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RESEARCH PAPER

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Fish biogeography in the "Lost World" of the Guiana Shield: Phylogeography of the weakly electric knifefish *Gymnotus carapo* (Teleostei: Gymnotidae)

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Abstract

Aim: The Guiana Shield region exhibits extraordinary topography that includes sheer, flat-topped mountains (tepuis) atop an upland platform. Rivers of the eastern Pakaraima Mountains descend to Atlantic coastal lowlands, often traversing spectacular rapids and waterfalls. For fish species distributed in both uplands and lowlands, it is unclear whether these rapids and waterfalls present population or biogeographical boundaries. We sought to test this using the geographically widespread banded-electric knifefish (*Gymnotus carapo*) as a model.

Location: The Guiana Shield region of South America.

Methods: We sampled 60 *Gymnotus carapo* specimens from the Guiana Shield region, and 75 *G. carapo* and closely related species from other parts of South America. We sequenced the mitochondrial cytochrome *b* gene and an intron from the nuclear S7 ribosomal protein gene, and used maximum likelihood and Bayesian tree-building approaches to generate phylogenetic trees of haplotypes.

Results: Haplotype sharing is minimal between populations separated by elevational barriers. We found evidence for two main haplotype clades in the Guiana Shield: one distributed in Atlantic coastal regions that includes most lowland samples, and one inland that includes most upland samples. Inland Guiana samples are more closely related to samples from the Amazon basin than to those of Atlantic coastal regions. A single sample from Tafelberg tepui in Suriname was most closely related to the Atlantic coastal lineages.

Main conclusions: Riverine barriers that result from steep elevational gradients in the Guiana Shield inhibit gene flow between uplands and lowlands, even for a widely distributed species. Biogeographical relationships of Guiana Shield *G. carapo* are complex, with most upland lineages showing affinities to the Amazon basin, rather than to nearby lowland drainages of the Atlantic coast.

KEYWORDS
Guiana Shield, *Gymotus carapo*, Pakaraima Mountains, phylogeography, South America, Tafelberg, tepui

1 | INTRODUCTION

The Guiana Shield region of north-eastern South America is famous for its striking topography. In particular, the Pakaraima Mountains of Guyana, Brazil, and Venezuela are extraordinary sheer-sided table-top mountains (tepuis) rising above an upland platform, which itself is elevated above the lowlands (Lujan & Armbruster, 2011; McConnell, 1968; Rull, 2005). These mountains are exceedingly remote, and this region has been referred to as a "Lost World" for this reason (e.g. Kok et al., 2016). The hydrogeography of this region is complex–upland regions are drained to the west and north by the Rio Orinoco, to the south by the Rio Amazonas via the Rio Branco and Rio Negro, and in the east by the Mazaruni and Essequibo drainages (Lujan & Armbruster, 2011). Rivers originating in the Pakaraima Mountains descend by as much as 1,000 m, often via spectacular waterfalls and rapids, before reaching the Atlantic coastal plain.

The uplands and highlands of the Guiana Shield exhibit remarkable biological endemism across taxonomic groups, including vascular plants (Berry & Riina, 2005), birds (Zyskowski et al., 2011), herpetofauna (McDiarmid & Donnelly, 2005) and mammals (Voss, Lim, Diaz-Nieto, & Jansa, 2013). Although sampling of fishes is generally sparse at higher altitudes of the Guiana Shield, surveys of the upper Mazaruni and upper Potaro rivers also indicate endemicity and biogeographical isolation. Alofs, Liverpool, Taphorn, Bernard, and López-Fernández (2014) estimated fish species endemicity in the upper Mazaruni, which is isolated from the lowland course of the river by a series of rapids and waterfalls, to be between 67–95%. The upper Mazaruni also hosts multiple endemic genera (in the families Cichlidae, Loricariidae, Crenuchidae and Lebiasinidae) suggesting a long period of isolation (Alofs et al., 2014). The upper Potaro, which is isolated by the Kaieteur Falls, contains the endemic *Lithogenes villosus*, a "relict" catfish species whose morphology is so distinct that its family-level phylogenetic placement has been subject to debate (Armbruster, 2004; Lujan, Armbruster, Lovejoy, & López-Fernández, 2015; Schaefer & Provenzano, 2008). Hardman, Page, Sabaj, Armbruster, and Knouft (2002) provided strong evidence that fish species distributions in the upper Potaro are affected by a series of rapids and waterfalls.

In many cases, the isolation imposed by Guiana Shield elevation changes and associated waterfalls and cataracts has limited fish species distributions, and resulted in endemism across a range of taxonomic levels. However, for other species, these barriers appear to be surmountable. For example, the banded-electric knifefish *Gymnotus carapo* L. is distributed in both uplands of the Pakaraima mountains and nearby lowlands (Albert & Crampton, 2003; Hardman et al., 2002). *Gymnotus carapo*, a member of the electric knifefish clade Gymnotiformes, is the most widely distributed species in its genus, occurring in the Amazon, Orinoco, Guianas and northeast Atlantic Brazilian drainages, as well as on Trinidad (Albert & Crampton, 2003). *Gymnotus carapo* has been collected from Guianas coastal lowland rivers including the Demerara, Berbice, Commewijne and Suriname rivers, as well as from upland Guiana Shield localities in the upper Mazaruni and upper Potaro drainages. Also, *G. carapo* was recently collected near the summit of Tafelberg, an isolated tepui in Suriname that has elevations ranging from 500 to 1,000 m (Figure 1). Because of its wide distribution in the Guianas, and its presence in both upland and lowland rivers, *G. carapo* is an interesting model for studying biogeographical relationships, genetic connectivity and dispersal pathways in this region.

