Biogeographic analysis of platyrrhines using updated taxonomic assessments to evaluate evidence for the Riverine Barrier Hypothesis

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The mechanisms that underlie the diversification of Neotropical primates remain contested. The Riverine Barrier Hypothesis (RBH) postulates that large rivers impede gene flow between populations on opposite riverbanks and promote allopatric speciation. A prediction of the RBH is that the strength of any river to act as a dispersal barrier should be a function of its width and flow – as demonstrated for the Amazon watershed in a classic study by Ayres and Clutton-Brock (1992). However, subsequent proliferation of Neotropical primate species, due to profound changes in taxonomy and species concepts, may have invalidated their results. Here we test whether, with the most recent taxonomic assessments and distribution maps, there is still evidence that similarity of opposite riverbank communities decreases with increasing river size. First, we conducted a literature review of primate taxonomy and developed a comprehensive spatial database, then applied GIS to query mapped primate ranges against the riverine geography of the Amazon watershed to produce a similarity index for opposite riverbank communities. Finally, we ran models to test how two measures of river size predicted levels of similarity. We found that, almost without exception, similarity scores were lower than scores from Ayres and Clutton-Brock (1992) for the same rivers. Our model showed a significant negative relationship between streamflow and similarity in all tests, and found width significant for the segmented Amazon, but not for multiple Amazon watershed rivers. We demonstrate that results of older biogeographic studies should be viewed with caution, because incorporating the greater number of species and subsequent changes in distributions now recognised, can alter conclusions drawn. These results still support the RBH insofar as they provide evidence for the prediction that rivers with higher streamflow act as more substantial barriers to dispersal, and accordingly exhibit greater variation in community composition between riverbanks. 2+

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Biogeographic analysis of platyrrhines using updated taxonomic assessments to evaluate evidence for the Riverine Barrier Hypothesis

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Keywords

Diversification, Amazon, Primates, Evolution, Distributions, Similarity index

Abstract

The mechanisms that underlie the diversification of Neotropical primates remain contested. The Riverine Barrier Hypothesis (RBH) postulates that large rivers impede gene flow between populations on opposite riverbanks and promote allopatric speciation. A prediction of the RBH is that the strength of any river to act as a dispersal barrier should be a function of its width and flow – as demonstrated for the Amazon watershed in a classic study by Ayres and Clutton-Brock (1992). However, subsequent proliferation of Neotropical primate species, due to profound changes in taxonomy and species concepts, may have invalidated their results. Here we test whether, with the most recent taxonomic assessments and distribution maps, there is still evidence that similarity of opposite riverbank communities decreases with increasing river size. First, we conducted a literature review of primate taxonomy and developed a comprehensive spatial database, then applied GIS to query mapped primate ranges against the riverine geography of the Amazon watershed to produce a similarity index for opposite riverbank communities. Finally, we ran models to test how two measures of river size predicted levels of similarity. We found that, almost without exception, similarity scores were lower than scores from Ayres and Clutton-Brock (1992) for the same rivers. Our model showed a significant negative relationship between streamflow and similarity in all tests, and found width significant for the segmented Amazon, but not for multiple Amazon watershed rivers. We demonstrate that results of older biogeographic studies should be viewed with caution, because incorporating the greater number of species and subsequent changes in distributions now recognised, can alter conclusions drawn. These results still support the RBH insofar as they provide evidence for the

prediction that rivers with higher streamflow act as more substantial barriers to dispersal, and accordingly exhibit greater variation in community composition between riverbanks.

Introduction

First conceived by Wallace (1845), the Riverine Barrier Hypothesis (RBH) was the earliest hypothesis of a biogeographical mechanism for species diversification in the Amazon. It stemmed from observations that range boundaries of primates, birds and insects often abut at rivers (Wallace 1854). It was more recently supported in a seminal study by Ayres and Clutton-Brock (1992) that provided evidence for one of the major predictions of the RBH - that river systems act as barriers which delineate species ranges, dividing populations, and causing isolation. However, recent, profound changes in taxonomy and species concepts resulting in major revisions to Neotropical primate taxonomy could invalidate the results of this study and reduce support for the RBH. Based on the latest species revisions and distribution data this study aims to determine whether one of the key predictions of the RBH still holds for Neotropical primates.

Numerous hypotheses have been proposed to account for Neotropical diversification, but which environmental feature was the vicariant agent that caused populations to become separated and subsequently genetically differentiated remains disputed (Robert 2000). The RBH postulates that river systems act as barriers which delineate species ranges, dividing populations, and causing isolation. Competing with the RBH, a model of allopatric speciation that originally received wide support is the Pleistocene Refugium hypothesis which posits that during ice age glacial maxima previously connected populations became separated, persisting in pockets of forest isolated from each other providing a vicariant mechanism for speciation (Haffer et al. 1969; Rull

2011). There are further vicariance-based diversification models, such as the Miocene marine incursion, structural arches, and disturbance vicariance (Aleixo 2004; Kay 2015; Leite & Rogers 2013). The different hypotheses for diversification are not necessarily mutually exclusive. The vicariant agents discussed above are estimated to have occurred at different points in geological time. Historically, mainly biogeographical and paleoecological approaches were available to draw conclusions about the origins of actual diversity and distribution. Based on this type of evidence, hypotheses promoting the importance of Quaternary climatic changes on speciation proliferated and the Pleistocene refugium hypothesis became the dominant theory. However, in the last few decades, the results from new molecular phylogenetic methods has not generally bolstered validity for Quaternary diversification and instead points toward a model involving a Tertiary (mainly Neogene) origin for Neotropical species, which provides support for the RBH (Rull 2011).

