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The trans-riverine genetic structure of 28 Amazonian frog species is dependent on life history

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Abstract: Among the hypotheses formulated to explain the origin of Amazonian biodiversity, two (the riverine-barrier and the river-refuge hypotheses) focus on the role that rivers play as biotic barriers promoting speciation. However, empirical results have both supported and refuted these hypotheses. This is likely due, at least in part, to river-specific hydrologic characteristics and the biology of the focal species. The rivers of the Guiana Shield represent a model system because they have had more stable courses over time than those of the western Amazon Basin, where most tests of riverine barrier effects have taken place. We tested whether life-history traits (body size, habitat and larval development), expected to be important in determining dispersal ability, of 28 frog species are associated with genetic structure and genetic distances of individuals sampled from both banks of the Oyapock River. Thirteen of these species displayed genetic structure consistent with the river acting as a barrier to dispersal. Surprisingly, body size was not correlated with trans-riverine population structure. However, leaf-litter dwellers and species lacking free-living tadpoles were found to exhibit higher river-associated structure than open habitat/arboreal species and those with exotrophic tadpoles. These results demonstrate that rivers play an important role in structuring the genetic diversity of many frog species though the permeability of such riverine barriers is highly dependent on species-specific traits.

Key Words: Amazonia, Anura, dispersal, diversity, Guiana Shield, mitochondrial DNA, phylogeography, river

INTRODUCTION

The astonishing extent of Amazonian diversity has fascinated biologists since the 19th century (Bates 1874, Wallace 1854). However, the actual number of species occurring in Amazonia (Fouquet *et al.* 2007, Funk *et al.* 2012), and the processes responsible for their origins (Haffer 1997, Hoorn *et al.* 2010) remain intensely debated. The first model proposed to explain animal diversity in Amazonia relied on the dissecting power of large Amazonian rivers acting as biogeographic barriers for many taxa (Wallace 1854), a hypothesis actually formulated even earlier by de Castelnau (1851).

Since then, many hypotheses have been put forward to explain these high levels of diversity in Amazonia (reviewed in Leite & Rogers 2013, Noonan & Wray 2006). Two of these hypotheses invoke the barrier effect of the rivers to explain patterns of diversity, the riverine-barrier hypothesis (Bates 1874, Capparella 1988, Mayr 1942, Wallace 1854) and the river-refuge hypothesis (Ayres & Clutton-Brock 1992). The first proposes that riverine barriers separated once continuous populations leading to differentiation and, eventually, speciation. The second argues that Pleistocene forest refugia and rivers interacted with forest fragmentation driven by the cold, dry periods of the Quaternary to reinforce this isolation during and after forest expansion. Empirical support for these hypotheses comes from the observation that the boundaries of closely related species or subspecies often coincide with major Amazonian rivers (e.g. Primates (Ayres & Clutton-Brock 1992); birds (Haffer 1997, Naka *et al.* 2012); lizards (Avila-Pires 1995, Souza *et al.* 2013)).

However, few studies have used molecular data to explicitly test the barrier effect of rivers in Amazonia and they provided contrasting evidence for the effectiveness

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of rivers as barriers (e.g. frogs: Fouquet *et al.* 2012a, Funk *et al.* 2007, Gascon *et al.* 1998, Kaefer *et al.* 2013, Lougheed *et al.* 1999; birds: Burney & Brumfield 2009, Naka *et al.* 2012, Ribas *et al.* 2011; mammals: Patton *et al.* 1994, Peres *et al.* 1996). Though conclusions about the role of rivers on diversification vary among these studies, it is clear that the permeability of rivers to gene flow is influenced by (1) river size and course variation over time (Bates *et al.* 2004) and (2) species' ability to disperse across them (Burney & Brumfield 2009).

Major rivers undoubtedly impede dispersal for many terrestrial organisms, but these rivers may not be sufficiently impenetrable or long-lived to generate lasting or significant genetic structure and ultimately lead to speciation (Slatkin 1987). One of the reasons is meander loop cut-off (Hayes & Sewlal 2004, Jackson & Austin 2013, Peres *et al.* 1996), a common phenomenon of the large rivers of Amazonia, which are known to regularly change course. However, this is thought to be less common for the more channelled and stable clear-water rivers of the Brazilian and Guiana Shields (Ayres & Clutton-Brock 1992, Bates *et al.* 2004, Lundberg *et al.* 1998), making these regions ideal for the study of the role of rivers in speciation.

The ability to disperse across rivers is also expected to vary substantially among species, and can depend on life-history traits such as body size, habitat preference and reproductive mode (Fouquet *et al.* 2012a, Gascon *et al.* 1998, Lampert *et al.* 2003, Newman & Squire 2001, Richardson 2012). In amphibians, species with large body size and free-living tadpoles deposited in lotic water are expected to disperse more readily than small-bodied, direct-developing species (Van Bocxlaer *et al.* 2010, Wollenberg *et al.* 2011). Amphibians are thus particularly valuable models to investigate processes shaping genetic structure (Zeisset & Beebee 2008), particularly in Amazonia where they display a high diversity of the aforementioned traits.

We hypothesize that genetic structure of anuran species across major rivers in the Guiana Shield is life-history dependent. Evaluating (1) the genetic structure and (2) the genetic distance among samples from opposite margins of the Oyapock River (a large, well-channelled river on Precambrian rock draining into the Atlantic Ocean), we tested whether variation in these two metrics are correlated with three traits that may influence dispersal: body size, habitat and development mode.

METHODS

Sampling

Samples (2–5 individuals from each of 28 species collected per locality, Appendix 1; taxonomy follows http://research.amnh.org/herpetology/amphibia/index.html) were gathered from opposite banks of the lower course of the Oyapock River, in the areas of St Georges (French Guiana) and Oiapoque (Amapá, Brazil) (Figure 1). In this area the Oyapock River width varies between 200 and 500 m. Its fast-flowing and well-channelled course is almost entirely without adjacent igapo (temporarily flooded areas) upriver of the coastal estuary, and as such is expected to have had a rather stable course over time (Bates *et al.* 2004). Samples were also collected from localities within the interfluvium of the Oyapock and the Approuague rivers (Savane Virgine) and from Lourenço and Serra do Navio in Amapá (Figure 1). No other major obstacles such as patches of open habitat or mountains occur between the localities.