Here, we present a biogeographical analysis that focuses on *Gymnotus carapo* from uplands (defined as elevations >300 m a.s.l.) and lowlands (elevations <300 m a.s.l.) of the north-eastern Guiana Shield region (hereafter Guianas), with three main objectives. First, we wanted to determine whether samples from upland and lowlands are genetically differentiated. Do the waterfalls and rapids that separate uplands and lowlands represent a barrier to *Gymnotus carapo*? Second, we investigated biogeographical relationships among upland and lowland Guianas lineages, in relation to samples from other parts of South America. Are individuals from uplands most closely related to individuals from nearby lowland river basins? Alternatively, uplands lineages from different rivers might form monophyletic groups, indicating biogeographical connectivity among upland

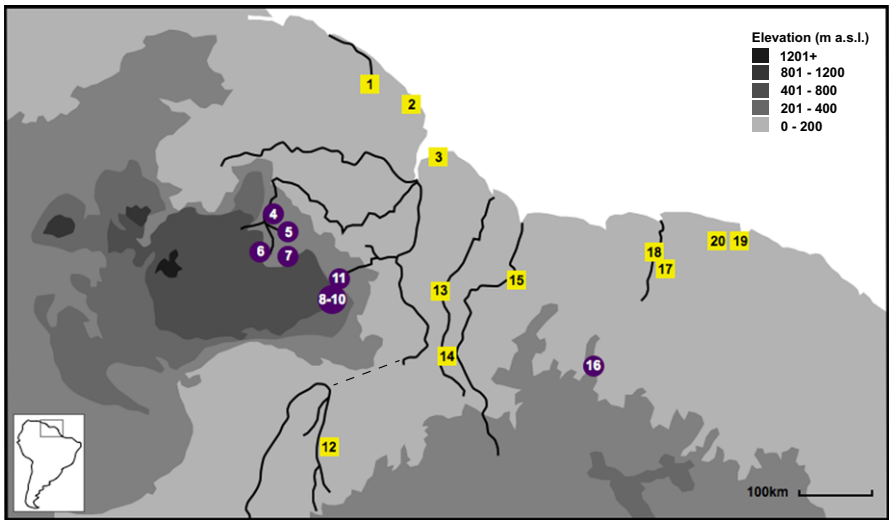


FIGURE 1 Collection localities of Guianas region *Gymnotus carapo* specimens used in this study. Yellow squares show lowland localities (<300 m a.s.l.) and purple circles show upland localities (300–1,500 m a.s.l.). Dashed line indicates seasonal connection between Rupununi and Potaro basins. Numbers indicate localities (more detailed information provided in Table 1): 1,2 Waini; 3, Demerera; 4–7, upper Mazaruni; 8–11, upper Potaro; 12, Rupununi; 13, middle Berbice; 14, upper Berbice; 15, Courantyne; 16, Tafelberg; 17–18, Suriname; 19–20, Commewijne [Colour figure can be viewed at wileyonlinelibrary.com]

sections of different river drainages. Finally, we sought to determine the phylogenetic affinity of the isolated Tafelberg tepui sample—would it be most closely related to upland populations of the Pakaraimas, or to geographically proximate lowland populations of *G. carapo*? To address these questions, we analysed sequence data from a mitochondrial coding gene (cytochrome *b*) and a nuclear intron (S7) from individuals representing both upland and lowland populations in the Guianas, as well as other regions of the species range in South America.

2 | MATERIALS AND METHODS

2.1 | Study region and taxon sampling

To explore the relationships of *Gymnotus carapo* in the Guianas region, we examined 60 samples obtained from upland (>300 m a.s.l.) and lowland localities (<300 m a.s.l.) between 2008 and 2014 (collection permits issued by the Suriname Nature Conservation Division and Guyana Environmental Protection Agency: 311—2007, 1194—2014; 300408 SP:004, 030211 BR149, 040414 SP:003, 190209 SP:010, 160410 SP: 020, 111413 SP:009). Our upland samples (Table 1; Figure 1) come from three main areas, two of which are in the Pakaraima mountains: the upper Potaro (localities 8–11) and the upper Mazaruni (localities 4–7). The third upland area is Tafelberg tepui (locality 16), which is geographically separated from the other upland regions by lowland habitat. In all three cases, upland localities are separated from lowland localities by rapids or waterfalls. The upper Mazaruni is separated from the lowlands by a series of steep rapids and waterfalls (Alofs et al., 2014); the upper Potaro is separated from the lowlands by the 226 m drop of Kaieteur Falls (Hardman et al., 2002); and streams on Tafelberg tepui flow over the sheer edge of the tepui, eventually draining to the right arm of the Coppename River. Although the upper Potaro and upper Mazaruni samples are geographically adjacent, there is no direct connection between these rivers (Lujan & Armbruster, 2011).

Our lowland Guianas samples were collected largely from coastal rivers in Guyana and Suriname (localities 1–3, 13–15, and 17–20) that drain in a north-easterly direction to the Atlantic (Table 1; Figure 1). An exception is the sample from the Sawariwau River (locality 12), which runs through the lowland Rupununi area between the Pakaraima and Kanuku mountain ranges, and forms a part of the upper Branco that flows southwards to the Amazon basin.

To provide geographical and phylogenetic context, we also included samples of *G. carapo* from other parts of South America, as well as samples of several closely related species (Appendix S1 in Supporting Information). Crampton, Lovejoy, and Albert (2003) defined *G. carapo* sensu stricto and we included samples from several allopatric populations delineated by these authors, including from the western and central Amazon, and the Orinoco. Phylogenetic studies based on morphological and molecular data have failed to support the monophyly of *G. carapo*, and have placed other described and undescribed *Gymnotus* species within *G. carapo* (Albert, Crampton, Thorsen, & Lovejoy, 2005; Brochu, 2011; Crampton, Rodríguez-Cattáneo, Lovejoy, & Caputi, 2013; Lovejoy, Lester, Crampton, Marques, & Albert, 2010; Maxime, 2013). We included representatives of as many of these species as possible (generally corresponding to members of "*G. carapo* clades" B, C, and D from Crampton et al., 2013). As outgroups, we included *G. obscurus*, *G. varzea*, *G. curupira* and *G. chaviro* (members of the "*G. carapo* clade A" from Crampton et al., 2013).