Primates colonised the Neotropics towards the end of the Oligocene (~23.03 – 28.1 Mya), before the final uplift of the Andes and subsequent reorganisation of the Amazonian drainage system (Hoorn et al. 2010; Latrubesse et al. 2010). Initially, platyrrhine taxa separated rapidly into discrete body-size niches (Kay 2015; Lynch Alfaro et al. 2015a). Within 15 Mya the ancestral lineage of platyrrhines diversified into 3 families comprising 15 genera and over the past 10 Ma those genera continued to expand (Jameson Kiesling et al. 2015; Rylands et al. 2012). The present Neotropical region, and particularly the Amazonian tropical rainforests, harbours a species diversity that is vastly disproportionate to its geographic area. According to the IUCN/SSC Primate Specialist Group there are 211 platyrrhine taxa. Molecular data from several systematics studies have converged on the genus-level phylogeny of extant platyrrhines. There are three monophyletic clades within the platyrrhines: Cebids, Atelids and Pitheciids (Kay 2015;

Opazo et al. 2006; Wildman et al. 2009). However, taxonomic inflation, from taxonomic revisions, rather than discovery of new species, over the last several decades has led to substantial increases in the number of species recognized (Groves 2014; Isaac & Purvis 2004; Zachos et al. 2013). This is largely due to a shift towards the Phylogenetic Species Concept (PSC) which tends to split rather than group taxa (Agapow et al. 2004; Frankham et al. 2012; Hausdorf 2011). It should be recognised that the details of platyrrhine taxonomy are widely disputed (Groves 2001b; Rylands & Mittermeier 2009; Rylands et al. 2012) and this instability presents a challenge to researchers seeking to decipher the history of species diversity and how it was assembled (Moritz et al. 2000; Opazo et al. 2006; Schneider & Sampaio 2015).

Studies across a range of taxonomic groups have demonstrated the inhibiting effect of rivers by showing how species assemblages vary on opposite riverbanks and by investigating the historical evolutionary relationships between them (Ribas et al. 2012; Leite & Rogers 2013; Boubli et al. 2015; Lynch Alfaro et al. 2015b). There are a few predictions from the RBH (Box. 1), and these are often a focus of research.

Box 1. Predictions of the Riverine Barrier Hypothesis

The following predictions would support river formation as the primary driver of primate speciation.

A. Reciprocally monophyletic taxa should exist on opposite riverbanks.

B. Sister taxa should exist on opposite riverbanks. Non-sister relationships suggest the river could be a meeting point for taxa that diverged elsewhere, and is only a dispersal barrier.

C. Similarity in species composition on opposing banks should be highest where the barrier effect is reduced.

D. Similarity in species composition on opposite banks should be higher for species that can colonise *várzea forest*, than for species restricted to *terra firme* forest.

E. Divergence times for all taxa on opposite banks should be similar, particularly in groups with similar characteristics.

F. Lineage divergence times should be congruent with estimated river formation times.

The seminal study by Ayres and Clutton-Brock (1992) provided compelling evidence for prediction 'C', showing that opposite bank similarity of primate assemblages declines significantly (and independently) with both increasing width and increasing annual discharge. Furthermore, they suggest similarity shows a secondary increase at the river mouth where sediment deposition produces islands that increase permeability. To address major changes in taxonomy and species concepts, recent revisions to Neotropical primate taxonomy and associated changes in species distributions, we repeat, and expand the scope of the classic biogeographic study of Ayres and Clutton-Brock (1992) to re-evaluate support for predictions of the RBH.

Methods

Study area

The Amazon drainage basin is a major component of the Neotropical region, comprising mostly lowland rainforest habitats. It extends across South America from the eastern Andean slopes to the Atlantic coast and across the Brazilian and Guiana plateaus, covering an area over 8 million km^2 (Sioli 1984). We selected twenty-five rivers for analysis, fifteen the same as analysed by Ayres and Clutton-Brock (1992), and ten additional rivers from the same watershed (Fig. 1; Supplemental Table 1).

Database development

To investigate primate community make-up along these riverbanks we conducted a major literature review into the current state of platyrrhine taxonomy. For this study we followed the classifications of Groves (2001a; 2005) and Mittermeier et al (2013). When these taxonomies disagreed over the classification of a species, or substantial taxonomic changes had occurred since publication, primary literature was used to include or exclude a given taxon. Data on the distributions of Neotropical primates were obtained from the Terrestrial Mammals Digital Distribution Maps of the IUCN Red List of Threatened Species Assessments 2008/2016 (IUCN 2016) and the online database of "All the World's Primates" (Rowe & Myers 2015). Shapefiles were imported into ArcMap 10.3.1 (ESRI, 2012) for exploration and comparison. All spatial records were screened and quality checked before inclusion. Taxonomic refinement has led to multiple identities for some taxa. The scientific name of each species was investigated to ascertain whether it was simply a duplicate masked by a synonym. Due to disagreement between authors on sub species we only included full species in analyses. We checked the distribution of all species by visual comparison to estimated primate distributions in Mittermeier et al (2013).

Geographical Information System (GIS) Model

Initial maps of species distributions indicated that distributions were generally spatially distributed within interfluvial areas, and clearly abutted by certain rivers. Despite distribution polygons in the model broadly following river lines, most distributions did not align perfectly with rivers, and we suspected many of these overlaps to be error, rather than true representations of primate ranges. We measured overlap areas, and a limit of 20,000km^2 was employed, so that any area smaller than this was discounted. To standardise the area of riverbank from which species were recorded we used a 60km buffer along each side of every river line. Distributions that crossed rivers at headwaters were not considered error, as headwaters are characteristically narrow with lower streamflow and pose less of a barrier to primates than river sections further downstream (Ayres & Clutton-Brock 1992). To avoid headwater permeability influencing results, distributions that appeared to have colonised the adjoining interfluve across the headwater only (defined as the first 20% of river length) were discounted.

Similarity Index

We calculated a similarity index using our GIS model (Fewster & Buckland 2001) for opposite bank primate communities of the twenty-five rivers. We measured similarity as (% species on side A common to side B) + (% species on side B common to side A) ÷ 2, as per Ayres and Clutton-Brock (1992). Additional to our analysis of similarity between riverbanks for multiple rivers of varying size, we tested how similarity changed between the headwater and the mouth of the Amazon River. To do this we divided the Amazon River into ten equal segments of 312 km and calculated similarity indices for each segment.

Measures of river size

We measured the average streamflow of rivers as the mean of all streamflow data points for a given river. River width, measured at the midpoint of each river during the dry season, was adapted from Ayres and Clutton-brock (1992). For additional rivers, we measured width at the midpoint of each river using Google Earth satellite imagery from the dry season. In our analysis of the segmented Amazon River we plotted all available streamflow data (GRDC, Germany) for the Amazon River against distance from the headwater and used the trendline to extrapolate streamflow values for each segment. Width for each segment was obtained by taking the average of ten within-segment measurements, using Google Earth satellite imagery from the dry season.