Molecular analysis

Genomic DNA was extracted from tissue samples preserved in 95% ethanol using the Wizard Genomic DNA Extraction kit (Promega; Madison, WI, USA) (Appendix 1). A portion of the mitochondrial 16S rDNA locus was amplified by standard PCR techniques using previously described primers and PCR conditions (Hillis *et al.* 1996, Salducci *et al.* 2005). Sequencing was performed using ABI Big Dye V3.1 (ABI, Foster City, CA, USA) and run on automated sequencers at Macrogen (Korea) and Beckman Coulter (UK). Sequences were edited and aligned with CodonCode Aligner v.3.5.2 (http://www.codoncode.com/aligner/download.htm). Novel sequences were deposited in GenBank (Appendix 1).

The 102 newly generated sequences were combined with 14 available sequences from GenBank and aligned with MAFFT v6 (Katoh & Standley 2013) under default parameters and the E-INS-i strategy, which is designed for sequences with one conserved domain and long gaps. Our final alignment consisted of 520 base pairs (bp) that was incomplete only for the last *c.* 140 bp of 15 terminals (those sequenced using 16SF and 16SR).

We used the software jModeltest version 2.1.1 (Darriba *et al.* 2012, Guindon & Gascuel 2003) to select the substitution model that best fit the data under the Bayesian Information Criterion. The resulting model (GTR + I + G) was employed in a Bayesian Analysis (BA) with MrBayes 3.2 (Ronquist & Huelsenbeck 2003). The BA consisted of 20×10^6 generations and 10 Markov chains (one cold) sampled every 1000 generations. Adequate burn-in was determined by examining likelihood scores of the heated chains for convergence and stationarity in Tracer 1.5 (http://beast.bio.ed.ac.uk/Tracer) and effective sample size of values were >200. We also calculated pairwise (p) genetic distances between samples from the opposite

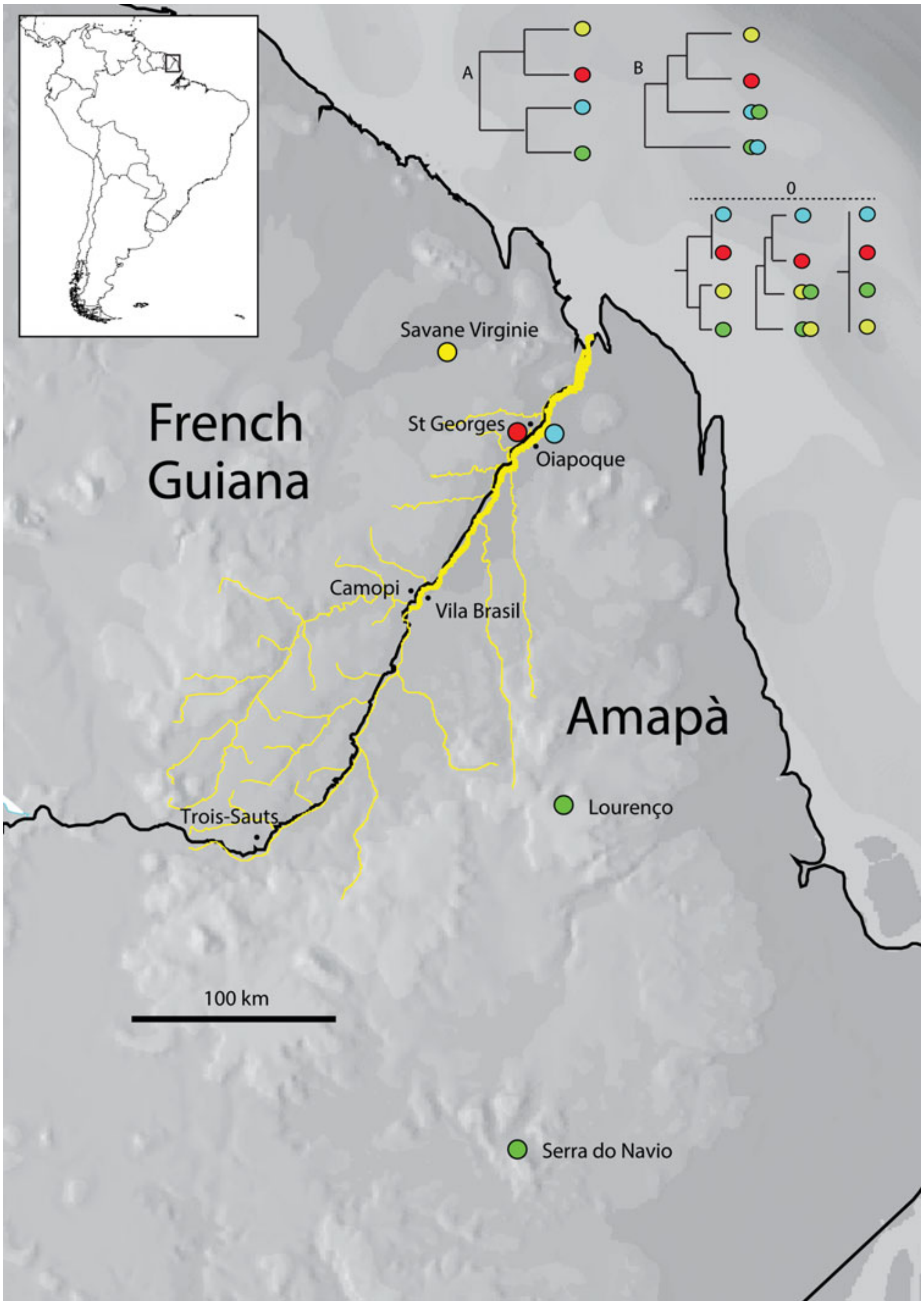

Figure 1. Map of the study area encompassing French Guiana and Amapá State (Brazil) with localities where frogs were sampled indicated with coloured circles and simplified drainage of the Oyapock River (yellow). Examples of two hypothetical topologies are illustrated on the top right corner indicative of a barrier effect of the river (topologies A and B) and three examples of topologies indicative of trans-riverine affinities (topology 0) are indicated below. Two dots in the same terminal indicate that the prediction will be the same regardless of the position of the samples.

margins of the Oyapock River (St Georges vs. Oiapoque only) with MEGA 5 (Tamura *et al.* 2011).