2.2 | DNA extraction, polymerase chain reactions and sequencing

DNA was extracted from muscle tissues using DNeasy Blood and Tissue kit, following manufacturer's instructions (Qiagen, Hilden, Germany). We amplified and sequenced fragments of the mitochondrial Cytochrome *b* (cyt *b*) gene and the first intron of the nuclear S7 gene, RP1 (Chow & Hazama, 1998). An approximately 1,100 base pair fragment of cyt *b* was amplified using PCR in 25 μl reaction volumes made up of 2.0 μl of DNA, 14.8 μl of de-ionized water (ddH_2O), 1× KCl Taq polymerase buffer, 2.0 mM $MgCl_2$, 0.15 μM each deoxynucleotide triphosphate (dNTPs), 0.4 μM forward primer, 0.4 μM reverse primer and 1U of Taq polymerase. Universal vertebrate primers GLUDG.L (5′-CGAAGCTTGACTTGAARAACCAYCGT T-3′), cyt*b*R (5′-CTCCGATCTTCGGATTACAAG-3′), and cyt*b*F (5′-TCYAWCATCTCAGCCTGATG-3′) were used for *cyt b* amplification. Primers specific to *Gymnotus* were developed for tissues that were difficult to amplify. Thermocycler conditions for cyt *b* were: 95°C for 30 s for initial denaturation followed by 35 cycles of 95°C for 30 s to denature DNA; 50.0°C for 60 s to anneal DNA, and 72°C for 90 s for elongation. This was followed by a final extension cycle for 300 s at 72°C.

An approximately 500 base pair fragment of the first intron of S7 was amplified using PCR in 25 μl reaction volumes of 11.875 μl ddH_2O, 1× of KCl Taq polymerase buffer, 1.5 mM of $MgCl_2$, 0.2 μM of each of dNTPs, 1.2 μM of forward and reverse primers, and 0.625 U of Taq polymerase. Thermocycler conditions for S7 were 95°C for 30 s of initial denaturation followed by 30 cycles of 95°C for 30 s denaturation; 50.6–55.5°C for 60 s annealing, and 72.0°C for 120s of elongation. An extension period of 600s followed the cycles. All PCR products were visualized using gel electrophoresis. Products were run on a 0.8% agarose gel pre-loaded with 5.0 μl of Amresco EZVision in-gel stain. 5.0 μl of DNA was pipette-mixed with 3.0 μl of Thermoscientific 6× loading dye. The electrophoresis was run for 30 min at 80 Volts and 70 milliAmperes. PCR products were purified using ExoSAP-IT according to the manufacturer protocol (Affymetrix), and sanger-sequenced at the Centre for Applied Genomics (Toronto, Canada).

2.3 | Sequence alignment and phylogenetic analyses

Sequences were imported into GENEIOUS 6.1.7 (Biomatters Ltd, Auckland, New Zealand) and aligned using default CLUSTALW parameters. For cyt *b*, we obtained 1,074 base pairs for 118 individuals. No

TABLE 1 Localities of Guianas *Gymnotus carapo* ($n = 60$) included in this study. Locality numbers refer to Figure 1. Approximate elevation is given in metres above sea level (m a.s.l.). To determine elevation, latitude and longitude were plotted using ArcGIS software on elevation maps acquired from DivaGIS 7.5.0 (Hijmans, Guarino, & Mathur, 2012). Dashes indicate sequence data unavailable

Locality	Number of individuals sampled	Drainage	Latitude	Longitude	Elevation category	Elevation	Cyt *b* haplotype	S7 haplotype
1	2	Waini	7.700	−59.233	Lowland	0–105	O	–
2	2	Waini	7.428	−58.676	Lowland	0–105	L	–
3	1	Demerera	6.734	−58.303	Lowland	0–105	Q	–
4	2	Upper Mazaruni	5.936	−60.614	Upland	493–646	A	bb
5	1	Upper Mazaruni	5.708	−60.360	Upland	335–492	A	bb
6	4	Upper Mazaruni	5.475	−60.779	Upland	493–646	A	bb
7	3	Upper Mazaruni	5.360	−60.371	Upland	493–646	A, B	bb
8	3	Upper Potaro	5.010	−59.637	Upland	493–646	C	cc
8	4	Upper Potaro	5.007	−59.631	Upland	493–646	C	cc
8	4	Upper Potaro	5.007	−59.636	Upland	493–646	C	–
9	1	Upper Potaro	5.108	−59.635	Upland	647–818	C	–
10	2	Upper Potaro	5.070	−59.653	Upland	493–646	C	cc
11	2	Upper Potaro	4.933	−59.799	Upland	493–646	C	dd
12	1	Rupununi	2.829	−59.808	Lowland	106–225	E	ee
13	1	Middle Berbice	4.905	−58.250	Lowland	0–105	P	–
14	2	Upper Berbice	4.156	−58.177	Lowland	0–105	D	ac, ab
15	7	Courantyne	5.097	−57.143	Lowland	0–105	F, G, H, I	fi, fh, fg
16	1	Tafelberg	3.919	−56.200	Upland	493–646	J	jj
17	4	Suriname	5.586	−54.285	Lowland	0–105	K, M	kk, gl
18	4	Suriname	5.452	−55.245	Lowland	0–105	L, N	kk, mn
19	2	Commewijne	5.582	−54.233	Lowland	0–105	L	kk, ok
20	7	Commewijne	5.586	−54.285	Lowland	0–105	L	dd, kk, ok, oo, pq, rs, tu

insertions or deletions were noted. For S7, we obtained 784 base pairs for 73 individuals, and seven indels were detected. For analysis of the diploid nuclear S7 dataset, we used Phase 2.1 (Stephens, Smith, & Donnelly, 2001) to determine the allele sequences (haplotypes) of any sequences with polymorphisms (heterozygotes). Phase was run for 100 iterations with a burn in of 100; the algorithm was set to run ten times. All other priors and parameters used the default settings. After analysis with Phase, our S7 dataset consisted of 103 haplotypes. We used Mega 7 (Kumar, Stecher, & Tamura, 2016) to calculate uncorrected pairwise distances between our sequences within the cyt *b* and *S7* matrices.

The cyt *b* and S7 matrices were analysed separately using Partitionfinder 1.1.0 (Lanfear, Calcott, Ho, & Guindon, 2012) to determine the most appropriate partition schemes and model of molecular evolution for each partition, based on the Akaike information criterion (AIC). Bayesian and maximum likelihood analyses were conducted on each gene independently, using MrBayes 3.2.2 (Huelsenbeck, Ronquist, Nielsen, & Bollback, 2001), and RAxML 1.31 (Stamatakis, 2006), respectively. For Bayesian analyses, each analysis was run until the average standard deviation of split frequencies was below 0.01. For Bayesian and maximum likelihood analysis of S7, the gene was not partitioned; an HKY model was used in MrBayes and a GTR model in RAxML.

