33340±0.0029
GUY0713	0.000	0.077	0.000	-0.171	0.000	0.000	0.000	1.000	1.000	0.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0710	0.000	0.351	0.385	0.020	0.000	0.000	0.000	1.000	1.000	0.000	0.385	0.000	-	0.20127±0.0021	0.20026±0.0020
GUY0723	1.000	0.000	0.000	0.571	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	-	1.00000±0.0000
GUY0719	1.000	0.000	0.000	0.571	1.000	1.000	1.000	1.000	1.000	1.000	0.000	1.000	1.000	1.000	-

* Significant P-values (<0.05).

Table 2.4j. Pairwise differentiation tests for populations of *Hoplias malabaricus* with CO1. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0503	GUY0504	GUY0516	GUY0517	GUY0703	GUY0708	GUY0709	GUY0734	GUY0738	GUY0739	GUY0740	GUY0512	GUY0508	GUY0719	GUY0720	GUY0723	GUY0724	GUY0725	GUY0726	GUY0728	GUY0730	GUY0731	GUY0736	GUY0509
GUY0503	-	1.000±0.000	1.00000±0.0000	0.33094±0.0023	0.39682±0.0039	1.00000±0.0000	1.00000±0.0000	0.33398±0.0017	1.00000±0.0000	0.59579±0.0027	0.14617±0.0034	0.49991±0.0032	0.24898±0.0013	-1.00000±1.0000	0.33423±0.0025	0.33443±0.0016	1.00000±0.0000	1.00000±0.0000	0.49859±0.0033	1.00000±0.0000	0.33995±0.0046	0.33161±0.0016	1.00000±0.0000	1.00000±0.0000
GUY0504	1.000	-	1.00000±0.0000	0.33211±0.0016	0.40421±0.0043	1.00000±0.0000	1.00000±0.0000	0.33504±0.0019	1.00000±0.0000	1.00000±0.0000	0.14234±0.0017	0.49645±0.0031	0.25082±0.0019	1.00000±0.0000	0.33297±0.0022	0.33043±0.0012	1.00000±0.0000	1.00000±0.0000	0.49511±0.0039	1.00000±0.0000	0.33540±0.0023	0.33342±0.0028	1.00000±0.0000	1.00000±0.0000
GUY0516	0.000	-1.000	-	1.00000±0.0000	0.59924±0.0025	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.28071±0.0032	1.00000±0.0000	0.09967±0.0030	1.00000±0.0000	0.33448±0.0033	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.60448±0.0021	1.00000±0.0000	0.52177±0.0029	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0517	1.000	1.000	0.000	-	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.09985±0.0020	0.33629±0.0015	0.33299±0.0019	-1.00000±1.0000	0.33297±0.0019	0.33246±0.0019	0.40106±0.0019	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	0.33160±0.0024	-1.00000±1.0000
GUY0703	0.500	0.500	-0.081	-0.263	-	1.00000±0.0000	0.59860±0.0032	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.46531±0.0029	1.00000±0.0000	0.05944±0.0021	0.39850±0.0033	0.06649±0.0014	1.00000±0.0000	0.39950±0.0045	0.39982±0.0056	0.25941±0.0028	0.59687±0.0046	1.00000±0.0000	1.00000±0.0000	0.40429±0.0051	1.00000±0.0000
GUY0708	1.000	1.000	-1.000	0.000	-1.000	-	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.25141±0.0018	1.00000±0.0000	0.33439±0.0014	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000
GUY0709	0.000	0.000	-0.333	0.000	-0.081	-1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.29186±0.0030	1.00000±0.0000	0.10202±0.0024	1.00000±0.0000	0.33068±0.0024	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.60356±0.0046	1.00000±0.0000	0.52055±0.0047	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0734	1.000	1.000	0.000	0.000	-0.263	0.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.09898±0.0008	0.33218±0.0019	0.32997±0.0021	-1.00000±1.0000	0.32932±0.0020	0.33464±0.0019	0.40214±0.0018	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	0.33181±0.0025	-1.00000±1.0000
GUY0738	1.000	1.000	-1.000	0.000	-1.000	0.000	-1.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24760±0.0018	1.00000±0.0000	0.33271±0.0025	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000
GUY0739	0.167	-0.111	-0.436	-0.081	-0.185	-0.667	-0.191	-0.081	-0.667	-	0.33373±0.0050	1.00000±0.0000	0.08549±0.0021	0.59424±0.0016	0.20274±0.0033	1.00000±0.0000	0.60039±0.0041	0.60021±0.0045	0.49018±0.0041	1.00000±0.0000	0.51634±0.0028	1.00000±0.0000	0.60154±0.0050	1.00000±0.0000
GUY0740	0.467	0.467	-0.015	-0.091	-0.036	-0.600	-0.015	-0.091	-0.600	-0.004	-	0.40084±0.0026	0.01073±0.0007*	0.14147±0.0016	0.07017±0.0010	1.00000±0.0000	0.14188±0.0022	0.14104±0.0020	0.16182±0.0028	0.29147±0.0018	0.45560±0.0023	1.00000±0.0000	0.14075±0.0032	1.00000±0.0000
GUY0512	0.333	0.333	-0.200	-0.200	-0.153	-1.000	-0.200	-0.200	-1.000	-0.136	-0.059	-	0.09790±0.0025	0.50503±0.0032	0.20012±0.0015	1.00000±0.0000	0.50579±0.0049	0.50110±0.0019	0.40008±0.0028	1.00000±0.0000	0.63987±0.0032	1.00000±0.0000	0.50232±0.0046	1.00000±0.0000
GUY0508	1.000	1.000	0.647	1.000	0.707	1.000	0.647	1.000	1.000	0.520*	0.642*	0.667	-	0.24893±0.0021	0.10096±0.0021	0.10115±0.0017	0.25274±0.0017	0.25185±0.0025	0.10313±0.0020	0.40125±0.0020	0.01761±0.0017*	0.10010±0.0016	0.25146±0.0017	0.24858±0.0015
GUY0719	0.000	1.000	0.000	1.000	0.500	1.000	0.000	1.000	1.000	0.167	0.467	0.333	1.000	-	0.33284±0.0013	0.33290±0.0016	1.00000±0.0000	1.00000±0.0000	0.50046±0.0041	1.00000±0.0000	0.33332±0.0039	0.33294±0.0014	1.00000±0.0000	1.00000±0.0000
GUY0720	1.000	1.000	0.500	1.000	0.642	1.000	0.500	1.000	1.000	0.420	0.586	0.571	1.000	1.000	-	0.33434±0.0021	-1.00000±1.0000	0.33147±0.0015	0.19910±0.0028	0.33193±0.0019	0.04883±0.0028*	0.33138±0.0016	0.33354±0.0017	0.33137±0.0013
GUY0723	1.000	1.000	0.000	0.000	-0.263	0.000	0.000	0.000	0.000	-0.081	-0.091	-0.200	1.000	1.000	1.000	-	0.33657±0.0014	0.33073±0.0019	0.39906±0.0017	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	0.33274±0.0013	-1.00000±1.0000
GUY0724	1.000	1.000	0.000	1.000	0.500	1.000	0.000	1.000	1.000	0.167	0.467	0.333	1.000	1.000	0.000	1.000	-	1.00000±0.0000	0.50760±0.0042	1.00000±0.0000	0.33118±0.0056	0.33383±0.0022	1.00000±0.0000	1.00000±0.0000
GUY0725	1.000	1.000	0.000	1.000	0.500	1.000	0.000	1.000	1.000	0.167	0.467	0.333	1.000	1.000	1.000	1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.33254±0.0014	0.33343±0.0023	-1.00000±1.0000	1.00000±0.0000
GUY0726	0.333	0.333	0.045	0.368	0.236	0.000	0.045	0.368	0.000	0.089	0.250	0.143	0.667	0.333	0.571	0.368	0.333	-1.000	-	0.59930±0.0030	0.46269±0.0018	0.39733±0.0024	1.00000±0.0000	1.00000±0.0000
GUY0728	0.000	0.000	-0.333	0.000	-0.081	-1.000	-0.333	0.000	-1.000	-0.191	-0.015	-0.200	0.250	0.000	0.500	0.000	0.000	0.000	0.045	-	0.52676±0.0050	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0730	0.600	0.600	0.015	-0.290	-0.119	-1.000	0.015	-0.290	-1.000	-0.007	-0.005	-0.104	0.742*	0.600	0.695	-0.290	0.600	0.500	0.152	0.015	-	1.00000±0.0000	0.33730±0.0023	1.00000±0.0000
GUY0731	1.000	1.000	0.000	0.000	-0.263	0.000	0.000	0.000	0.000	-0.081	-0.091	-0.200	1.000	1.000	1.000	0.000	1.000	1.000	0.368	0.000	-0.290	-	0.33256±0.0018	-1.00000±1.0000
GUY0736	1.000	1.000	0.000	1.000	0.500	1.000	0.000	1.000	1.000	0.167	0.467	0.333	1.000	1.000	1.000	1.000	1.000	1.000	0.000	-1.000	0.000	0.500	1.000	-	1.00000±0.0000
GUY0509	1.000	1.000	-1.000	0.000	-1.000	0.000	-1.000	0.000	0.000	-0.667	-0.600	-1.000	1.000	1.000	1.000	0.000	1.000	1.000	0.000	-1.000	-1.000	0.000	1.000	-

* Significant P-values (<0.05).

Table 2.4k. Pairwise differentiation tests for populations of *Hoplias malabaricus* with Cyt b. Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0503	GUY0504	GUY0516	GUY0517	GUY0703	GUY0708	GUY0734	GUY0738	GUY0739	GUY0740	GUY0512	GUY0508	GUY0719	GUY0723	GUY0725	GUY0726	GUY0728	GUY0730	GUY0731	GUY0736	GUY0509
GUY0503	-	1.00000±0.0000	0.33316±0.0032	0.10189±0.0020	0.09895±0.0016	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.20261±0.0048	0.07040±0.0024	1.00000±0.0000	0.10152±0.0017	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.40283±0.0040	1.00000±0.0000	0.14511±0.0024	0.09980±0.0031	1.00000±0.0000	1.00000±0.0000
GUY0504	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24793±0.0018	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.50181±0.0043	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0516	0.500	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.09954±0.0010	0.33235±0.0015	0.33386±0.0026	0.33389±0.0030	0.20205±0.0023	0.33264±0.0022	1.00000±0.0000	-1.00000±1.0000	0.33373±0.0017	0.33321±0.0016
GUY0517	0.647	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	0.49899±0.0017	0.39851±0.0035	0.09987±0.0019	0.24999±0.0023	0.10098±0.0016	0.25246±0.0022	0.09921±0.0018	0.10276±0.0021	1.00000±0.0000	-1.00000±1.0000	0.24743±0.0013	0.25035±0.0016
GUY0703	0.647	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	0.50120±0.0020	0.40134±0.0022	0.10058±0.0013	0.24654±0.0022	0.09691±0.0021	0.24904±0.0013	0.09938±0.0014	0.09803±0.0025	1.00000±0.0000	-1.00000±1.0000	0.25217±0.0016	0.24958±0.0031
GUY0708	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24866±0.0026	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.50069±0.0042	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0734	0.000	0.000	0.000	0.000	0.000	0.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.24637±0.0016	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.50321±0.0029	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0738	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.25022±0.0016	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.49971±0.0042	1.00000±0.0000	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000
GUY0739	0.344	-1.000	-0.263	-0.091	-0.091	-1.000	-1.000	-1.000	-	0.47031±0.0029	0.48631±0.0030	0.05583±0.0011	0.40091±0.0039	0.20271±0.0020	0.39854±0.0050	0.08550±0.0030	0.20189±0.0033	1.00000±0.0000	1.00000±0.0000	0.40255±0.0063	0.39813±0.0018
GUY0740	0.347	-0.600	-0.091	0.040	0.040	-0.600	-0.600	-0.600	-0.036	-	0.16368±0.0049	0.01115±0.0006*	0.14572±0.0027	0.06903±0.0024	0.14111±0.0026	0.02320±0.0010*	0.07320±0.0017	0.45321±0.0025	0.50197±0.0016	0.14145±0.0029	0.14383±0.0023
GUY0512	0.000	-0.500	0.077	0.250	0.250	-0.500	-0.500	-0.500	0.034	0.077	-	0.10401±0.0039	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	0.40834±0.0039	1.00000±0.0000	0.27780±0.0042	0.39886±0.0024	1.00000±0.0000	1.00000±0.0000
GUY0508	0.647	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.707	0.642*	0.500	-	0.24707±0.0024	0.09673±0.0017	0.25311±0.0031	0.10288±0.0018	0.39947±0.0019	0.01777±0.0010*	0.10071±0.0017	0.25039±0.0025	0.24790±0.0009
GUY0719	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.500	0.467	0.000	1.000	-	1.00000±0.0000	1.00000±0.0000	0.50096±0.0039	1.00000±0.0000	0.33708±0.0025	0.24888±0.0025	1.00000±0.0000	1.00000±0.0000
GUY0723	0.000	0.000	0.500	0.647	0.647	0.000	0.000	0.000	0.344	0.347	-0.200	0.647	0.000	-	1.00000±0.0000	0.40148±0.0047	1.00000±0.0000	0.14606±0.0041	0.10177±0.0037	1.00000±0.0000	1.00000±0.0000
GUY0725	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.500	0.467	0.000	1.000	1.000	0.000	-	1.00000±0.0000	1.00000±0.0000	0.33493±0.0024	0.24680±0.0019	-1.00000±1.0000	1.00000±0.0000
GUY0726	0.208	0.333	0.571	0.667	0.667	0.333	0.333	0.333	0.429	0.419*	0.167	0.667	0.333	0.208	-1.000	-	0.39683±0.0042	0.07379±0.0032	0.09789±0.0020	1.00000±0.0000	0.50125±0.0044
GUY0728	0.000	0.000	0.500	0.647	0.647	0.000	0.000	0.000	0.344	0.347	0.000	0.250	0.000	0.000	0.000	0.208	-	0.14589±0.0051	0.10039±0.0014	1.00000±0.0000	1.00000±0.0000
GUY0730	0.440	-1.000	-0.290	-0.132	-0.132	-1.000	-1.000	-1.000	-0.119	-0.005	0.118	0.742*	0.600	0.440	0.500	0.420	0.440	-	1.00000±0.0000	0.33240±0.0016	0.33094±0.0037
GUY0731	0.647	0.000	0.000	0.000	0.000	0.000	0.000	0.000	-0.091	0.040	0.250	1.000	1.000	0.647	1.000	0.667	0.647	-0.132	-	0.25038±0.0023	0.24877±0.0026
GUY0736	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.500	0.467	0.000	1.000	1.000	0.000	0.000	-1.000	0.000	0.500	1.000	-	1.00000±0.0000
GUY0509	0.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.333	0.467	0.000	1.000	1.000	0.000	1.000	0.333	0.000	0.600	1.000	1.000	-

* Significant P-values (<0.05).

Table 2.4l. Pairwise differentiation tests for populations of *Hoplias malabaricus* with S7 (1st intron). Pairwise ΦST comparisons (below the diagonal) using Tamura & Nei distance and gamma correction (0.59), and P-values for exact tests of pairwise haplotype frequency comparisons (above the diagonal).

	GUY0503	GUY0516	GUY0703	GUY0709	GUY0734	GUY0738	GUY0740	GUY0739	GUY0508	GUY0512	GUY0720	GUY0725	GUY0726	GUY0728	GUY0730	GUY0731
GUY0503	-	0.33170±0.0022	0.33446±0.0014	0.33022±0.0024	0.33056±0.0023	0.33070±0.0024	0.33354±0.0027	0.33306±0.0018	0.33462±0.0021	0.33316±0.0017	0.33368±0.0030	0.33015±0.0019	0.33379±0.0024	0.33050±0.0034	0.33039±0.0025	0.33338±0.0017
GUY0516	1.000	-	-1.00000±1.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0703	1.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0709	0.500	-1.000	-1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0734	0.500	0.000	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0738	1.000	1.000	1.000	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0740	0.500	-1.000	-1.000	-0.333	0.000	0.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0739	1.000	1.000	1.000	0.000	0.000	1.000	0.000	-	1.00000±0.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0508	1.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0512	1.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	-	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0720	1.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	-	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000	1.00000±0.0000
GUY0725	1.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	-	-1.00000±1.0000	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0726	1.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	-	1.00000±0.0000	-1.00000±1.0000	-1.00000±1.0000
GUY0728	0.500	0.000	0.000	0.000	0.000	0.000	0.000	-1.000	0.000	-1.000	0.000	-1.000	-1.000	-	1.00000±0.0000	1.00000±0.0000
GUY0730	1.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	-1.000	-	-1.00000±1.0000
GUY0731	1.000	1.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	-1.000	0.000	-

* Significant P-values (<0.05).

Table 2.5. Mantel tests & Partial Mantel tests of *Hypostomus squalinus*, *Potamorrhaphis guianensis*, *Geophagus surinamensis*, and *Hoplias malabaricus*.

Species	Geographic vs. Genetic distance	Genetic distance vs. Binary matrix (Rupununi portal)
Potamorrhaphis guianensis	r = 0.251; p-value = 0.082	r = -0.135; p-value = 0.815
Geophagus surinamensis	r = 0.115; p-value = 0.890	r = 0.036; p-value = 0.312
Hoplias malabaricus	r = 0.2253; p-value = 0.090	r = 0.1432; p-value = 0.918
Hypostomus squalinus	r = -0.1787; p-value = 0.966	r = 0.899; p-value = 0.0004***

*** = P < 0.001

Table 2.6a. Analysis of molecular variance (AMOVA) for the cytochrome oxidase subunit I (COI) sequences of *Hypostomus squalinus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	11.641	0.38665 V_a	84.74	Φ_{CT} = 0.84744***
Among populations within drainages	14	0.750	-0.00592 V_b	-1.30	Φ_{SC} = -0.08498
Within populations	48	3.625	0.07552 V_c	16.55	Φ_{ST} = 0.83448***
Total	63	16.016	0.45626		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6b. Analysis of molecular variance (AMOVA) for the cytochrome *b* sequences of *Hypostomus squalinus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	0.185	0.00653 Va	8.16	ΦCT = 0.08157
Among populations within drainages	19	0.648	-0.02220 Vb	-27.74	ΦSC = -0.30200
Within populations	41	3.925	0.09573 Vc	119.58	ΦST = -0.19579
Total	61	4.758	0.08006		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6c. Analysis of molecular variance (AMOVA) for the S7 (1st intron) sequences of *Hypostomus squalinus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	0.294	-0.00638 Va	-2.86	ΦCT = -0.02863
Among populations within drainages	13	4.364	0.07686 Vb	34.52	ΦSC = 0.33559
Within populations	23	3.500	0.15217 Vc	68.34	ΦST = 0.31657
Total	37	8.158	0.22266		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6d. Analysis of molecular variance (AMOVA) for the cytochrome oxidase subunit I (COI) sequences of *Potamorrhaphis guianensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	0.662	-0.00205 Va	-1.13	ΦCT = -0.01128
Among populations within drainages	9	3.048	0.03836 Vb	21.16	ΦSC = 0.20926
Within populations	64	9.277	0.14495 Vc	79.97	ΦST = 0.20034**
Total	74	12.987	0.18126		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6e. Analysis of molecular variance (AMOVA) for the cytochrome *b* sequences of *Potamorrhaphis guianensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	1.573	0.02352 Va	8.38	ΦCT = 0.08378
Among populations within drainages	7		0.04507 Vb	16.06	ΦSC = 0.17524
Within populations	52	2.872	0.21213 Vc	75.57	ΦST = 0.24433**
Total	60	11.031	0.28072		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6f. Analysis of molecular variance (AMOVA) for the S7 (1st intron) sequences of *Potamorrhaphis guianensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	2.456	0.08443 Va	22.14	ΦCT = 0.22138
Among populations within drainages	6	1.377	-0.01984 Vb	-5.2	ΦSC = -0.06682
Within populations	50	15.839	0.31679 Vc	83.06	ΦST = 0.16936
Total	57	19.672	0.38137		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6g. Analysis of molecular variance (AMOVA) for the cytochrome oxidase subunit I (COI) sequences of *Hoplias malabaricus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	0.836	0.01212 Va	3.54	ΦCT = 0.03537
Among populations within drainages	22	9.518	0.09022 Vb	26.34	ΦSC = 0.27303**
Within populations	29	6.967	0.24023 Vc	70.13	ΦST = 0.29874**
Total	52	17.321	0.34257		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6h. Analysis of molecular variance (AMOVA) for the cytochrome *b* sequences of *Hoplias malabaricus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	1.916	0.05610 Va	14.97	ΦCT = 0.14969
Among populations within drainages	19	8.483	0.10262 Vb	27.38	ΦSC = 0.32200***
Within populations	28	6.05	0.21607 Vc	57.65	ΦST = 0.42349***
Total	48	16.449	0.37479		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6i. Analysis of molecular variance (AMOVA) for the S7 (1st intron) sequences of *Hoplias malabaricus* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	1.417	0.10150 Va	21.05	ΦCT = 0.21050**
Among populations within drainages	14	5.25	-0.01933 Vb	-4.01	ΦSC = -0.05077
Within populations	5	2.00	0.40000 Vc	82.96	ΦST = 0.17042
Total	20	8.667	0.48217		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6j. Analysis of molecular variance (AMOVA) for the cytochrome oxidase subunit I (COI) sequences of *Geophagus surinamensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	0.802	0.00504 Va	1.49	ΦCT = 0.01489
Among populations within drainages	16	7.418	0.09640 Vb	28.51	ΦSC = 0.28937**
Within populations	29	6.865	0.23673 Vc	70	ΦST = 0.29996**
Total	46	15.085	0.33816		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6k. Analysis of molecular variance (AMOVA) for the cytochrome *b* sequences of *Geophagus surinamensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	3.606	0.10339 Va	24.32	ΦCT = 0.24323**
Among populations within drainages	16	8.388	0.13730 Vb	32.3	ΦSC = 0.42682***
Within populations	33	6.084	0.18438 Vc	43.38	ΦST = 0.56624***
Total	50	18.078	0.42506		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

Table 2.6l. Analysis of molecular variance (AMOVA) for the S7 (1st intron) sequences of *Geophagus surinamensis* in Rupununi Savanna

Source of variation	d.f.	Sum of squares	Variance component	% variation	Φ statistic
Among drainages	1	1.327	0.01696 Va	4.06	ΦCT = 0.04064
Among populations within drainages	16	9.036	0.08737 Vb	20.94	ΦSC = 0.21827***
Within populations	40	12.516	0.31291 Vc	75	ΦST = 0.25004***
Total	57	22.879	0.41724		

Va, Vb and Vc are the associated covariance components
** = P < 0.01; *** = P < 0.001.