We assume that the absence of direct affinity and genetic distance >0.2% (minimal distance observed for species displaying reciprocal monophyly across the river) between the populations from opposite margins can be interpreted as a barrier effect of the Oyapock river. The most straightforward case will be species displaying reciprocally monophyletic lineages from each side (Figure 1; e.g. topology A). Alternative scenarios include species displaying monophyly of only one interfluvium which may result from gene flow in headwater areas (Figure 1; e.g. topology B). Results were coded as a binary character reflecting the presence (1) or absence (0) of genetic structure suggestive of a barrier effect. These two metrics (coded topologies and p-distances) were used as proxies of the extent to which the Oyapock River acts as a barrier.

For each species, we also coded three life-history traits: body size (max SVL: small = 10–30 mm; medium = 30–60 mm; large = >60 mm), habitat of adults (forest litter, arboreal and open habitats) and larval development (free exotrophic tadpoles in lentic or lotic waters vs. direct developing, endotrophic and phytotelmic tadpoles) (Figure 2). These data were collected from Lescure & Marty (2001) and personal observations. For each of these traits we tested for an association with the presence/absence of genetic structure and genetic distance across the Oyapock River.

Notably our study does not address whether this river currently acts as a barrier to dispersal, as this would require greater population-level sampling and multiple fast-evolving molecular markers. Instead, we used a single slow-evolving mtDNA locus, which will allow us to detect (robustly) historical events (e.g. fragmentation of the range). Observations of structure from our sampling can be the result of either the river acting as a primary barrier or secondary contact zone between demes originally isolated by other barriers. However, the maintenance of such structure is still informative with respect to the effects of rivers on gene flow.

Statistical analysis

We first tested whether p-distances display a significant phylogenetic signal by calculating Blomberg's K (Blomberg *et al.* 2003). This statistic is a representation of the phylogenetic signal in the tip data relative to the expectation for a trait evolving by Brownian motion along the specified phylogeny. Values near 0 indicate a lack of a phylogenetic signal, and values approaching 1 indicate Brownian character evolution (i.e. a tendency for closely related species to display similar trans-riverine p-distance). Significance of K was assessed by 1000 replicates of randomly shuffling trans-riverine p-distance values among species.

Using the binary character states resulting from the phylogenetic analysis (presence/absence of genetic structure across Oyapock River), we tested whether the proportion of species for which the river represents a barrier is correlated with (1) body size (2) habitat and (3) larval development using Chi-square tests.

We also tested whether p-distances between samples from opposite margins are dependent on species' (1) body size (non-parametric Kruskal–Wallis test) (2) habitat (non-parametric Kruskal–Wallis test) and (3) mode of development (non-parametric Wilcoxon test). Non-parametric tests were used because p-distance were not normally distributed and samples sizes were low. All statistical analysis were performed with the R software v 2.15.3 (http://cran.r-project.org/) using the package Picante (Kembel *et al.* 2010).

RESULTS

Phylogenetic analysis and genetic distances

Relationships among conspecific samples ranged from deeply structured to uniform (Appendix 2). Thirteen species displayed genetic structure consistent with isolation by the Oyapock River (e.g. either reciprocal monophyly of the A/O interfluvium and Oiapoque/Amapà populations and p distance >1%; or monophyly of only the A/O interfluvium with genetic distances >0.2%) (Figure 2). The topologies recovered within the other 15 species did not possess genetic structure reflective of a barrier effect of the Oyapock River. Genetic distance between conspecific samples from St Georges and Oiapoque ranged from 0 in nine species to 2.7% in *Pristimantis chiastonotus* and *Anomaloglossus baeobatrachus* (Table 1).

Analyses of life-history traits

Genetic distances did not display a significant phylogenetic signal (Blomberg's K, P = 0.16, N = 28 species). Smaller-bodied species were not found to display higher genetic distance across the Oyapock River than medium or large body-sized species (Kruskal–Wallis, K = 1.83, P = 0.40, df = 2). Nor did they display more cases of genetic structure matching the course of the river (Chi-square test, $\chi^2 = 3.5$, P = 0.18, df = 2). However, forest-litter species did display greater genetic differentiation across the Oyapock River than arboreal and open-habitat species (Figure 3, Kruskal–Wallis, K = 5.8, P = 0.05, df = 2) and were more prone to display genetic structure across the river (Figure 3, Chi-square test, $\chi^2 = 7.0$, P = 0.03, df = 2). Similarly, species with free-living larvae displayed lower genetic differentiation across the river than species with terrestrial larvae (Figure 3, Wilcoxon test, W = 45.5, P = 0.05, df = 1), but the proportion of species displaying structure across the river did not differ significantly among larval types (Figure 3, Chi-square test, $\chi^2 = 3.5$, P = 0.06, df = 1).