For analysis of cyt *b*, the gene was partitioned by codon position as determined by Partitionfinder. Codon positions one and two were assigned the GTR+G+I model, and codon position three was assigned the GTR+G model. The cyt *b* analysis in MrBayes was run for 50 million generations sampling every 10,000 generations. Our RAxML analysis included 50 searches to infer the best ML tree. Node support was estimated by 1,000 bootstrap replicates. To analyze S7, MrBayes was run for 50 million generations sampling every 10,000 generations. For RAxML, a total of 50 searches were used to generate the best ML tree. Node support was estimated by 1,000 bootstrap replicates.

We used *Beast 2.1.3 (Drummond, Suchard, Xie, & Rambaut, 2012) to implement a Bayesian species tree approach using the concatenated cyt *b* and S7 data. We used geographically defined lineages as proxies for species, as the species identities within the *Gymnotus carapo* complex are unclear. Though it is possible to estimate divergence times in *Beast using geological events or fossil data, neither of these are readily available for the Guianas region or *Gymnotus*, and we chose not to include it in our analysis. Three separate runs were conducted for 100 million generations sampling every 10,000 generations, using unlinked trees and models for each gene, and an additional run was conducted using only priors. We used a GTR+G+I model for cyt *b* and a HKY model for S7, with a

strict clock imposed and a birth-death process prior for rates of cladogensis. Log files of each separate run were examined in Tracer 1.6 (Rambaut et al., 2014) to assess convergence of parameter estimates before combining all three runs to generate a single tree in TreeAnnotator 1.8.1, a part of the Beast package (Drummond et al., 2012).

3 | RESULTS

3.1 | Distributions of haplotypes across the Guianas region

GenBank numbers for sequences used in this study are provided in Appendix S1. We observed a total of four different cyt *b* haplotypes across 27 individuals from upland rivers, including the upper Mazaruni and upper Potaro Rivers from the Pakaraima mountains in Guyana, as well as from Tafelberg tepui in Suriname (Table 1). Cyt *b* haplotypes were shared between localities within the same river drainage, but not across upland river drainages (Table 1). All 10 individuals from localities 4–7 within the upper Mazaruni shared haplotype A, except for a single individual from locality 7 with unique haplotype B. All fish from the upper Potaro (localities 8–11) shared haplotype C. The single individual from the upland Tafelberg site had a unique haplotype (J).

We collected cyt *b* sequences from 33 individuals from rivers of lowland Guyana and Suriname, and these exhibited considerably more haplotypes (17) than upland individuals (Table 1). Also, in contrast to uplands, lowland rivers showed haplotype sharing across river basins. For example, haplotype L is found in the Waini River (locality 2), the Suriname River (locality 18) and the Commewijne River (localities 19 and 20). Compared to upland localities, more haplotype diversity was observed within lowland localities. For example, four haplotypes were observed among the seven sequenced individuals from the Courantyne River (locality 15).

For cyt *b*, we found no shared haplotypes between sites from upland and lowland regions of the Guiana Shield. The mean uncorrected sequence divergence between individuals from the upland rivers of the eastern Pakaraimas (upper Potaro and upper Mazaruni) and lowland Guianas was 1.2%. The average sequence divergence between the Tafelberg individual and lowland Guianas individuals was 1.5%, and the average sequence divergence between the Tafelberg individual and the Pakaraima *G. carapo* was 1.9%.

We sequenced S7 from 35 fish from the Guianas, including 13 from upland localities and 22 from lowland localities, and recorded a total of 18 haplotypes (Table 1). Many of the patterns observed in cyt *b* were repeated in this nuclear locus. In general, fish from upland localities had fewer haplotypes (four), compared to lowland localities (14). We observed no haplotype sharing across basins in upland localities, but haplotypes are shared across basins in lowlands.

We observed minimal haplotype sharing between upland and lowland localities. Eastern Pakaraima fish exhibited three haplotypes (b, c and d), and the Tafelberg *G. carapo* had a unique haplotype (j). These haplotypes were not observed at lowland localities, except that haplotype c (present in both the upper Potaro and upper Mazaruni) was recorded in a single individual from the upper Berbice (locality 14), as well as in a single individual of *Gymnotus arapaima* from the central Amazon.

The mean uncorrected sequence divergence between individuals from the upland Pakaraima rivers and Guianas lowlands was 0.7%. Average sequence divergence between the Tafelberg individual and lowland Guianas individuals was 0.8%; the average sequence divergence between the Tafelberg individual and Pakaraima individuals (upper Potaro and upper Mazaruni) was 0.3%.