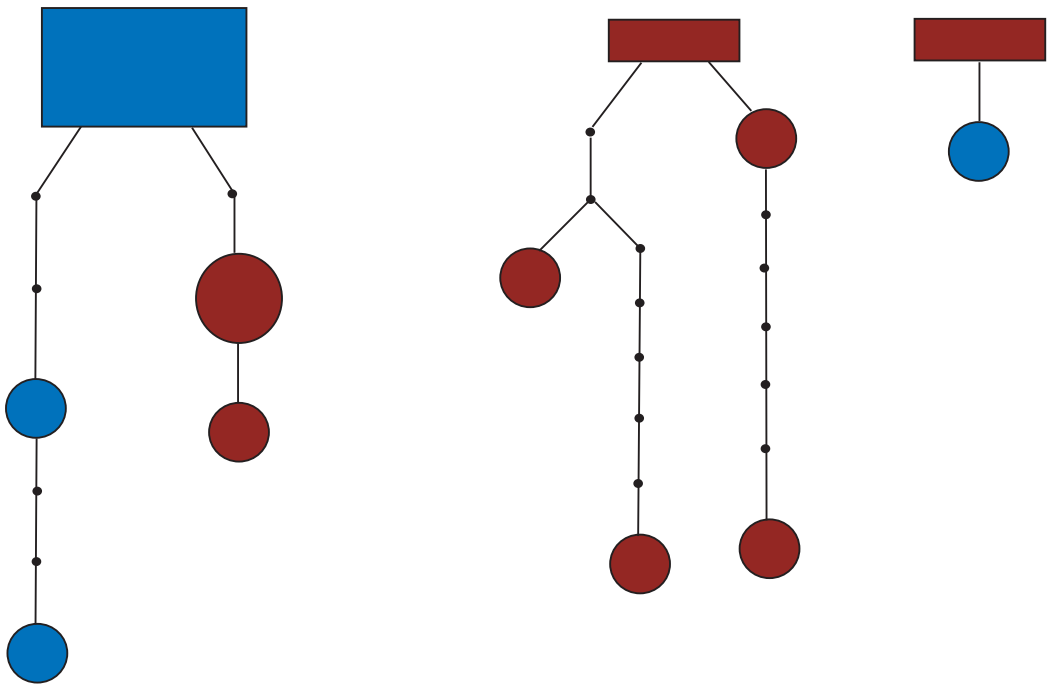

Figure 2.1a. Haplotype network of *Ancistrus* sp. 'white spot' in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

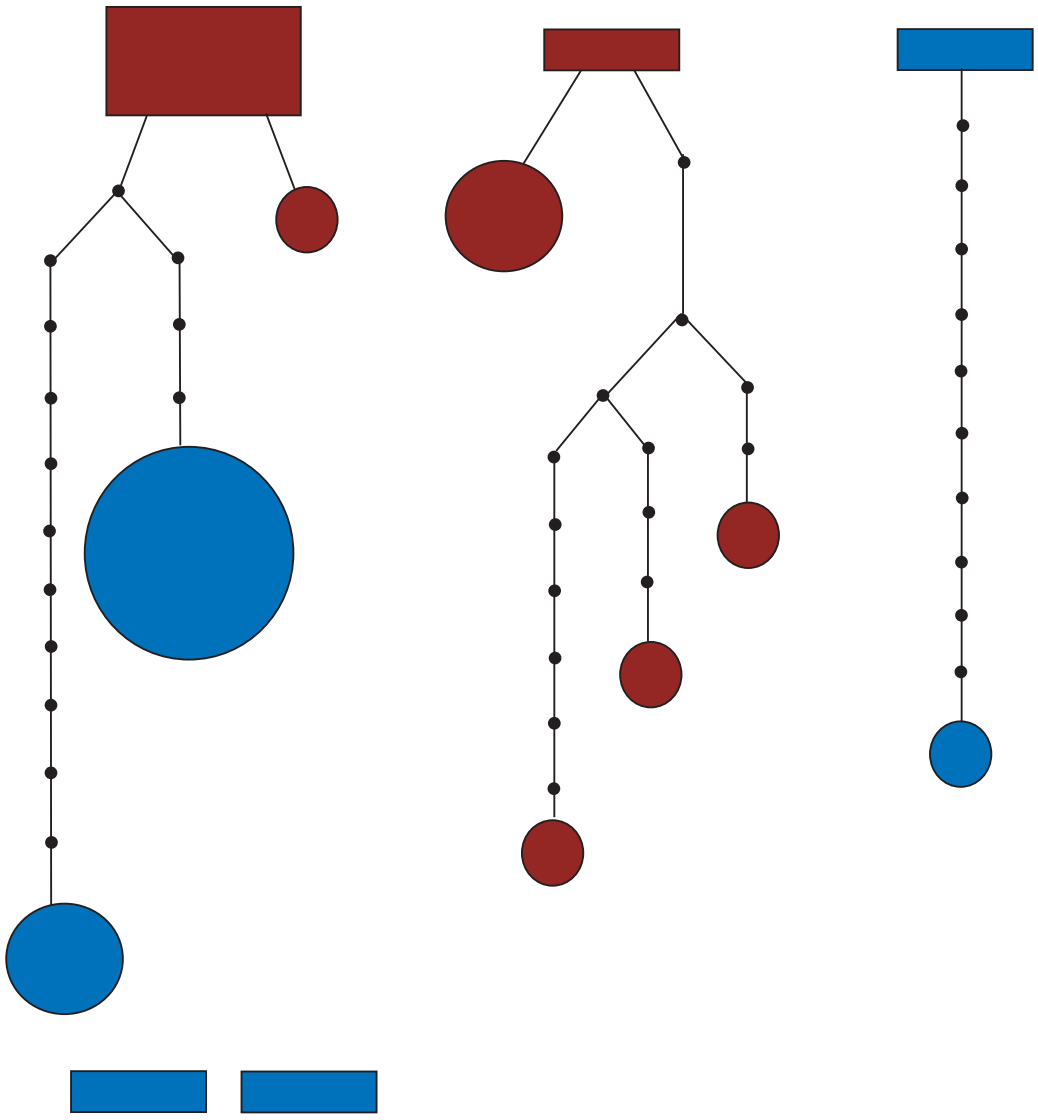

Figure 2.1b. Haplotype network of *Ancistrus* sp. 'white spot' in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome b (Cyt b) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

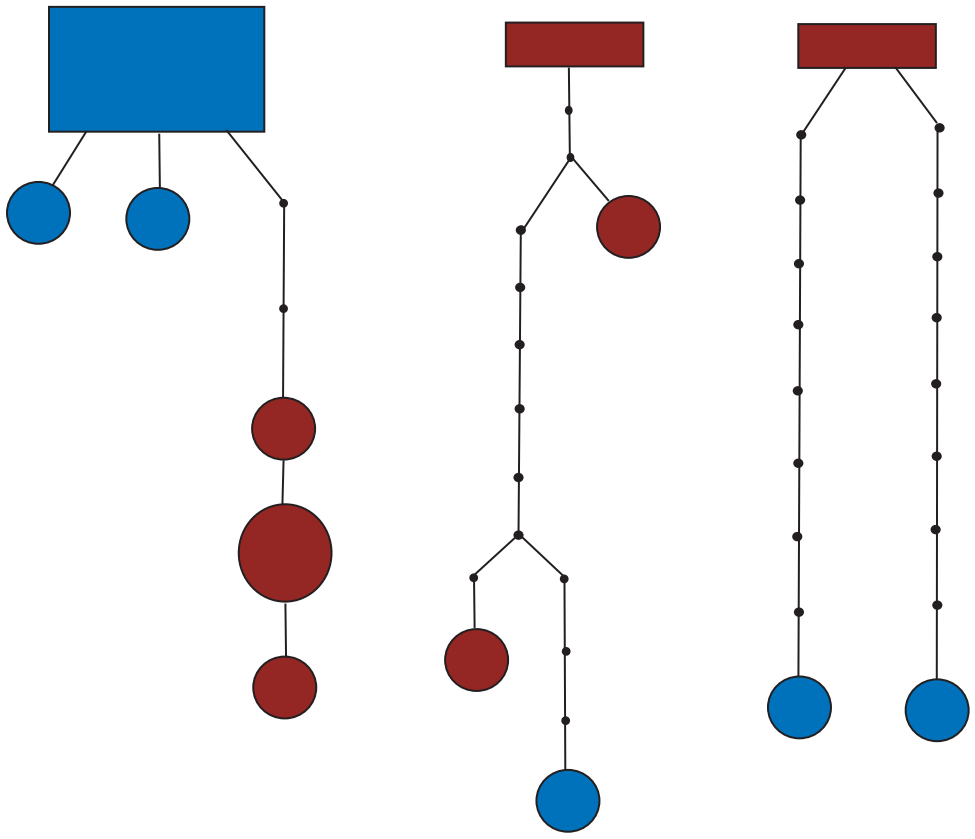

Figure 2.1c. Haplotype network of *Ancistrus* sp. 'white spot' in the Rupununi savanna. Each circle in a network represents a unique nuclear DNA (nDNA) S7 (1st intron) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

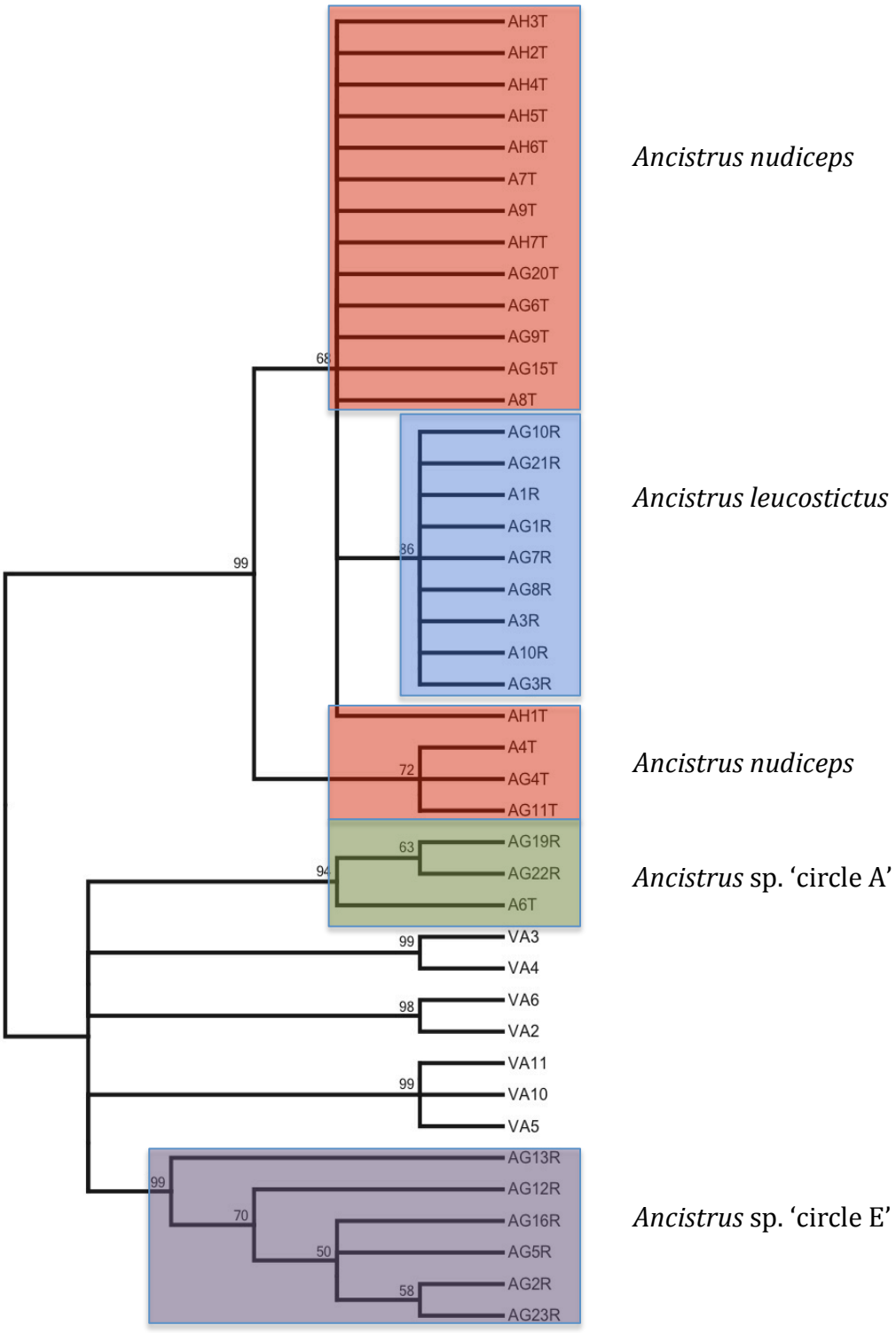

Figure 2.2a. Maximum likelihood (ML) tree based on 669 bp aligned sites of COI for 42 taxa of *Ancistrus* sp. 'white spot'. Number on nodes indicate bootstrap support values. Rupununi and Takutu denoted at end of taxon label with R or T to indicate drainage. *Ancistrus nudiceps* highlighted in red, *Ancistrus leucostictus* highlighted in blue, *Ancistrus* sp. 'circle A' highlighted in green, and *Ancistrus* sp. 'cicle E' highlighted in purple. VA taxa are Venezuela samples not labeled or discussed in this study.

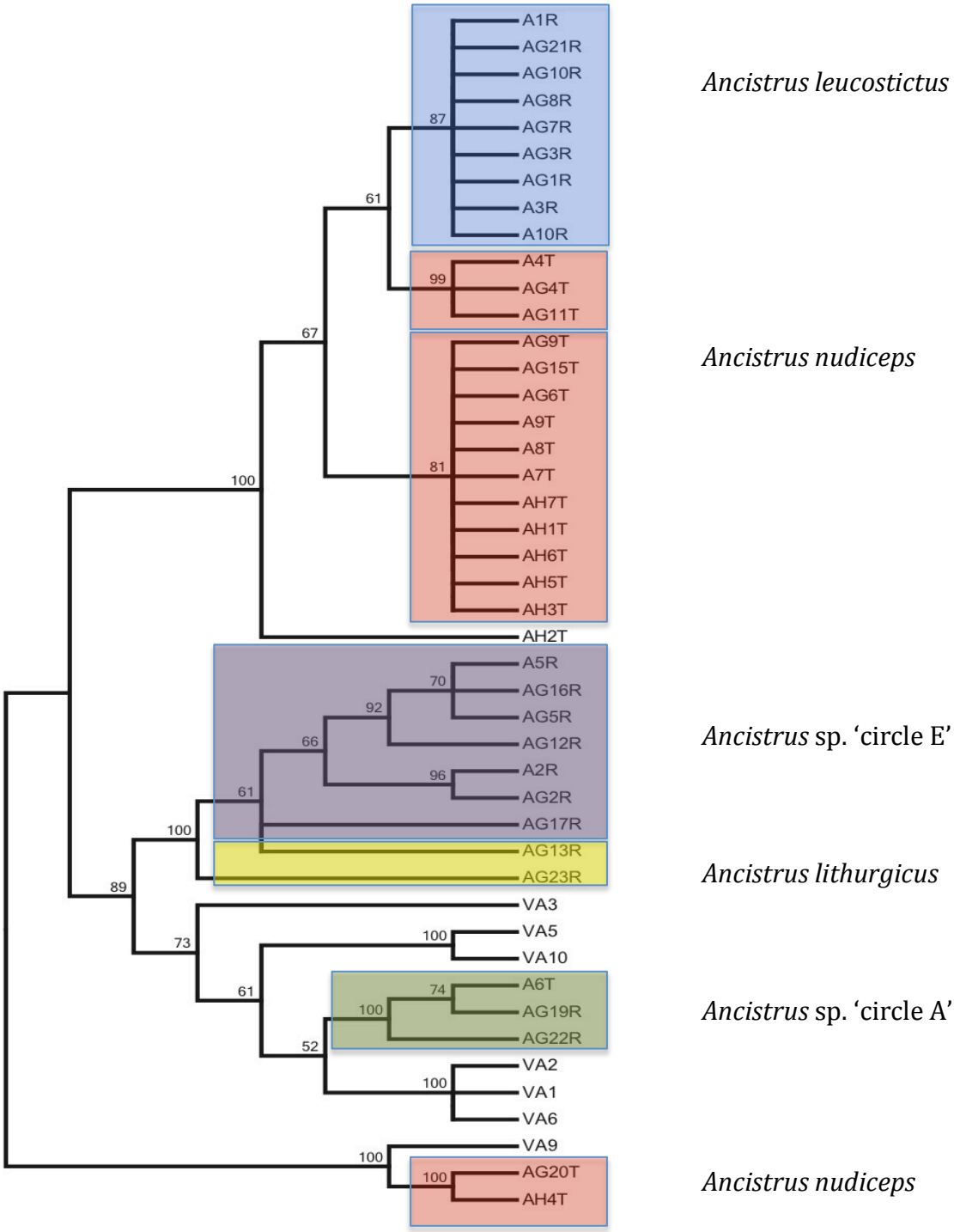

Figure 2.2b. Maximum Likelihood (ML) tree based on 1107 bp aligned sites of Cyt b for 45 taxa of *Ancistrus* sp. 'white spot'. Number on nodes indicate bootstrap support values. Rupununi and Takutu denoted at end of taxon label with R or T to indicate drainage. *Ancistrus nudiceps* highlighted in red, *Ancistrus leucostictus* highlighted in blue, *Ancistrus* sp. 'circle A' highlighted in green, *Ancistrus lithurgicus* highlighted in yellow, and *Ancistrus* sp. 'cicle E' highlighted in purple. VA taxa are Venezuela samples not labeled or discussed in this study.

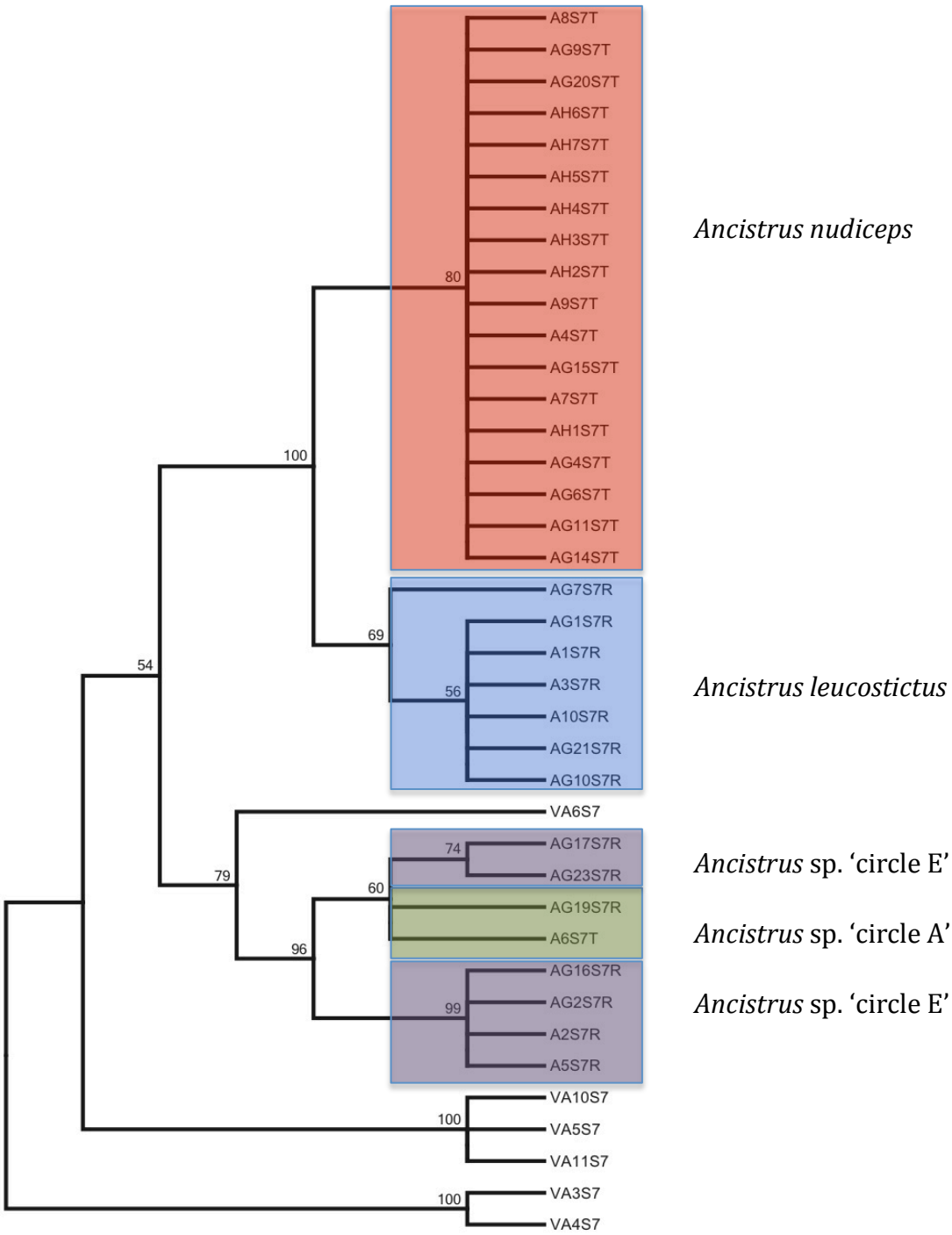

Figure 2.2c. Maximum likelihood (ML) tree based on 640 bp aligned sites of S7 for 39 taxa of *Ancistrus* sp. 'white spot'. Number on nodes indicate bootstrap support values. Rupununi and Takutu denoted at end of taxon label with R or T to indicate drainage. *Ancistrus nudiceps* highlighted in red, *Ancistrus leucostictus* highlighted in blue, *Ancistrus* sp. 'circle A' highlighted in green, and *Ancistrus* sp. 'cicle E' highlighted in purple. VA taxa are Venezuela samples not labeled or discussed in this study.

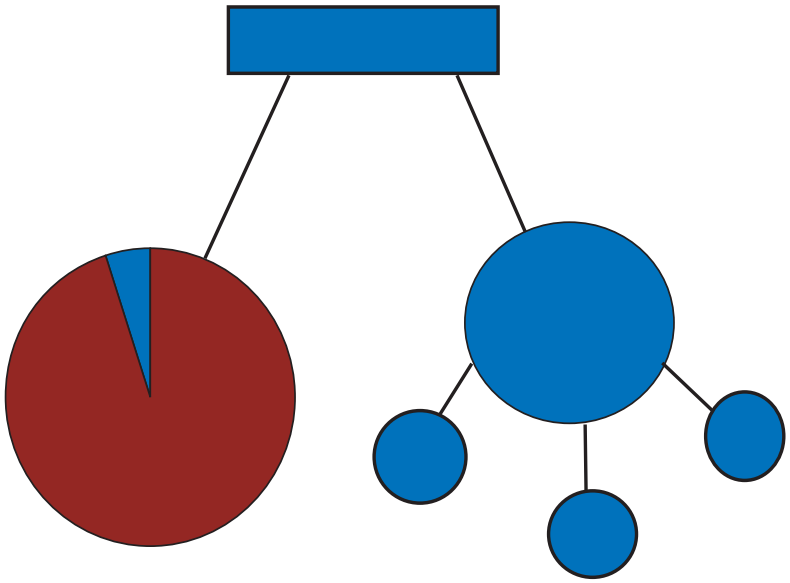

Figure 2.3a. Haplotype network of *Hypostomus squalinus* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

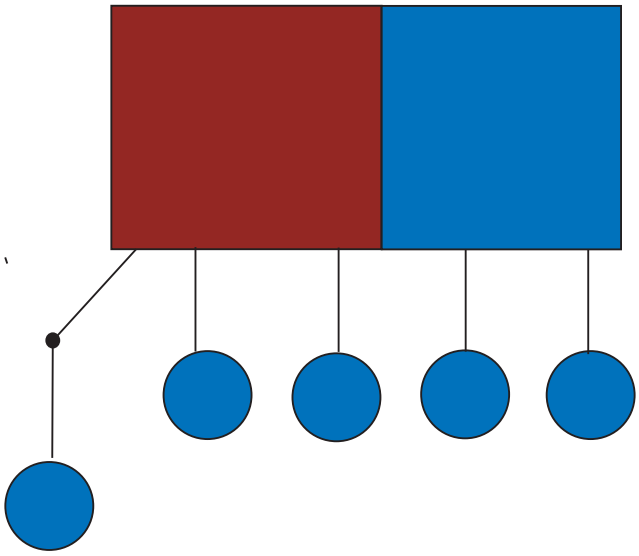

Figure 2.3b. Haplotype network of *Hypostomus squalinus* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome b (Cyt b) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

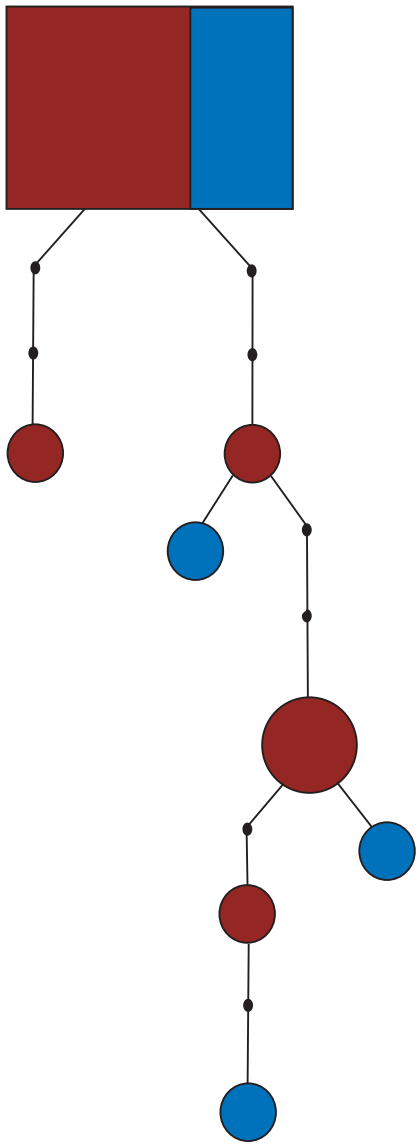

Figure 2.3c. Haplotype network of *Hypostomus squalinus* in the Rupununi savanna. Each circle in a network represents a unique nuclear DNA (nDNA) S7 (1st intron) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