DISCUSSION

The lower Oyapock River is a barrier for many taxa

The lower course of the Oyapock River corresponds to phylogeographic breaks in 13 of 28 species examined,

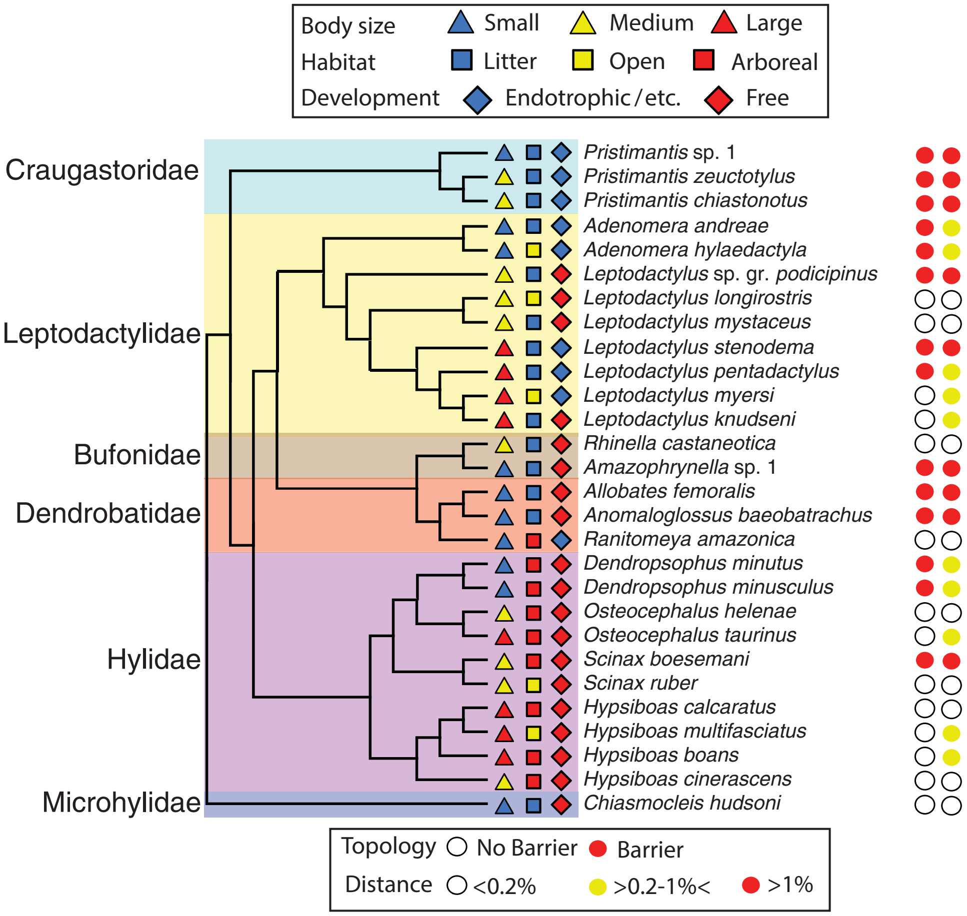

Figure 2. Phylogenetic relationship among the focal frog species sampled from opposite banks of the Oyapock River (from Pyron & Wiens (2011) and Fouquet *et al.* (2013)). For each species, the coded life-history traits are indicated before the species name with coloured symbols (triangle, square, diamond) and the categorical results from the molecular analyses analyses are indicated after the species names with coloured circles.

a pattern similar to the findings of previous studies of amphibians in the region (Fouquet *et al.* 2012a, 2012b) and birds along the Branco and Negro rivers (Naka *et al.* 2012). The fact that such structure was not recovered in frogs (*Scinax*, *Scarthyla*, *Allobates*, *Engystomops*) studied in central Amazonia (Gascon *et al.* 1998, Lougheed *et al.* 1999) is likely due to the highly dynamic nature of the courses of Amazonian floodplain rivers, allowing populations to shift from one bank to the other via recurrent cutting-off of meanders (Jackson & Austin 2013). Guiana Shield rivers on the other hand, while not as large, are channelled on Precambrian rocks and are more stable over time (Bates *et al.* 2004).

Fouquet *et al.* (2012b) hypothesized that the congruent phylogeographic structure observed in frogs of the region originated during periods of climatic instability. Cold/dry periods of the Pleistocene are thought to have aridified the central Guiana Shield restricting wet-forest patches to the coastal plain, where the lower courses of the rivers would have fragmented species' distributions. During these times, the narrower headwaters would not have been traversable as they lay in inhospitably arid, savanna habitat. This is the basis of a hypothesis analogous to the river refuge hypothesis (Ayres & Clutton-Brock 1992), but shaped and scaled to the unique hydrology of the eastern Guiana Shield (not all of Amazonia). Notably, these Pleistocene events are likely to have produced the type of intraspecific structure (not speciation, Rull 2011) we see here. Our results suggest this hypothesis has significant explanatory power for forest-litter-dwelling species with terrestrial larval development. This hypothesis is further supported by the suggestion of upstream dispersal in several species (e.g. *Amazophrynella* sp. and *Leptodactylus pentadactylus*) where the Oiapoque population is sister to all others (Figure 1).

While the slowly evolving nature of the locus employed here and the limited within-population sampling precludes estimates of gene flow across the river per se, our data do provide insight into the historical isolation of anuran populations across the Oyapock River. In other

Table 1. Table summarizing the results from the molecular analyses and the coding of the life history traits for each focal frog species sampled on each bank of the Oyapock River: species identification (sample size = n), the three trait modalities (Development, Habitat, Body size), p-distances between samples from opposite margins, topology recovered from the phylogenetic analysis (A and B are indicative of a genetic structure matching the course of the river while 0 are not).