Statistical Analysis

We used R statistical software, version 3.3.2 (R Core Team 2016), for all statistical analyses. We tested data for normality with Shapiro-Wilk and Spearman's Rank Correlation tests for multicollinearity. We used Generalized Linear Models (GLMs) to examine the 'opposite-bank similarity response variable' as a function of explanatory variables, streamflow and river width, with binomial distribution of errors and the logit-link function (Warton & Hui 2011). All model variations were compared using the Akaike information criterion (AIC) and goodness of fit assessed by visual inspection of residual plots to detect violations of homogeneity of variance, normality of residuals and independence of both explanatory variables and residuals. We chose the model with the lowest AIC score as the best description of the observed data.

Results

Spatial database for Neotropical primates

Data screening began with 421 spatial records from three different datasets (Figure 2). We identified and removed 155 duplicates, 113 synonym name duplicates, 83 sub species and 2 erroneous records to generate a database containing shapefiles suitable for use in our model.

Similarity Index

The difference between similarity indices calculated for fourteen rivers, twenty-five years apart, is shown in Figure 3. Ayres and Clutton-Brock's (1992) index of similarity ranged from 38% to 100% with our revised index of similarity ranging from 34% to 100%. Although the range of similarities between studies is comparable, our opposite riverbank similarity percentages are generally lower than those calculated by Ayres and Clutton-Brock (1992). The Jari is the only river which maintained the same percentage similarity from both studies, and the river with the largest disparity between the studies is the Juruá which now exhibits 35% less similarity than previously calculated.

Multiple rivers: Generalized Linear Model

Binomial GLM output showed a highly significant negative relationship between streamflow (m^3/s) and the proportion of opposite bank similarity (GLM, $N = 25$, $Z = -6.05$, $P = <0.001$) (Fig. 4). The association between width and the proportion of opposite bank similarity was not significant (GLM, $N = 25$, $Z = 1.24$, $P = 0.21$, Goodness of fit residual deviance/null deviance = 0.52).

Amazon River: Generalized Linear Models

Binomial GLM output (Fig. 5) showed a significant negative relationship between streamflow and the proportion of opposite bank similarity across ten Amazon River segments (GLM, $N = 10$, $Z = -3.03$, $P = < 0.001$, Goodness of fit residual deviance/null deviance = 0.12). It also showed (Fig. 6) a significant negative relationship between river width and the proportion of opposite bank similarity across the ten river segments (GLM, $N = 10$, $Z = -2.40$, $P = < 0.01$ Goodness of fit residual deviance/null deviance = 0.52).

Discussion

Controversy surrounding the extent to which rivers are drivers of platyrrhine speciation through vicariance is ongoing. Key issues include: uncertainty over how to define species (Frankham et al. 2012; Groves 2001b; Isaac & Purvis 2004), species divergence estimates (Rull 2008; Rull 2011; Rylands et al. 2016) and river formation timing (Hoorn et al. 2010; Latrubesse et al. 2010). Research aiming to resolve platyrrhine phylogeny is ongoing (Jason et al. 2009; Osterholz et al. 2009; Perelman et al. 2011; Ray et al. 2005; Schrago 2007) and genetic and biogeographic investigations of the RBH have produced contrasting conclusions (Aleixo 2004; Ayres & Clutton-Brock ; Boubli et al. 2015; Claude et al. 2000; Díaz-muñoz 2012). In this study, based on the latest Neotropical primate species revisions and distribution data, we show a key prediction for the RHB still holds - that opposite bank dissimilarity increases with increases in discharge for multiple Amazonian Rivers and for width for the Amazon itself.

The main mechanism underlying species richness and endemism in the Amazon Basin is allopatric speciation. There are three principal ways in which rivers can function as landscape barriers and promote allopatric speciation (Ribas et al. 2012). Firstly, evolution proceeds along independent trajectories in distinct blocks due to river formation dissecting the landscape and dividing previously continuous populations, stranding primates on opposite riverbanks through vicariance. The RBH does not provide a strictly allopatric model because while genetic flow is hindered, there is not zero migration (Leite & Rogers 2013). Secondly, rivers inhibit dispersal of species from their centres of origin, causing them to be restricted to only one bank (Link et al. 2015). Finally, (compared to a landscape structure without barriers) when a species goes locally extinct on one riverbank, the probability of subsequent re-colonisation is lower. The role of the RBH in primate speciation through vicariance remains controversial and patterns should be broadly congruent between species with shared characteristics, i.e. ecological requirements and vagility (Moritz et al. 2000; Rocha et al. 2015). Recent research on Amazonian drainage evolution shows the complexity of past geologic events, and there is ongoing controversy over the dating of river formation (Hoorn et al. 2010; Latrubesse et al. 2010). Underlying the RBH in the Neotropics is the assertion that the Amazonian river system formed before the speciation that produced extant species (Kay 2015). Tectonic behaviour of the Central Andes led to the evolution of the Amazon drainage basin. Based on geological evidence, Hoorn et al. (2010) place the origin of major Amazonian rivers in the Miocene (~23.03 – 5.3 Ma), but others have determined younger dates and there are differences between rivers (Latrubesse et al. 2010). These discrepancies have implications for the interpretation of historical diversification. Concordance between estimated divergence times for multiple species on opposite river banks in the Amazon can provide support to both river formation timing arguments and the RBH (Boubli et al. 2015).