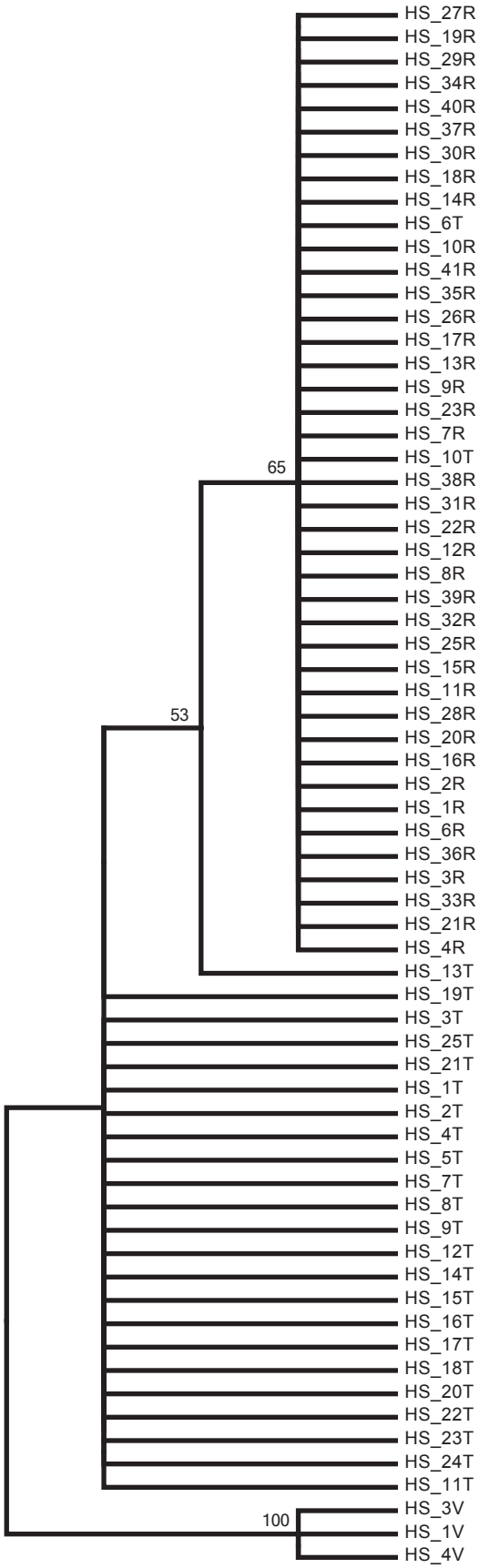

Figure 2.4a. Maximum likelihood (ML) tree based on 673 bp aligned sites of COI for 67 taxa of *Hypostomus squalinus*. Number on nodes indicate bootstrap support values. HS, *Hypostomus squalinus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

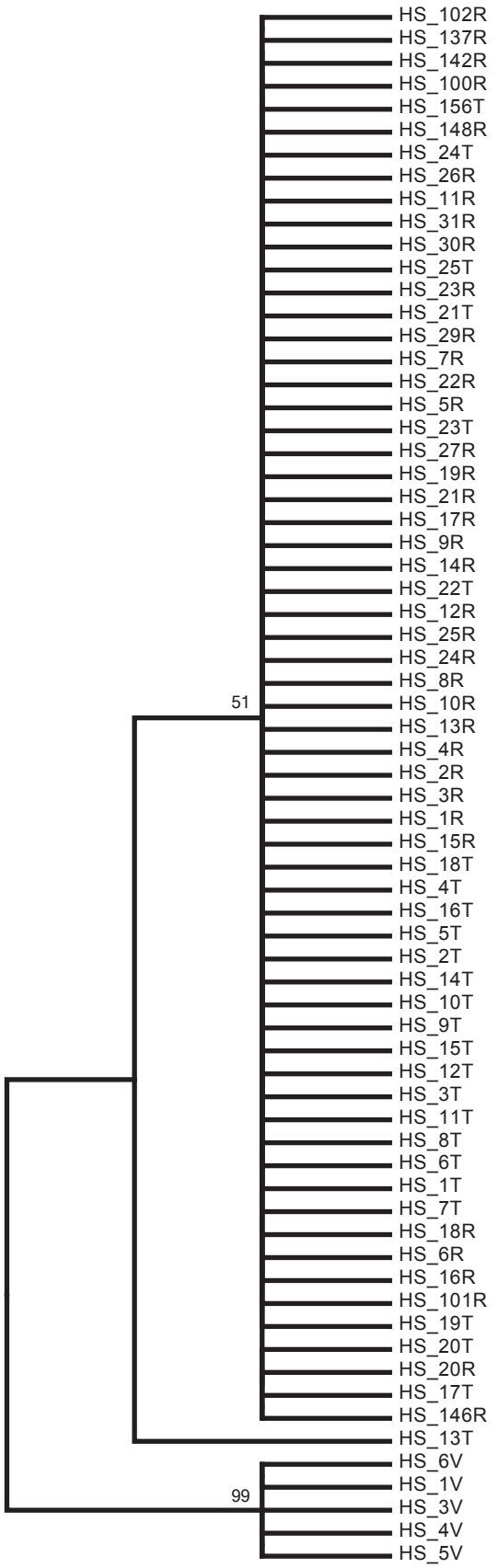

Figure 2.4b. Maximum likelihood (ML) tree based on 1152 bp aligned sites of Cyt b for 68 taxa of *Hypostomus squalinus*. Number on nodes indicate bootstrap support values. HS, *Hypostomus squalinus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

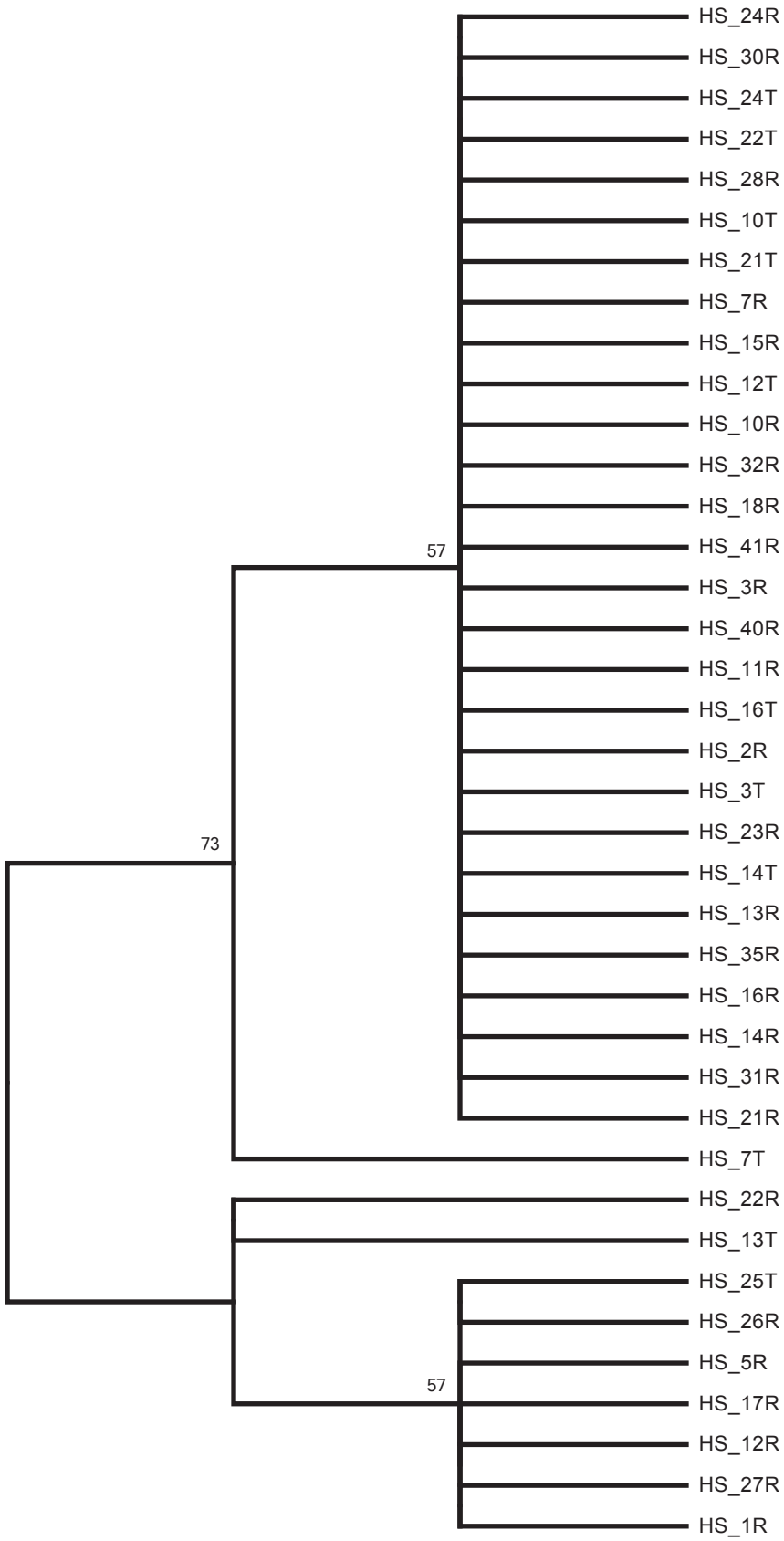

Figure 2.4c. Maximum likelihood (ML) tree based on 623 bp aligned sites of S7 (1st intron) for 38 taxa of *Hypostomus squalinus*. Number on nodes indicate bootstrap support values. HS, *Hypostomus squalinus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

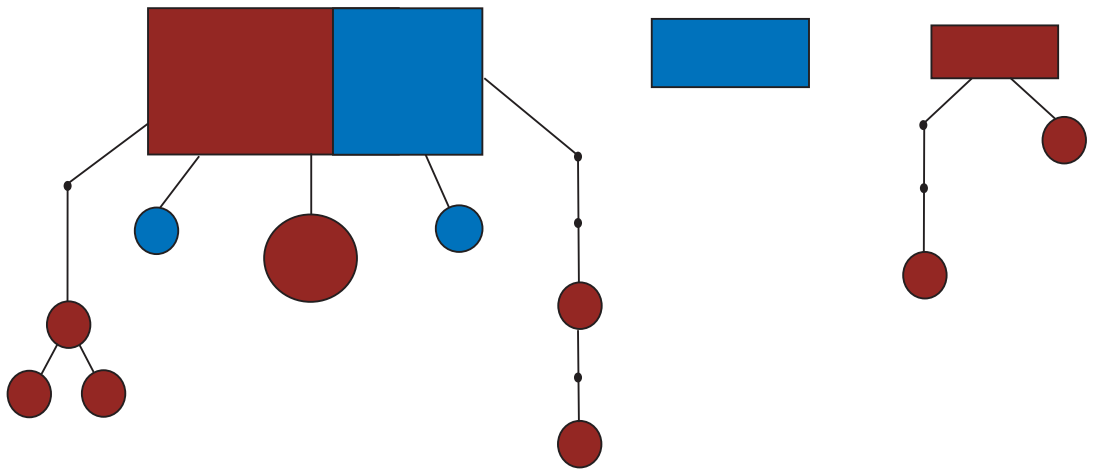

Figure 2.5a. Haplotype network of *Geophagus surinamensis* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

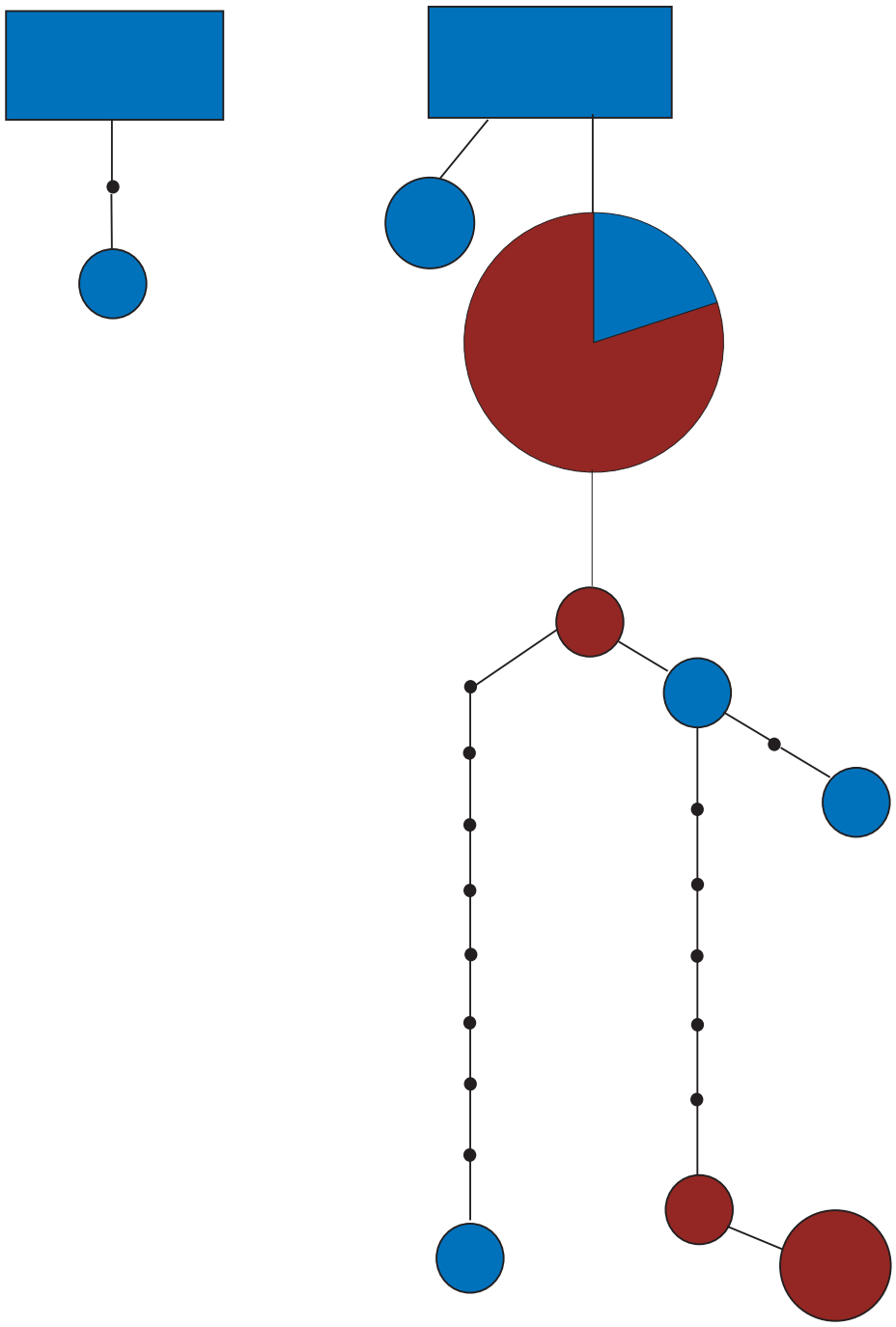

Figure 2.5b. Haplotype network of *Geophagus surinamensis* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome b (Cyt b) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

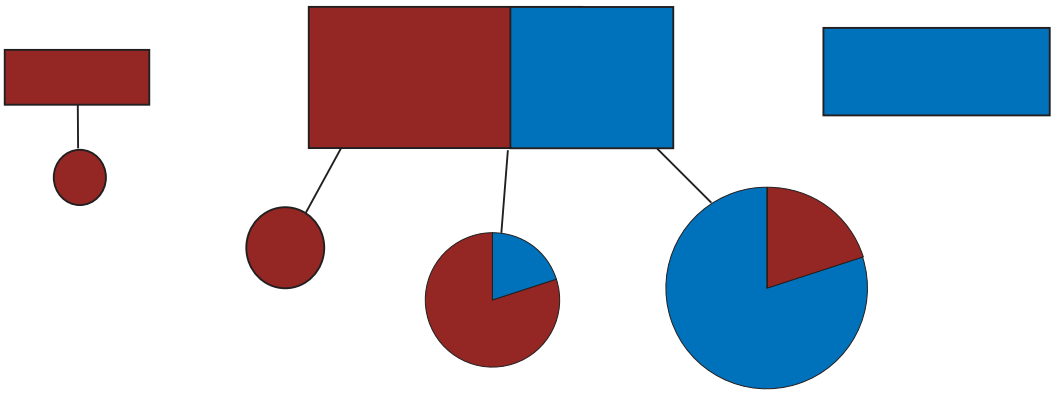

Figure 2.5c. Haplotype network of *Geophagus surinamensis* in the Rupununi savanna. Each circle in a network represents a unique nuclear DNA (nDNA) S7 (1st intron) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

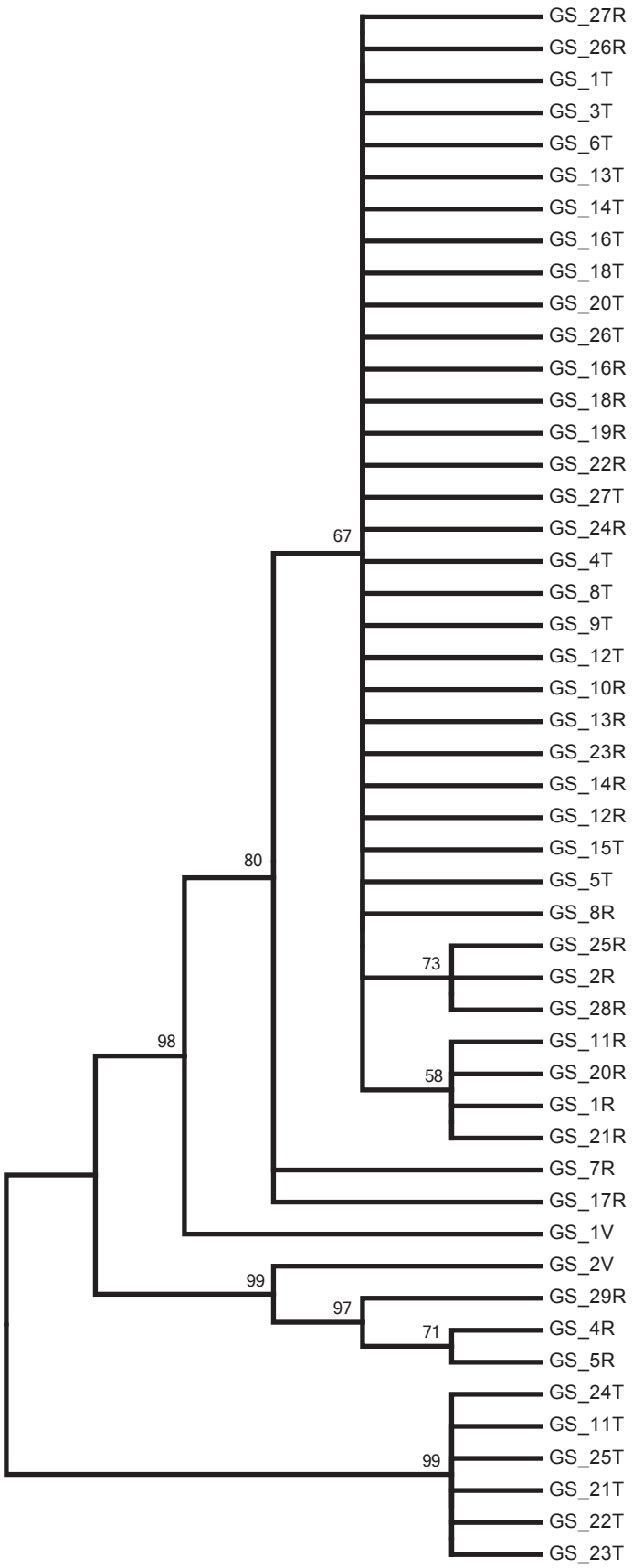

Figure 2.6a. Maximum likelihood (ML) tree based on 678 bp aligned sites of COI for 49 taxa of *Geophagus surinamensis*. Number on nodes indicate bootstrap support values. GS, *Geophagus surinamensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

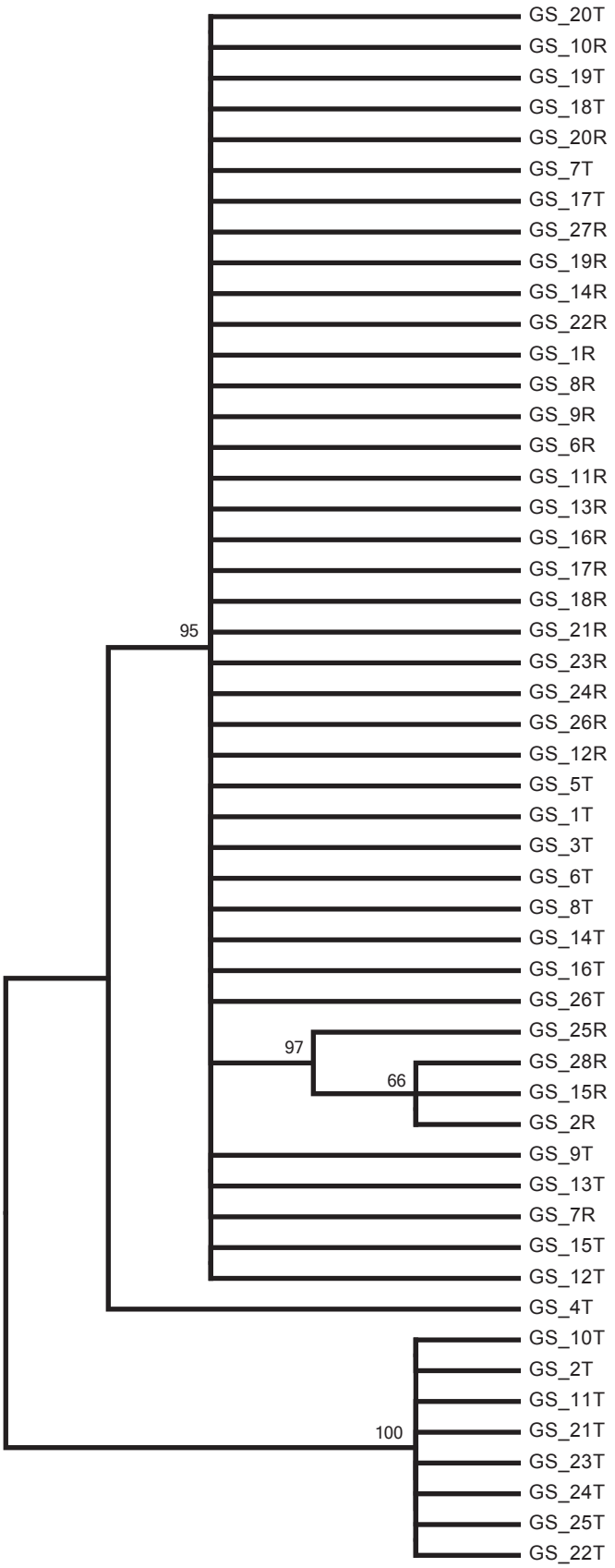

Figure 2.6b. Maximum likelihood (ML) tree based on 1047 bp aligned sites of Cyt b for 51 taxa of *Geophagus surinamensis*. Number on nodes indicate bootstrap support values. GS, *Geophagus surinamensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