Species	Development	Habitat	Body	p-dist	Topology
Leptodactylus longirostris (n = 4)	Classical	Open	Medium	0	0
Leptodactylus mystaceus (n = 4)	Classical	Litter	Medium	0	0
Rhinella castaneotica (n = 4)	Classical	Litter	Medium	0	0
Ranitomeya amazonica (n = 4)	Direct/Endo./Phyt.	Arboreal	Small	0	0
Osteocephalus helenae (n = 2)	Classical	Arboreal	Medium	0	0
Scinax ruber (n = 5)	Classical	Open	Medium	0	0
Hypsiboas calcaratus (n = 3)	Classical	Arboreal	Large	0	0
Hypsiboas cinerascens (n = 4)	Classical	Arboreal	Medium	0	0
Chiasmocleis hudsoni (n = 4)	Classical	Litter	Small	0	0
Leptodactylus myersi (n = 3)	Direct/Endo./Phyt.	Open	Large	0.0020	0
Dendropsophus minutus (n = 5)	Classical	Arboreal	Small	0.0020	0
Dendropsophus minusculus (n = 5)	Classical	Arboreal	Small	0.0020	A
Leptodactylus knudseni (n = 3)	Classical	Litter	Large	0.0040	0
Hypsiboas boans (n = 4)	Classical	Arboreal	Large	0.0041	0
Leptodactylus pentadactylus (n = 4)	Direct/Endo./Phyt.	Litter	Large	0.0060	B
Hypsiboas multifasciatus (n = 4)	Classical	Open	Large	0.0060	0
Adenomera hylaedactyla (n = 5)	Direct/Endo./Phyt.	Open	Small	0.0061	B
Osteocephalus taurinus (n = 4)	Classical	Arboreal	Large	0.0080	0
Amazophrynella sp. 1 (n = 4)	Classical	Litter	Small	0.0101	B
Leptodactylus sp. gr. podicipinus (n = 5)	Classical	Litter	Medium	0.0121	B
Pristimantis sp. 1 (n = 4)	Direct/Endo./Phyt.	Litter	Small	0.0123	A
Allobates femoralis (n = 5)	Classical	Litter	Small	0.0144	AB
Pristimantis zeuctotylus (n = 5)	Direct/Endo./Phyt.	Litter	Medium	0.0151	A
Scinax boesemani (n = 4)	Classical	Arboreal	Medium	0.0159	A
Leptodactylus stenodema (n = 3)	Direct/Endo./Phyt.	Litter	Large	0.0161	AB
Adenomera andreae (n = 6)	Direct/Endo./Phyt.	Litter	Small	0.0162	A
Anomaloglossus baeobatrachus (n = 4)	Classical	Litter	Small	0.0267	A
Pristimantis chiastonotus (n = 5)	Direct/Endo./Phyt.	Litter	Medium	0.0274	A

words, the Oyapock River may represent a barrier for species in which this structure is not detectable with the evolving mtDNA used here. It is also possible that gene flow may go undocumented in some species for which we report significant structure across the river due to our sampling limitations. This latter, type-two error, seems unlikely given the results of previous phylogeographic studies (Fouquet *et al.* 2012a, 2012b) which report a very low incidence of polyphyletic populations.

A limitation of our dataset is the limited sampling from each locality and we thus cannot rule out the occurrence of polyphyletic populations that we recovered as monophyletic. Nevertheless, this is the case only for 12 species out of 28 because more extensive datasets are already available in that area for 16 species included herein (Fouquet 2008, 2012a, 2012b; Funk *et al.* 2012, Gehara *et al.* 2014, Jungfer *et al.* 2013, Peloso *et al.* 2014) which confirmed the existence or absence of phylogeographic breaks matching the lower course of the Oyapock river. Among these 12 species, only four displayed obvious trans-riverine genetic structure; i.e. occurrence of undetected polyphyly that would indicate recent dispersal across the river cannot be ruled out for only four species. However, these four species are represented by pairs of populations sampled on each side of the Oyapock river. Additionally, even though undetected polyphyletic populations may occur in our sampling, the very existence of such structure remains meaningful even if secondarily admixed. We therefore choose to favour the taxonomic sampling (i.e. more species but fewer individuals) to assess whether the genetic structure of a greater number of frog species corresponds to this river.

Riverine barriers are life-history dependent

Our results suggest that the extent to which the Oyapock River acts as a barrier depends on species-specific life-history traits. These ecological differences are reflected in our recovered patterns of genetic structure and, consequently, the amount of genetic variation between demes. Fouquet *et al.* (2012b) hypothesized that idiosyncrasies among the phylogeographic patterns of 12 forest-litter frog species of the Eastern Guiana Shield were partly due to variation in specific traits such as body size, implying differences in generation time and population size. Wollenberg *et al.* (2011)