Ribas et al. (2012) analysed molecular data and found support for the RBH predictions 'E' and 'F' (Box 1) by estimating divergence times and showing *Psophia* (trumpeter birds) were unaffected by glacial cycles, but patterns of speciation were strongly associated with rivers suggesting river formation, not refugia, were the main cause of diversification. Similarly, Boubli et al. (2015), who focused on the Rio Negro and its largest tributary the Rio Branco, demonstrated divergence times were concordant for allopatric species of three primate genera separated by the Rio Negro: *Cacajao, Cebus and Callicebus* (0.83 – 1.85 Ma). These also coincide with the divergence times of *Psophia* studied by Ribas et al. (2012), adding validity to the argument for the RBH and supporting a Plio-Pleistocene origin for Amazonian drainage. Results for the Rio Branco were more ambiguous, with the Rio Branco providing an important physical barrier to primates, delimiting the ranges of six primate genera. However, they could not establish the extent to which the river was a vicariant agent. In contrast, Morales Jimenez et al. (2015) found spider monkey (*Ateles)* divergence times to be between 6.7 and 4 Ma, implying that Amazonian river barriers could not have been implicated in these divergences. Attaining estimates for speciation times from molecular phylogenies has increased chronological and explanatory power, allowing for more rigorous testing of alternative diversification hypotheses, though there is no overall spatial or temporal trend (Moritz et al. 2000; Rosenberger 1992; Rull 2008). Uncertainties surrounding paleogeographic events, combined with a scarcity of rigorous tests for mechanisms promoting speciation, have led to a lack of consensus, with many studies finding little congruence between species and few generalisations have emerged. Several studies have supported prediction 'B' (Box 1), showing sister lineages across opposite riverbanks - sharing a most recent common ancestor (Boubli et al. 2015; Leite & Rogers 2013; Lynch Alfaro et al. 2015b). However, phylogenetic analysis of tamarin species recovered non-sister relationships between taxa from opposite banks of the Juruá River, implying a lack of influence from river barriers on speciation on these primates (Jacobs et al. 1995). Conversely, a more recent assessment by Diaz-Munoz (2012) in the Panama Canal watershed provided support for the role of rivers in shaping genetic structure. Similarly, opposite-bank saddleback tamarins have been shown to have increased gene flow toward the headwater streams of the Juruá River, Brazil (Peres et al. 1996). However, because opposite-bank populations are not reciprocally monophyletic, the river was not described as a primary barrier for diversification. Shifting river courses may have resulted in occasional passive transfer of individuals across rivers.

Furthermore, predictions 'A' and 'B' (Box 1) are supported by research showing sister relationships and reciprocal monophyly between opposite-bank bird populations, supporting rivers as drivers of allopatric speciation (Aleixo 2004; Bates et al. 2004; Capparella 1987). Prediction 'C' (Box 1) states that the strength of any river to act as a barrier is a function of its width and flow. Accordingly, similarity between opposite riverbanks should be greatest where the barrier effect is least, such as for smaller rivers, at river headwaters, or at the river mouth. Similarity analyses conducted on opposite bank communities of birds, concluded that rivers played a vital role in shaping present day patterns of species composition (Hayes & Sewlal 2004; Oliveira et al. 2017). Alternatively, Gascon et al. (2000) performed the same analysis on frogs and small mammals between opposite banks of the Juruá river (a major tributary of the Amazon River) and found no evidence to support the RBH. Between taxonomic groups there is variation in sensitivity to vicariant mechanisms and this might explain contrasting diversification histories. Since the publication of Ayres and Clutton-Brock (1992) there have been significant methodological advancements in primate systematics. Taxonomic assessments were previously largely underpinned by the study of primate morphology. However, cytogenetic and molecular phylogenetic studies have provided increased detail on evolutionary relationships, often resulting in taxonomic revisions that increase species numbers (Link et al. 2015). Notably, in conjunction with advances in phylogenetics since the 1990s, there has been a shift in species concepts used in primatology. Testing hypotheses such as the RBH requires clear taxonomic and distributional species data, and similarity indices rest entirely on the notion of species. The established Biological Species Concept (BSC) has been criticised for the indeterminate status of allopatric species and an over-reliance on reproductive isolation to define species (Defler & Bueno 2007; Frankham et al. 2012). The increasing adoption of PSC is implicated in rising species numbers

(Groves 2001b; Groves 2004; Groves 2013). PSC is widely applied in most recent primate taxonomy research, and provided the foundation for our database. Our dataset included distribution information for primates at the species level which could represent a limitation, as it has been suggested that more recently diverged lineages could provide a more detailed picture of biogeographic processes (Oliveira et al. 2017). However, subspecies delimitation is prone to disagreement between authors and taxonomic assessments.

Our results, as compared with Ayres and Clutton-Brock (1992), illustrate how greater species numbers can impact conclusions reached in biogeographical research. Using the latest classifications, the percentage of similarity in primate community composition for nearly every river analysed is lower than similarity percentages based on older taxonomies. The Juruá river shows the largest discrepancy between the studies, with 35% less similarity than previously calculated. The evidence we present here prompts the conclusion that Ayres and Clutton-Brock (1992) underestimated the effect of the RBH on Neotropical primates.

Imprecision of mapped primate distributions in our model meant that some areas of estimated distribution overlap were considered unrepresentative of real world primate ranges and were consequently disregarded. To avoid increased similarity specifically across headwaters (due to increased permeability) from obscuring patterns of similarity more broadly, we discounted any distribution that crossed to the other side at the headwater only. Haffer (2008) criticises authors invoking the RBH for overlooking problems associated with the lack of spatial separation of populations in headwater regions. Nonetheless, scale is of critical importance and, where headwaters do allow localised gene flow, this does not prevent the application of the RBH for river sections further downstream.

Here we report evidence to support prediction 'C' of the RBH (Box 1), that similarity in the composition of opposite bank communities should be highest where the barrier effect is lowest. Our analyses showed streamflow to be a highly significant predictor of opposite bank similarity in primates. Congruent with Ayres and Clutton-brock (1992), the results of our models demonstrate that rivers with higher streamflow act as more substantial barriers to dispersal, exhibiting greater variation in community composition. Because the Amazon River is so large and spatially configurated with many tributaries, community composition was not only variant between opposite banks but was also variable along the length of the river, which was not the case for most other rivers in the watershed. Segmentation captured this more complex arrangement of species distributions, enabling us to show that the barrier effect is not constant. Segments towards the river mouth which are wider and have greater streamflow exhibit less similar opposite bank community composition than segments nearer the headwater. Our streamflow data was taken as the average of several monitoring stations per river and should therefore be more accurate than that used by Ayres and Clutton-Brock (1992). Notably, in their study there was a secondary increase in similarity towards the mouth of the Amazon River. This pattern of similarity might be expected due to decreased water speed and associated sedimentation which creates islands that facilitate dispersal between opposite riverbanks. However, our model did not capture this as we did not extend our analysis that far through the delta due to a lack of streamflow data for that area.