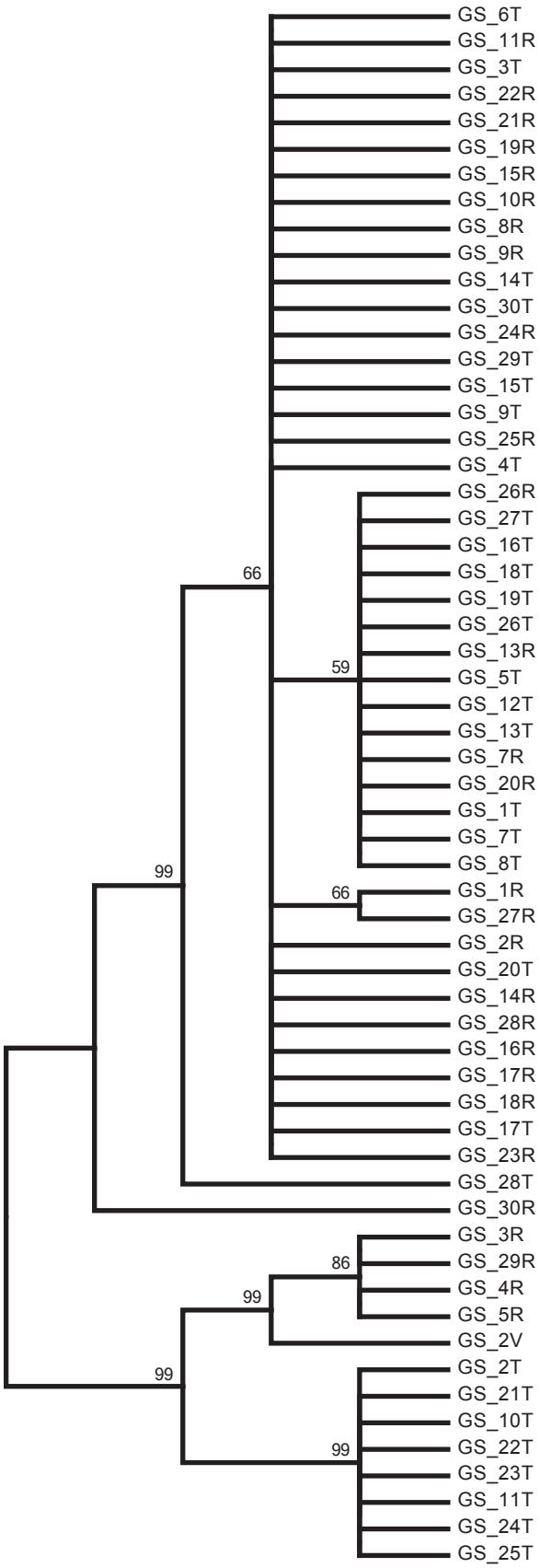

Figure 2.6c. Maximum likelihood (ML) tree based on 604 bp aligned sites of S7 (1st intron) for 59 taxa of *Geophagus surinamensis*. Number on nodes indicate bootstrap support values. GS, *Geophagus surinamensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

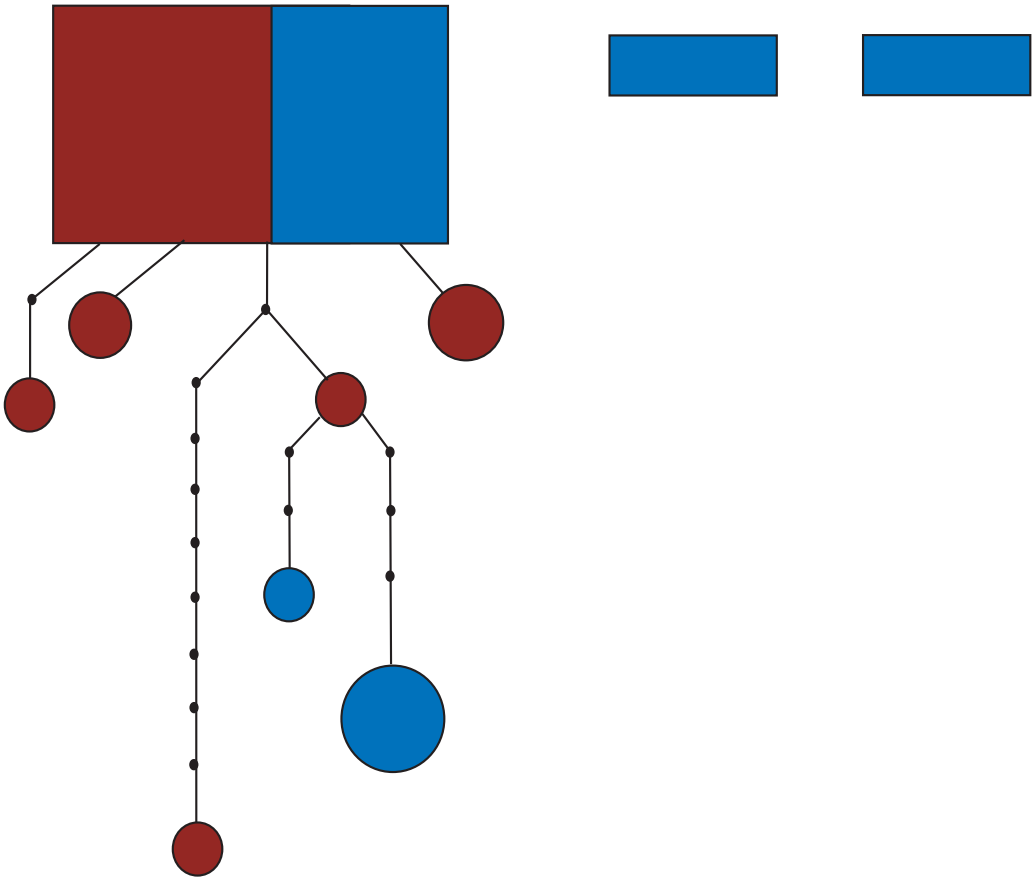

Figure 2.7a. Haplotype network of *Hoplias malabaricus* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

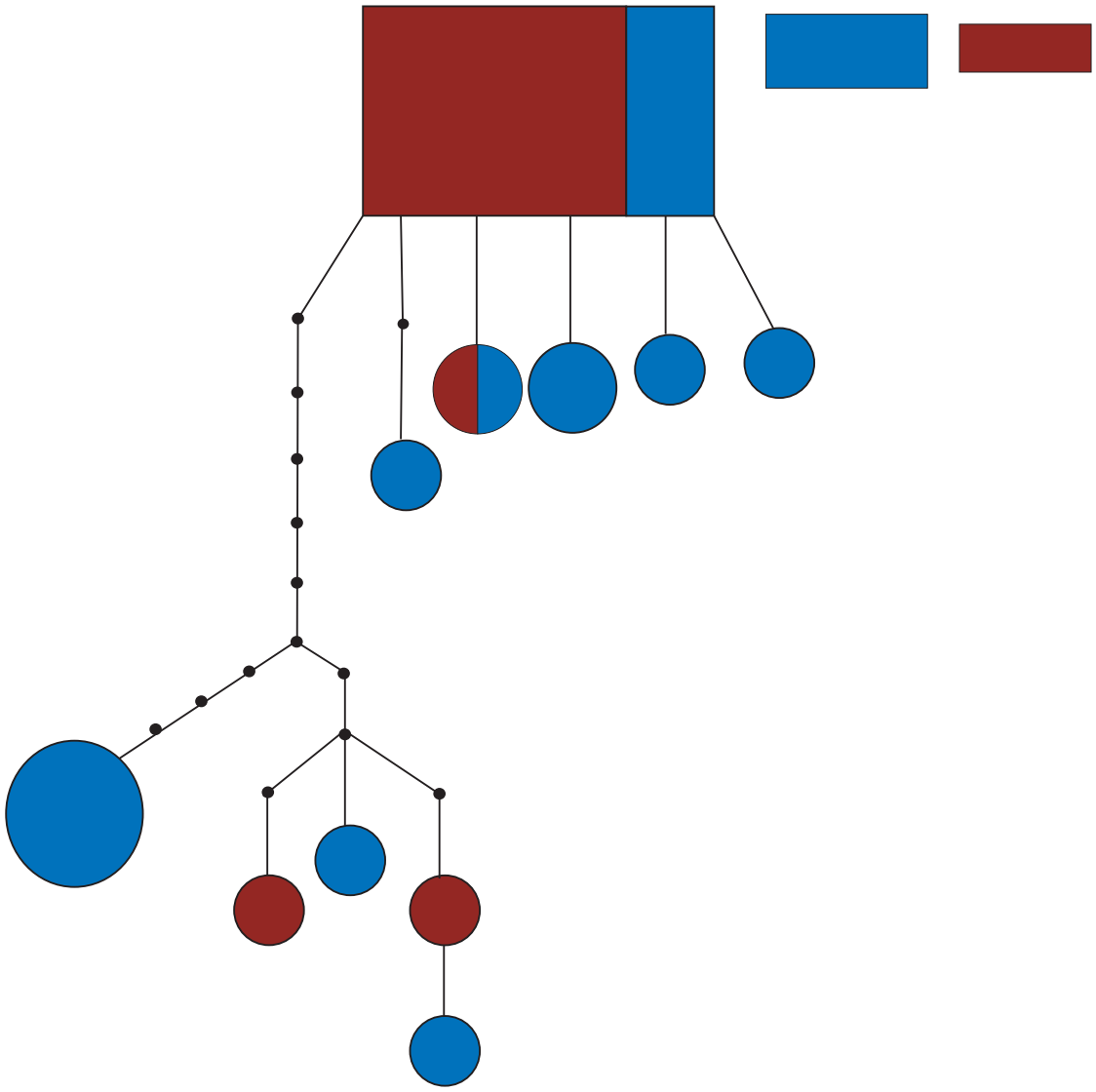

Figure 2.7b. Haplotype network of *Hoplias malabaricus* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome b (Cyt b) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

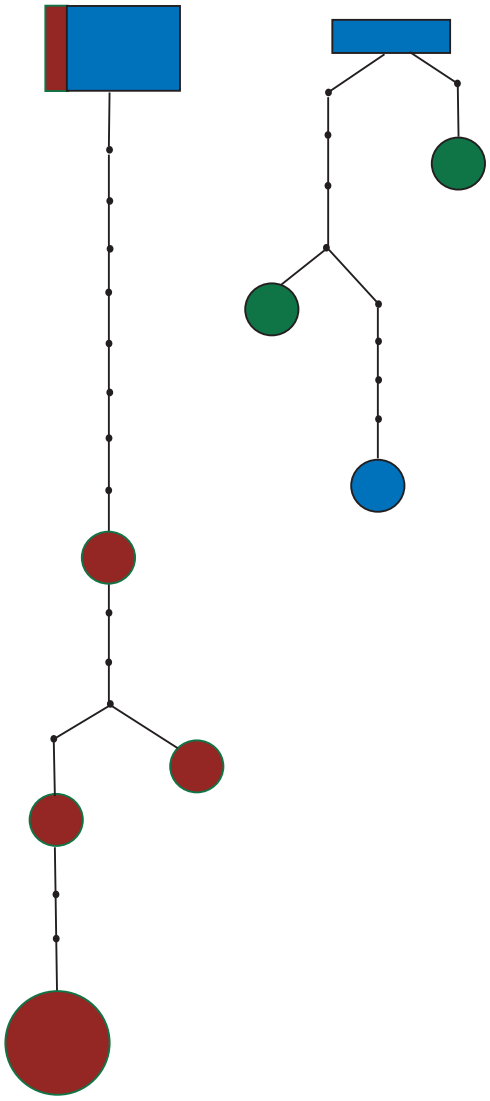

Figure 2.7c. Haplotype network of *Hoplias malabaricus* in the Rupununi savanna. Each circle in a network represents a unique nuclear DNA (nDNA) S7 (1st intron) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi, Blue =Takutu and Green = Venezuela.

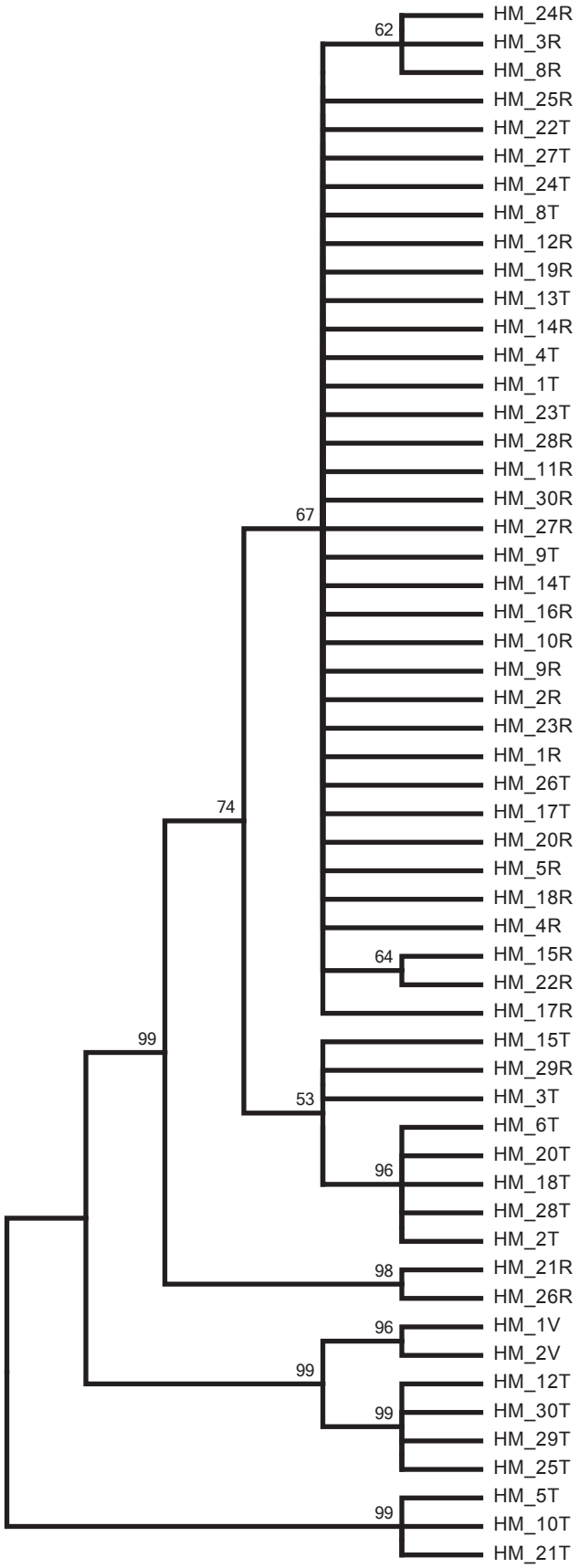

Figure 2.8a. Maximum likelihood (ML) tree based on 672 bp aligned sites of COI for 55 taxa of *Hoplias malabaricus*. Number on nodes indicate bootstrap support values. HM, *Hoplias malabaricus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

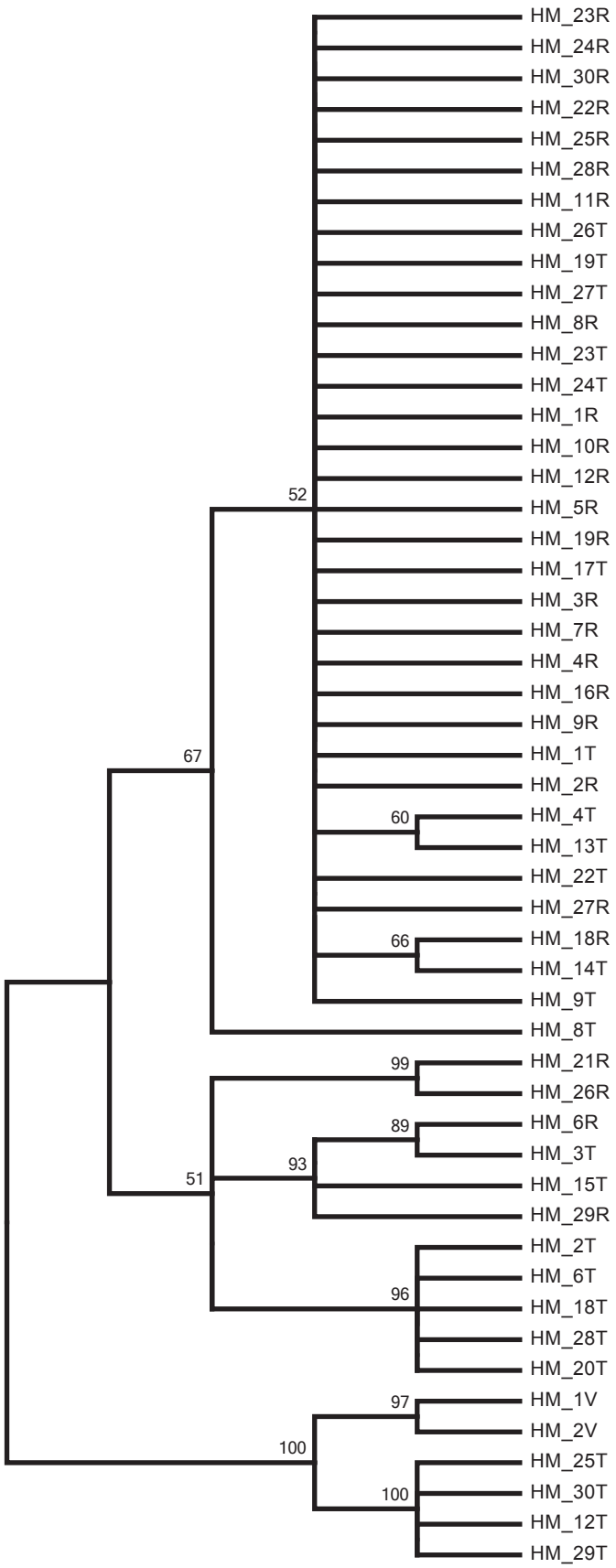

Figure 2.8b. Maximum likelihood (ML) tree based on 996 bp aligned sites of Cyt b for 51 taxa of *Hoplias malabaricus*. Number on nodes indicate bootstrap support values. HM, *Hoplias malabaricus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

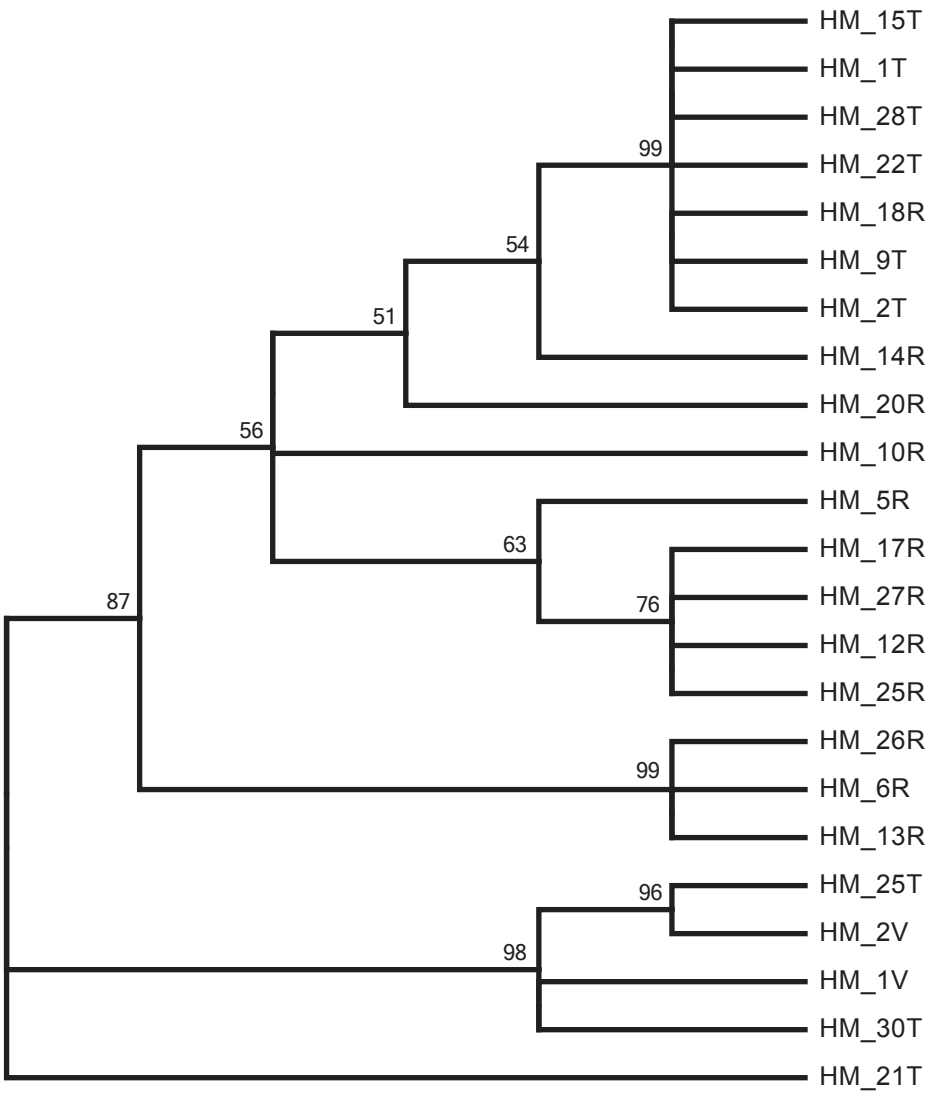

Figure 2.8c. Maximum likelihood (ML) tree based on 704 bp aligned sites of S7 (1st intron) for 23 taxa of *Hoplias malabaricus*. Number on nodes indicate bootstrap support values. HM, *Hoplias malabaricus*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

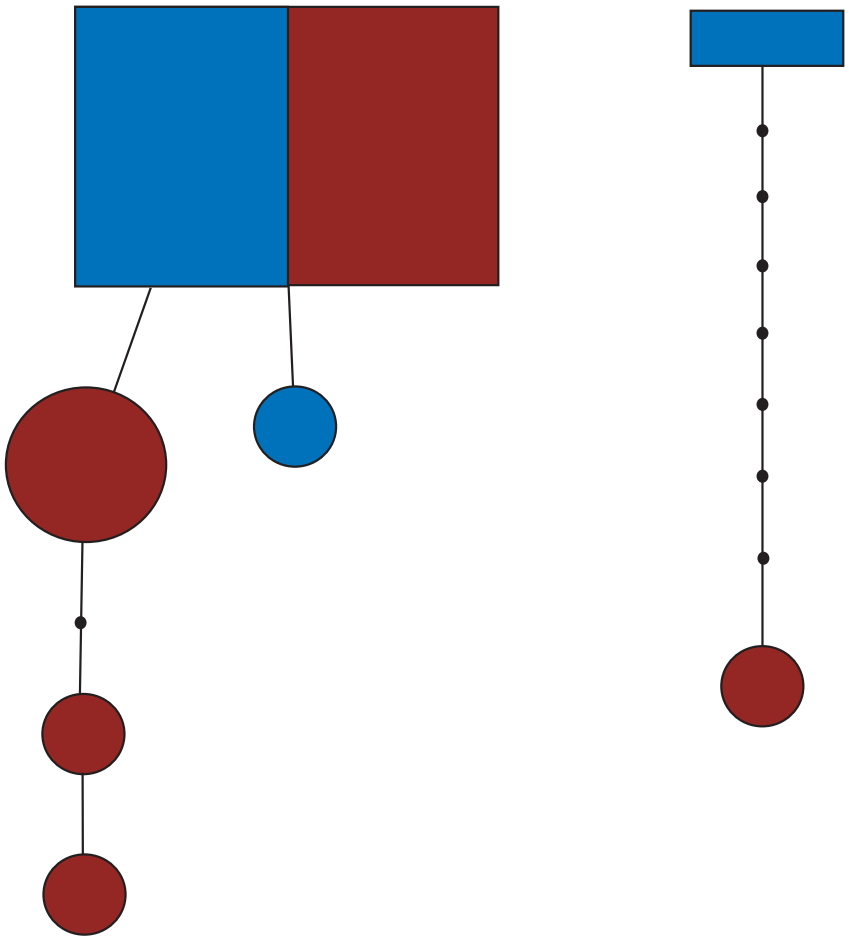

Figure 2.9a. Haplotype network of *Potamorrhaphis guianensis* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

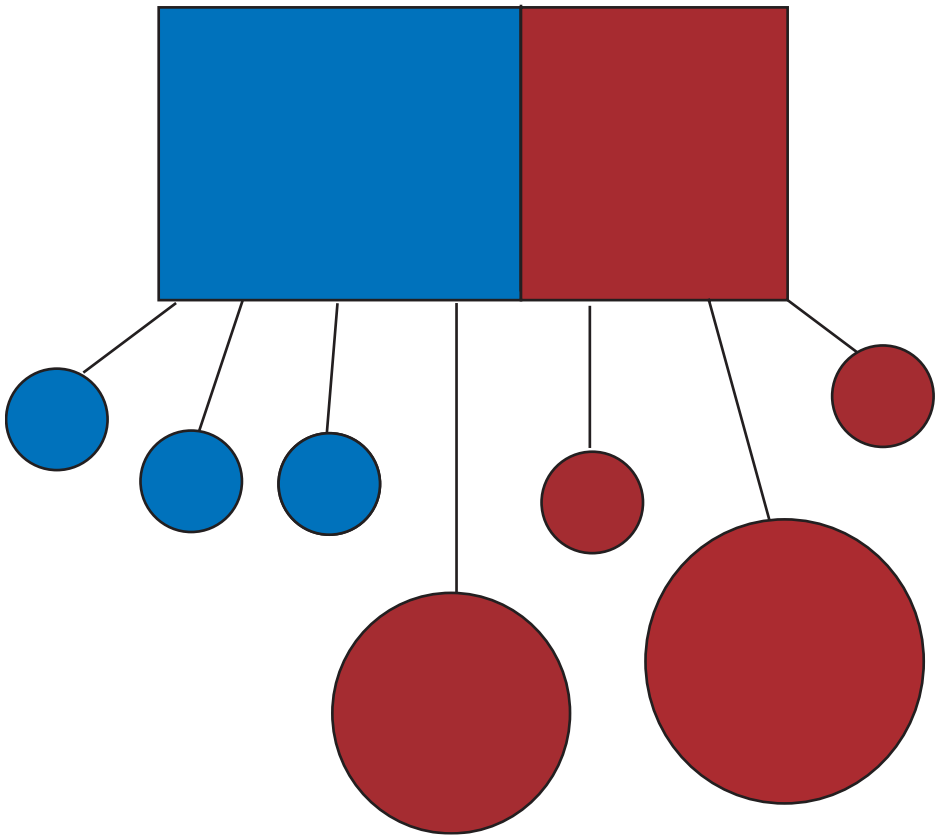

Figure 2.9b. Haplotype network of *Potamorrhaphis guianensis* in the Rupununi savanna. Each circle in a network represents a unique mitochondrial DNA (mtDNA) cytochrome b (Cyt b) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