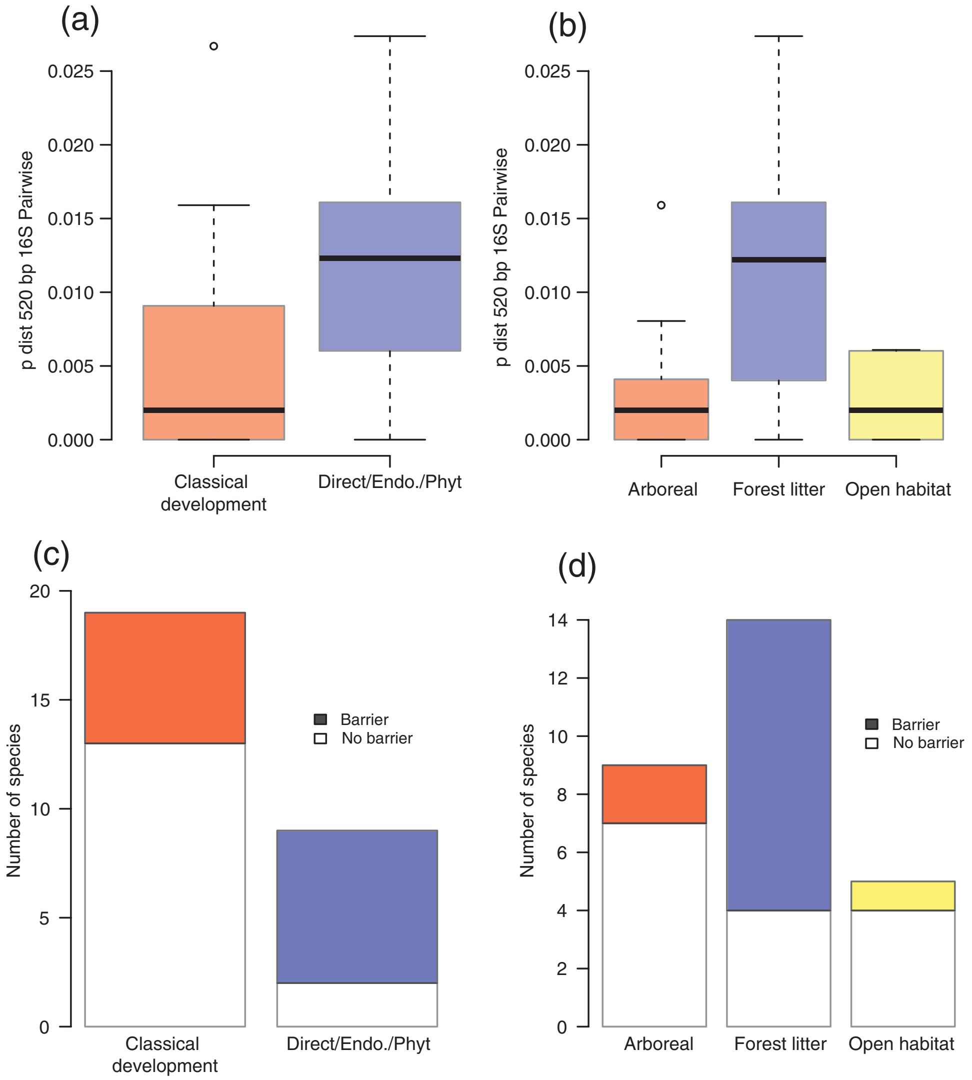

Figure 3. Box plots comparing genetic distances to developmental (classical in red and direct/endo./phyt. in blue) (a) and habitat traits (arboreal in red, forest litter in blue and open habitat in yellow) (b); and genetic structure classification to developmental (c) and habitat traits (d) of focal frog species sampled from opposite banks of the Oyapock River. The proportion of species with barrier effect is indicated with solid colours and the proportion of species with no barrier effect in white (c, d).

also established a link between small body size, low dispersal and high speciation rate in Malagasy frogs. Body size has also been shown to be positively correlated with dispersal ability and geographic distribution (Van Bocxlaer *et al.* 2010), again revealing a role of life-history and morphology in population structure. Our findings reveal no significant link between body size and isolation across the Oyapock River. This may be due to the fact that our sampling is phylogenetically highly heterogeneous (seven families) rather than focusing on one specific clade, as in former studies. This may also highlight the distinction between the dispersal ability over distance through homogeneous landscape and across a barrier such as a river. The latter may be more dependent on life-history traits other than the body size. Our study instead highlights the importance of habitat and reproductive mode.

Studies of frog species sampled along the Juruá River found greater genetic differentiation among species associated with terra firme than flooded areas, though this structure was not coincident with the course of the river (Gascon *et al.* 1998, Lougheed *et al.* 1999). Fouquet *et al.* (2007) also demonstrated that open-habitat and aquatic species display less genetic structure/divergence than forest-dwelling species over vast distances in Amazonia. The extent to which population structure of birds is explained by rivers has also been demonstrated to be highly influenced by ecology, with canopy species exhibiting lower genetic divergence across Amazonian rivers than understorey birds (Burney & Brumfield 2009). Similarly, arboreal species in our study display lower genetic distances across the river and fewer of these species possess genetic structure concordant with the Oyapock than forest-litter species. In frogs at least, arboreal species may be more prone to cross rivers via tree fall. Higher dispersal ability of arboreal species may also be due to a greater tolerance to temperature variation and desiccation inherent to this microhabitat than forest-litter species for which the hygrometry and temperature of the forest floor is more stable. Therefore, arboreal species may be able to disperse more easily through the dry, or at least drier, climate areas of the central Guiana Shield (Gond *et al.* 2011). The only arboreal species in our study to display evidence of isolation across the river utilize stagnant water at the edge of forest (*Dendropsophus minusculus, Scinax boesemani*).

Open-habitat species also exhibit little if any genetic structure coincident with the river course. In fact, none of the open-adapted species displays structure across the river except *A. hylaedactyla*, which has endotrophic larvae. This may be the result of recent historical expansion of open landscapes during the Quaternary allowing these species to disperse, possibly in the headwater regions (Fouquet *et al.* 2011). Open landscape connections have been hypothesized in the interior of the Guiana Shield, but expansion may also have occurred along the coast during periods of lowered sea levels. The coastal region of the Guiana Shield is currently a patchwork of savannas, mangroves, swamps and forests facing a very shallow 100-km continental shelf. During cold/dry periods of the Quaternary, this area was likely prone to harbour a vast extent of open habitat which may have favoured dispersal, as exemplified by the occurrence of relictual savannas peppered throughout the coast of the region and the distribution of open habitat species. As they are more tolerant of climatic conditions of open areas, open habitat specialists may be also more prone to dispersal during the dry season across a reduced-width river (e.g. across rocky rapids).

Reproductive mode also seems to be an important factor determining dispersal ability across the river. This pattern may be directly related to larval dispersal, at least for the species with large tadpoles that breed directly in the rivers (*Hypsiboas boans, Osteocephalus helenae*) or in flooded areas (*Leptodactylus mystaceus*). On the other hand, all species with direct developing (*Pristimantis*) or endotrophic larvae (*Adenomera*) display strong differentiation across the river. Though there have been few studies that explicitly examine barrier effects of multiple taxa with varying larval development strategies, such characteristics have been shown to affect the distribution and connectivity of populations (Van Bocxlaer *et al.* 2010).

This study represents the first attempt to test the riverine barrier hypothesis with a large multitaxon dataset. In the Guiana Shield, the historical stability of the river courses has allowed us to explore the influence of life-history traits on the dispersal ability of anurans across a river barrier. Our findings clearly demonstrate that these traits predict the extent to which the Oyapock River, and likely other stable, major rivers in the tropics, acts as a barrier to dispersal in anurans.

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Appendix 1. Frog samples from French Guiana (left bank of the Oyapock River) and Amapá State (Brazil) (right bank of the Oyapock River) included in the molecular analyses: species identification, field numbers, locality, geographic coordinates and GB accession numbers.