Our results did not support a significant influence of width on similarity across the twenty-five rivers tested. This result contradicts our findings for the segmented Amazon River and is at odds with the findings of Ayres and Clutton-brock (1992), and several studies of Amazonian bird composition (Hayes & Sewlal 2004; Leite & Rogers 2013; Oliveira et al. 2017). Some of the width data used in this analysis was obtained through measurement of satellite imagery, to provide mean estimates. This measure could be ineffective when attempting to identify predictors, as river width is highly variable. This limitation provides a possible explanation for the non-significant result and is supported by the finding of width as significant along the Amazon River, which used more robust width measures.

We made several methodological adjustments, in addition to the use of up-dated taxonomic and distribution information, compared with Ayers and Clutton-Brock (1992). Our use of GIS provides advantages over non-digital techniques, such as the use of finer-scale environmental data and the incorporation of intricately mapped distributions, especially useful as species ranges have been broken up through taxonomic splitting.

Due to significant variation in river characteristics and between taxonomic groups, it would be inappropriate to over generalize the barrier-effect of rivers on community composition (Link et al. 2015; Lynch Alfaro et al. 2015b). Mixed results demonstrate the extent of the complexities behind diversification. The capacity of a river to act as a barrier to species distributions and their capacity to prevent dispersal (Mitchell et al. 2015) is probably reduced when a meander loop is cut off or a new river course is carved out within the floodplain, transferring a portion of land to the opposite side of the river. Present understanding of this process is limited in terms of the extent of land that gets transferred or the frequency with which it happens (Haffer 2008). We suggest this process on geological timescales could allow even poorly dispersing primates to be passively transported across most small rivers and possibly large ones, convoluting RBH arguments or acting as a vicariance mechanism itself. Although beyond the scope of this study, further research should examine how the strength of a river to act as a barrier might be mediated by species-specific traits, such as the ability to colonise *várzea* forest and body size. Hayes and

Sewlal (2004) provided evidence for the former, showing that the barrier effect was enhanced for birds restricted to *terra firme* and Ayres and Clutton-brock (1992) found evidence for the latter by identifying a relationship between river size and the maximum size of species whose geographic range was restricted by the river.

Based on our results, rivers do broadly limit the distributions of Neotropical primates and appear to maintain diversity in the Amazon Basin by isolating populations. We have provided evidence in support of the RBH, showing that river width and streamflow separating communities on opposite riverbanks can explain variation in composition. This was a broad scale spatial analysis investigating patterns of community similarity within the context of riverine geography. Further phylogenetic research into the presence of reciprocal monophyly and sister taxa between riverbanks is required to determine whether rivers were the vicariant agent in the rapid diversification of Neotropical primates. To understand if rivers prompted allopatric speciation by dissecting previously continuous populations, consideration of timing is key. Neotropical diversification is associated with complex historical scenarios involving a range of spatial and temporal scales. Therefore, we argue that it is unlikely that any one theory can fully explain this diversity (Bush 1994; Cortés-Ortiz et al. 2003; Rull 2011). The vagaries of taxonomy make testing diversification theories challenging. We have demonstrated that results of older biogeographic studies should be viewed with caution, as incorporating the greater number of species now recognised can alter results. Accurate taxonomic and biogeographic information is essential for understanding the history of platyrrhine diversification and the processes that shaped their distributions.

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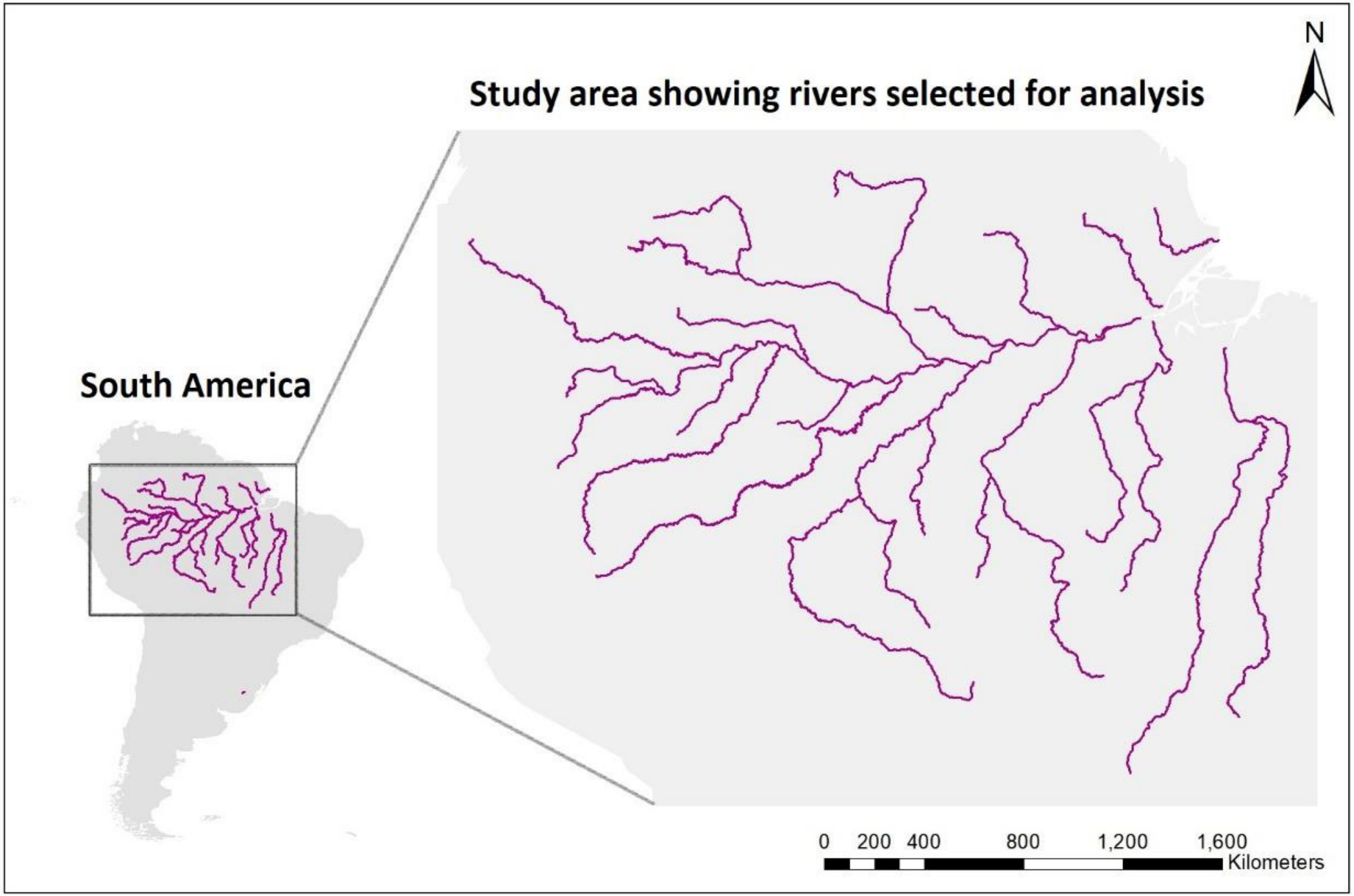
Figure 1(on next page)