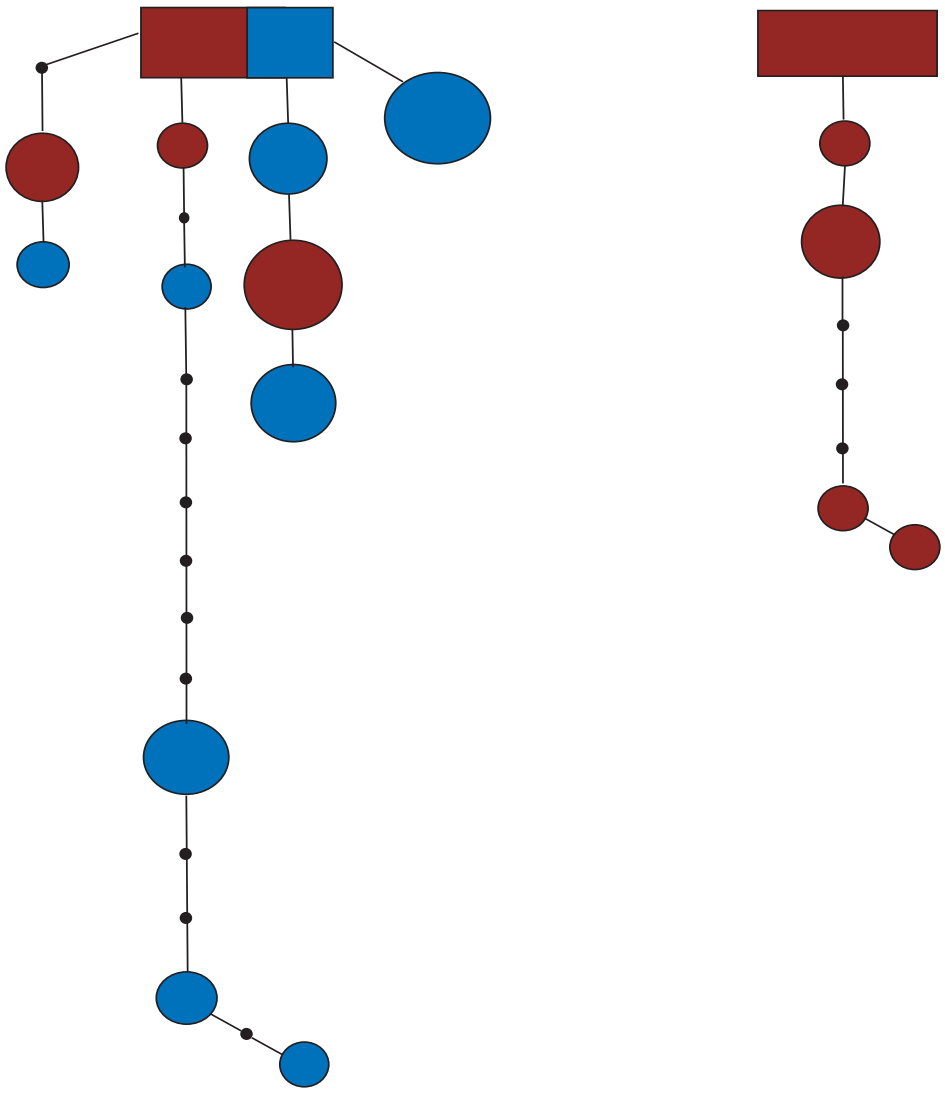

Figure 2.9c. Haplotype network of *Potamorrhaphis guianensis* in the Rupununi savanna. Each circle in a network represents a unique nuclear DNA (nDNA) S7 (1st intron) haplotype, with circle size corresponding to haplotype frequency. Rectangles represent the inferred ancestral haplotype or the haplotype with the highest outgroup probability according to the TCS analyses. Each branch, in spite of length, represents one mutational difference between haplotypes, with black circles indicating unsampled (i.e., missing) haplotypes. Colors represent locality of haplotype, Red = Rupununi and Blue =Takutu.

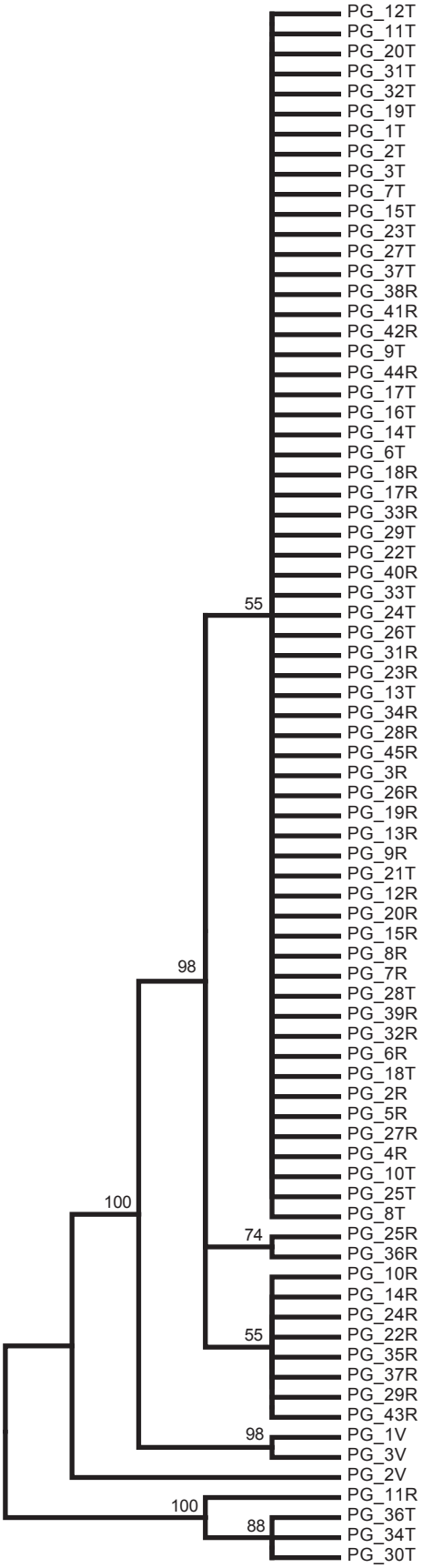

Figure 2.10a. Maximum likelihood (ML) tree based on 660 bp aligned sites of COI for 78 taxa of *Potamorrhaphis guianensis*. Number on nodes indicate bootstrap support values. PG, *Potamorrhaphis guianensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

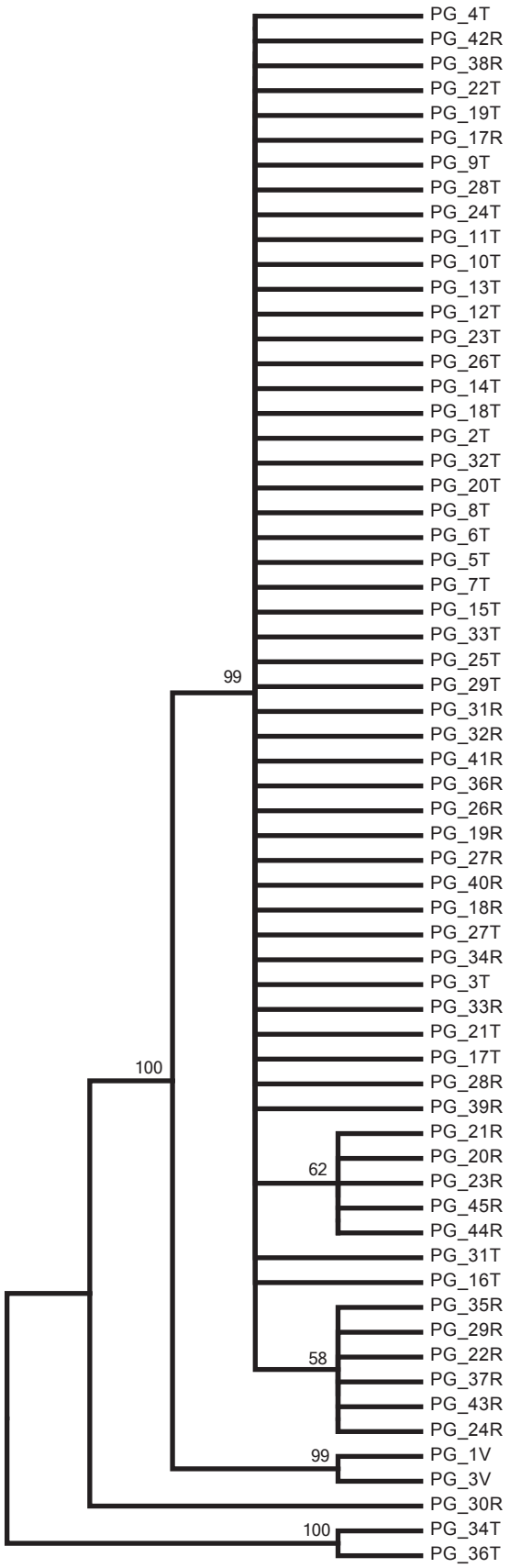

Figure 2.10b. Maximum likelihood (ML) tree based on 777 bp aligned sites of Cyt b for 63 taxa of *Potamorrhaphis guianensis*. Number on nodes indicate bootstrap support values. PG, *Potamorrhaphis guianensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

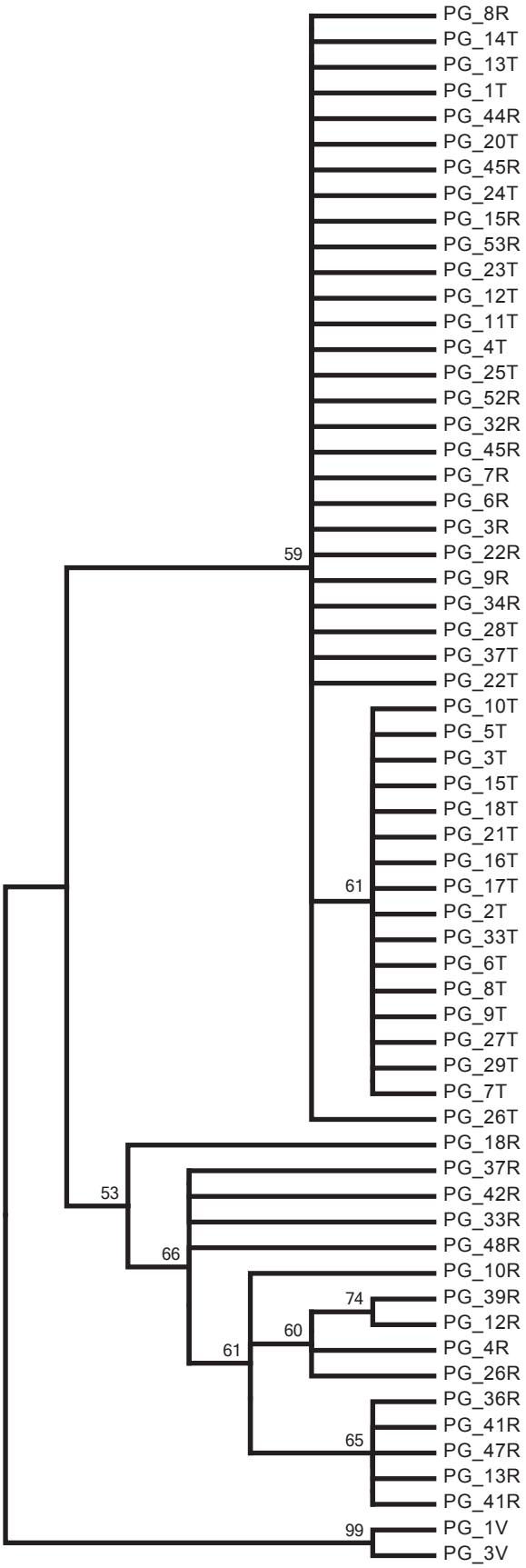

Figure 2.10c. Maximum likelihood (ML) tree based on 746 bp aligned sites of S7 (1st intron) for 63 taxa of *Potamorrhaphis guianensis*. Number on nodes indicate bootstrap support values. PG, *Potamorrhaphis guianensis*; Number, identification #; R, T or V, Rupununi, Takutu or Venezuela drainage.

CHAPTER 3 – Loricariidae phylogeny predicts a pre-Pleistocene break up of the proto-Berbice

INTRODUCTION

Prior to recent advances in understanding Neotropical diversification, a common perception supported hypotheses of major diversification events occurring during the climatic fluctuations of the Pleistocene. Studies beginning two decades ago (Weitzman & Weitzman 1982; Vari 1988, 1989a,b; Vari & Weitzman 1990) began to dispel these hypotheses, suggesting that important diversifications of Neotropical fishes predated the Pleistocene. Currently, the consensus among Neotropical ichthyologists is the great antiquity of the major lineages of Neotropical fishes (Lundberg 1998; Lundberg and Aguilera 2003; Lundberg 2005; Malabarba et al. 1998; M. Malabarba and Malabarba, 2008, 2010; Reis et al. 2003; Sabaj-Perez et al. 2007; Vari 1988; Weitzman and Weitzman 1982). Additionally, recent advances in the earth sciences of Amazonia have shed new light on understanding the temporal context and paleographic circumstances for diversification of Neotropical fishes (Lundberg et. al 1998; Hoorn and Wesselingh 2010). These include geological data on paleoclimates, paleoenvironments, discovery of new fossils, and the relatively recent (Miocene) time frame for the modern day assembly of the Amazon Basin (Hoorn et. al 1995, Räsänen and Linna 1996, Lundberg et. al 1998, Lundberg 2005). Therefore, the present-day configuration of the major river drainages in South America are based on major historical events associated with the tectonic evolution of the Amazon Basin.

The South American platform, of Gondwanan origin, is the largest geological feature of the continent. Embedded within this platform are two large areas of exposed Precambrian crystalline igneous and metamorphic rocks, the Guianan and Brazilian shields. Shields are

ancient and tectonically stable portions of the continental crust that have endured the dynamics of tectonic events forming and dividing continents and supercontinents for at least 500 million years. This is in contrast to regions of recent geological origin that are subject to subsidence, uplift, downwarping, and rifting (Almeida et. al 2000). The highland regions of the shields contain a distinct ichthyofauna, which is attributed to a complex association of ancient history and ecological constraints. Studies suggest that these highland regions have acted as evolutionary museums for species during the marine incursions of the interglacial periods (Nores 1999, 2004). The subsequent regression of the seawater allowed for dispersal of fish through the lowlands. The expansion and contraction of habitat available in the lowlands surrounding these highlands seems to have contributed to the diversification of many taxa.

In the lowlands between the western and eastern Guiana shield is a depression referred to as the Takutu graben. This is a sediment-filled ENE-WSW trending rift that extends 280km long and 40km wide at the border of Brazil and Guyana, centered over the town of Lethem, Guyana (Hammond, 2005). A large endorheic lake, Lake Maracanata, filled the graben approximately 100m deep during the early Cretaceous then by the Paleogene began transitioning to a fluvial system (Crawford *et al*., 1985). This fluvial system became the main-stem of the proto-Berbice, which during the majority of the Cenozoic, was a large northeast flowing river that drained most of the central Guiana Shield, draining into the Atlantic somewhere between present day towns of New Amsterdam, Guyana and Nickerie, Suriname (McConnell, 1959) (Figure 3.1). A series of stream-capture events by the Rio Branco shifted the drainage patterns of the upper proto-Berbice. Initially the Branco captured the Cotinga and Uraricoera then subsequently capturing the Takutu and Ireng Rivers (Crawford *et al*., 1985; Gibbs & Barron, 1993). The lower proto-Berbice shifted away from present day Berbice joining the Essequibo River as evidenced by the sharp

elbow curve north of the confluence with the Rupununi River. Subsequent to this break up was the relatively recent development of the Rupununi portal in this ancient corridor. The Rupununi portal is a seasonal connection of the Essequibo River drainage and Amazon River drainage via the inundation of the Rupununi savannas.

Aging the final break-up of the proto-Berbice is important in understanding speciation in the region, in addition to testing the hypothesis of diversification events occurring in the Pleistocene. No study has attempted to assess the timing of this historical event. Species cognates and significant community differences in the Essequibo and Branco basins suggest that the final break-up of the proto-Berbice occured a long time ago (chapter 1), with subsequent formation of the Rupununi Portal dated to about 100,000 years ago (Turner et al. 2004). It is clear that some taxa are able to move across the portal (Chapter 2), and thus would obscure any genetic patterns that were left by the breakup of the proto-Berbice. Although this could be true for taxa able to disperse across the corridor (i.e. prochilodontids in Turner et. al 2004), it is likely the genetic signal of the proto-Berbice breakup might be detected in species cognates across the portal or taxa unable to cross. For instance, *Peckoltia braueri* and *P. cavatica* are sister species found only in drainages on either side of the portal. These sister taxa are typically found in swift-flowing, lotic habitats and are unlikely to disperse across the lentic conditions of the flooded savanna. Their divergence could be explained by the proto-Berbice break up splitting and isolating the ancestral populations. A large, ancient river system having shifted its drainage pattern certainly had consequences on aquatic taxa, likely influencing speciation, dispersal and extinction of lineages. Therefore, accurate timing of this historical event has profound implications to evolutionary processes that govern the diversification of Neotropical biota;

further allowing a better understanding of the distributional patterns of the Guiana shield flora and fauna.

The objective of this study is to date the break up of the proto-Berbice utilizing a Bayesian relaxed molecular clock on a phylogeny of the Loricariidae (with emphasis on Ancistrini) based on cytochrome *b*. Loricariidae is a good group to use to age the final break-up of the proto-Berbice because of the sister species pair across the divide, previously discussed (*Peckoltia cavatica* in the Essequibo and *P. braueri* in the Amazon; (Armbruster, 2008; Armbruster & Werneke, 2005), and the great diversity of the family in the region. The dataset includes two species pairs that were split due to the rise of the Andes that provide calibration points for the molecular clock. *Chaetostoma anomalum* and a very similar, undescribed species occur on either side of the Andes and were presumably separated at the rise of the Merida Andes ~ 8 MYA (Albert *et al.*, 2006; Hardman & Lundberg, 2006; Hoorn *et al.*, 1995). *Hemiancistrus maracaiboensis* was presumably split from *He. aspidolepis* with the rise of the Sierra de Perijá ~ 11.8 MYA (Hardman & Lundberg, 2006). These dates were used as calibration points to estimate divergence times.

MATERIALS AND METHODS

Taxon sampling, DNA data and Phylogenetic reconstructions

A large reference data set for cytochrome *b* has been established for the Loricariidae. The ingroup includes sequence data from the subfamily Hypostominae with focus on tribes Ancistrini and Hypostomini. Members of two related catfish families were used as outgroups (Callichthyidae and Astroblepidae). Total genomic DNA was extracted from muscle tissue using the method described by Coffroth et. al (1992). The template was utilized to amplify ~1100bp

fragment of the mitochondrial cytochrome *b* gene. Polymerase chain reaction (PCR) was conducted in 25 μL volumes containing ~10-30ng of template DNA, 10mM Tris-HCl (pH 8.3), 2.0mM KCl, 200μM dNTPs and 0.4μM each of primers Glu-2 (5'-AACCACCGTTGTTATTCAACTA-3') and Pro-R1 (5'-TAGTTTAGTTTAGAATTCTGGCTTTGG-3') (Hardman 2005), and 1 U *Taq* DNA polymerase. PCRs were conducted in a PTC-100™ thermocycler (MJ Research) under the following conditions: initial denaturing step of 94° C for 3 min, 34 cycles of 94° C for 30s, 45° C for 30s, 72° C for 45s and a final extension of 72° C for 5 min. Amplifications were visualized via electrophoresing 3μL of PCR product in a 1% agarose gel. Amplified products were sequenced by High-Throughput Genomics Unit (HTGU) genomics facility at the University of Washington. All sequences were aligned using SEQUENCHER 4.1.4 (Gene Codes Corporation, Ann Arbor, MI).

Phylogenetic reconstructions were performed using Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI) criteria. The MP reconstruction was conducted in PAUP* v. 4.0b10 (Swofford 2003) via heuristic searches using 1000 replicates with random addition sequences and tree-bisection-reconnection (TBR) branch-swapping algorithm. Clade support was evaluated with 1,000 pseudoreplicates of non-parametric bootstrapping using random addition of sequences (10 replicates) and TBR. For ML and BI, the best-fit models of sequence evolution was estimated using the Akaike information criterion (AIC) in JModelTest version 0.1.1 (Posada 2008). The model selected was GTR + I + Γ sequence evolution. ML analyses were conducted in programs Garli v. 0.951 and RAxML v. 7.04 via the CIPRES portal v. 1.13 under default settings. Garli and RAxML searches were performed from several

randomly starting seeds to ensure convergence of likelihood scores. ML nodal support was evaluated in RAxML using the rapid bootstrapping algorithm.

BI analyses were performed using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003) using four chains, one cold and three incrementally heated. Markov chain Monte Carlo (MCMC) analyses were conducted to run for at least 6.0 x 10^6 generations, sampling trees every 100 generations. The first 25% of trees (15,000 trees) sampled in each MCMC run were discarded as burn-in. Marginal probabilities of summary parameters, consensus phylograms, and posterior probabilities of nodes were estimated from post burn-in samples of the independent runs combined.

Divergence time estimations

The protein-coding gene, Cytochrome *b*, dataset for loricariids was used for chronological estimations. Relative rates test based on likelihood using software r8s version 1.71 (Sanderson, 2003) suggested a significant departure from clock-like behavior. To estimate timing of divergence events without assuming a strict molecular clock, estimations were performed using a Bayesian relaxed clock analysis as implemented in BEAST v. 1.6.1 (Drummond & Rambaut, 2007). Currently, few loricariid fossils are known, and most are not easily identifiable (Malabarba and Lundberg, 2007). Therefore, biogeographic calibration points were used to place priors on the age of nodes within the tree. These calibration points include: the rise of the Merida Andes (~ 8 mya; (Albert *et al.*, 2006; Hoorn *et al.*, 1995) that is hypothesized as the vicariant event leading to divergence of *Chaetostoma anomalum* and a very similar, undescribed species occur on either side of the Andes; the rise of the Sierra de Perija (~ 11.8 Mya; Hardman & Lundberg, 2006) which is hypothesized to have led to the divergence of *Hemiancistrus maracaiboensis* that was split from *He. aspidolepis*. The split between the

Loricariidae and Astroblepidae, estimated from previous work (Lundberg et. al 2007), was set as the maximum calibration point ~ 90 Mya (Table 3.1).

The BEAST XML file was generated in BEAUti v.1.6.1 (Drummond & Rambaut 2007). The substitution model was GTR + I + Γ (as selected by AIC) with bases frequencies estimated empirically, using three partitions (separate codon positions). All parameters were unlinked and substitution rate unfixed utilizing a relaxed uncorrelated lognormal clock. The starting tree used in the analysis was the topology recovered from the ML and BI reconstructions. The tree prior assigned was the Yule prior, that assumes a constant rate of speciation and most appropriate for species-level phylogenies (Drummond et. al 2007). The calibration nodes were constrained using normal distributions, which is most appropriate when applying a biogeographical date or secondary calibration (a node age estimate from a previous study) (Drummond et. al 2007). The tree root was set to 140, a conservative assignment based on previous work on divergences estimates in Siluriformes (Lundberg et. al 2007). Other priors and operators were set to their default settings. Three consecutive MCMC analyses were run for 20,000,000 generations, sampling trees every 10^3 generations. MCMC log files were combined in TRACER v. 1.5 and LOGCOMBINER v. 1.6.1 to verify that the three runs converged on the posterior distributions and reached stationarity, the first 25% were discarded as burn-in. TREEANNOTATOR v. 1.6.1 was used to identify the topology with the best support calculating the posterior probabilities and 95% HPD (highest posterior density limits) intervals for node-specific parameters. The effective sample size (ESS) values were all greater than 200, indicating good mixing during MCMC runs. To examine whether the priors were having a strong effect on the posterior divergence estimates, a BEAST XML file was generated in BEAUti v.1.6.1 with an empty alignment, running the MCMC

analysis sampling only from the priors. The analysis indicated priors placed on the parameters did not overwhelm the signal in the data.