Species	Field number	Locality	Latitude	Longitude	GB accession
Adenomera andreae	AF0543	S. Virginie	4.19988	−52.13644	KR811145
Adenomera andreae	AF1248	Mont. Bruyer	4.20043	−51.63725	KR811146
Adenomera andreae	MTR13807	Serra do Navio	0.91805	−52.00278	JN690847
Adenomera andreae	MTR24083	Oiapoque	3.85956	−51.75951	KR811144
Adenomera andreae	MTR24232	Lourenço	2.32361	−51.64528	KR811143
Adenomera andreae	PG711	Saut Maripa	3.80696	−51.87849	KR811147
Adenomera hylaedactyla	BM238	St Georges	3.86667	−51.8	JN690918
Adenomera hylaedactyla	CM092	Mont. d'Argent	4.38333	−51.7	EU201052
Adenomera hylaedactyla	MTR13813	Serra do Navio	0.91805	−52.00278	JN690905
Adenomera hylaedactyla	MTR24102	Oiapoque	3.85956	−51.75951	KR811148
Adenomera hylaedactyla	MTR24216	Lourenço	2.32361	−51.64528	KR811149
Allobates femoralis	AF0557	S. Virginie	4.19988	−52.13644	KR811190
Allobates femoralis	AF0753	Saut Maripa	3.80696	−51.87849	KR811191
Allobates femoralis	MTR13802	Serra do Navio	0.91806	−52.00278	JN690958
Allobates femoralis	MTR24184	Oiapoque	3.85956	−51.75951	KR811192
Allobates femoralis	MTR13936	Lourenço	2.32361	−51.64528	JN690959
Amazophrynella sp. 1	AF0761	Saut Maripa	3.80696	−51.87849	KR811168
Amazophrynella sp. 1	MTR13918	Lourenço	2.32361	−51.64528	KR811166
Amazophrynella sp. 1	MTR24057	Oiapoque	3.85956	−51.75951	KR811165
Amazophrynella sp. 1	PG776	S. 14 Juillet	3.96764	−51.87225	KR811167
Anomaloglossus baeobatrachus	MTR24144	Oiapoque	3.85956	−51.75951	KR811196
Anomaloglossus baeobatrachus	MTR24210	Lourenço	2.32361	−51.64528	KR811195
Anomaloglossus baeobatrachus	PG720	Saut Maripa	3.80696	−51.87849	KR811194
Anomaloglossus baeobatrachus	PG790	S. Virginie	4.19988	−52.13644	KR811193
Chiasmocleis hudsoni	AF0758	Saut Maripa	3.80696	−51.87849	KR811107
Chiasmocleis hudsoni	AG397	Kourouaie	4.23788	−52.03817	KR811106
Chiasmocleis hudsoni	MTR24296	Oiapoque	3.85956	−51.75951	KR811109
Chiasmocleis hudsoni	AG396	Kourouaie	4.23788	−52.03817	KR811108
Dendropsophus minusculus	AF0736	Saut Maripa	3.80696	−51.87849	KR811133
Dendropsophus minusculus	AF1083	Oiapoque	3.85956	−51.75951	KR811136
Dendropsophus minusculus	MTR13795	Serra do Navio	0.91806	−52.00278	KR811134
Dendropsophus minusculus	MTR13910	Lourenço	2.32361	−51.64528	KR811135
Dendropsophus minusculus	PG735	RN2 PK169	3.86667	−51.8	KR811137
Dendropsophus minutus	AF0088	St Georges	3.86667	−51.8	KR811132
Dendropsophus minutus	AF0560	S. Virginie	4.19988	−52.13644	KR811131
Dendropsophus minutus	AF1078	Oiapoque	3.85956	−51.75951	KR811130
Dendropsophus minutus	MTR13739	Serra do Navio	0.91806	−52.00278	KJ833304
Dendropsophus minutus	MTR13905	Lourenço	2.32361	−51.64528	KJ833305
Hypsiboas boans	BM100	S. Virginie	4.19988	−52.13644	KR811171
Hypsiboas boans	MTR24182	Oiapoque	3.85956	−51.75951	KR811170
Hypsiboas boans	PG767	Saut Maripa	3.80696	−51.87849	KR811169
Hypsiboas boans	MTR24187	Lourenço	2.32361	−51.64528	KR811172
Hypsiboas calcaratus	DAN12	St Georges	3.86667	−51.8	KR811159
Hypsiboas calcaratus	MTR24072	Oiapoque	3.85956	−51.75951	KR811158
Hypsiboas calcaratus	PG105	Kaw	4.58943	−52.25246	KR811160
Hypsiboas cinerascens	AF0556	S. Virginie	4.19988	−52.13644	KR811175
Hypsiboas cinerascens	AF0747	St Georges	3.86667	−51.8	KR811174
Hypsiboas cinerascens	MTR24062	Oiapoque	3.85956	−51.75951	KR811173
Hypsiboas cinerascens	MTR24205	Lourenço	2.32361	−51.64528	KR811176
Hypsiboas multifasciatus	AF0749	St Georges	3.86667	−51.8	KR811157
Hypsiboas multifasciatus	MTR24173	Oiapoque	3.85956	−51.75951	KR811155
Hypsiboas multifasciatus	PG779	S. 14 Juillet	3.96764	−51.87225	KR811154
Hypsiboas multifasciatus	MTR24194	Lourenço	2.32361	−51.64528	KR811156
Leptodactylus knudseni	AF0562	S. Virginie	4.19988	−52.13644	KR811114
Leptodactylus knudseni	AF1063	Saut Maripa	3.80696	−51.87849	KR811115
Leptodactylus knudseni	AF1066	Oiapoque	3.85956	−51.75951	KR811113
Leptodactylus longirostris	MTR13796	Serra do Navio	0.91806	−52.00278	KR811124
Leptodactylus longirostris	MTR24148	Oiapoque	3.85956	−51.75951	KR811125
Leptodactylus longirostris	PG786	S. 14 Juillet	3.96764	−51.87225	KR811123
Leptodactylus longirostris	MTR24097	Oiapoque	3.85956	−51.75951	KR811126
Leptodactylus myersi	PG199	S. Virginie	4.19988	−52.13644	KR811116
Leptodactylus myersi	PG771	S. 14 Juillet	3.967634	−51.87225	KR811117

Appendix 1. Continued.