3.2 | Biogeographical relationships between upland and lowland Guianas

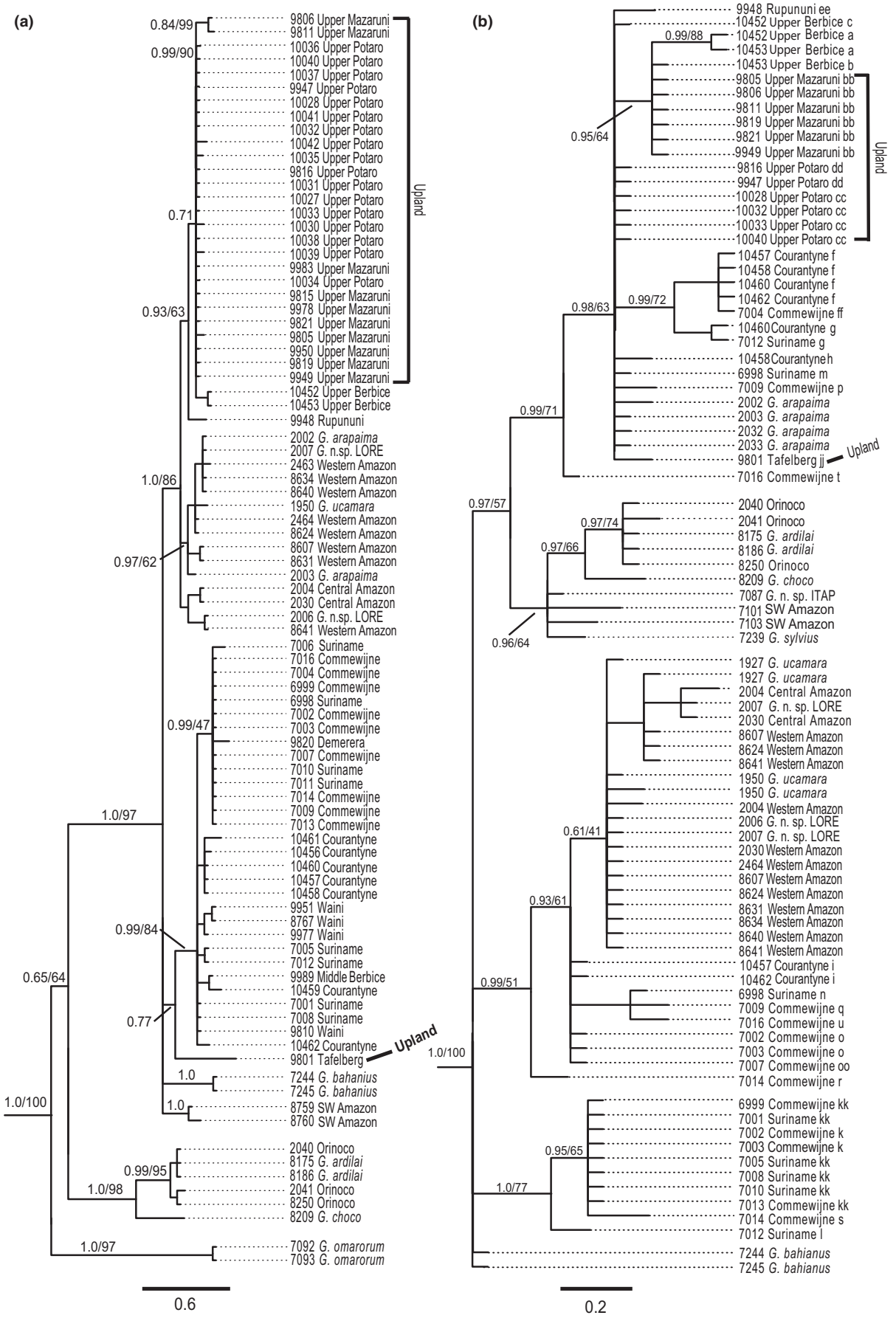
In the cyt *b* topology (Figure 2), haplotypes from upland and lowland Guianas are for the most part, but not exclusively, positioned in different clades. Haplotypes from the upper Mazaruni (uplands), upper Potaro (uplands) and upper Berbice (lowlands) form a monophyletic clade that has moderate support (pp = 0.71). This clade is the sister group of a haplotype from the Rupununi (lowlands) (pp = 0.93). We name this entire group the "Inland Guianas Clade" (IGC), based on its relatively inland geographical distribution. The IGC is sister (pp = 1.0) to a group of *G. carapo* haplotypes and *Gymnotus* species (*G. arapaima*, *G. ucamara* and *G.* n. sp. LORE) haplotypes from the Amazon (hereafter, the "Amazon Clade"). All other *G. carapo* haplotypes from the Guianas are part of a large "Atlantic Coastal Clade" (ACC) (pp = 0.99), with the exception of the single haplotype from Tafelburg tepui, which is positioned as the sister lineage of the ACC (pp = 0.77). The *BEAST tree (Figure 3) largely matches the cyt *b* tree, indicating a monophyletic IGC that is sister to an Amazon Clade, and a monophyletic ACC that is sister to the Tafelberg lineage.

Compared to cyt *b*, the S7 topology (Figure 2) shows shallower branches and less correspondence between inferred clades and geographical distributions. The S7 tree has a clade that includes inland haplotypes, but this clade also includes several haplotypes from coastal regions and Tafelberg, as well as several representatives of the closely related species *Gymnotus arapaima*. Other lowland/coastal haplotypes are distributed in other parts of the tree.

4 | DISCUSSION

4.1 | Genetic divergence across the Guianas—elevational barriers isolate the upland rivers

A key finding of our study is that for the electric knifefish *Gymnotus carapo*, haplotypes are not generally shared between upland and lowland Guianas localities. This provides evidence that barriers between these habitats likely restrict gene flow. In most cases, rivers that flow across planation surfaces of the Guianas experience a steep gradient as they pass from uplands to lowlands (Hammond, 2005; McConnell, 1968). For example, as the Mazaruni River leaves the uplands, it passes through 60 km of rapids and waterfalls (Alofs