A final method that employs a more general approach for estimating divergence was conducted by direct translation of genetic distances into time based on reported molecular clock rates in catfishes using protein-coding genes. For cyt *b*, it is estimated 0.5-0.8%/my/lineage in pimelodid fishes (Hardman & Lundberg, 2006). Genetic distances were estimated under the Kimura two-parameter model in MEGA5 (Tamura et. al 2011).

RESULTS

Data sequence characteristics and Phylogenetic inference

The final alignment of protein coding gene cytochrome *b* dataset included 969 bp from 134 taxa. There were no ambiguous positions within the chromatograms. Furthermore, frameshifts or stop codons were absent following translation into amino acids, suggesting these sequences are not likely pseudogenes. The primary goal for reconstructing the phylogeny of loricariid taxa based on cytochrome b, is to recover lower level relationships. Thus, allowing us to estimate divergence times of clades found across the portal. Specific clades of interest include: *Peckoltia cavatica* and *P. braueri*, *Ancistrus* sp. (Takutu vs. Rupununi), *Peckoltia sabaji* (Venezuela) and *P. sabaji* (Guyana), *Chaetostoma anomalum* and *C.* sp LMar, *Hemiancistrus maracaiboensis* and *H. aspidolepis*.

All reconstructions recovered the same topology and strong support at cladal nodes of interest. There was low resolution at deeper nodes, which is not unexpected with a mitochondrial marker. Both ML and BI analyses were run under GTR+I+Γ model, as selected by AIC. Three independent ML analyses resulted in a single tree with similar optimal scores (best

lnL -22584.59), rapid bootstraps were terminated at 1000 runs (Figure 3.2). A majority rule (50%) consensus tree recovered in the BI analysis after 15,000 post burn-in (mean lnL -21766.72) (Figure 3.3). The MP search resulted in 35 most parsimonious trees, consensus tree of 5259 steps with consistency index (CI) of 0.164 and retention index (RI) of 0.657 (Figure 3.4). These analyses consistently had well resolved relationships at the interspecific level, but low-support at some generic and most family level clades. This is expected with the use of a mitochondrial marker, but suitable for measuring more recent divergence events. These deeper relationships are beyond the scope of this study and will be addressed in future work.

Divergence time estimations

Node A, B, and C in Figure 4 denotes clades, sister species *Peckoltia cavatica* and *Peckoltia braueri* (node A), *Peckoltia sabaji* (Venezuela) and *Peckoltia sabaji* (Guyana) (node B) and *Ancistrus* sp. (Takutu) and *Ancistrus* sp. (Rupununi) (node C) with divergence across the Rupununi portal. The BEAST analysis resulted in high effective sample sizes (ESS) for all parameters and the three runs converged on the posterior distributions and reached stationarity. Maximum clade credibility trees for the independent runs were identical in topology. Estimated divergence times for nodes A, B and C were 4.3 mya (2.1-6.5 mya, 95% high posterior density interval), 5.3 mya (2.3-8.6 mya, 95% HPD interval) and 8.9 mya (4.8-13.5 mya, 95% HPD interval) respectively, all supported an initial Pliocene diversification (Figure 3.5).

Divergence ages were also estimated by a direct translation of genetic distances into time based on reported molecular clock rates in fishes using cytochrome *b* (0.5-0.8%/my/lineage in catfish; Hardman & Lundberg 2006). This rate was applied to the *p* genetic distance between

clades and resulted in averaged estimates of 4.2-6.7 mya, again supporting a Pliocene diversification of clades across the portal.

DISCUSSION

Divergence estimates using two independent approaches indicate that the break up of the proto-Berbice occurred during the Pliocene, providing further evidence of important diversification events occurring prior to the Pleistocene. This vicariant event offers a strong explanation for the prevalence of species cognates and the significant community differences found on either side of the portal (Chapter 1). This is the first study attempting to date the fragmentation of the proto-Berbice and correlate this drainage modification to speciation events. The influence of shifting ancient river drainages on fish diversification has been well documented in North America (Mayden 1988, Burr & Page 1986, Hocutt et. al 1986). For example, the Mississippi-Teays River is an ancient river system that drained a once extensive highland province of central North America and present distributions of Central Highland fishes are the result of a series of geological events that fragmented it (Mayden 1988). The role of the Mississippi-Teays break up on North American ichthyofauna is not unlike the potential influence of the proto-Berbice on Guiana shield fish diversification.

The extent of the proto-Berbice basin encompassed many of the paleofluvial headwaters of the modern day drainages in the Guiana shield, including headwaters of the Branco River, Uraricoera River, Takutu River, Ireng River, Essequibo River, Potaro River, Cuyuni River, Corentyne River and Orinoco tributaries: Caroni River, Caura River and Erebato River. The large area encompassed by the proto-Berbice would allow for a broad distribution of shield endemics or shield specialists typically found in rheophilic habitats. During the early Cenozoic

(prior to Andean uplift), the shield regions contained the highest concentrations of high gradient habitat available. Based on the phylogeny of the Loricarioidea, the Loricariidae likely had its origins in the fast-waters of the shield regions of South America.

Relictual fauna and disjunct distributions of several groups of loricariids could be explained by the prevalence of this ancient drainage and its broad extent. These include members of the genera *Lithoxus, Exastilithoxus, Neblinichthys,* and *Harttia*. *Lithoxus jantjae* is found in the upper Ventuari (Orinoco), while its nearest neighbor, *L. lithoides,* is found in the Essequibo, upper Branco, and Trombetas in the east (Lujan 2008). Sister to *Lithoxus*, *Exastilithoxus* is represented by *E. hoedemani* in the Marauiá River (upper Negro), and in the east by *E. fimbriatus* in the Caroni. *Neblinichthys* is represented by *N. pilosus* in the Baria River (lower Casiquiare), *N. roraima* and *N. yaravi* in the tributaries of the upper Caroni River, and *N. brevibracchium* and *N. echinasus* in tributaries of the Upper Mazaruni (Essequibo) (Taphorn et al., 2010). *Harttia* is represented in the upper Caura and upper Ventuari Rivers by *H. merevari* and to the east in the Essequibo River by *H. platystoma* (Lujan & Armbruster 2011; Taphorn et al., 2010).

A taxon of particular fascination found in the Guianan shield is *Lithogenes*. The three species within this genus have disjunct distributions, *Lithogenes villosus* is in the Potaro-Essequibo, *L. wahari* is in the Cuao-Orinoco and *L. valencia* is likely from Lago Valencia in northern Venezuela (the species is thought to be extinct; Provenzano et. al 2003). Its relationship within Loricarioidea is debated, and *Lithogenes* may be sister to loricariids (Shaeffer 2003a), or astroblepids (Armbruster 2004, 2008; Hardman, 2005). Both of these hypotheses confirm the antiquity of this genus, sister to astroblepids would suggest that this lineage arose ~20 mya and

sister to loricariids ~65-70 mya (Lundberg 2007). There are also shield specialists like some species of *Pseudancistrus* and *Pseudacanthicus* with ranges that traverse the proto-Berbice basin.

The upper Pliocene was marked by a period of global cooling and aridity resulting in an expansion of the coastal floodplains and lowland savannas. The Neotropics was experiencing the 8th marine regression (Albert et. al 2011). The opening of the vast lowlands during this period would provide new habitat for organisms that have been confined to the uplands during the marine incursions. The museum hypothesis has taken on many different definitions, but at its inception (Stebbins 1974) was the idea that, during the marine highstands, the uplands functioned as museums harboring taxa and preventing lineages from extinction, while the lowlands functioned as cradles promoting speciation. The reduction of extinction over long periods of time is central to Stebbins (1974) hypothesis. Once the marine waters regressed, taxa invaded the lowlands and occupied new niches. This dispersal served as a diversifying agent among many taxonomic groups. For loricariids with a suggested shield origin, this is a likely scenario explaining diversification into the lowlands.

The uniqueness of the Guiana shield ichthyofauna can be seen in the level of the region's endemism. In particular, the eastern Guiana shield that encompassed many of the headwaters of the *proto*-Berbice has among the highest numbers of endemic fish on the continent, at 126-206 species (Albert et. al 2011). Its neighboring ecoregion, which would have included the western tributaries of the proto-Berbice, has substantially fewer endemics, 26-75 species (Albert et. al 2011). The border of the two ecoregions align with the ancient corridor of the proto-Berbice. The separation of the two main ecoregions in the Guiana shield by an ancient river channel could have resulted in the relative isolation of fauna in drainages of the eastern Guiana shield and in concert with regional dynamics of geological activity (tilting, uplift, subsidence, etc.) promoted

diversification processes in fishes. River systems have been suggested as important barriers to gene flow for both terrestrial and aquatic taxa (Colwell 2000; Gascon et. al 1998, 2000; Hayes and Sewlal 2004). Additionally, eastern Guiana shield ecoregion rivers in the past and present have no hydrological connections to large river systems like the western Guiana shield ecoregion, which has hydrological connections to the Amazon and Orinoco River drainages, the two largest river systems in South America. The connection to a larger drainage basin can facilitate dispersal, while smaller drainages in isolation would likely promote diversification. The unique phylogeographic patterns in the eastern Guiana shield have been demonstrated by various taxa including frogs (Noonan & Gaucher 2005, 2006), snakes (Wüster et. al 2005), mammals (Steiner & Catzeflis 2004) and fish (Cardoso & Montoya-Burgos 2009) supporting an independent trajectory of evolutionary processes.

In conjunction with paleogeological events shifting river drainages between eastern and western Guiana shields is the recent development of the Rupununi portal (RP). The seasonal connection at the RP brings additional complexity to understanding the processes influencing diversification, because this allows for a reconnection of portions of the ancient proto-Berbice. Therefore, two processes at work influencing fish diversity and distribution are vicariance of the proto-Berbice break-up that was complete in the Pliocene and/or recent dispersal via the RP (the role of these processes on gene flow in fish is discussed more thoroughly in chapter 2). The divergence estimates based on loricariids in this study allow us to elucidate patterns of diversification in aquatic taxa in rivers traversing the complex landscape of the Guiana shield. Although in order to confirm these findings, this pattern of a pre-Pleistocene vicariance hypothesis shaping aquatic taxa in the Guiana shield should be replicated in other aquatic organisms.

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Table 3.1 Calibration points used to estimate divergence time.

Calibration (node)	**Minimum time estimate**	**Reference**
Chaetostoma split (*Chaetostoma anomalum*, *Chaetostoma* sp.)	~8mya (Merida Andes)	Albert et. al 2006 Rodríguez-Olarte et. al 2011
Hemiancistrus split (*Hemiancistrus maracaiboensis*, *Hemiancistrus aspidolepis*)	~11.8mya (Sierra de Perija)	Albert et. al 2006 Rodríguez-Olarte et. al 2011
	Maximum time estimate	
Astroblepidae vs. Loricariidae	~90mya	Lundberg et al. 2007

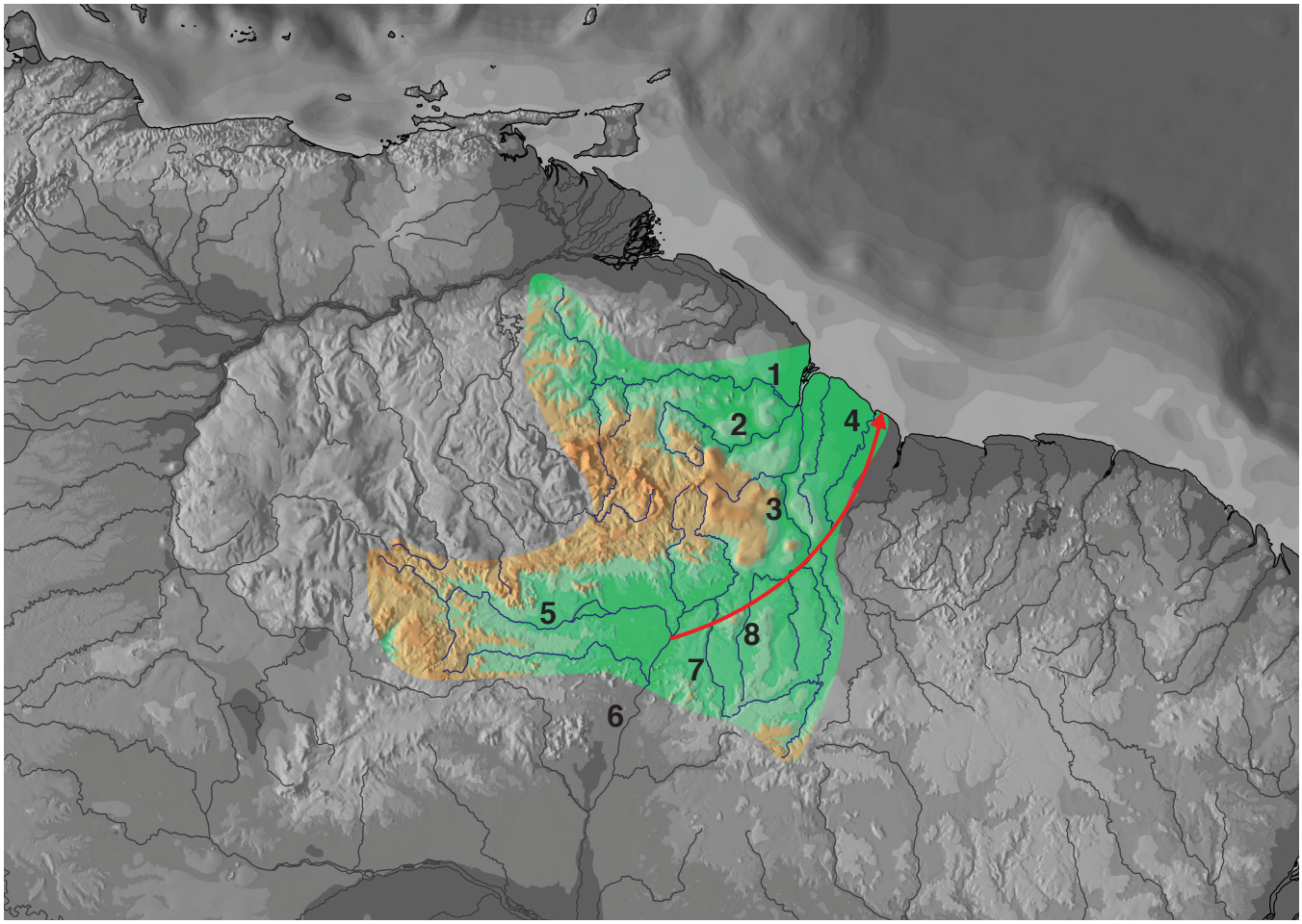


Figure 3.1. Map of the ancient proto-Berbice River basin (highlighted in green). Red arrow denotes the drainage course. 1- Cuyuni River; 2- Mazaruni River; 3- Essequibo River; 4- Berbice River; 5- Uraricoera River; 6- Branco River; 7- Takutu River; 8- Rupununi River.

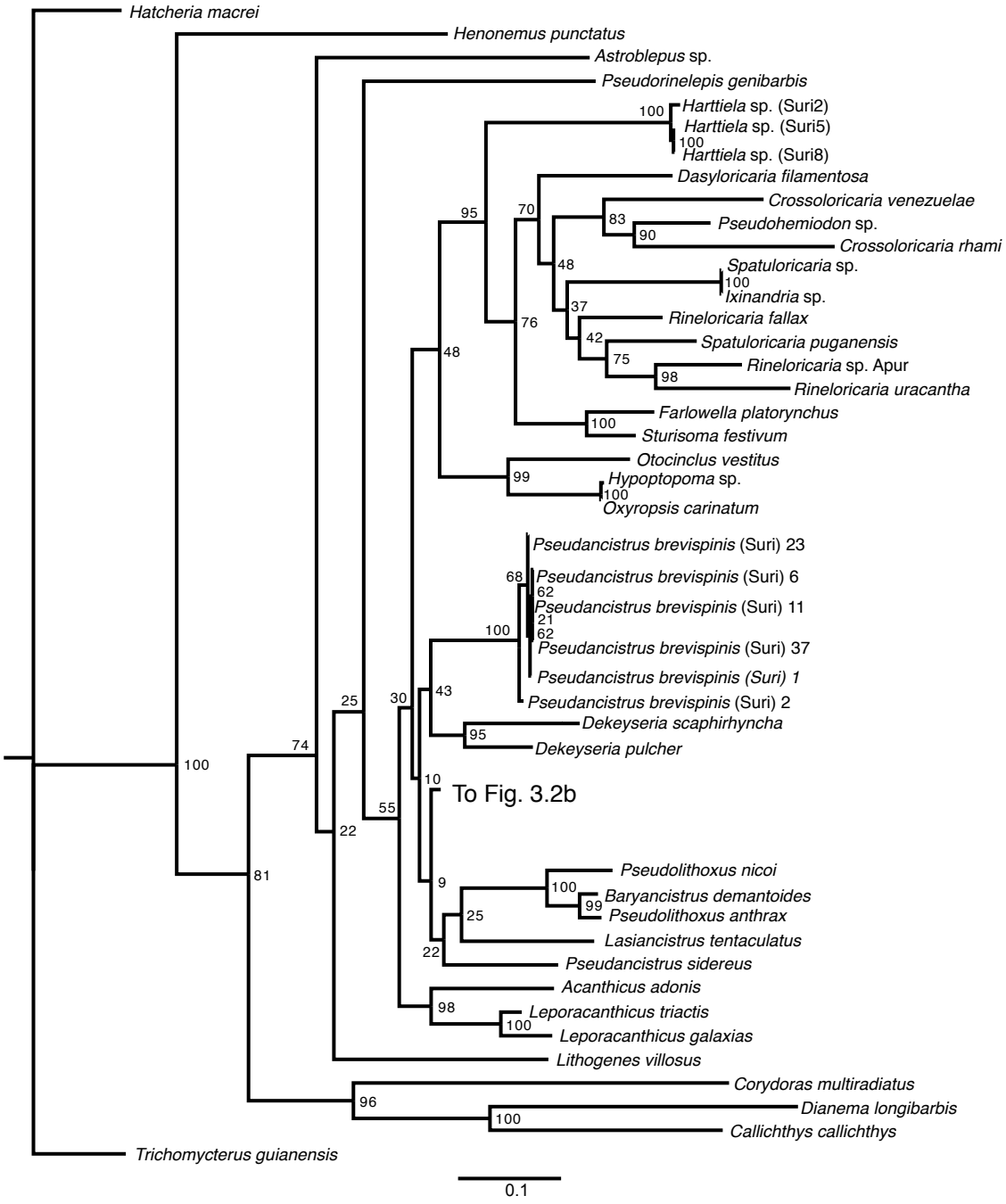


Figure 3.2a. Maximum-Likelihood (ML) tree based on model GTR+I+Γ as selected by AIC; number on nodes indicate bootstrap support values from RAxML analysis. Based on 969 aligned sites of cytochrome *b* for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

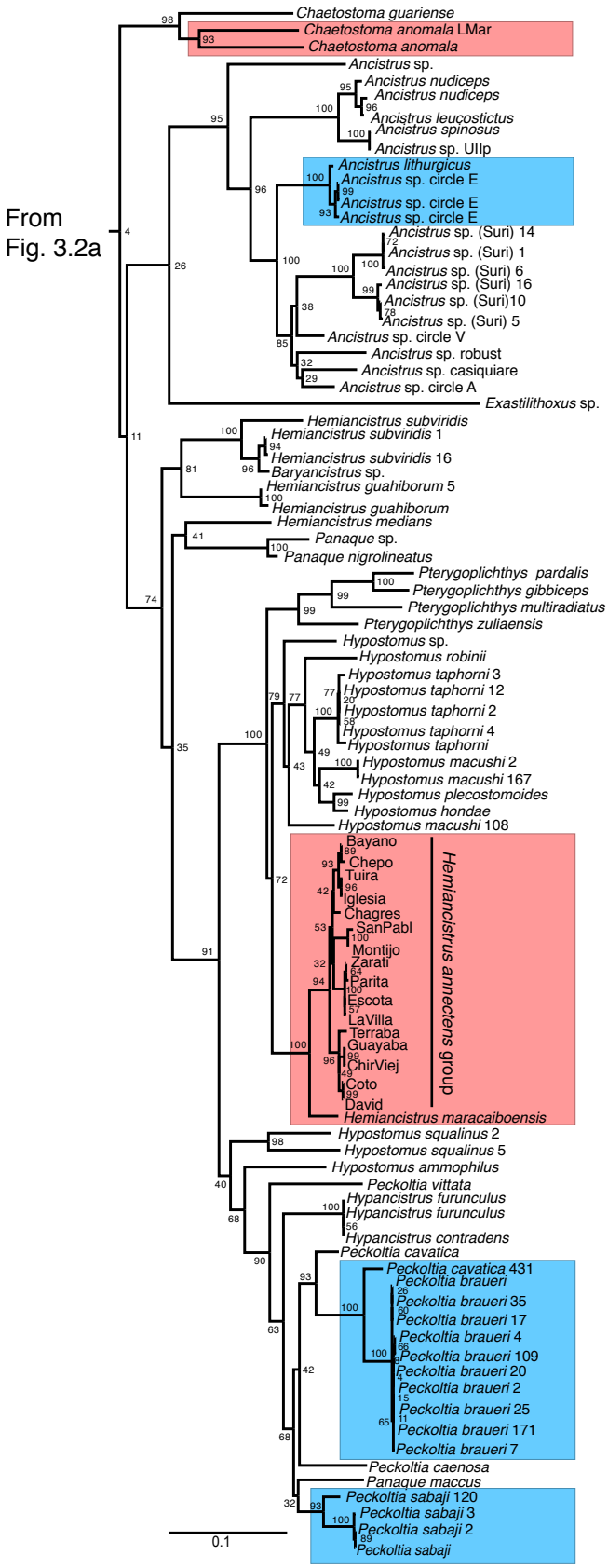


Figure 3.2b. Maximum-Likelihood (ML) tree based on model GTR+I+Γ as selected by AIC; number on nodes indicate bootstrap support values from RAxML analysis. Based on 969 aligned sites of cytochrome *b* for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

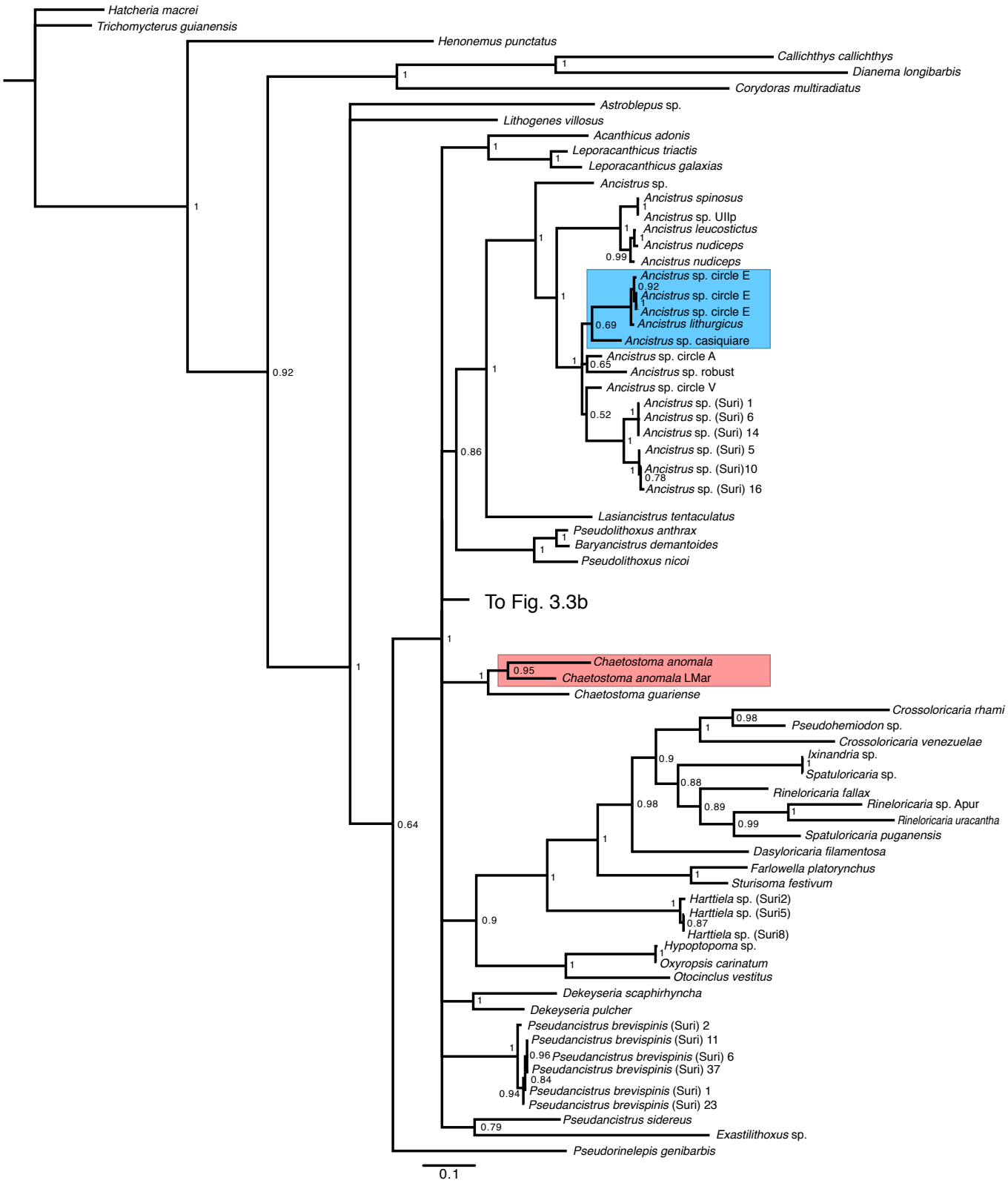


Figure 3.3a. 50% majority rule consensus phylogram resulting from Bayesian analysis of 78,319 post burn-in trees; numbers on nodes indicate posterior probabilities. Based on 969 aligned sites of cytochrome b for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