Species	Field number	Locality	Latitude	Longitude	GB accession
Leptodactylus myersi	MTR24303	Oiapoque	3.85956	−51.75951	KR811118
Leptodactylus mystaceus	MTR13787	Serra do Navio	0.91806	−52.00278	JN691159
Leptodactylus mystaceus	PG741	St Georges	3.86667	−51.8	KR811127
Leptodactylus mystaceus	PG766	Saut Maripa	3.80696	−51.87849	KR811128
Leptodactylus mystaceus	MTR24175	Oiapoque	3.85956	−51.75951	KR811129
Leptodactylus pentadactylus	AF1065	Oiapoque	3.85956	−51.75951	KR811122
Leptodactylus pentadactylus	DAN18	St Georges	3.86667	−51.8	KR811119
Leptodactylus pentadactylus	MTR24302	Lourenço	2.32361	−51.64528	KR811120
Leptodactylus pentadactylus	CM109	Kaw	4.58943	−52.25246	KR811121
Leptodactylus sp. gr. podicipinus	AF0735	Saut Maripa	3.80696	−51.87849	KR811152
Leptodactylus sp. gr. podicipinus	AF0810	S. Virginie	4.19988	−52.13644	KR811150
Leptodactylus sp. gr. podicipinus	MTR13809	Serra do Navio	0.91806	−52.00278	JN691205
Leptodactylus sp. gr. podicipinus	MTR24141	Oiapoque	3.85956	−51.75951	KR811153
Leptodactylus sp. gr. podicipinus	PG783	S. 14 Juillet	3.96764	−51.87225	KR811151
Leptodactylus stenodema	AF0553	S. Virginie	4.19988	−52.13644	KR811112
Leptodactylus stenodema	AF0760	Saut Maripa	3.80696	−51.87849	KR811111
Leptodactylus stenodema	AF1071	Oiapoque	3.85956	−51.75951	KR811110
Osteocephalus helenae	DAN20	St Georges	3.86667	−51.8	KR811141
Osteocephalus helenae	MTR24176	Oiapoque	3.85956	−51.75951	KR811142
Osteocephalus taurinus	AF0077	St Georges	3.86667	−51.8	KR811140
Osteocephalus taurinus	MTR13797	Serra do Navio	0.91806	−52.00278	KF002160
Osteocephalus taurinus	PG778	S. 14 Juillet	3.96764	−51.87225	KR811138
Osteocephalus taurinus	AF1074	Oiapoque	3.85956	−51.75951	KR811139
Pristimantis chiastonotus	AF0083	St Georges	3.86667	−51.8	KR811101
Pristimantis chiastonotus	AF0550	S. Virginie	4.19988	−52.13644	KR811100
Pristimantis chiastonotus	MTR13765	Serra do Navio	0.91806	−52.00278	JN691267
Pristimantis chiastonotus	MTR24101	Oiapoque	3.85956	−51.75951	KR811098
Pristimantis chiastonotus	MTR24266	Lourenço	2.32361	−51.64528	KR811099
Pristimantis sp. 1	AF0563	S. Virginie	4.19988	−52.13644	KR811102
Pristimantis sp. 1	AF0757	Saut Maripa	3.80696	−51.87849	KR811105
Pristimantis sp. 1	MTR24285	Lourenco	2.32361	−51.64528	KR811104
Pristimantis sp. 1	MTR24300	Oiapoque	3.85956	−51.75951	KR811103
Pristimantis zeuctotylus	AF0081	St Georges	3.86667	−51.8	JN691258
Pristimantis zeuctotylus	AF0565	S. Virginie	4.19988	−52.13644	KR811197
Pristimantis zeuctotylus	AF1075	Oiapoque	3.85956	−51.75951	KR811199
Pristimantis zeuctotylus	MTR13827	Serra do Navio	0.91806	−52.00278	JN691229
Pristimantis zeuctotylus	MTR24199	Lourenço	2.32361	−51.64528	KR811198
Ranitomeya amazonica	AF1526	S. Virginie	4.19988	−52.13644	KR811187
Ranitomeya amazonica	MTR24118	Oiapoque	3.85956	−51.75951	KR811189
Ranitomeya amazonica	PG714	Saut Maripa	3.80696	−51.87849	KR811186
Ranitomeya amazonica	MTR24245	Lourenço	2.32361	−51.64528	KR811188
Rhinella castaneotica	MTR24050	Oiapoque	3.85956	−51.75951	KR811162
Rhinella castaneotica	MTR24189	Lourenço	2.32361	−51.64528	KR811161
Rhinella castaneotica	PG740	Saut Maripa	3.80696	−51.87849	KR811163
Rhinella castaneotica	PG789	S. Virginie	4.19988	−52.13644	KR811164
Scinax boesemani	AF0559	S. Virginie	4.19988	−52.13644	KR811184
Scinax boesemani	AF0746	St Georges	3.86667	−51.8	KR811185
Scinax boesemani	MTR24162	Oiapoque	3.85956	−51.75951	KR811182
Scinax boesemani	MTR13903	Lourenço	2.32361	−51.64528	KR811183
Scinax ruber	AF0089	St Georges	3.86667	−51.8	KR811180
Scinax ruber	MTR13738	Serra do Navio	0.91806	−52.00278	KR811181
Scinax ruber	MTR24052	Oiapoque	3.85956	−51.75951	KR811179
Scinax ruber	PG763	Saut Maripa	3.80696	−51.87849	KR811178
Scinax ruber	MTR24219	Lourenço	2.32361	−51.64528	KR811177

Appendix 2. Subtrees obtained from a Bayesian analysis of a single alignment of a 16S fragment for each frog species sampled at the border between French Guiana and Amapà State, Brazil, i.e. on each side of the Oyapock River. Posterior probabilities (×100) of each branch are indicated, with asterisks when posterior probability = 1. Coloured symbols correspond to the localities (cf. Figure 1). Species names in red (left) are the ones displaying trans-riverine population structure (topologies A, B). Species names in blue (right) are the ones with no trans-riverine population structure.