Map of South America showing Amazon Basin watershed and rivers selected for analysis (Source data: Natural Earth Data).



Study area showing rivers selected for analysis
South America
N
0 200 400 800 1,200 1,600
Kilometers

Figure 2 (on next page)

Flow diagram summarising process of screening and selection of spatial records for development of a comprehensive neotropical primate distribution database (Flow diagram adapted from PRISMA 2009).

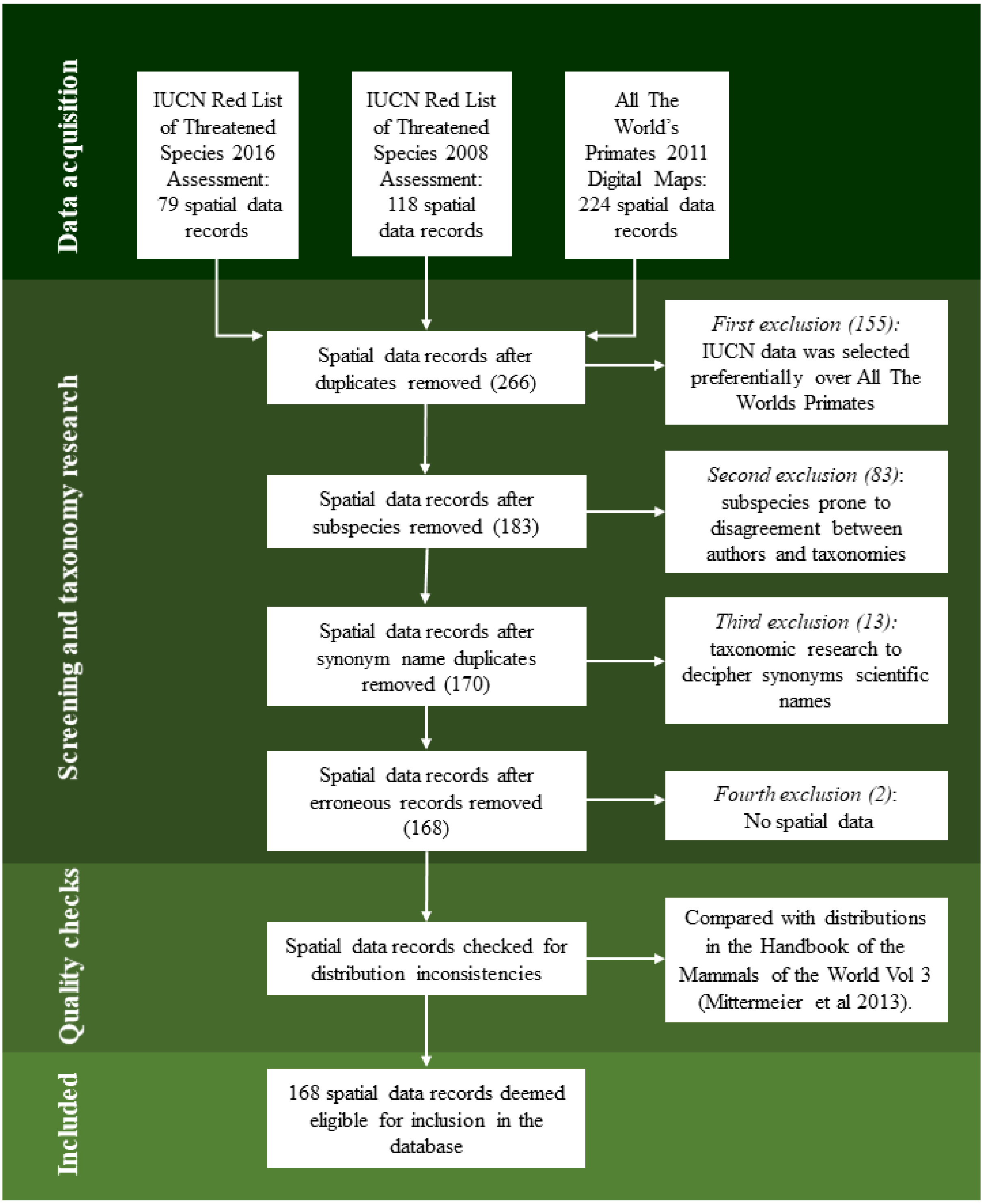

Data acquisition
IUCN Red List of Threatened Species 2016 Assessment: 79 spatial data records
IUCN Red List of Threatened Species 2008 Assessment: 118 spatial data records
All The World's Primates 2011 Digital Maps: 224 spatial data records
Screening and taxonomy research
Spatial data records after duplicates removed (266)
First exclusion (155): IUCN data was selected preferentially over All The Worlds Primates
Spatial data records after subspecies removed (183)
Second exclusion (83): subspecies prone to disagreement between authors and taxonomies
Spatial data records after synonym name duplicates removed (170)
Third exclusion (13): taxonomic research to decipher synonyms scientific names
Spatial data records after erroneous records removed (168)
Fourth exclusion (2): No spatial data
Quality checks
Spatial data records checked for distribution inconsistencies
Compared with distributions in the Handbook of the Mammals of the World Vol 3 (Mittermeier et al 2013).
Included
168 spatial data records deemed eligible for inclusion in the database

Figure 3(on next page)

Comparison of similarity indices for opposite riverbank primate communities in the Amazon watershed. Similarity measured as (% species side A common to B) + (% species side B common to A) ÷ 2.