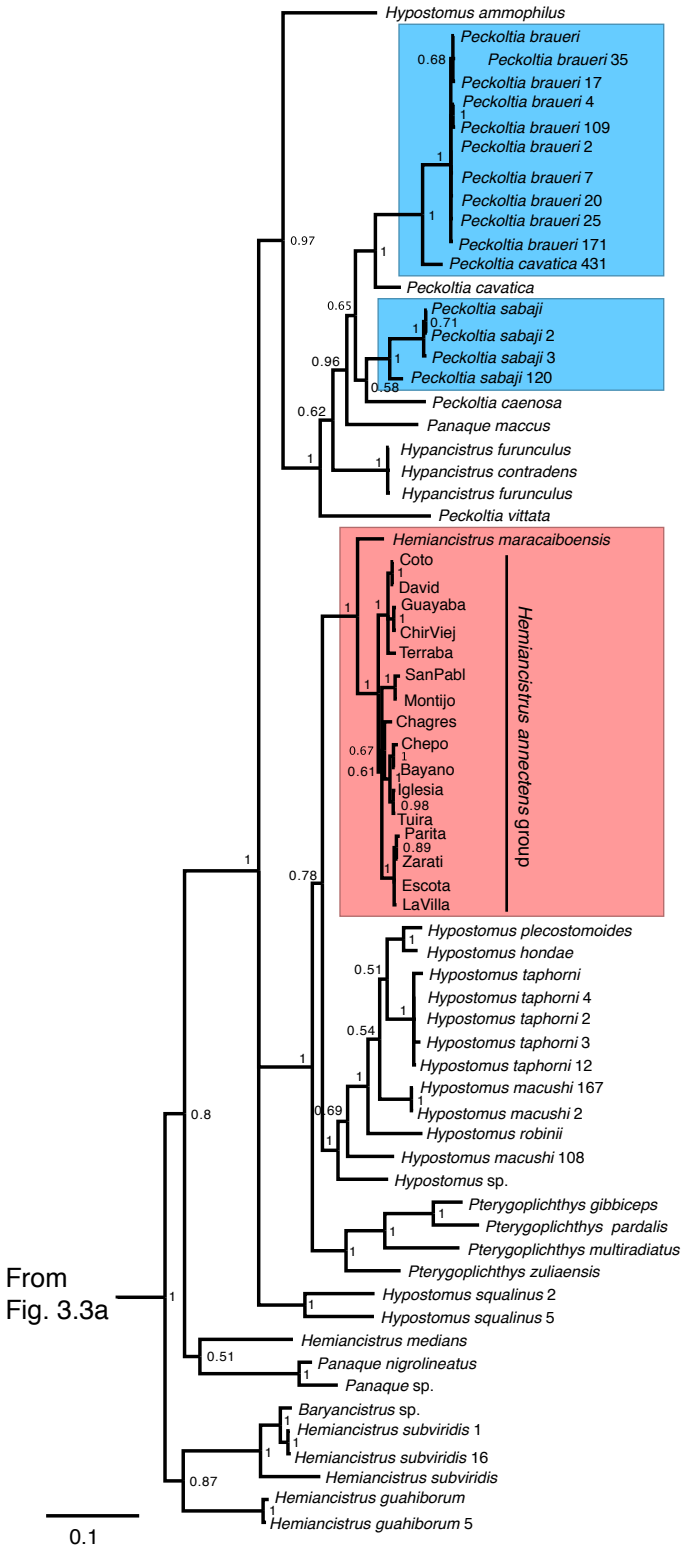


Figure 3.3b. 50% majority rule consensus phylogram resulting from Bayesian analysis of 78,319 post burn-in trees; numbers on nodes indicate posterior probabilities. Based on 969 aligned sites of cytochrome b for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

Figure 3.4a. Maximum parsimony (MP) strict consensus cladogram of 35 most parsimonious topologies of 5259 steps (CI=0.164, RI=0.657); numbers on nodes indicate bootstrap support values. Based on 969 aligned sites of cytochrome b for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

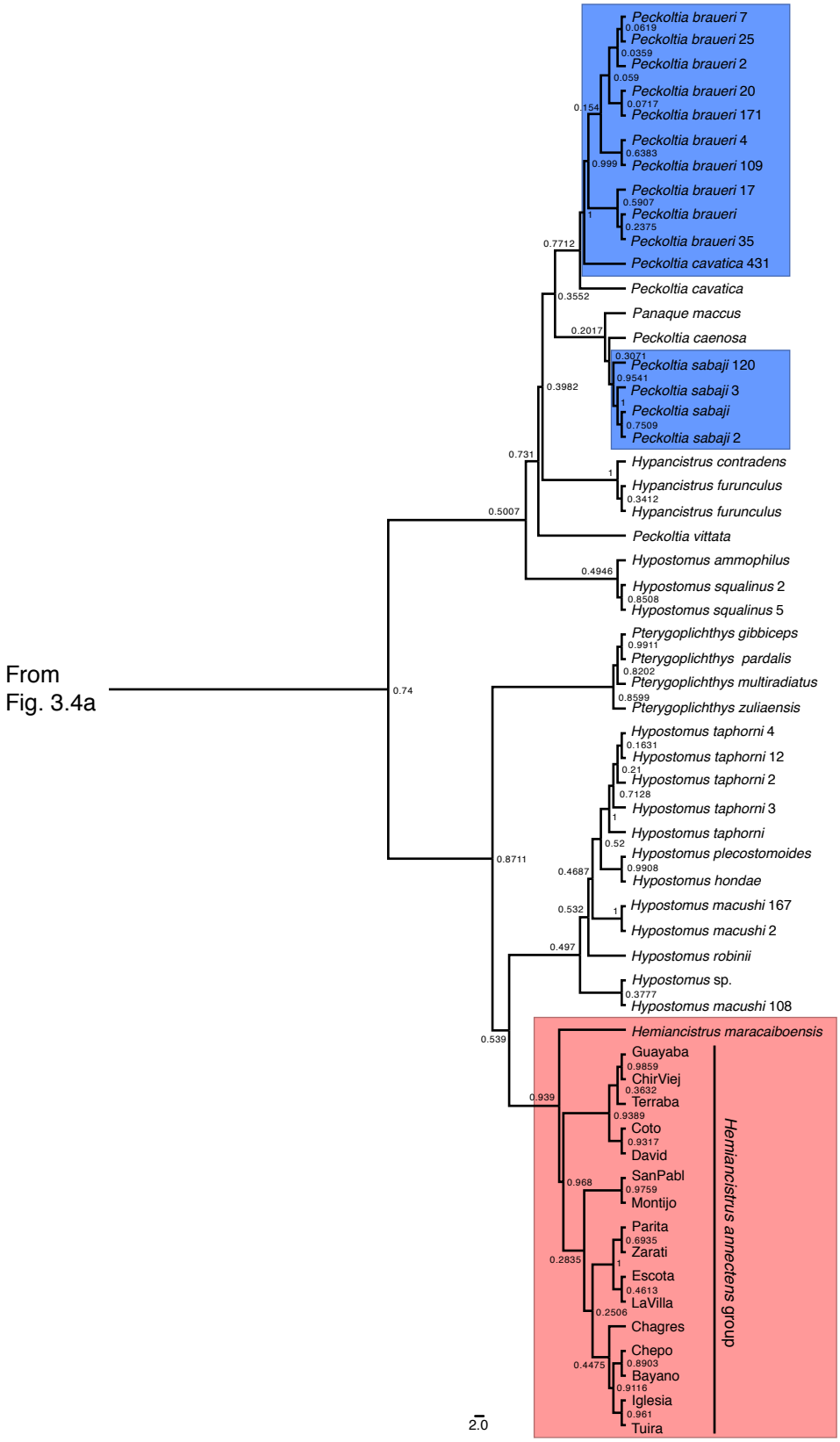


Figure 3.4b. Maximum parsimony (MP) strict consensus cladogram of 35 most parsimonious topologies of 5259 steps (CI=0.164, RI=0.657); numbers on nodes indicate bootstrap support values. Based on 969 aligned sites of cytochrome b for 134 taxa. Clades highlighted in red indicate clades used for calibration points and blue indicate clades of interest for divergence estimations.

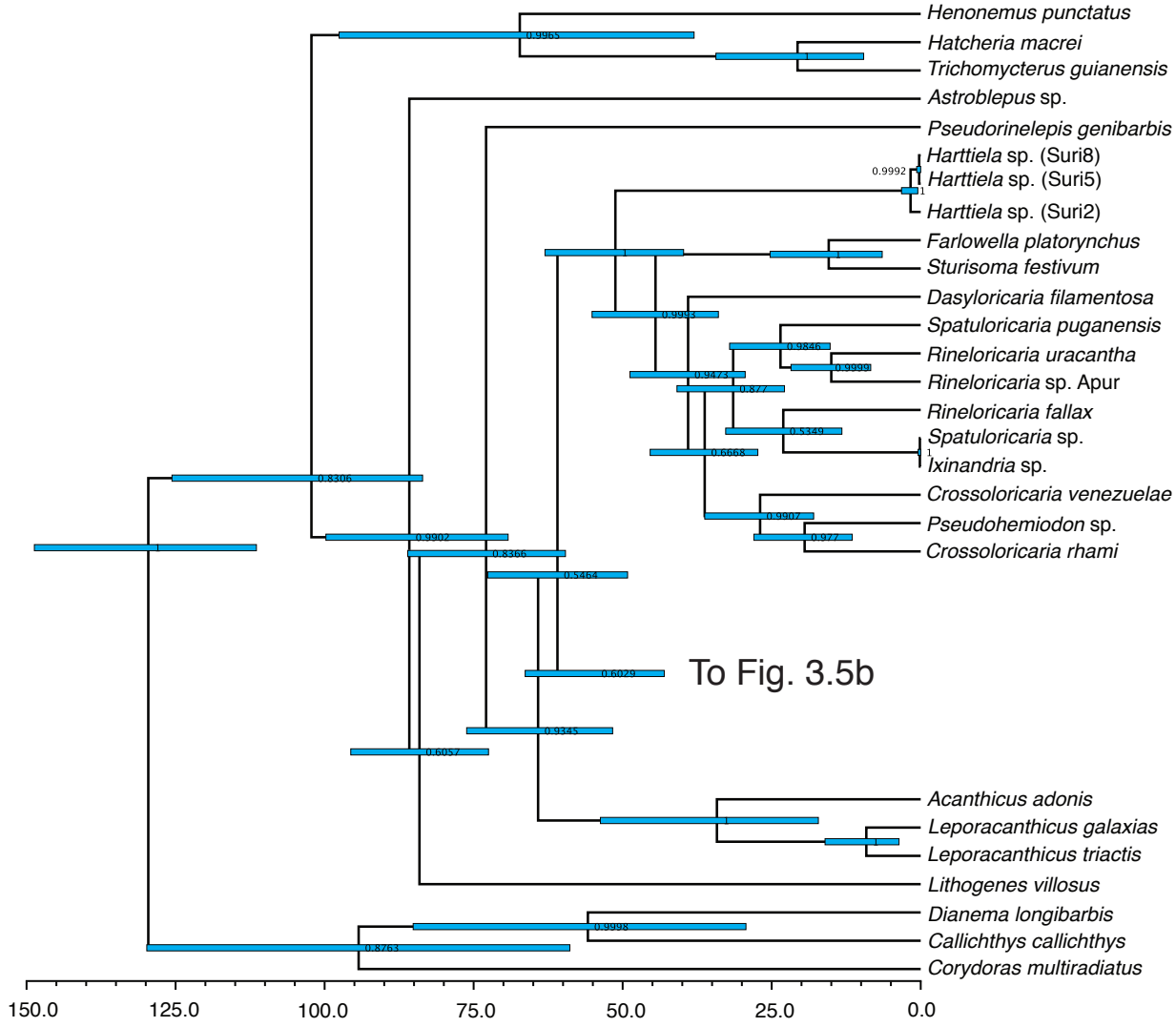


Figure 3.5a. Bayesian consensus tree illustrating divergence time estimations within Loricariidae across the portal. Divergence times were estimated from a cytochrome *b* dataset with four biogeographical calibration points (listed in Table 1). Labeled nodes (A-C) correspond to divergence dates of interest. Horizontal bars indicate 95% credibility intervals of joint prior and posterior estimate of divergence times.

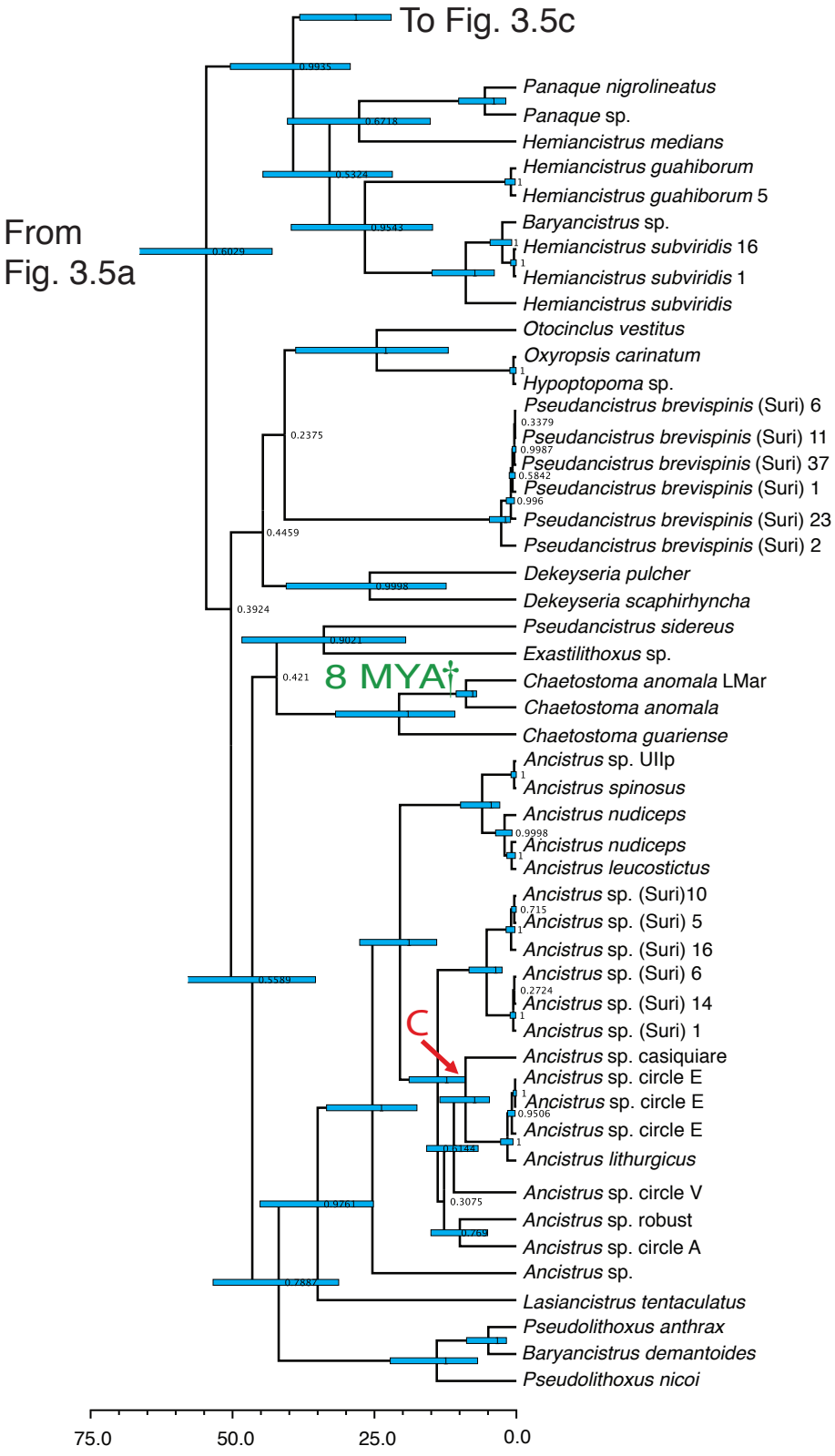


Figure 3.5b. Bayesian consensus tree illustrating divergence time estimations within Loricariidae across the portal. Divergence times were estimated from a cytochrome *b* dataset with four biogeographical calibration points (listed in Table 1). Labeled nodes (A-C) correspond to divergence dates of interest. Horizontal bars indicate 95% credibility intervals of joint prior and posterior estimate of divergence times.

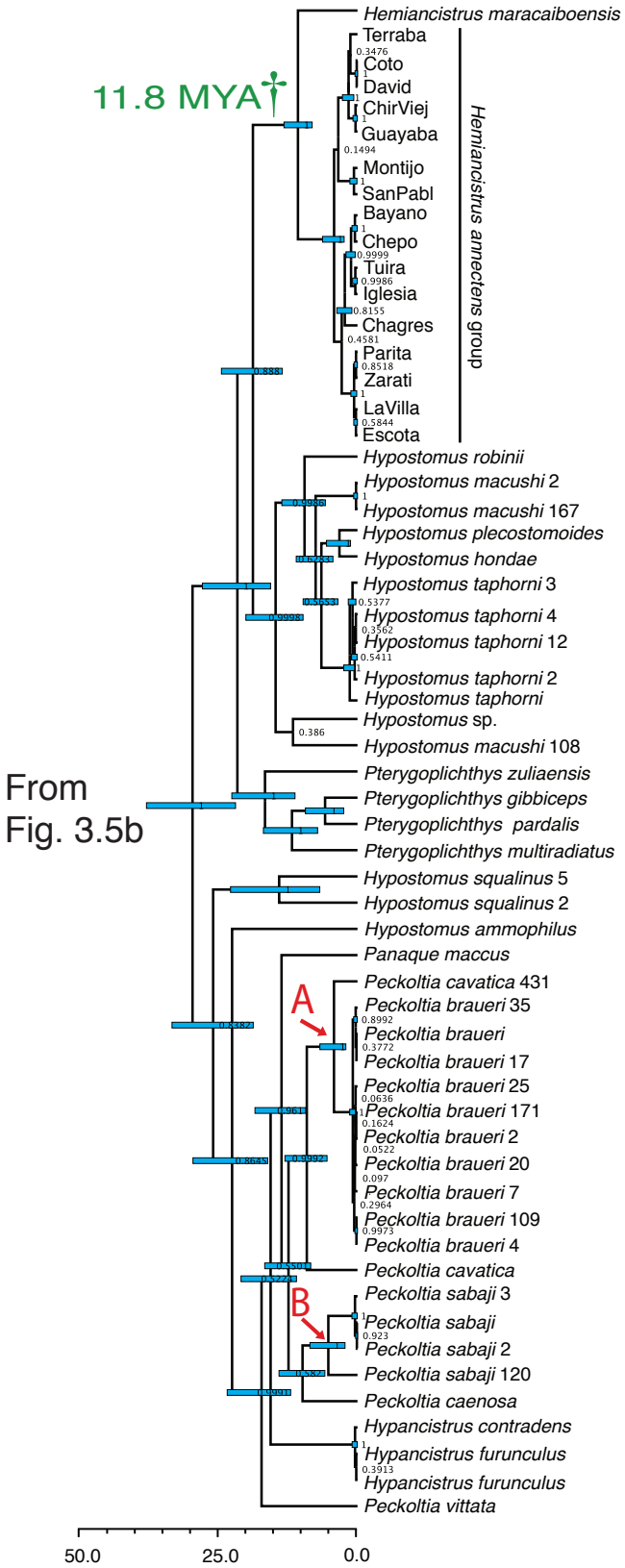


Figure 3.5c. Bayesian consensus tree illustrating divergence time estimations within Loricariidae across the portal. Divergence times were estimated from a cytochrome *b* dataset with four biogeographical calibration points (listed in Table 1). Labeled nodes (A-C) correspond to divergence dates of interest. Horizontal bars indicate 95% credibility intervals of joint prior and posterior estimate of divergence times.

Appendix 1. Data attributes for taxa in this study.

Hoplias malabaricus	**CO1**	**Cyt b**	**S7 (1st intron)**
# of individuals	53	49	22
# of haplotypes	10	13	11
# of basepairs	672	996	704
Ti/Tv			
Codon 1+2+3	R=1.94	R=2.26	R=0.78
Codon 1	R=5.58	R=2.41	R=0.70
Codon 2	R=2.04	R=2.22	R=0.69
Codon 3	R=22.03	R=14.20	R=0.97
Pairwise distance			
Overall	0.043	0.028	0.058
Between groups	0.046	0.031	0.068
Within groups	Takutu = 0.074 Rupununi = 0.003	Takutu = 0.050 Rupununi = 0.004	Takutu = 0.103 Rupununi = 0.016
Nucleotide divergence of clades in ML trees			
Rupununi vs Takutu	0.0946	0.1202	0.1883
RT vs Takutu	0.0913	0.0279	0.1908
RT vs Rupununi	0.0064	0.1202	0.0217

Potamorrhaphis guianensis	**CO1**	**Cyt b**	**S7 (1st intron)**
# of individuals	75	61	61
# of haplotypes	7	11	31
# of basepairs	660	777	746
Ti/Tv			
Codon 1+2+3	R=4.89	R=1.92	R=0.43
Codon 1	R=5.08	R=1.58	R=0.99
Codon 2	R=1.53	R=1.72	R=0.25
Codon 3	R=18.82	R=29.10	R=0.85
Pairwise distance			
Overall	0.022	0.024	0.003
Between groups	0.023	0.023	0.004
Within groups	Takutu = 0.035 Rupununi = 0.011	Takutu = 0.030 Rupununi = 0.017	Takutu = 0.001 Rupununi = 0.003
Nucleotide divergence of clades in ML trees			
Rupununi vs Takutu	0.116	0.139	0.0048
RT vs Takutu	0.114	0.109	0.0008
RT vs Rupununi	0.056	0.0465	0.0039

Geophagus surinamensis	**CO1**	**Cyt b**	**S7 (1st intron)**
# of individuals	45	51	58
# of haplotypes	13	11	9
# of basepairs	678	1047	604
Ti/Tv			
Codon 1+2+3	R=3.66	R=2.49	R=1.103
Codon 1	R=3.02	R=1.36	R=1.927
Codon 2	R=3.47	R=2.37	R=6.128
Codon 3	R=37.80	R=35.58	R=1.086
Pairwise distance			
Overall	0.006	0.051	0.037
Between groups	0.061	0.058	0.039
Within groups	Takutu = 0.071 Rupununi = 0.037	Takutu = 0.082 Rupununi = 0.002	Takutu = 0.030 Rupununi = 0.041
Nucleotide divergence of clades in ML trees			
Rupununi vs Takutu	0.1009	0.0597	0.0879
RT vs Takutu	0.1237	0.0573	0.0622
RT vs Rupununi	0.1908	0.0045	0.0659

Hypostomus squalinus	**CO1**	**Cyt b**	**S7 (1st intron)**
# of individuals	64	63	26
# of haplotypes	6	6	9
# of basepairs	673	1052	623
Ti/Tv			
Codon 1+2+3	R=2.22	R=4.91	R=1.266
Codon 1	R=0.67	R=4.59	R=0.437
Codon 2	R=1.34	R=2.76	R=0.395
Codon 3	R=5.07	R=24.91	R=0.210
Pairwise distance			
Overall	0.002	0.002	0.002
Between groups	0.003	0	0.002
Within groups	Takutu = 0.001 Rupununi = 0	Takutu = 0 Rupununi = 0	Takutu = 0.002 Rupununi = 0.003
Nucleotide divergence of clades in ML trees			
Rupununi vs Takutu	-	0.0020	0.0042
RT vs Takutu	0.001	0.0011	0.0049
RT vs Rupununi	-	-	0.0031

***Ancistrus* spp.**	**CO1**	**Cyt b**	**S7 (1st intron)**
# of individuals	42	45	39
# of haplotypes	16	19	19
# of basepairs	669	1107	640
Nucleotide divergence between putative species			
A. nudiceps vs *A. leucostictus*	0.0042	0.0265	0.0063
A. nudiceps vs *A*. sp. circle A	0.0877	0.1176	0.0596
A. nudiceps vs *A*. sp. circle E	0.0915	0.1272	0.0673
A. nudiceps vs *A. lithurgicus*	0.0891	0.1260	-
A. sp. circle A vs *A*. sp. circle E	0.0693	0.0764	0.0149
A. sp. circle A vs *A. leucostictus*	0.0901	0.1114	0.0596
A. sp. circle E vs *A. leucostictus*	0.0954	0.1229	0.0674
A. sp. circle E vs *A. lithurgicus*	0.0124	0.0099	-
A. leucostictus vs *A. lithurgicus*	0.0923	0.1201	-

Appendix 2. Base composition for taxa per gene.