(a)
0.84/99
0.99/90
0.71
0.93/63
1.0/86
0.97/62
0.99/47
1.0/97
0.99/84
0.65/64
0.77
1.0
1.0
1.0/100
0.99/95
1.0/98
1.0/97
9806 Upper Mazaruni
9811 Upper Mazaruni
10036 Upper Potaro
10040 Upper Potaro
10037 Upper Potaro
9947 Upper Potaro
10028 Upper Potaro
10041 Upper Potaro
10032 Upper Potaro
10042 Upper Potaro
10035 Upper Potaro
9816 Upper Potaro
10031 Upper Potaro
10027 Upper Potaro
10033 Upper Potaro
10030 Upper Potaro
10038 Upper Potaro
10039 Upper Potaro
9983 Upper Mazaruni
10034 Upper Potaro
9815 Upper Mazaruni
9978 Upper Mazaruni
9821 Upper Mazaruni
9805 Upper Mazaruni
9950 Upper Mazaruni
9819 Upper Mazaruni
9949 Upper Mazaruni
Upland
10452 Upper Berbice
10453 Upper Berbice
9948 Rupununi
2002 G. arapaima
2007 G. n.sp. LORE
2463 Western Amazon
8634 Western Amazon
8640 Western Amazon
1950 G. ucamara
2464 Western Amazon
8624 Western Amazon
8607 Western Amazon
8631 Western Amazon
2003 G. arapaima
2004 Central Amazon
2030 Central Amazon
2006 G. n.sp. LORE
8641 Western Amazon
7006 Suriname
7016 Commewijne
7004 Commewijne
6999 Commewijne
6998 Suriname
7002 Commewijne
7003 Commewijne
9820 Demerera
7007 Commewijne
7010 Suriname
7011 Suriname
7014 Commewijne
7009 Commewijne
7013 Commewijne
10461 Courantyne
10456 Courantyne
10460 Courantyne
10457 Courantyne
10458 Courantyne
9951 Waini
8767 Waini
9977 Waini
7005 Suriname
7012 Suriname
9989 Middle Berbice
10459 Courantyne
7001 Suriname
7008 Suriname
9810 Waini
10462 Courantyne
9801 Tafelberg
Upland
7244 G. bahanius
7245 G. bahanius
8759 SW Amazon
8760 SW Amazon
2040 Orinoco
8175 G. ardilai
8186 G. ardilai
2041 Orinoco
8250 Orinoco
8209 G. choco
7092 G. omarorum
7093 G. omarorum
0.6
(b)
0.99/88
0.95/64
0.98/63
0.99/72
0.99/71
0.97/57
0.97/74
0.97/66
0.96/64
1.0/100
0.61/41
0.93/61
0.99/51
0.95/65
1.0/77
9948 Rupununi ee
10452 Upper Berbice c
10452 Upper Berbice a
10453 Upper Berbice a
10453 Upper Berbice b
9805 Upper Mazaruni bb
9806 Upper Mazaruni bb
9811 Upper Mazaruni bb
9819 Upper Mazaruni bb
9821 Upper Mazaruni bb
9949 Upper Mazaruni bb
Upland
9816 Upper Potaro dd
9947 Upper Potaro dd
10028 Upper Potaro cc
10032 Upper Potaro cc
10033 Upper Potaro cc
10040 Upper Potaro cc
10457 Courantyne f
10458 Courantyne f
10460 Courantyne f
10462 Courantyne f
7004 Commewijne ff
10460 Courantyne g
7012 Suriname g
10458 Courantyne h
6998 Suriname m
7009 Commewijne p
2002 G. arapaima
2003 G. arapaima
2032 G. arapaima
2033 G. arapaima
9801 Tafelberg jj
Upland
7016 Commewijne t
2040 Orinoco
2041 Orinoco
8175 G. ardilai
8186 G. ardilai
8250 Orinoco
8209 G. choco
7087 G. n. sp. ITAP
7101 SW Amazon
7103 SW Amazon
7239 G. sylvius
1927 G. ucamara
1927 G. ucamara
2004 Central Amazon
2007 G. n. sp. LORE
2030 Central Amazon
8607 Western Amazon
8624 Western Amazon
8641 Western Amazon
1950 G. ucamara
1950 G. ucamara
2004 Western Amazon
2006 G. n. sp. LORE
2007 G. n. sp. LORE
2030 Western Amazon
2464 Western Amazon
8607 Western Amazon
8624 Western Amazon
8631 Western Amazon
8634 Western Amazon
8640 Western Amazon
8641 Western Amazon
10457 Courantyne i
10462 Courantyne i
6998 Suriname n
7009 Commewijne q
7016 Commewijne u
7002 Commewijne o
7003 Commewijne o
7007 Commewijne oo
7014 Commewijne r
6999 Commewijne kk
7001 Suriname kk
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7003 Commewijne k
7005 Suriname kk
7008 Suriname kk
7010 Suriname kk
7013 Commewijne kk
7014 Commewijne s
7012 Suriname l
7244 G. bahianus
7245 G. bahianus
0.2

FIGURE 2 Reduced phylogenies of *Gymnotus carapo* lineages and closely related species, based on Bayesian analyses of two loci (complete phylogenies included in Supplementary Materials). a) Phylogeny based on mitochondrial cyt *b* gene. b) Phylogeny based on intron of nuclear S7 gene. Upland Guianas samples are indicated in trees, all other samples are from lowlands (<300 m a.s.l.). Species names replaced by locality names for *G. carapo* samples. Numbers above branches indicate posterior probabilities followed by bootstrap values. For S7 tree, haplotypes determined by PHASE are indicated by lower case letters and only shown for Guianas individuals

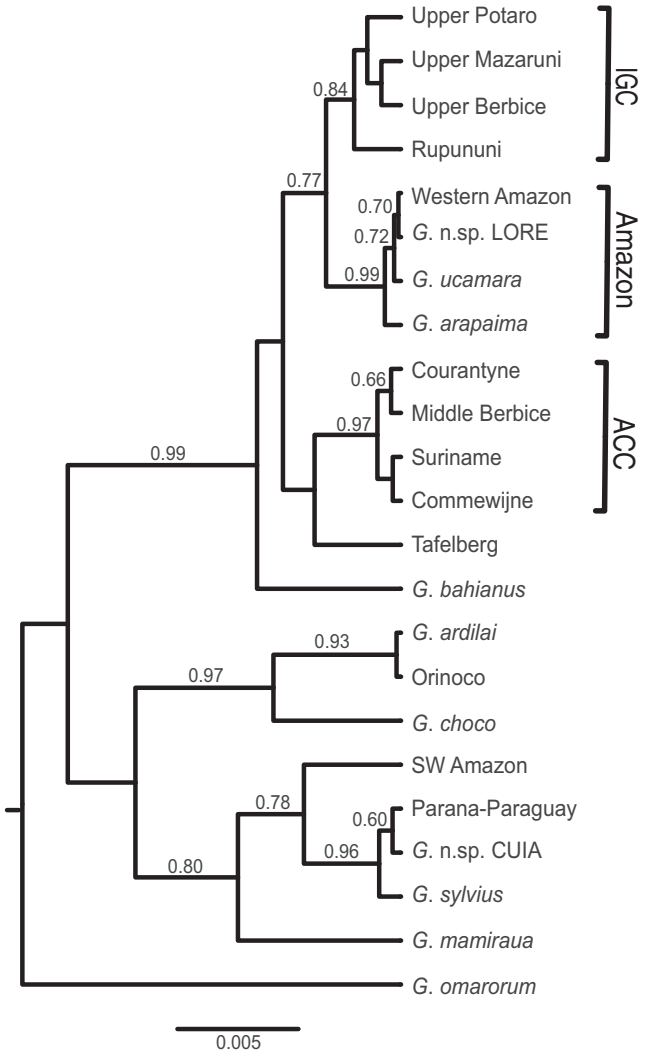

FIGURE 3 *BEAST phylogeny for geographically defined lineages of *G. carapo* and close relatives, generated using cytochrome *b* and nuclear S7 sequences. Taxa without species names are *G. carapo* lineages. Localities from which S7 sequences could not be obtained were excluded from this analysis. IGC = *G. carapo* inland Guianas clade; ACC = *G. carapo* Atlantic coastal clade

et al., 2014; Gery & Zarske, 2002), while the Potaro River flows over Kaieteur Falls, a spectacular 226-m drop in elevation from the Pakaraima uplands (Hardman et al., 2002), then crosses another barrier called the Tumatumari cataract, before traversing the coastal lowlands of the Guianas. While the effect of these physical barriers on fish communities and species distributions has been discussed (e.g. Alofs et al., 2014; Hardman et al., 2002; Lujan & Armbruster, 2011), our study provides the first genetic evidence that these barriers influence genetic connectivity within a species.