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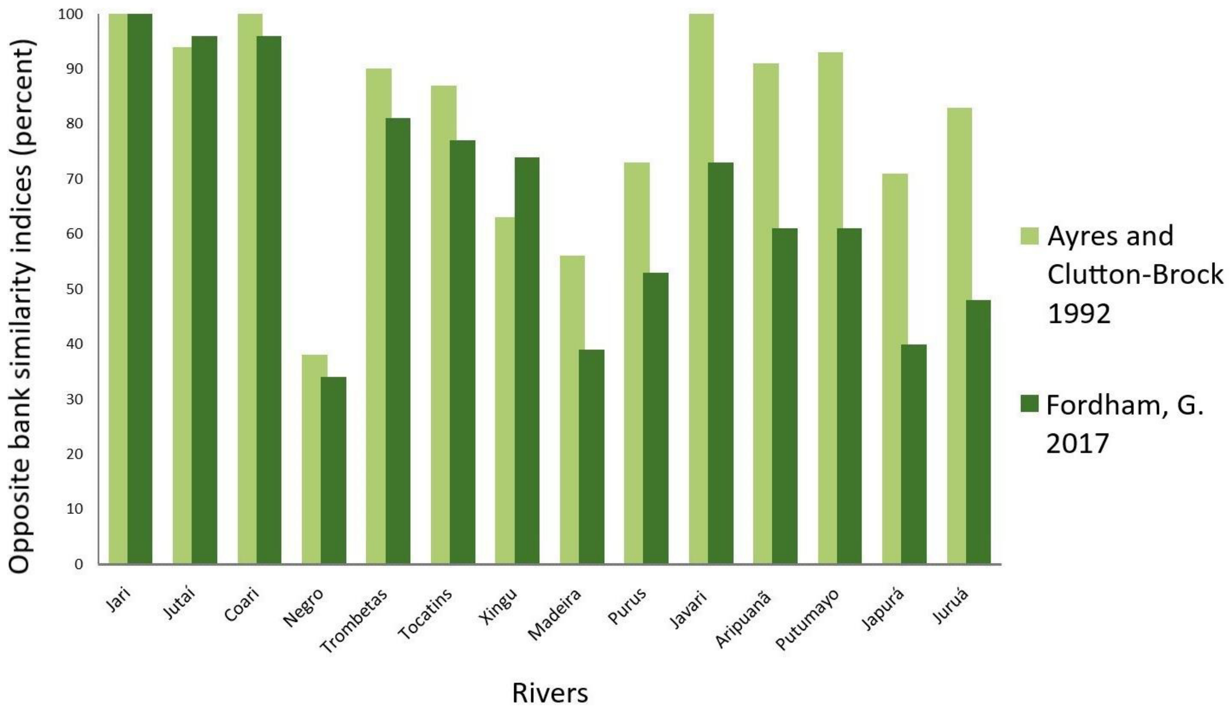
Opposite bank similarity indices (percent)
100
90
80
70
60
50
40
30
20
10
0
Jari
Jutaí
Coari
Negro
Trombetas
Tocatins
Xingu
Madeira
Purus
Javari
Aripuanã
Putumayo
Japurá
Juruá
Rivers
Ayres and Clutton-Brock 1992
Fordham, G. 2017

Figure 4(on next page)

Logistic regression curve and 95% confidence limits for the effect of streamflow on the proportion of similarity between opposite river banks of twenty-five rivers in the Amazon Basin.

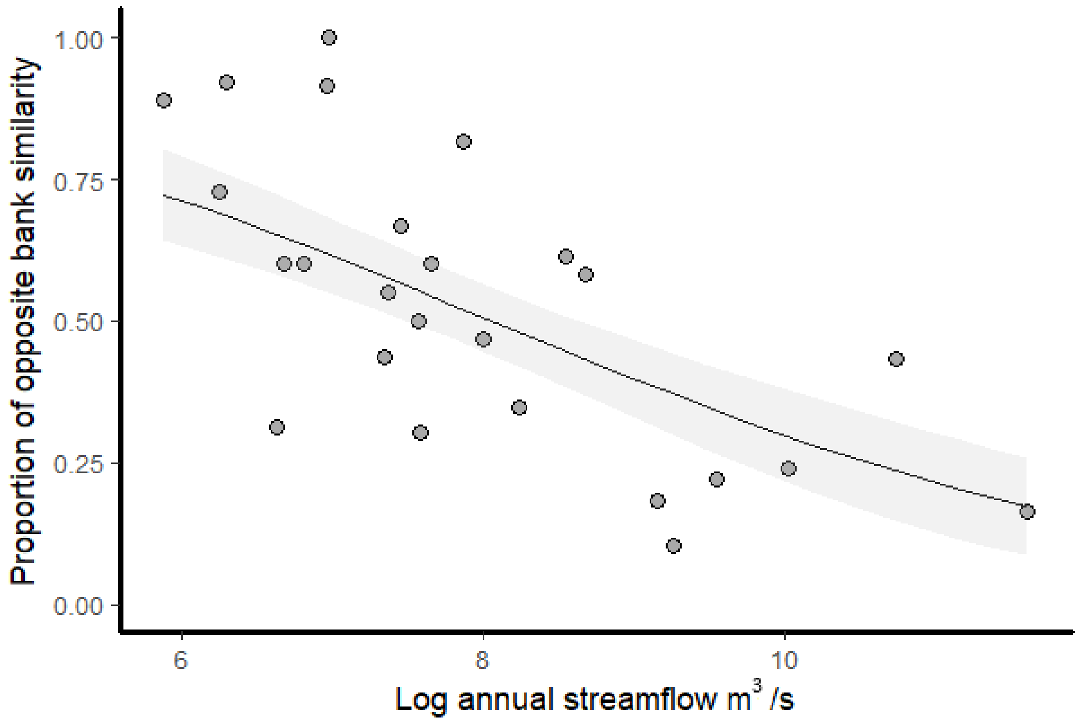
1.00
0.75
0.50
0.25
0.00
Proportion of opposite bank similarity
6
8
10
Log annual streamflow m3 /s

Figure 5(on next page)

Logistic regression curve and 95% confidence limits for the effect of streamflow on the proportion of similarity between opposite river banks across ten 312 km segments of the Amazon River.