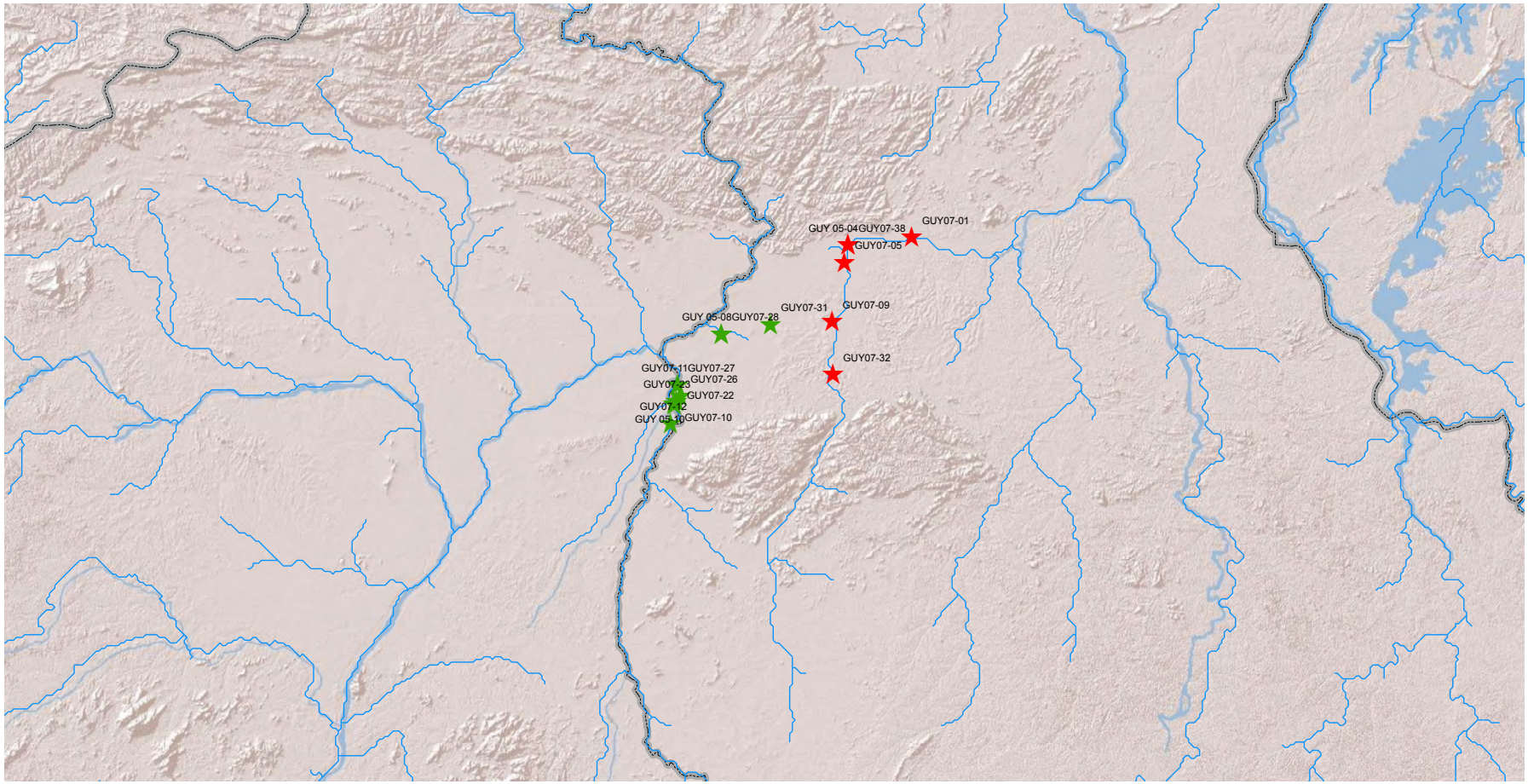
	T(U)	C	A	G	T-1	C-1	A-1	G-1	T-2	C-2	A-2	G-2	T-3	C-3	A-3	G-3
Ancistrus sp. 'white spot'																
COI	31.1	25.8	25.1	18.0	20	24.2	24.3	31.3	42	27.8	14.8	15.3	31	25.3	36.3	7.4
Cyt *b*	29.8	28.1	28.3	13.8	26	24.4	24.0	25.3	43	24.3	20.3	12.6	20	35.5	40.7	3.4
S7 (1st intron)	24.7	27.3	33.9	14.0	-	-	-	-	-	-	-	-	-	-	-	-
Potamorrhaphis guianensis																
COI	31.4	25.9	26.5	16.2	19	23.9	28.5	28.7	42	28.2	15.0	15.0	34	25.5	36.0	4.9
Cyt *b*	31.9	27.7	27.3	13.2	27	21.6	28.5	23.3	40	25.1	21.2	13.9	29	36.3	32.1	2.4
S7 (1st intron)	31.7	20.0	24.0	24.4	-	-	-	-	-	-	-	-	-	-	-	-
Geophagus surinamensis																
COI	30.8	27.6	24.3	17.4	42	27.3	15.5	15.1	30	30.4	32.6	6.7	20	24.9	24.7	30.5
Cyt *b*	30.8	30.2	25.1	13.8	25	26.3	24.8	24.3	42	24.8	19.5	13.8	26	39.6	31.2	3.2
S7 (1st intron)	30.3	20.8	23.3	25.6	-	-	-	-	-	-	-	-	-	-	-	-
Hoplias malabaricus																
COI	30.2	28.6	23.2	18.0	18	25.4	24.6	31.6	43	27.3	14.7	15.2	29	33.0	30.2	7.3
Cyt *b*	28.9	31.5	25.5	14.1	25	25.5	23.7	26.3	42	25.3	20.5	12.7	21	43.7	32.2	3.4
S7 (1st intron)	31.1	15.1	26.5	27.2	-	-	-	-	-	-	-	-	-	-	-	-
Hypostomus squalinus																
COI	31.8	25.4	24.5	18.3	21	23.1	24.4	31.6	42	27.7	14.7	15.2	32	25.3	34.2	8.2
Cyt *b*	29.5	27.2	29.3	13.9	26	24.7	24.4	25.3	42	24.5	20.6	13.0	21	32.6	42.9	3.4
S7 (1st intron)	33.7	13.9	24.9	27.5	-	-	-	-	-	-	-	-	-	-	-	-

Appendix 3. Populations per taxa.

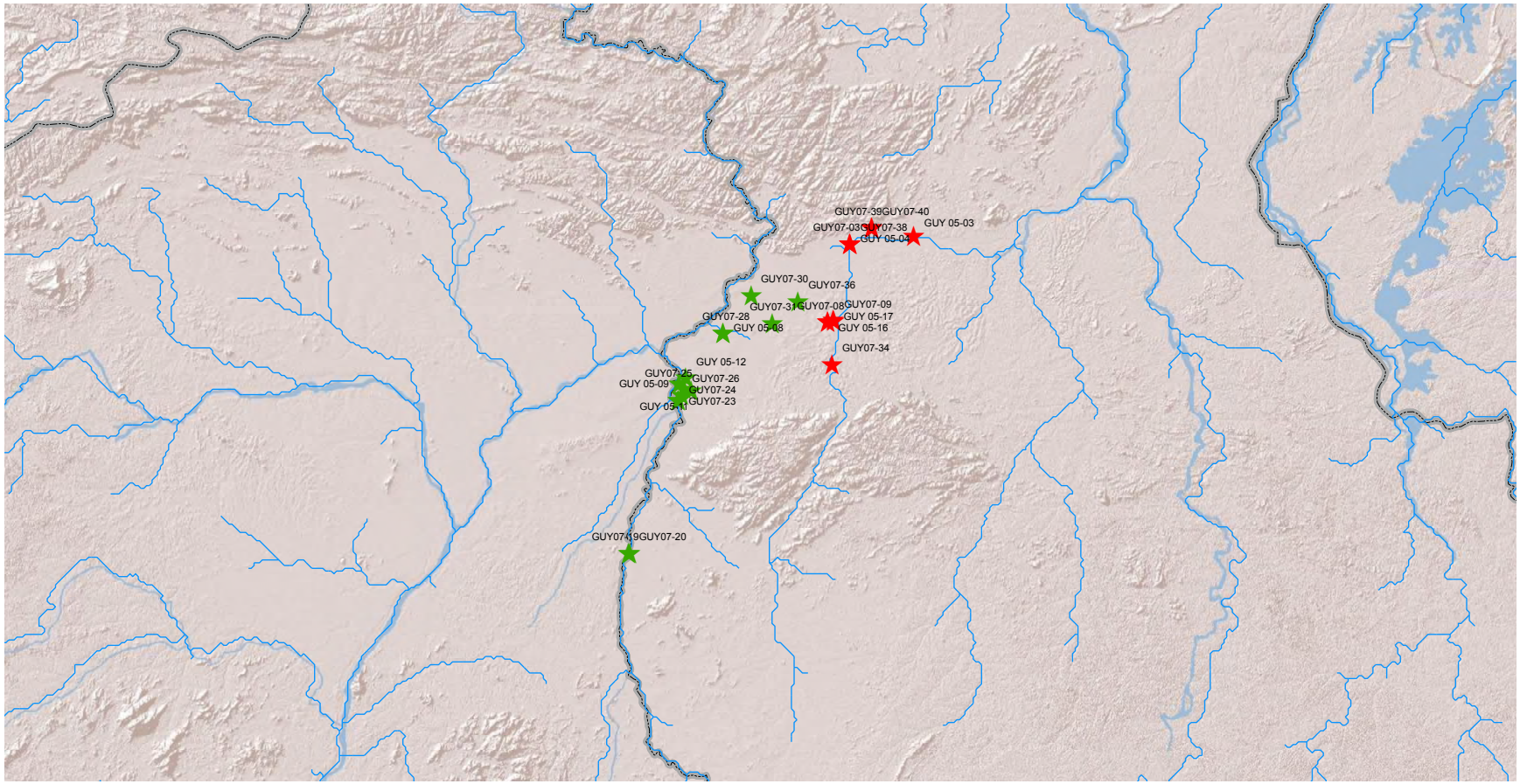
Potamorrhaphis guianensis	
GUY 0731	creek, between Amuku Lake and Pirara Creek
GUY 0510	Takutu River, beach near Lethem
GUY 0728	Pirara Creek, at Pirara Ranch
GUY 0516	Pond at Yukupari
GUY 0722	Takutu River, Garlic landing, beach N of Lethem
GUY 0521	Rupununi River at Kwaimatta
GUY 0522	Essequibo River, at Kwaimatta, beach in main channel and mouth of side channel
GUY 0503	Rupununi River, at Kwatamang
GUY 0517	Rupununi River, at Yukupari
GUY 0701	Rupununi River, at Kwatamang landing
GUY 0738	Rupununi River, at Massara landing
Geophagus surinamensis	
GUY 0731	creek, between Amuku Lake and Pirara Creek
GUY 0726	Mangeer Creek, isolated pond near Lethem
GUY 0738	Rupununi River, at Massara landing
GUY 0701	Rupununi River, at Kwatamang landing
GUY 0705	Rupununi River, at Kwaimatta landing
GUY 0709	Rupununi River, upstream of Yupukari landing on beach
GUY 0727	Takutu River, at rock beach
GUY 0711	Takutu River, rock beach
GUY 0732	Aruwa Falls, on Rupununi River, 15 miles upstream of Yupukari
GUY 0710	Takutu River, Tom's beach
GUY 0508	Pirara River, at Pirara Ranch
GUY 0712	Takutu River, upstream of Lethem, sand beach
GUY 0722	Takutu River, Garlic landing, beach N of Lethem
GUY 0723	Takutu River, Garlic landing, beach N of Lethem
GUY 0504	Rupununi River, at Massara
GUY 0728	Pirara Creek, at Pirara Ranch
GUY 0510	Takutu River, beach near Lethem
Hoplias malabaricus	
GUY 0503	Rupununi River, at Kwatamang
GUY 0504	Rupununi River, at Massara
GUY 0512	Manari Pond
GUY 0516	Pond at Yukupari
GUY 0517	Rupununi River, at Yukupari
GUY 0703	Rupununi River, at Massara landing
GUY 0708	Awaraku Lake, near Yupukari
GUY 0709	Rupununi River, upstream of Yupukari landing on beach

GUY 0734	gravel beach, downstream of Aruwa Creek
GUY 0738	Rupununi River, at Massara landing
GUY 0739	borrow pit, 12 miles S of Annai
GUY 0740	Parimoco Creek, behind borrow pit
GUY 0508	Pirara River, at Pirara Ranch
GUY 0511	Manari Creek, near Lethem
GUY 0719	Takutu River , N of Sand Creek
GUY 0720	Takutu River, Kaburra Falls
GUY 0723	Takutu River, Garlic landing, beach N of Lethem
GUY 0724	pond, near Garlic landing, N of Lethem
GUY 0725	oil rigg pond, near Manari
GUY 0726	Mangeer Creek, isolated pond near Lethem
GUY 0728	Pirara Creek, at Pirara Ranch
GUY 0730	borrow pit, 2.5 miles E of Good Hope, on the road to Meritizero
GUY 0731	creek, between Amuku Lake and Pirara Creek
GUY 0736	Pirara Head, aka Lake Amuku
GUY 0509	Takutu River, near Lethem
Hypostomus squalinus	
GUY 0504	Rupununi River, at Massara
GUY 0517	Rupununi River, at Yukupari
GUY 0521	Rupununi River at Kwaimatta
GUY 0522	Essequibo River, at Kwaimatta, beach in main channel and mouth of side channel
GUY 0701	Rupununi River, at Kwatamang landing
GUY 0702	Rupununi River, at Kwatamang landing, night sample
GUY 0703	Rupununi River, at Massara landing
GUY 0705	Rupununi River, at Kwaimatta landing
GUY 0707	Rupununi River, at Yupukari, sidewater bay
GUY 0709	Rupununi River, upstream of Yupukari landing on beach
GUY 0717	Rupununi River, at Dadanawa Ranch
GUY 0718	Rupununi River, at Waichibai
GUY 0732	Aruwa Falls, on Rupununi River, 15 miles upstream of Yupukari
GUY 0734	gravel beach, downstream of Aruwa Creek
GUY 0509	Takutu River, near Lethem
GUY 0710	Takutu River, Tom’s beach
GUY 0713	Takutu River, at sand beach
GUY 0719	Takutu River , N of Sand Creek
GUY 0722	Takutu River, Garlic landing, beach N of Lethem
GUY 0723	Takutu River, Garlic landing, beach N of Lethem

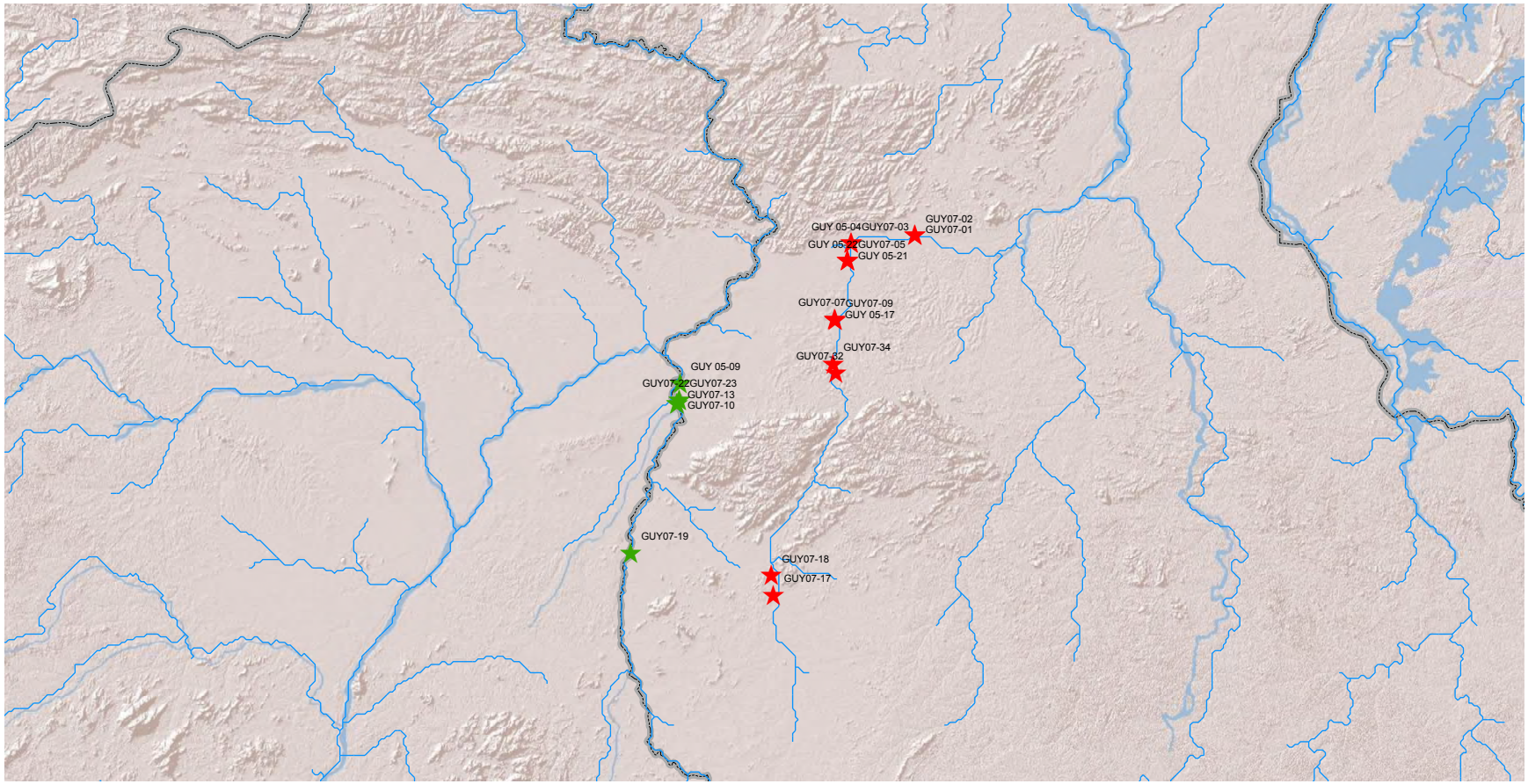
Appendix 4. Map of fish populations in the Rupununi Savanna.



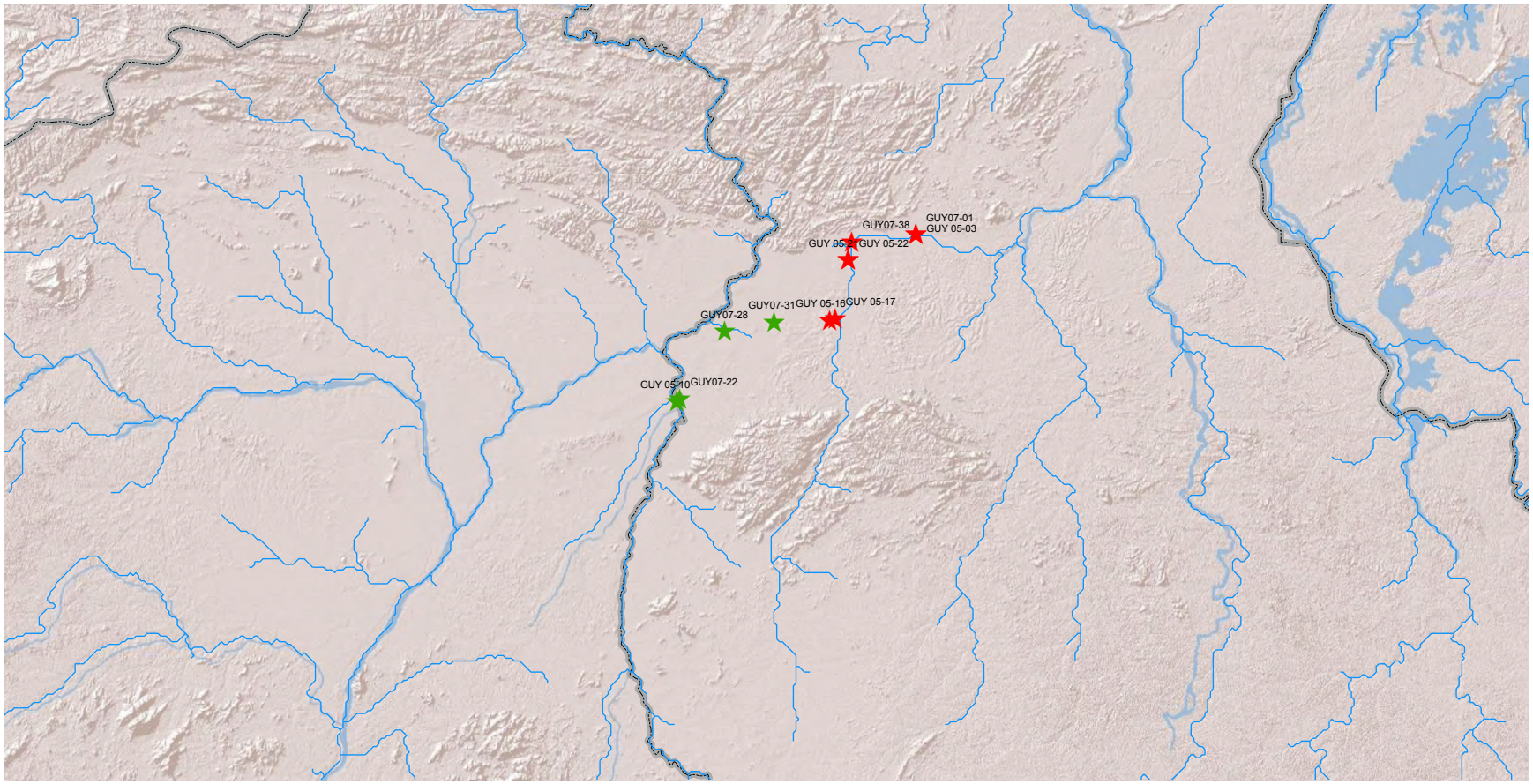
Geophagus surinamensis populations in the Rupununi savannas. Red stars indicate Rupununi drainage populations. Green stars indicate Takutu drainage populations.



Hoplias malabaricus populations in the Rupununi savannas. Red stars indicate Rupununi drainage populations. Green stars indicate Takutu drainage populations.



Hypostomus squalinus populations in the Rupununi savannas. Red stars indicate Rupununi drainage populations. Green stars indicate Takutu drainage populations.



Potamorrhaphis guianensis populations in the Rupununi savannas. Red stars indicate Rupununi drainage populations. Green stars indicate Takutu drainage populations.