The distinctiveness of upland and lowland populations reflects a pattern of endemicity that has been reported from the Pakaraima Mountains and associated upland areas. In fishes, there are several species endemic to upland rivers of the Guianas, including members of the families Hypopomidae, Cichlidae and Loricariidae, as well as four families of Characiformes (Armbruster & Taphorn, 2011; Gery & Zarske, 2002; López-Fernández, Taphorn, & Liverpool, 2012; Lujan, 2008; Lujan, Agudelo-Zamora, Taphorn, Booth, & López-Fernández, 2013; Maldonado-Ocampo et al., 2014; Taphorn, Lopez-Fernandez, & Bernard, 2008). Although the antiquity of these endemic species is not well known, some may date to the Oligocene or earlier (López-Fernández, Arbour, Winemiller, & Honeycutt, 2013; Lujan & Armbruster, 2011), indicating a very long period of isolation. We did not attempt to estimate a time-tree for *G. carapo* because we do not have precise fossil or biogeographical calibration points. However, we calculated cyt *b* sequence divergence between upland taxa and other populations to be 1.5%. Based on a rate of mitochondrial sequence divergence of approximately 1% per million years, this would provide an age estimate of 1.5 Myr for the divergence of upland *G. carapo* from nearby lowland populations. This suggests that *G. carapo* is a more recent addition to the fauna of upland Guiana, in line with ages of some amphibians found on tepui summits (Kok et al., 2012, 2016). Based on these results, we suggest that upland Guianas ichthyofauna consist of a mosaic of relatively ancient endemics combined with more recent arrivals like *G. carapo*.

4.2 | Two distinct *G. carapo* lineages in the Guianas

Our analyses indicated imperfect concordance between gene trees for cyt *b* and S7. This pattern has been observed in other taxa where mitochondrial and nuclear genes have been compared (e.g. Bensch, Irwin, Irwin, Kvist, & Åkesson, 2005; Monsen & Blouin, 2003; Wiens, Kuczynski, & Stephens, 2010). As expected, S7 is less divergent between populations than cyt *b*, and this is likely due to lower rates of molecular evolution and larger effective population sizes for nuclear versus mitochondrial genes. Given the very low levels of divergence for S7 between *G. carapo* populations, we suggest that this gene is less likely to track recent dispersal and genetic isolation of populations, with observed variation potentially representing inherited ancestral polymorphisms that have not yet "sorted" based on geography and population barriers. In light of this, we emphasize cyt *b* in our interpretation of biogeographical patterns, as well as the *BEAST tree, which most closely resembles the cyt *b* gene genealogy.

Our phylogenetic analysis of *G. carapo* lineages shows a mismatch with our initial upland and lowland site categorization. Instead, we find evidence that two major lineages in the Guianas are

(1) an inland Guianas clade (IGC) that is composed of *G. carapo* from the upper Mazaruni and Potaro rivers, as well as the upper Berbice and Rupununi; and (2) an Atlantic coastal clade (ACC) that includes fish from the coastal rivers and the Tafelberg *G. carapo* (Figure 4). Surprisingly, the IGC and ACC are not each other's closest relatives. Instead, the IGC is most closely related to an Amazon clade that includes samples from the upper and middle Amazon. Overall, these patterns suggest that while elevational barriers such as waterfalls and rapids play a significant role in shaping phylogeographical patterns in *G. carapo* in the Guianas, they are not the only factor determining relationships among populations; it is likely that continent-scale biogeographical connections and routes of dispersal between areas are also key drivers.

The *G. carapo* ACC is in agreement with an Atlantic Coastal biogeographical corridor, evidence for which is summarized by Lujan and Armbruster (2011). This corridor encompasses coastal Atlantic drainages from the mouth of the Orinoco to the mouth of the Amazon River. Dispersal within the eastern part of the corridor is likely facilitated by north-western movements of the Amazon discharge causing reduced salinity in coastal habitats, thereby allowing fishes to move from mouth to mouth of separated drainages (Jégu & Keith, 1999). Sea level changes that connect rivers in coastal plains, and headwater captures and connections are also likely enhancers of dispersal. The ACC geographical pattern of *G. carapo* cyt *b* and S7 haplotype distributions fit this scenario of dispersal and gene flow, as several haplotypes are distributed across multiple river drainages. Future inclusion of *G. carapo* from the lower Amazon, as well as from other Amazon drainage rivers that have been proposed as "sources" for coastal corridor dispersers (such as the Jari River; Cardoso & Montoya-Burgos, 2009), would clarify the biogeographical affinities of the *G. carapo* ACC.

The biogeographical basis for the *G. carapo* IGC is less clear. This clade includes a group of strongly geographically demarcated individuals from the Pakaraimas, but also includes individuals from the upper Berbice and Rupununi (both low elevation sites). Low levels of genetic divergence between IGC individuals imply recent genetic connectivity between these localities, but dispersal routes, in the absence of more extensive sampling, remain unclear. We note that a similar biogeographical pattern has been observed in a species of the characiform family Crenuchidae—*Skiotocharax meizon* is found in the upper Mazaruni, but has been reported from a single locality in the Berbice as well (Presswell, Weitzman, & Bergquist, 2000).