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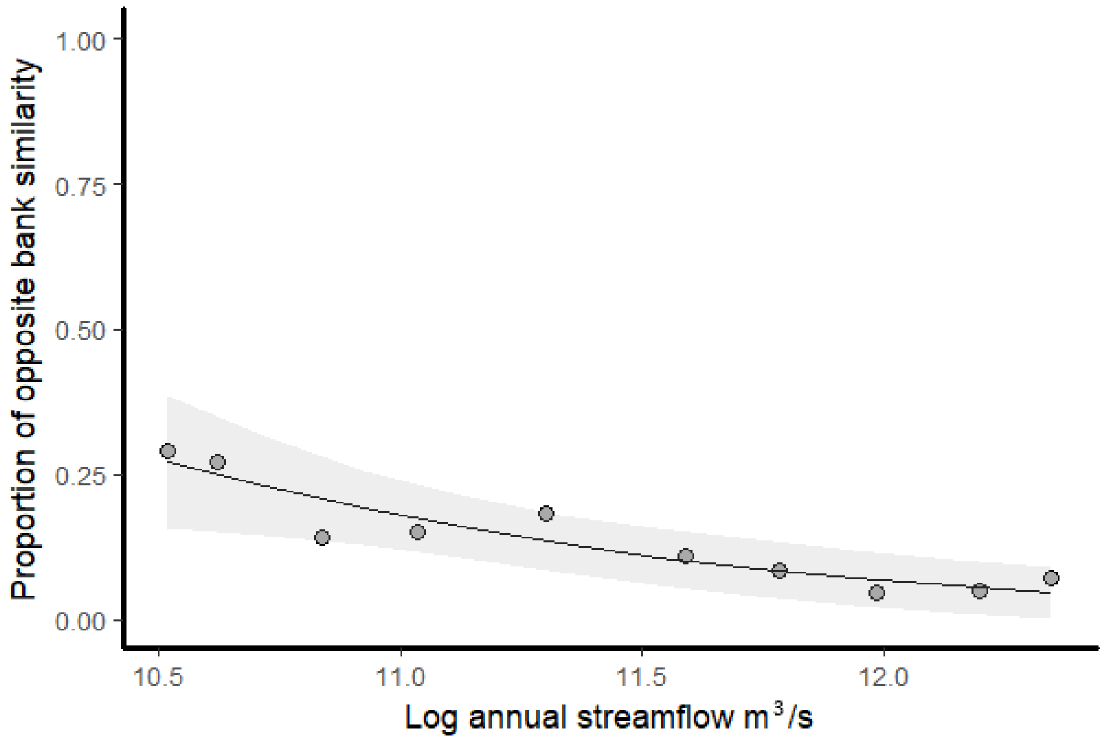

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Figure 6 (on next page)

Logistic regression curve and 95% confidence limits for the effect of river width on the proportion of similarity between opposite river banks across ten 312 km segments of the Amazon River.

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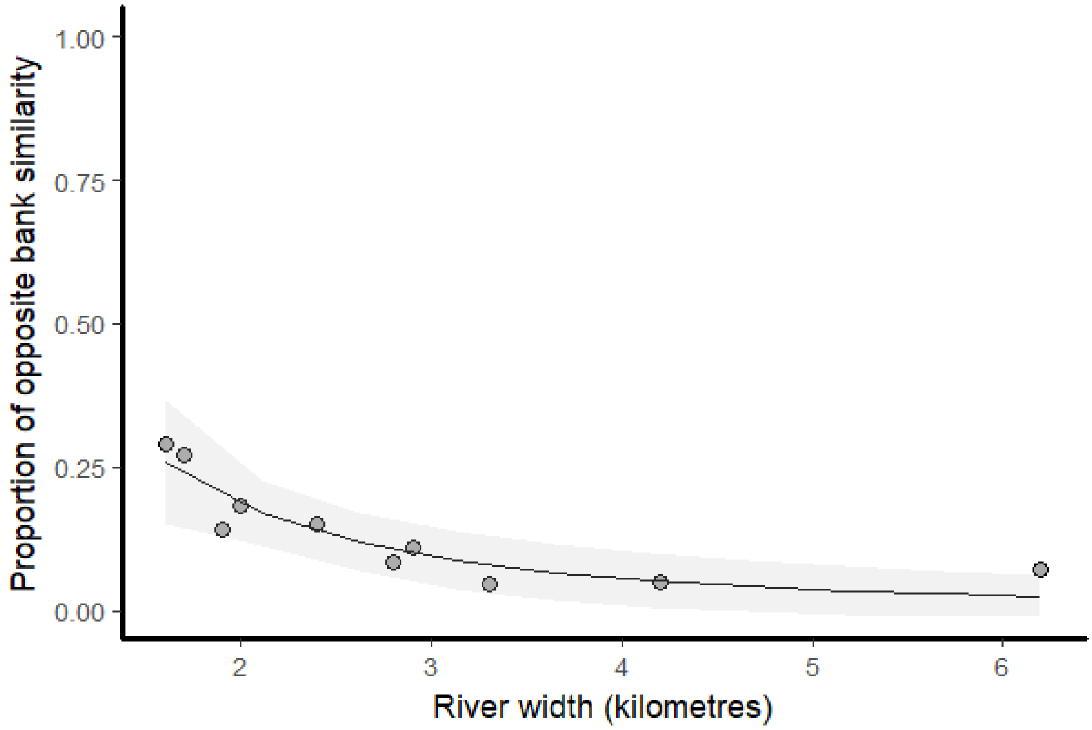

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