Lujan and Armbruster (2011) have previously highlighted the importance of the proto-Berbice palaeo-drainage for the biogeography of the Guiana Shield region. The proto-Berbice was a large river that, until the Plio-Pleistocene, is thought to have drained much of the eastern Guiana Shield region, including the southern Guiana Shield uplands, the Rupununi Savannas, and the current Berbice basin. The fact that the IGC occupies these regions suggests that it dispersed throughout the proto-Berbice. However, the sister–taxon relationship between the IGC and Amazon clade suggests, alternatively, that the IGC is the product of relatively recent dispersal from the Amazon.

While not the focus on this study, these results provide insight regarding taxonomic and evolutionary aspects of the *Gymnotus carapo* species. Albert and Crampton (2003) defined *Gymnotus carapo* sensu stricto, but phylogenetic studies using both morphological and molecular datasets have failed to resolve *G. carapo* as a monophyletic lineage (Albert et al., 2005; Lovejoy et al., 2010; Brochu, 2011; Crampton et al., 2013). The present study, which includes increased molecular sampling of *G. carapo* populations, confirms that there is a complex relationship between geographical isolates of this species and their close relatives. Our analyses show that several described species, including *G. arapaima*, *G. ucamara*, *G. ardilai* and *G. bahianus* are nested within *Gymnotus carapo* lineages. In addition, our results demonstrate discordance with Albert and Crampton's (2003) morphology-based population boundaries of *G. carapo*; notably, the putative GO (Guiana Shield and Orinoco) population defined by Albert and Crampton (2003) encompasses at least three genetic lineages (the ACC, IGC and a lineage in the Orinoco/Andes region) that do not make up a monophyletic group.

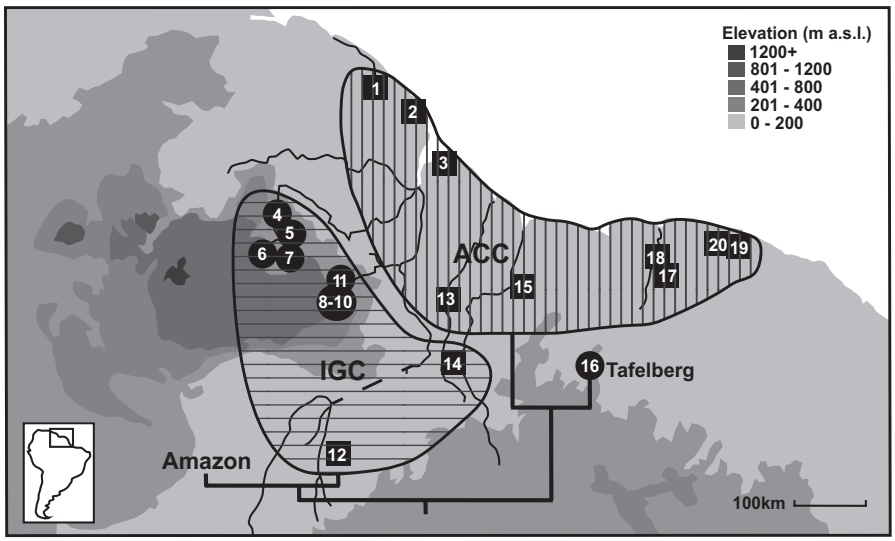

FIGURE 4 Distribution and phylogenetic relationships of Guianas *G. carapo* lineges. Squares denote lowland localities (300–1,500 m a.s.l.) and circles denote upland localities (<300 m a.s.l.). IGC = *G. carapo* inland Guianas clade; ACC = *G. carapo* Atlantic coastal clade. Dashed line indicates seasonal connections between Rupununi and Potaro basins. Numbers indicate localities (more detailed information provided in Table 1): 1,2 Waini; 3, Demerera; 4–7, upper Mazaruni; 8–11, upper Potaro; 12, Rupununi; 13, middle Berbice; 14, upper Berbice; 15, Courantyne; 16, Tafelberg; 17–18, Suriname; 19–20, Commewijne

4.3 | Tafelberg tepui *Gymnotus carapo*

Tafelberg tepui is a table-top mountain in Suriname, and is geographically isolated from the Pakaraima mountain range of Guyana, Brazil and Venezuela by approximately 300 km of lowland habitat. Our single *G. carapo* specimen was collected from a small pool on the summit of Tafelberg, at approximately 600 m elevation. Our analyses suggest that this sample is genetically distinct, with unique cyt *b* and S7 haplotypes that are, respectively, minimally 1.1% and 0.2% diverged from other haplotypes in our study. Our cyt *b* and *BEAST phylogenies (Figures 2 and 4) place the Tafelberg sample as the sister to the ACC, indicating an independent dispersal to this high elevational habitat from lowland coastal habitat. The question of whether there is a genetically isolated, self-sustaining population of *Gymnotus* at the summit of Tafelberg, and what its age might be, is deferred until samples can be procured from lowland drainages in the immediate vicinity of this tepui.

4.4 | *Gymnotus carapo*, a super-dispersing, mountain-climbing species complex

Our results highlight the continental-scale population connectivity of a widespread Neotropical fish species. Especially impressive is the minimal genetic divergence detected between upper Amazon individuals and members of the IGC. In fact, we identified an S7 haplotype (b) that is shared across the upper Mazaruni, middle Berbice and upper Amazon (in *G. arapaima*), despite the maximum separation of these basins by roughly 2,000 km. Also, unlike most other Neotropical fish taxa, *G. carapo* has gained access to extremely isolated upland river drainages of the Guiana Shield, including rivers of the Pakaraima mountains and Tafelberg tepui. In contrast to other taxa present in these isolated regions, which are often endemic species or genera (Alofs et al., 2014), we find that *G. carapo* is only diverged at a population level (<2% cyt *b* divergence) from nearby conspecifics. We hypothesize that this species has life-history traits that differentiate it from other *Gymnotus* species (and indeed most other Neotropical fish species) with more local distributions, and that enable both extreme long-distance dispersal and the ability to surmount daunting elevational river barriers. Variation in dispersal ability among different fish lineages likely contributes to the spectrum of antiquities exhibited by inhabitants of Guianas "Lost World" rivers.

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CONFLICT OF INTEREST

The authors state no conflict of interest in a financial or research capacity in the publication of this paper. All authors were made aware of this statement and have acknowledged it to be true.

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BIOSKETCH

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SUPPORTING INFORMATION

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